



Iran's 25th International Congress of

# MICROBIOLOGY

Mazandaran University of Medical Sciences

Sari

28-30 August 2024



IRAN'S 25<sup>TH</sup> INTERNATIONAL  
CONGRESS OF MICROBIOLOGY 2024

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Booklet of Abstracts

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## **The statement of the President of the Iranian Society of Microbiology**

Dear professors, respected colleagues and dear students

We held the 25th International Congress of Microbiology as our beloved country is in a difficult economic situation under the black shadow of sanction which has casted a shadow over all scientific and humanitarian activities. Despite difficulties, thanks to the grace of God and the efforts of loyal companions, the congress venue was successfully moved outside the capital city. The 25th Congress was well received in terms of the quality and the number of articles and participants.

This year's congress was scientifically designed according to the variety of subspecialties of microbiology in form of different organized scientific panels for the interested researchers. Today, the importance of water and wastewater microbiology as one of the main specialized branches of environmental microbiology and the realization of sustainable development is inevitable to everyone. This year's Congress focused on this matter, in cooperation with Mazandaran Province Water Resources Organization relying on many other basic sciences to interpret and solve problems related to the health and safety of the society. Furthermore, this year's Congress in cooperation with national and international experts in artificial intelligence managed to organize important and innovative fields of AI application in microbiology.

Fortunately, the 25th Congress communicating with active national and international institutions, including the European Society of Microbiology, has played a significant role in the development of microbiologists' activities and has taken effective steps in coordination and promotion of professional activities in Asia as well as worldwide.

In this challenging time in the history of science, when diagnosis and treatment in all fields and branches of medical science in the world is unimaginable without the help of microbiology, the value science and ethics of professors is doubled and it is hoped that all Respected members of the Iranian Society of Microbiology of Iran, with a longing for the past and the proud predecessors of this field, walk the path of science and ethics by maintaining the integrity and protection of the position of this field more firmly than before.

It is obvious to everyone that our veteran professors are shining stars who have given greatness and importance not only to the field of microbiology, but also to the level of health and treatment of our country. Therefore, on behalf of

myself and the board members of the association, I would like to thank all the veteran professors who participated in this scientific event and enlightened the gathering.

I will leave the discussion about the scientific contents to the summary booklet of the presented articles.

I would like to express my gratitude to the honorable board of Mazandaran University of Medical Sciences, especially the honorable president of the university, Dr. Gholami for his support for the presence of the country's scientists and microbiology elites in Sari. May God bless you all.

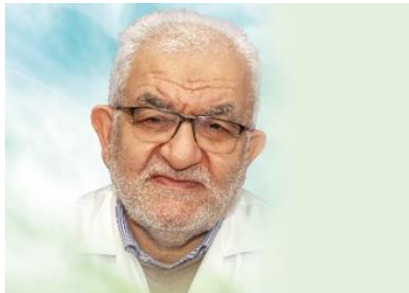


**Dr. Mohammad Mehdi Feizabadi**

## Scientific secretaries Welcome

Praise and thank God that it was possible to hold Iran's 25th International Congress of Microbiology in 2024 in the academic community of Mazandaran University of Medical Sciences. Education of knowledgeable and skilled individuals, production of science, creation of the national authority, and social welfare are the mission of universities and disease associations, therefore, considering the prevalence of infectious and emerging diseases and the resistance of pathogenic organisms including Superbugs, it is necessary to decide to prevent this terrifying problem and protect the world from a dreadful situation for the future generations.

In order to overcome the microbes, in addition to improving the tools, efforts should be made to reduce the unnecessary use of antibiotics. Now is the time to invest and implement solutions to prevent this terrifying global health problem. Therefore, all researchers, professors, and students in related fields are invited to actively participate and help us in this important congress, which is held in collaboration with the Iranian Society of Microbiology and Mazandaran University of Medical Sciences. So, holding such scientific congresses will contribute to the exchange of experiences among researchers. In this opportunity, we would like to express our gratitude to the Honorable President of Mazandaran University of Medical Sciences Dr. Farhad Gholami, and his esteemed assistants and also Dr. Mohammad Mehdi Feizabadi, the Honorable President of the Iranian Society of Microbiology, for their emphasis on and support for organizing this scientific event.



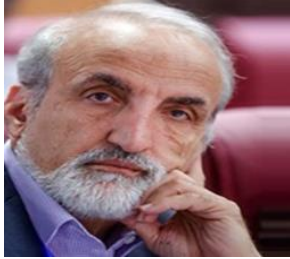
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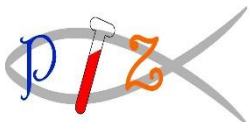
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# Assessment of the Synergistic Antibacterial Activity of Snail Mucus, Thymol, and Ferulic Acid in a Rat Model of Burn Wounds Infected with *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Burn is still one of the most devastating injuries in emergency medicine while improvements in wound healing knowledge and technology have resulted in the development of new antibiotics and dressings. This study was undertaken to evaluate the antimicrobial effect of snail slime, Thymol and ferulic acid in *Pseudomonas aeruginosa* and *Staphylococcus aureus* infected burn wounds of experimental rat model.

**MATERIALS AND METHODS:** Forty rats were subjected to random allocation across 10 groups of equal size. Groups A and B were respectively infected with *Staphylococcus aureus* ATCC25923 and *Pseudomonas aeruginosa* ATCC27853, while groups C and D were exposed to multi-drug resistant isolates of these bacteria. These infected groups were treated with a newly developed ointment. Each group was paired with a control group (groups E, F, G, and H), which received treatment with silver sulfadiazine ointment. Additionally, two groups (groups J and I) were designated as controls, receiving no medication and solely undergoing burn injury. A standardized second-degree burn wound was inflicted using a hot plate, covering approximately 20% of the total body surface area (TBSA) and maintained at a consistent temperature. Following 24 hours post-burn, 10<sup>8</sup> colony-forming units (CFU) of *P. aeruginosa* and *S. aureus* strains were inoculated onto the burned area. During the following 3, 7, 14, 21, and 28 days

**RESULTS AND DISCUSSION:** During the initial week following therapy, groups A to D exhibited limited reduction in burn wound size, inflammation, and reepithelialization. Nevertheless, by the 14th day, all groups demonstrated satisfactory and desired outcomes. The efficacy of silver sulfadiazine ointment in wound healing and antimicrobial activity did not significantly surpass that of the newly developed ointment. Our natural-based antimicrobial ointment has demonstrated remarkable efficacy in the healing of burn wounds infected with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The application of this ointment not only effectively eradicated bacterial infections but also accelerated the healing process, leading to significant improvements in wound closure and tissue regeneration.

**Keywords:** Snail Mucus, Thymol, Ferulic Acid, Burn, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

# Bioterrorism and artificial intelligence opportunities and treats

Bacteriology

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Bioterrorism and artificial intelligence (AI) are two rapidly evolving fields that intersect in complex and concerning ways. This paper explores the potential opportunities and threats posed by the convergence of these domains. The intersection of bioterrorism and AI presents both opportunities and threats that require careful consideration and proactive measures. Policymakers, researchers, and the public must work together to harness the potential of AI for biodefense while mitigating the risks of misuse and unintended consequences. Ongoing research, robust governance frameworks, and international cooperation are essential to address this complex and evolving challenge.

**MATERIALS AND METHODS:** "This paper is a review article based on research from databases covering the years 2010 to 2023."

**RESULTS AND DISCUSSION:** Opportunities AI-assisted pathogen detection and monitoring: AI algorithms can analyze vast amounts of data from various sources to rapidly detect and track the spread of biological threats. AI-driven vaccine and drug development: AI can accelerate the process of designing and testing new vaccines and drugs by simulating molecular interactions and predicting drug efficacy. AI-powered biosecurity systems: AI-enabled biosecurity systems can enhance physical and digital security measures to protect against bioterrorism threats. Threats AI-assisted bioweapon design: Adversaries could potentially use AI to optimize the design and production of bioweapons, making them more potent and difficult to detect. AI-powered disinformation campaigns: Malicious actors could leverage AI to create convincing fake news and propaganda related to bioterrorism, sowing panic and confusion. AI system vulnerabilities: AI systems used for biodefense could be vulnerable to hacking, manipulation, or unintended consequences, potentially amplifying the impact of a bioterrorism attack.

**Keywords:** Bioterrorism, Artificial Intelligence, Pathogen Detection, Biosecurity , Bioweapons

# Characterization of virulence factors, antimicrobial susceptibility, biofilm production, and molecular epidemiology of *Klebsiella pneumoniae* clinical isolates

Bacteriology

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**BACKGROUND AND OBJECTIVES:** The rise in multidrug-resistant pathogens poses a significant challenge in treating hospital-acquired infections, particularly those caused by *Klebsiella pneumoniae*. Biofilm formation is a critical factor contributing to antibiotic resistance, enhancing bacterial adherence and persistence. *K. pneumoniae* strains exhibit diverse virulence factors, influencing their pathogenicity and resistance profiles. This study aimed to characterize the virulence factors, antimicrobial susceptibility, biofilm formation, and molecular epidemiology of *K. pneumoniae* isolates from clinical samples in Hamdan hospitals.

**MATERIALS AND METHODS:** A total of 402 *K. pneumoniae* isolates were collected from various hospital departments. Standard laboratory methods confirmed their identity, followed by antimicrobial susceptibility testing and biofilm strength assessment using the microplate method. PCR analysis targeted a panel of virulence genes, including *fimH1*, *mrkD*, *kpn*, *ycfM*, *entB*, *iutA*, *irp-1*, *irp-2*, *ybtS*, *fyuA*, *traT*, *rmpA*, *magA*, *iroN*, *hlyA*, and *cnf-1*. Multilocus sequence typing (MLST) was employed to determine the sequence types (ST) of 10 selected isolates based on their virulence gene profiles and antibiotic resistance patterns.

**RESULTS AND DISCUSSION:** The findings revealed a high prevalence of *K. pneumoniae* (88.15%), predominantly isolated from tracheal samples (62%). Resistance rates varied, with ceftazidime being the most prevalent (305 isolates), while amikacin showed the lowest resistance (165 isolates). Biofilm formation was observed in a minority, with 17 isolates exhibiting strong biofilm production. Virulence gene distribution was diverse, with *fimH1* (87.31%) and *mrkD* (98.5%) being the most prevalent, while *hlyA* and *cnf-1* were absent. MLST analysis identified a variety of sequence types, notably ST147 (50%) and ST11 (30%). The study characterized the virulence factors, antimicrobial susceptibility, biofilm formation, and molecular epidemiology of *Klebsiella pneumoniae* isolates from clinical samples in Hamdan hospitals. The high prevalence of *K. pneumoniae* (88.15%) and diverse virulence gene profiles highlight the pathogen's heterogeneity and potential for varied pathogenicity. The predominance of *fimH1* and *mrkD* genes, associated with adhesion and biofilm formation, is concerning. The study also found that ceftazidime was the

**Keywords:** *Klebsiella pneumoniae*, biofilm, antibiotic resistance, virulence factors, molecular epidemiology



# Diphtheria toxin-based immunotoxin as therapeutic vaccine for lymphoma/leukemia

Bacteriology

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Therapeutic toxin-based cancer vaccines are relatively new potential and efficacious approach to assist patients with other resistant usual immunotherapies. Monoclonal antibodies are efficacious in leukemia treatment, however their potency as a sole agent is doubtful. For this reason, the binding domains of monoclonal antibodies are attached to protein toxins to intensify their effectiveness. Exploration of the pharmaceutical potential of some toxins to efficiently damage crucial cellular processes and/or destroy the target cell integrity, shed light on new way for toxin-based therapeutic vaccines in patients resistant to conventional treatments. Immunotoxins, are attached on and released into the leukemia/lymphoma cells depended on the cell surface target antigens. Recombinant immunotoxins have revealed a highly cytotoxic impact on leukemic/lymphoblastic cells in vitro and in early and late clinical trials phases in humans where there is no doubt that will play an important role in the treatment of leukemia/lymphoma.

**MATERIALS AND METHODS:** CD19 is a 95-kDa which categorized as a type I transmembrane glycoprotein and belonging to the B-cell-specific immunoglobulin superfamily that controls B-cell outcome at multiple stages of development by playing a critical role in keeping the balance between humoral, antigen-induced response and tolerance induction. CD19 is prominently expressed in almost all B-NHLs which makes it an outstanding aim in immune-based therapies. CD19 is widely expressed on both normal and malignant B cells including chronic lymphatic lymphoma, non-Hodgkin's B-cell lymphoma (NHL), and B-cell acute lymphoblastic leukemia. However anti-human CD19 monoclonal antibody drugs are previously in the clinic or in clinical trials, effective antibody treatment as an approach, requires assistance of the host effector mechanisms such as complement and NK cells.

**RESULTS AND DISCUSSION:** In contrary to the involved host effector cells, immunotoxins have the ability to remove target cells even when these mechanisms due to immunosuppressed agents or malnutrition are not functioning appropriately. Some chemical-conjugated anti-human CD19 immunotoxins have attained FDA approval (Ontak) and some are previously in different clinical trial phases. Fusion proteins designated as immunotoxins using truncated and genetically reduced diphtheria toxin immunogenicity and conjugated or genetically fused to anti-CD19 or other -CDs (25, 122, 132, ...) are currently under clinical evaluations for hematological malignancies.

**Keywords:** Immunotoxins, diphtheria toxin

# Evaluating the Clinical Efficacy of Two Meropenem Dosages in Critically Ill Patients with Hospital-Acquired or Ventilator-Associated Pneumonia: A Single-Blind Randomized Trial

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**BACKGROUND AND OBJECTIVES:** Hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) are critical infections in intensive care units, leading to higher illness, mortality, and healthcare costs. Meropenem, a broad-spectrum antibiotic, is essential for treatment, but its optimal dosing is unclear. This randomized, single-blind trial compares two different doses of meropenem in critically ill patients with HAP or VAP. By assessing patient recovery, microbial eradication rates, and side effects, the study aims to identify the more effective dose. The goal is to provide evidence-based recommendations to improve treatment outcomes, enhance patient recovery, and manage antibiotic resistance in critical care.

**MATERIALS AND METHODS:** In this randomized clinical trial, 24 out of 34 eligible patients were assigned to receive either a high dose of meropenem (3 g every 8 hours, 11 patients) or a standard dose (2 g every 8 hours as a 3-hour infusion, 13 patients). The primary outcome measured was clinical success after 7 days, defined by patient survival, stable hemodynamics, improved Sequential Organ Failure Assessment (SOFA) score, and stable or improved PaO<sub>2</sub>/FiO<sub>2</sub> ratio. Secondary outcomes included 28-day mortality, length of ICU and hospital stay, and time to weaning from mechanical ventilation. The trial protocol is registered at IRCT.ir (number IRCT20100107003014N19).

**RESULTS AND DISCUSSION:** The clinical success rates were similar between the high dose and standard dose groups (54.5% vs. 38.5%, P=0.431). However, there was a significant reduction in the clinical pulmonary infection score in the high dose group compared to the standard dose group (P=0.038). The SOFA score also significantly decreased in the high dose group throughout the study (P=0.006). Secondary outcomes were comparable between the groups, except for a shorter duration of VAP treatment in the high dose group (11.8 ± 8.6 days vs. 19.1 ± 9.4 days, P=0.061). No significant adverse events related to meropenem were observed. The most frequently isolated bacteria from tracheal aspirations were *Acinetobacter* spp. (34.8%), *Klebsiella* spp. (32.6%), and *Pseudomonas aeruginosa* (19.5%), with 78% showing meropenem resistance according to E-test results. High dose meropenem appears to be safe and achieves a similar clinical success rate as the standard dose, potentially within a shorter timeframe.

**Keywords:** meropenem, critically ill, ventilator-associated pneumonia, gram-negative bacteria

# FliD-Urea B chimeric antigen in serodiagnosis of *H. pylori* infections

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Helicobacter pylori* (*H. pylori*), a Gram-negative, spiral-shaped bacterium, is prevalent worldwide, infecting over 90% of people in developing countries and half of the global population. Key virulence factors include FlaB, FlaA, VacA, CagA, HspB, UreA, and Omp18. *H. pylori* is linked to diseases such as gastritis, peptic ulcers, gastric cancer, atrophic gastritis, lymphoma, and non-Hodgkin's lymphoma. The genetic diversity of *H. pylori* and potential cross-reactivity necessitate using antigens from local strains for better test sensitivity and specificity. Recent research focuses on serological tests using chimeric proteins by expressing recombinant multi-antigens. UreB and FliD have shown high sensitivity and specificity in serological detection.

**MATERIALS AND METHODS:** Blood samples from 200 patients and 200 controls were used for evaluation of a home-made ELISA using chimeric recombinant FliD-UreaB antigen. An *H. pylori* strain isolated from an Iranian patient was verified biochemically and molecularly. Genomic DNA was extracted and used for PCR amplification of UreB and FliD coding sequences. The PCR products were joined by soieing PCR and cloned into the pET 28a expression vector. Positive clones were identified by colony PCR, and the pET28a-Fli-D-UreaB plasmid was isolated. The expression construct was transformed into *E. coli* BL21 cells. The bacteria containing plasmids were cultured, induced with IPTG, and the expression was analyzed by SDS-PAGE. The purification of recombinant chimeric FliD-UreaB was conducted using Nickel-NTA Affinity column. The sensitivity and specificity of purified antigen were analyzed by ELISA method.

**RESULTS AND DISCUSSION:** PCR amplification of FliD-UreaB resulted in PCR products of 624 bp and 597 base pairs. The sequencing results confirmed the identity of amplified products. Expression of chimeric recombinant FliD-UreaB protein resulted in a protein of about 40 kDa. Purification resulted in a highly purified protein appearing as a single band in SDS-PAGE. Analysis by homemade ELISA using a panel of *H. pylori* positive and negative sera revealed that chimeric FliD-UreaB antigen can detect *H. pylori* infection with a sensitivity of 91% and specificity of 85%. In conclusion, the results showed that chimeric antigens derived from FliD and UreB can be effectively utilized in serological tests to detect *H. pylori* infection

**Keywords:** *H. pylori*, Serodiagnosis, Chimeric antigen, FliD, UreaB

# Histone Deacetylase Inhibitor (HDACi) and Oncolytic Respiratory Syncytial Virus A2 Strain (RSV-A2) Combination in a Mouse Model for HPV-related Tumors

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Today, cervical cancer, which has a lot to do with HPV infections, is the second cause of cancer-related deaths among women. Due to the disadvantages of conventional treatments, oncolytic virotherapy is one of the new treatments. Considering the effect of each HDACi drug (SAHA) and oncolytic RSV-A2 virus in inducing different apoptosis pathways in tumor cells, this study aims to demonstrate their synergistic impact on inducing apoptotic cell death. The study investigated TC-1 cells as a cancer model caused by HPV in vitro and in vivo conditions and promises to be an effective and safe treatment method in the future.

**MATERIALS AND METHODS:** The study used the MTT ELISA test to investigate the cytotoxic responses of RSV-A2 and SAHA on TC-1 cells (RSV-A2, SAHA, and PBS) in vitro. Also, the apoptotic responses were assessed using flow cytometry and real-time PCR. In vivo, TC-1 cells were used to evaluate antitumor effects in the five groups (PBS, Vehicle, RSV, SAHA, and RSV-SAHA). For this purpose, subcutaneous injections of  $9 \times 10^5$  TC-1 cells into C57BL/6 mice were conducted. After 10 days, the RSV-A2 was injected twice with an interval of one week, and SAHA was injected every other day until the 28th day. The spleens of three mice were removed for immunity tests (LDH, MTT, cytokines (IL-4, IL10, IFN- $\gamma$ )) by ELISA method, and TRAIL expression in tumor tissue was evaluated. The mice were then followed up for six weeks.

**RESULTS AND DISCUSSION:** The in vitro results demonstrated that the RSV-A2 and SAHA are cytotoxic for TC-1 cells and can induce apoptosis in them. In the in vivo study, the combined RSV-SAHA group had a better immune response than the RSV, SAHA, and controls (P0.0001). Tumor size in the combined group showed a significant difference compared to other groups (P0.001). Also, TRAIL expression increased in the RSV-SAHA group compared to the monotherapy and control groups (P0.0001). According to the in vitro and in vivo results, the combined group (RSV-SAHA) in inducing anti-tumor immune responses and the expression of apoptotic factors can have a high potential in cervical cancer therapy.

**Keywords:** Cervical cancer †Papillomavirus† RSV-A2† HDACi

# personalized bacteriophage therapy till 2024

Bacteriology

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** In spite of reports on successfully treatment of infections using personalized bacteriophage therapy (BT), non personalized Bt didn't show expected results in studies.

**MATERIALS AND METHODS:** In this review, the cases of successful Bt on difficult-to-treat infections and their outcomes were expressed. The infections were related to lower respiratory tract, skin and soft tissue and bone infection.

**RESULTS AND DISCUSSION:** Eradication of infected bacteria and also clinical improvement of infections in 70% of cases were revealed clearly. Bacteriophage-antibiotic synergy was significant in the treatment. Low rate of adverse effects was observed. Based on findings, Bt in combination with antibiotic can be very effective in designing clinical trials.

**Keywords:** personalized bacteriophage therapy, infection outcome, difficult to treat infections

# Potential of *Lactobacillus acidophilus* to modulate cytokine production by peripheral blood monocytes in patients with endometriosis

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Endometriosis is defined as the presence of endometrial tissue outside the uterine cavity. Peripheral blood monocyte cells (PBMCs) may have altered function to some extent in women with endometriosis. *Lactobacillus acidophilus* is a probiotic bacterium within the human body with the ability to alleviate many inflammatory diseases. Here, we examined the effect of *L. acidophilus* on PBMCs of endometriosis patients.

**MATERIALS AND METHODS:** In this study, peripheral blood samples were obtained from endometriosis patients (n=11) and non-endometriosis individuals (n=11). After isolation of peripheral blood mononuclear cells with Ficoll, cells were cultured in the presence and absence of phytohemagglutinin. Also, these cells were co-cultured with  $1 \times 10^6$  CFU/ml of *L. acidophilus*. IL-6 and IL-1 cytokines were measured by ELISA method and the two groups were evaluated and compared.

**RESULTS AND DISCUSSION:** The results showed that in endometriosis patients, the production of pro-inflammatory cytokines, including IL-1 and IL-6, by PBMC was increased compared to non-endometriosis subjects, and stimuli such as PHA intensified this elevation. Also, *L. acidophilus* increased the levels of pro-inflammatory cytokines including IL-1 and IL-6. However, the production of these cytokines decreased due to the modulatory properties of bacterial cells after 48 h. According to the results of the current study, IL-1 and IL-6 production was significantly increased in PMBCs of endometriosis patients compared to that of the healthy controls. Also, *Lactobacillus acidophilus* was considered as an antigenic compound and induced IL-1 and IL-6 production. According to these results, probiotics can be further used for the treatment of endometriosis patients and more investigations are needed to confirm these results.

**Keywords:** Endometriosis, probiotics, cytokines

# Principles of common diseases between animals and humans

Bacteriology

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** The intersection of human and animal health has garnered increasing attention in recent years, particularly in the context of zoonotic diseases—those that can be transmitted between animals and humans. This paper explores the fundamental principles underlying common diseases affecting both populations, emphasizing the shared biological, environmental, and behavioral factors that contribute to disease emergence and transmission. We analyze key zoonotic diseases, such as rabies, influenza, and COVID-19, highlighting their pathways of transmission and the role of wildlife, domestic animals, and human activities in their spread. Furthermore, we discuss the implications of these shared diseases for public health, veterinary medicine, and ecological conservation, advocating for a One Health approach that integrates human, animal, and environmental health strategies. By fostering interdisciplinary collaboration and enhancing surveillance systems, we aim to improve disease prevention and response efforts. This paper ultimately underscores the necessity of understanding the interconnectedness of human and animal health to mitigate

**MATERIALS AND METHODS:** This study employs a comprehensive literature review and meta-analysis approach to investigate the principles of common diseases between animals and humans. We systematically collected data from peer-reviewed journals, governmental health organization reports, and relevant textbooks to synthesize existing knowledge on zoonotic diseases.

**RESULTS AND DISCUSSION:** Investigating emerging zoonotic diseases: Identifying novel pathogens with the potential for cross-species transmission and developing early detection and response mechanisms. Evaluating the effectiveness of One Health interventions: Assessing the impact of integrated approaches to zoonotic disease prevention and control in various settings. Exploring the role of climate change and environmental factors: Examining how changes in climate and ecosystem dynamics may influence the emergence and spread of zoonotic diseases. This study highlights the fundamental principles underlying common diseases between animals and humans, emphasizing the need for a One Health approach to effectively prevent and control zoonotic diseases. By fostering interdisciplinary collaboration, strengthening disease surveillance systems, and promoting public awareness, we can work towards a healthier future for both human and animal populations. Continued research and policy development in this area are crucial to mitigate the risks posed by zoonotic diseases and ensure global health security.

**Keywords:** Zoonotic diseases, One Health, disease transmission, public health, veterinary medicine,

# Relationship between gut microbiota and inflammatory cytokines in inflammatory bowel disease

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract, including Crohn's disease and ulcerative colitis. One of the effective factors for its occurrence is the loss of homeostasis between the immune system and the gut microbiota. This study aimed to determine the correlation between gut microbiota and inflammatory cytokines including Interleukin-1, Interleukin-6, and TNF- $\alpha$  in IBD.

**MATERIALS AND METHODS:** In this study, DSS (dextran sulfate sodium) was used to induce inflammation in a mouse model every day and mice sacrificed on day 28. The gut microbiota composition and inflammatory cytokines levels were studied using real-time PCR and ELISA. SPSS Statistics 26 software and Kendall's tau-b test were used to investigate the correlation between the results of changes in the intestinal microbiota population and the inflammatory cytokines.

**RESULTS AND DISCUSSION:** The results of this study showed that there was almost a significant relationship between the changes in the microbiota composition of mice and inflammatory cytokines. In some phyla including Bacteroidetes and Proteobacteria, a positive relationship was seen, and with the increase in the population of bacterial groups, the amount of cytokines also increased. On the other hand, there was an opposite relationship between Firmicutes and inflammatory cytokines. According to the results, gut microbiota is one of the most important agents for the development of IBD. Some of inflammatory cytokines has increased in IBD and had a significant relationship with the changes in the bacterial population. Enterobacteriaceae, had a significant relationship with the inflammatory cytokines and caused an increase in the amount of inflammatory cytokines and severity of inflammation.

**Keywords:** Inflammatory bowel disease, gut microbiota, inflammatory cytokines, dextran sulfate sodium



# Zoogeographic survey of protozoan infectious diseases in the Caspiansea sea area of northern Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Many geographical phenomena affect the distribution of parasites, vectors, hosts and human health. Since thousands of years before the knowledge of microbes, attention has been paid to the role of climate in disease and health. Iran's climate and its impact on endemic diseases have always been and still are the focus of experts. The north of the country has a Mediterranean climate due to its proximity to the sea and the Alborz Mountain range. The dominant climatic factor in the region is precipitation and humidity. These favorable conditions can affect the spread of parasitic diseases, rodents and vectors. This study tries to review the geographical distribution of zoonotic protozoan infectious diseases in the Caspiansea area of northern Iran by reviewing the available data, and to achieve this goal some statistics on the prevalence of zoonotic protozoan infections in different cities the northern strip of Iran is mentioned.

**MATERIALS AND METHODS:** In this study, the studies conducted in the field of the prevalence of zoonotic protozoan parasitic infections in Gilan, Mazandaran and Golestan provinces (on the Caspian Sea area) were conducted. Also, the important role of geographical conditions on the spread of zoonotic protozoan infectious diseases, hosts (intermediate and definitive), vectors and reservoirs are discussed.

**RESULTS AND DISCUSSION:** According to various studies conducted in the cities near the Caspian Sea, the highest prevalence of zoonotic protozoa in humans is Toxoplasma and Giardia, and Cryptosporidium protozoa has a lower prevalence in humans than in animals. Also, the prevalence of leishmaniasis in the cities of the eastern part of Mazandaran province is higher than the two central and western parts. In addition, the results of the investigation showed a high level of contamination of animals, dogs and rodents in these areas with zoonotic protozoa compared to humans.

**Keywords:** Zoogeographic-protozoan infectious- Caspiansea area

# Severe Candidemia infections in critically Burn patients with COVID-19

Mycology

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Burn patients are highly susceptible to invasive fungal infections due to their compromised skin barrier, the widespread use of broad-spectrum antibiotics, and weakened immune systems. The COVID-19 pandemic has further complicated this situation by contributing to a rise in fungal infections, including candidemia. *Candida* is a common pathogen encountered in intensive care units (ICUs), and candidiasis significantly contributes to high mortality in ICUs patient. Therefore, we aimed to evaluate fungal blood infection in burn patients underlining COVID-19.

**MATERIALS AND METHODS:** Out of 335 burn patients admitted to the ICU. Blood samples were collected from patients suspected of having an infection. The initial identification of isolates was performed using CHROMagar *Candida* based on morphological characteristics. Further identification was conducted through PCR and Restriction Fragment Length Polymorphism (RFLP) analysis to ensure accurate species determination. Antifungal susceptibility testing of the identified fungal isolates was performed based on the CLSI-M27-S4 guidelines.

**RESULTS AND DISCUSSION:** Out of the 56 patients with concurrent COVID-19 enrolled in this study, a total of 29 isolates were obtained from blood cultures. Out of the 56 patients, 29 had candidemia while 27 experienced colonization. *Candida parapsilosis* emerged as the most frequently isolated species candidemia patients. During the patients' stay in the ICU, 21 fatalities were recorded, with a mortality rate of 43.8% among colonized patients and 69.0% among those with candidemia. Fluconazole and itraconazole exhibited the highest minimum inhibitory concentrations (MIC), whereas luliconazole and amphotericin B demonstrated the greatest efficacy. In conclusion, our study revealed a high prevalence of candidemia and *Candida* colonization among burn patients with concurrent COVID-19, emphasizing the importance of early diagnosis and prompt antifungal treatment in this vulnerable patient population.

**Keywords:** COVID-19, Candidemia, *Candida* species, Antifungal susceptibility

# Network Analysis of Altered MicroRNAs in Helicobacter pylori-Infected Gastric Cancer

Diagnostic Bacteriology

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**BACKGROUND AND OBJECTIVES:** Helicobacter pylori (H. pylori) infection is a well-established risk factor for gastric cancer, contributing to the alteration of various cellular pathways through changes in microRNA (miRNA) expression. This study aims to perform a comprehensive network analysis of miRNAs altered in H. pylori-infected gastric cancer and their regulatory effects on target genes. By leveraging advanced bioinformatics tools, we will elucidate the complex interactions between these miRNAs and their target genes, highlighting potential biomarkers and therapeutic targets for gastric cancer.

**MATERIALS AND METHODS:** We extracted miRNAs associated with H. pylori-infected gastric cancer from the study by Xu et al. and conducted a network analysis using Cytoscape software. Key hub genes and pathways were identified through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses.

**RESULTS AND DISCUSSION:** The findings reveal significant alterations in several miRNAs, including let-7a, miR-31, miR-101, miR-141, miR-203, miR-210, miR-218, miR-375, and miR-449, which are downregulated, and miR-17, miR-20a, miR-21, miR-146a, this integrative network analysis provides a detailed understanding of the miRNA-mediated regulatory landscape in H. pylori-infected gastric cancer, offering novel insights into potential diagnostic and therapeutic avenues. The identified miRNAs and their target networks serve as promising candidates for further investigation in the context of gastric cancer treatment.

**Keywords:** Helicobacter pylori, Gastric Cancer, Network analysis, subnetwork analysis, Promoter motif

# The epidemiological situation of Aedes borne Diseases in Iran and the world

Epidemiology of infectious diseases & antimicrobial resistance

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Aedes-borne diseases, such as dengue fever, chikungunya, and Zika virus, have been a growing concern in recent years due to their increasing global spread. In Iran, the presence of Aedes mosquitoes has been documented, raising concerns about the potential for outbreaks of these diseases. The objective of this study is to assess the epidemiological situation of Aedes-borne diseases in Iran and compare it to the global trends.

**MATERIALS AND METHODS:** Data on reported cases of Aedes-borne diseases in Iran were collected from the Ministry of Health and Medical Education. Global data on the incidence and distribution of these diseases were obtained from the World Health Organization and other relevant sources. The data were analyzed to determine the prevalence and distribution of Aedes-borne diseases in Iran and compare it to the global situation.

**RESULTS AND DISCUSSION:** The study results showed that while Aedes-borne diseases are relatively rare in Iran compared to other regions, there has been an increasing trend in recent years. The most common Aedes-borne disease reported in Iran was dengue fever. The global situation showed a similar trend worldwide. The findings highlight the need for continued surveillance and control measures to prevent outbreaks of Aedes-borne diseases in Iran and globally.

**Keywords:** Dengue, Aedes, Emerging

# New Strategies to Combat Resistant Bacteria in Healthcare Facilities

Microbial resistance and infection control

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Human strategies in interaction and take advantage of different organisms has often led to the domestication and breeding of animals and plants that had more benefit to human, while human interaction with pathogenic bacteria in the last 100 years has led to a completely opposite result. Misuse of antibiotics making bacteria wilder and more resistant in such a way that human finds himself unable to control these bacteria.

**MATERIALS AND METHODS:** In analysis of the causes of this failure, a comparison has been made between the human strategies used to interact with animals/plants and human strategies to interact with pathogenic bacteria.

**RESULTS AND DISCUSSION:** In this comparison, the first difference that comes to mind is that in plants or animals, humans have tried to choose/change their desired trait so that the organism becomes more favorable. In combatting diseases and health promotion human tried to eliminate all bacteria. In that way they used as much disinfectants and antibiotics as possible which results selecting more and more resistant strains. Actually, human action has led to pressure for selecting wilder and worse pathogenic bacteria. It is time to change our strategies and choose multidisciplinary strategies to manage pathogenic bacteria in medical and health centers. Pathogenic bacteria can be better managed with multidisciplinary. Among the new solutions are the use of antimicrobial peptides, the use of bacteriophages, and the use of non-pathogenic or less pathogenic strains in the environment.

**Keywords:** New Strategies, Resistant Bacteria, Healthcare Facilities

# “Utilization of Whole Genome Sequencing approach for surveillance of Mycobacterium tuberculosis in Lombardy Region in 2017-2019.”

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Since 1993, the year World Health Organization (WHO) declared tuberculosis (TB) a global public health emergency, has been estimated that 9.9 million people fell ill with TB. Although the incidence of TB has decreased in the European Union/European Economic Area (EU/EEA) in recent decades, certain subgroups of the population, such as immigrants, continue to be at high risk of the disease. According to the ECDC (European Centre for Disease Prevention and Control), there were an estimated 21,000 TB deaths in 2020, equating to 2.3 deaths per 100,000 people. In Italy, the Lombardy region has the highest number of hosted asylum seekers (13% of the national territory) of any Italian region.

**MATERIALS AND METHODS:** The main goal of this research project was to demonstrate the utility of Whole Genome Sequencing (WGS) based methodologies in routine diagnostic and epidemiological surveillance in the Lombardy Region of Italy. For this purpose, 1043 Mycobacterium Tuberculosis complex (MTBC) culture isolates were used in this study. Specifically in this research Library construction, Cluster Generation, and Sequencing were done after the extraction of DNA from TB samples. In the Final step, WGS analysis was performed by using of MTBseq pipeline on 999 MTBC strains collected in the Lombardy region during 2018-2020 to screen genetic mutations in drug-resistance-related genes, cluster analysis, and strain typing.

**RESULTS AND DISCUSSION:** The results revealed that the MDR, PRE-XDR/XDR clustered strains were more prevalent in the LAM and Mainly T families. In addition, drug-resistant strains are more distributed among L4 and L2. This study revealed lineages 2 to 4 have been linked to more disease and drug resistance than lineages 1, 5, and 6. Furthermore, it was discovered that L2 and L4 had higher proportions of clustered strains. The results in this study support the claim of the theory that WGS allows the simultaneous detection of DR to all first- and second-line anti-TB drugs throughout the genome, which could provide more information for clinical treatment, particularly for MDR/TB. This research suggests that WGS genotyping identifies cases with genetically similar TB isolates and this data helps us to detect clusters of possible recent and future transmissions.

**Keywords:** Whole Genome Sequencing, AMR

# The incidence of Health-care associated infections caused by *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolates and evaluation of antibiotic resistance among ICU patients of two hospitals in Tehran

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Healthcare-associated infections (HAIs) are a major public health concern in healthcare settings [1]. Multidrug-resistant bacteria, like *Klebsiella pneumoniae* and *Acinetobacter baumannii*, are the leading cause of the rise in nosocomial infections [2]. These infections are associated with increased mortality rates and treatment costs [3].

**MATERIALS AND METHODS:** In a cross-sectional study, 292 non-repetitive respiratory samples were collected from ICU-admitted patients of two teaching hospitals of Tehran University of Medical Sciences. *A.baumannii* and *K.pneumoniae* were identified using biochemical and molecular tests. The incidence of HAI caused by so-called bacteria was assessed. The antibiotic resistance pattern was evaluated and compared with demographic data such as mortality rate.

**RESULTS AND DISCUSSION:** Of 292 respiratory specimens, the HAI-causing *K.pneumoniae* and *A.baumannii* was 54.8%. Full antibiotic resistance against *A.baumannii* isolates was reported for carbapenems, cephalosporins, and  $\beta$ -lactam inhibitors. Moreover, a high antibiotic resistance rate to cephalosporins (100%) and  $\beta$ -lactam inhibitors (70%) was detected among *K.pneumoniae* isolates. The resistance to colistin was 12.2% and 15.9% among *A.baumannii* and *K.pneumoniae*, respectively. Almost, 83% of patients with HAI were expired ( $p \leq 0.05$ ). The appearance of a high HAI rate of MDR-isolates and subsequent increased mortality of patients who acquired HAI, highlights the importance of focusing on antibiotic stewardship practices. This involves accurate microbial diagnostic and drug susceptibility tests followed by rational antimicrobial prescription to alleviate the speed of antimicrobial resistance.

**Keywords:** *Acinetobacter baumannii*, *Klebsiella pneumoniae*, HAI, Multi-drug resistance

# Enhanced anti-biofilm activity of the minocycline-and-gallium-nitrate using niosome wrapping against *Acinetobacter baumannii* in C57/BL6 mouse pneumonia model

Respiratory infections

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**BACKGROUND AND OBJECTIVES:** *Acinetobacter baumannii* is a worldwide health issue in terms of its high antibiotic resistance and ability to form biofilms. Nanoparticles (NPs) with high biocompatibility, high penetrating ability, and low medication dose can successfully treat the antibiotic-resistant infections.

**MATERIALS AND METHODS:** In this research, several clinical samples were isolated from the lungs of patients hospitalized at Loghman hospital, Tehran, Iran. The biofilm formation of most lethal clinical isolates of *A. baumannii* was analyzed. The anti-biofilm activity of niosomes containing minocycline and gallium nitrate (GaN) against *A. baumannii* biofilm was determined. In order to improve their anti-biofilm properties, minocycline and GaN were encapsulated in niosomes as biocompatible drug carriers. The niosomes' size, zeta potential, shape, stability, drug entrapment efficacy, FTIR, drug release pattern, antibacterial activity, hemolysis assay and biocompatibility investigations were assessed. The pneumonia model was generated by intranasally administering *A. baumannii* suspension to anesthetized mice whose immune systems was compromised twice by cyclophosphamide. Lung infection of the mouse with *A. baumannii* was confirmed using PCR. After treatment, the lungs were excised under sterile conditions and stained with hematoxylin and eosin (H&E) to determine histological symptoms, inflammation, and intercellular secretions.

**RESULTS AND DISCUSSION:** The niosomes containing minocycline and GaN had an average size of 230 nm and a zeta potential of -40 mV, respectively. The percentage of drug entrapment and delayed drug release was both high in niosomal formulations. FTIR showed that two drugs had been entrapment in the niosome and had kept their nature. Niosomes containing minocycline and GaN dispersed 1, 3 and 5 days old biofilms. Medications not only did not have cytotoxicity but also increased cell proliferation compared to the control group. The mice given the combination of two compounds required less time to be treated than the animals given the single medication (minocycline). The minocycline& GaN-loaded niosomes could be considered as promising candidates to treat the infections caused by *A. baumannii* biofilm.

**Keywords:** Minocycline; Gallium Nitrate; Pneumonia Model; *Acinetobacter baumannii*;



# The efficacy of two triple therapy regimens and one quadruple regimen (bismuth, omeprazole, metronidazole with amoxicillin) in eradicating *Helicobacter pylori* in patients with peptic ulcer: a randomized clinical trial.

Abdominal/gastrointestinal, urinary tract & genital infections

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**BACKGROUND AND OBJECTIVES:** The eradication of *Helicobacter pylori* (*H. pylori*) is crucial in treating peptic ulcers (PU). Various regimens have been suggested to eradicate this organism. In this study, the effectiveness of three anti-*H. pylori* regimens were compared in patients with dyspepsia or PP.

**MATERIALS AND METHODS:** This randomized, open-label clinical trial included 136 patients with *H. Pylori* infection without a history of *H. Pylori* treatment. Patients were randomly divided into Three groups. The OAC group received 20 mg Omeprazole capsules twice a day, two 500 mg Amoxicillin capsules twice a day, and 500 mg Clarithromycin capsules twice a day, for 14 days. The OAL group received 20 mg Omeprazole capsules twice a day, two 500 mg Amoxicillin capsules twice a day, and Levofloxacin 500 mg capsules twice a day, for 14 days. The OAMB group received 20 mg Omeprazole capsules twice a day, two 500 mg Amoxicillin capsules twice a day, Metronidazole 500mg three times a day, and Bismuth 240 mg twice a day, for 14 days. Evaluation for compliance and drug-related adverse effects were assessed at the end of two weeks. *H. Pylori* eradication was evaluated eight weeks after treatment using the C14urease breath test(UBT).

**RESULTS AND DISCUSSION:** Result: A total of 136 patients participated in this study, and their groups were matched based on age and sex. The results of the UBT test showed that the eradication rate of *H. Pylori* was 82.2%, 91.3%, and 97.3% for the three-drug OAC, OAMB, and OAL treatment regimens, respectively. Moreover, all the regimens showed high compliance among the patients. Conclusion: The OAL regime achieved an acceptable rate of *H. Pylori* infection eradication with good tolerance in patients with PU without acute side effects.

**Keywords:** *H.Pylori*, Antimicrobial, eradication therapy, randomized trial, Triple therapy, *Helicobacter pylori*

# Antibiotic stewardship and emergence of MDR, XDR, and PDR bacteria

Resistance surveillance & epidemiology

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Many different definitions for multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacteria are being used in the medical literature. The European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) came together to create a standardized international terminology in all healthcare-associated bacteria. On the other hand, unappropriated use of antibiotics in medical centers and easy access to drugs for people can increase the emergence of antibiotic resistant strains in different parts of the world. This study aimed to evaluate the role of antibiotic stewardship in the emergence of resistant strains of bacteria.

**MATERIALS AND METHODS:** We searched for randomized controlled trials (RCTs) and observational studies published from 2020 to 2023 on PUBMED, Google scholar, and other scientific sites using the following keywords: “MDR” AND “XDR”, AND “PDR”, AND “Gram-negative bacteria”, AND “Definition” AND “Antibiotic stewardship”. Title and abstract screening were performed in order to check for consistency with the selected topic. Only studies published as full-text documents were reviewed.

**RESULTS AND DISCUSSION:** Kadri et al. retrospectively analyzed a cohort of 29474 inpatients with gram-negative blood infection at 173 US hospitals. A total of 46521 isolates were recorded: 28640 (61.6%) *Escherichia coli*, 9168 (19.7%) *Klebsiella* spp., 3221 (6.9%) *Enterobacter* spp., 4493 (9.6%) *P. aeruginosa*, 999 (2.14%) *A. baumannii*. Among the isolates, the difficult to treat (DTR) prevalence was 1.1% (n = 471), compared with 1048 (2.3%) carbapenem-resistant (CR); 4165 (9.0%) extended-spectrum cephalosporin resistance (ESCR); and 10240 (22%) fluoroquinolone-resistant (FQR). *P. aeruginosa* had a CR/DTR prevalence ratio of 4.5, reflecting the underlying susceptibility of many CR isolates to piperacillin-tazobactam (85.1% susceptible) and/or aztreonam (49.5% susceptible). Prevalence differences between CR and DTR were smaller but still significant for the other Gram-negative bacteria. Unadjusted mortality rate was 43% (202 of 471) in patients with DTR, 35% for CR (183 of 526), 22% for ESCR (609 of 2756) and 18% (795 of 4342) for FQR.

**Keywords:** MDR, XDR, PDR, Antibiotic, Stewardship

# Evaluation of antibiotic resistance pattern of *Staphylococcus aureus* isolated from clinical specimens in Qaemshahr Razi Hospital

Resistance surveillance & epidemiology

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**BACKGROUND AND OBJECTIVES:** *S. aureus* is one of the most important bacteria that cause hospital infections. Improper prescription and use of antibiotics causes resistant *S. aureus*. The aim of this study was to determine the prevalence and resistance patterns of *S. aureus* isolates from clinical specimens in hospital.

**MATERIALS AND METHODS:** This cross-sectional study conducted in during April 2023 to March 2024 in inpatients and outpatients Razi hospital. The culture was performed on the samples of abscess, urine, blood, sputum and biological fluids. Antibiotic susceptibility testing was performed by using the disk diffusion technique on Mueller-Hinton agar. Ten antibiotics recommended by the Clinical Laboratory and Standards Institute (CLSI) underwent testing.

**RESULTS AND DISCUSSION:** Results: Among the 363 culture-positive *Staphylococcus* samples, 89 (23%) were identified as *S. aureus* isolates. Out of these, 53 (59.5%) were determined to be Methicillin antibiotic-resistant *Staphylococcus aureus* (MRSA). High resistance was related to penicillin (100%) and high sensitivity was recorded for Rifampin (96%). The Abscess drain culture showed the highest number of culture-positive *S. aureus* samples among all the specimens. Conclusion: This study has shown an increase in antibiotic resistant *S. aureus* strains, particularly those resistant to penicillins. Therefore, early diagnosis is crucial in order to choose the appropriate treatment and prevent the further spread of resistance.

**Keywords:** antibiotic resistance, *Staphylococcus aureus*, Methicillin resistant *S. aureus*

# Pitfalls of polymyxin antimicrobial susceptibility testing: The new phenotypic methods for implementation in routine clinical laboratories

Susceptibility testing methods

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**BACKGROUND AND OBJECTIVES:** Polymyxins [polymyxin B and polymyxin E (colistin)], as antimicrobial cationic polypeptides, are last-resort antibiotics to treat serious infections caused by multidrug-resistant Gram-negative bacilli, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*. Due to the increased need for polymyxin treatment in critically ill patients, a significant effort is required to maintain antibacterial properties of these antibiotics. Updating the susceptibility data on polymyxins, including standardization of in vitro, and determining correct breakpoints is a critical issue both for patient care and epidemiological surveillance purposes.

**MATERIALS AND METHODS:** The in vitro susceptibility testing of polymyxins is influenced by various factors, such as the lack of a reliable reference susceptibility method, multicomponent composition of polymyxins, poor diffusion into agar, their cationic properties, the effect of polysorbate-80, as well as others (e.g. quality controls, the impact of medium, subcultures and storage). Currently, the reference test to evaluate the susceptibility of isolates to polymyxins is the broth microdilution (BMD) method. However, this method is associated with some technical issues. Several phenotypic methods have been reported as being promising for implementation in routine diagnostics, including polymyxin elution test, culture medium with polymyxins, rapid polymyxin NP test, and the Polymyxin Drop Test.

**RESULTS AND DISCUSSION:** It is expected that, in the future, laboratories will have access to these technologies via the reduction in equipment required and the input costs.

**Keywords:** Antimicrobial susceptibility testing, Gram-negative bacilli, Phenotypic methods, Polymyxins

# Introduction of Novel Drug Targets against *Staphylococcus aureus* and Proposing Putative Inhibitors against Adenine N1 (m1A22)-tRNA Methyltransferase (TrmK) using Computer-aided Drug Discovery

Resistance mechanisms

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**BACKGROUND AND OBJECTIVES:** Abstract: Background: Nowadays, the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA) strains has dramatically restricted the treatment options against this microorganism. Aim: In this study, we aimed to discover new drug targets and inhibitors against *S. aureus*.

**MATERIALS AND METHODS:** Methods: This study consists of two major sections. In the upstream evaluation, after a comprehensive core- proteome analysis, essential cytoplasmic proteins with no similarity to the human proteome were selected. Then the *S. aureus* metabolome-specific proteins were selected, and novel drug targets were identified using the DrugBank database. In the downstream analysis, a structure-based virtual screening approach was performed to reveal potential hit compounds against adenine N1 (m1A22)-tRNA methyltransferase (TrmK) using the StreptomeDB library and AutoDock Vina software. The compounds with a binding affinity -9 kcal/mol were analyzed based on AD- MET properties. Finally, the hit compounds were selected based on Lipinski's rule of five (RO5).

**RESULTS AND DISCUSSION:** Three proteins, including glycine glycosyltransferase (FemA), TrmK, and heptaprenyl pyrophosphate synthase subunit A (HepS1), were selected as feasible and promising drug targets based on PDB file availability and their essential role in the survival of the *S. aureus*. Finally, seven hit compounds, including Nocardiazine\_A, Geninthiocin\_D, Citreamicin\_delta, Quinaldopeptin, Rachelmycin, Di-AFN\_A1 and Naphthomycin\_K were introduced against the binding cavity of TrmK, as a feasible drug target. Conclusion: The results of this study provided three feasible drug targets against *S. aureus*. In the following, seven hit compounds were introduced as potential inhibitors of TrmK, and Geninthiocin\_D was identified as the most desirable agent. However, in vivo and in vitro investigations are needed to confirm the inhibitory effect of these agents on *S. aureus*.

**Keywords:** *Staphylococcus aureus*, structure-based virtual screening, TrmK, Quinaldopeptin, ADMET, VRSA.

# Epidemiology of Antimicrobial Resistance (AMR) in Iran

## Policy aspects of AMR

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**BACKGROUND AND OBJECTIVES:** In our country, Iran, the antimicrobial resistance mirrors global trends but shows distinct regional challenges. Here, we present the current state of AMR in Iran, focusing on the most prevalent pathogens and their resistance mechanisms. The misuse and overuse of antibiotics, facilitated by easy over-the-counter availability, have intensified this problem.

**MATERIALS AND METHODS:** The high prevalence of AMR in Iran significantly complicates public health management, particularly in healthcare settings. Resistant pathogens (especially, MDR, XDR and PDR phenotypes) limit treatment options, resulting in longer hospital stays, higher healthcare costs, and increased mortality.

**RESULTS AND DISCUSSION:** In Iran in 2019, AMR was responsible for 6,900 deaths and associated with an additional 24,900 deaths. The main pathogens of concern in Iran, along with the number of deaths associated with AMR, are *Acinetobacter baumannii* (4,500), *Staphylococcus aureus* (3,600), *Escherichia coli* (3,400), *Streptococcus pneumoniae* (3,300), and *Klebsiella pneumoniae* (2,600). Efforts to combat AMR in Iran must focus on improving antibiotic stewardship, enhancing infection control protocols, and maintaining rigorous surveillance systems. Public awareness campaigns are also crucial to educate the population on the dangers of antibiotic misuse and the importance of following prescribed treatments.

**Keywords:** Antimicrobial Resistance, Epidemiology, Surveillance, Iran

# Brucellosis Control and eradication programs in livestock

Veterinary microbiology/ Zoonotic diseases

Panel Oral Presentation

Karim Amiri <sup>1</sup> © P

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**BACKGROUND AND OBJECTIVES:** An important problem encountered by the veterinary authorities in countries affected by brucellosis is to select the sanitary strategy to be applied against the disease. Adequate organization of veterinary services is, without any doubt, the most important element to be taken into consideration by decision-makers previous to any potential selection of a sanitary programme. The economic costs of eradication programs are very important, and financial resources should be allocated to support the programs as an essential requisite prior to the selection of any eradication strategy. The adequate organization and involvement of Farmers is also another essential requisite for success in the implementation of even the simplest control strategy based on mass vaccination. Once the professional organization and the economic resources are fully adequate, the epidemiological unit of intervention should be defined.

**MATERIALS AND METHODS:** Collective prevalence (percentage of infected flocks)

**RESULTS AND DISCUSSION:** When the collective prevalence of Brucellosis (percentage of infected flocks) is very low (always less than 1% of flocks infected), a strategy based on a test and slaughter programmed and a ban on vaccination could be applied to eradicate the disease in the short to medium term in that particular epidemiological unit. In the case where prevalence is moderate (1-10 %), a combined eradication programmed based on the simultaneous application of vaccination in young replacements and a test and slaughter in adult animals could be recommended to eradicate the disease in the medium to long term. However, when the disease is highly prevalent (more than 10% of flocks are infected), even though the professional organization and the economic resources be fully adequate, the mass vaccination of all animals is the only reasonable strategy that can be applied to control the disease.

**Keywords:** Brucellosis, Control, Eradication, livestock, Strategy, Mass vaccination, Test and slaughter

# Isolation of *Burkholderia cepacia* complex (BCC) and *Streptococcus equi* subsp *zooepidemicus* from a suspected equine case of Glanders: A Case report

Veterinary microbiology/ Zoonotic diseases

Panel Oral Presentation

Nader Mosavari <sup>1</sup> © ©, Pejvak Khaki <sup>1</sup>, Shojaat Dashtipour <sup>1</sup>, Karim Amiri <sup>2</sup>, Seyed Mohammad Barani <sup>2</sup>, Alireza Ahmadi <sup>2</sup>, Arman Taherzadeh Eydani <sup>1</sup>, Mehdi Gare Khani <sup>1</sup>, Abbas Zarei <sup>1</sup>, Golamreza Kazemi <sup>1</sup>, Zahra Baradaran Seyed <sup>1</sup>, Mohsen Bashashati <sup>1</sup>

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<sup>2</sup> Iran Veterinary Organization

**BACKGROUND AND OBJECTIVES:** The Iran Veterinary Organization requested a Razi Vaccine and Serum Research Institute (RVSRI) research team to diagnose a suspected Glanders case in a male horse from Qom Province. The horse showed clinical symptoms such as swelling of lymphatic vessels with thick yellow pus discharge, severe respiratory distress, clear snoring sound, ulcers in the nostrils, and hemorrhagic and purulent nasal discharge. The complement fixation and mallein tests both resulted in negative findings, indicating the need for further investigation.

**MATERIALS AND METHODS:** Samples, including discharged pus from lymph nodes and nostril swabs, were taken and cultured on-site using biphasic media containing glycerin, TSA, and TSB, with and without antibiotics. The cultured samples were then transferred to the diagnostic laboratory at RVSRI and incubated at 37 °C for 48 hours. The next step involved extracting DNA from cultivated bacteria. We first performed PCR to identify the *Burkholderia* genus and then conducted recA PCR and TTS1 real-time PCR to identify *Burkholderia* species. Subsequently, a four-stage culture process was utilized to isolate single colonies from the bacterial growth in biphasic media.

**RESULTS AND DISCUSSION:** The *Burkholderia* was isolated from the lymph node pus culture. Both biochemical and molecular identification tests did not confirm the presence of *Burkholderia mallei* and *Burkholderia pseudomallei* species, indicating that the isolate belonged to the *Burkholderia cepacia* complex (BCC). Additionally, BCC and *Streptococcus equi* subsp *zooepidemicus*, were isolated from nostril swabs. This is the first report of the isolation of *Burkholderia cepacia* complex from a horse with glanders-like symptoms. It is crucial to consider the saprophytic pathogen of *Burkholderia* and use modern techniques in the differential diagnosis of glanders and melioidosis. Additionally, isolation and identification should be carried out according to standard operating procedures for human, animal, and environmental samples.

**Keywords:** Glanders, *Burkholderia*, *Burkholderia cepacia* complex, melioidosis



# Interactions of Bacteriophages with Eukaryotic cells

Phage therapy

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Bacteriophages or bacterial viruses infect and kill the bacterial hosts and were thought to have no specific receptors on eukaryotic cells, and were for a long time considered to be neutral to animals and humans. However, studies of recent years provided clear evidence that bacteriophages can interact with eukaryotic cells, significantly influencing the functions of tissues, organs, and systems of mammals, including humans. In this article, we summarize and discuss recent discoveries in the field of interactions of phages with eukaryotic cells.

**MATERIALS AND METHODS:** Possibilities of penetration of bacteriophages into eukaryotic cells, tissues, and organs can affect on functions of the immune system, respiratory system, central nervous system, gastrointestinal system, urinary tract, and reproductive system. Modulations of cancer cells by bacteriophages are indicated.

**RESULTS AND DISCUSSION:** It seems these interactions are crucial for understanding and developing bacteriophages as the therapeutic agents and pharmaceutical delivery systems. With the advancement and combination of in silico, in vitro, and in vivo approaches and clinical trials, bacteriophages definitely serve as useful repertoire for biologic target-based drug development to manage many complex diseases in the future.

**Keywords:** Bacteriophage, phage therapy, Eukaryotic cells

# New strategies for the production of recombinant bacteriophages and phage-encoded endolysins

Phage therapy

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** This study introduces innovative methods for producing genetically engineered bacteriophages, which are gaining traction in the food and pharmaceutical industries and phage therapy. These methods manipulate the phage genome through various advanced techniques, including mutagenic treatments, DNA recombination, and CRISPR-Cas systems.

**MATERIALS AND METHODS:** Mutagenic Agents involve treating phages with mutagens to induce mutations in the phage genome. While sequencing can identify the phenotypes of mutated phages, the effects of these mutations can be unpredictable. Recombination Techniques: Co-infection in bacterial hosts with two distinct phages can lead to novel phenotypes in the progeny. Also, Plasmids are utilized to facilitate the deletion, insertion, and replacement of specific nucleotides for homologous recombination between the phage genome and plasmid. However, this method often suffers from low recombination efficiency. CRISPR-Cas systems can enhance selection against wild-type phages. Type I systems, such as I-E and I-D, have shown effectiveness in counter-selecting recombinant phages, while type II systems, though less abundant, are studied for engineering purposes. Type III systems provide robust immunity against phages, making them ideal for selecting recombinant mutants. Genome Synthesis, Recent advancements allow the synthesis of phage genomes that can be propagated in bacterial hosts or cell-free

**RESULTS AND DISCUSSION:** This study highlights the potential of engineered phages in various applications, including enhancing antibacterial properties and targeting specific bacterial strains. Researchers can improve phages' host range and efficacy by modifying receptor-binding proteins. Strategies to improve enzyme properties include removing the cell wall binding domain, changing the catalytic domain's net charge, adding domains, DNA mutagenesis, chimerization of domains, and fusion with domains targeting outer membrane receptors or transport systems. Moreover, combining CRISPR-mediated techniques with traditional methods offers a promising avenue for developing phages with specific mutations. The efficiency of these methods can significantly reduce phage titers, thus improving the therapeutic potential of engineered phages. The ongoing research in phage engineering presents exciting opportunities for creating a new class of biological agents with diverse applications in medicine, agriculture, and biotechnology, paving the way for innovative solutions to combat antibiotic-resistant bacteria and enhance food safety.

**Keywords:** Bacteriophage, Endolysin, Genetic Engineering, Phage Therapy, Recombination

# Developing a Targeted Endolysin Fusion Protein for the Treatment of MRSA Infections

Antimicrobial peptides

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**BACKGROUND AND OBJECTIVES:** Introduction: Antibiotic resistance has increased in bacterial species, including *Staphylococcus aureus*. The shortage of new antibiotics has worsened the problem. *S. aureus* has developed ways to evade the immune response and survive antibiotic treatment. Endolysins, enzymes produced by bacteriophages, have shown promise in killing antibiotic-resistant bacteria like MRSA. The gene LY22 from phage  $\phi$ MR11 can lyse *S. aureus* but it lacks specificity. In this study, an engineered version of LY22 was created by combining it with a *Staphylococcus aureus* cell wall-binding domain to specifically target methicillin-resistant *S. aureus*.

**MATERIALS AND METHODS:** Methods and Materials: LY22 -CBD (CSTENZ) was constructed and its tertiary structure was predicted. The CSTENZ was then cloned, expressed, and purified. The antibacterial activity of the endolysins was evaluated by disk diffusion assay, turbidity reduction assay, and antimicrobial susceptibility testing. The Cytotoxicity of the CSTENZ was assessed against Human Skin Fibroblasts. The binding of the CSTENZ to the cell wall of *S. aureus* was evaluated. Thermal and pH stability of the CSTENZ was also assessed.

**RESULTS AND DISCUSSION:** Result and discussion: The 38.2 kDa CSTENZ fusion protein contains 343 amino acids with 22 negatively charged and 42 positively charged residues. Secondary structure prediction shows it is composed of 55.39% coil, 32.65% alpha-helix, and 11.95% strand. The recombinant CSTENZ was expressed in soluble form and purified, yielding 1.5 mg/L. CSTENZ exhibited potent antibacterial activity against MRSA, able to reduce titers by up to 3.39 log at 10  $\mu$ g/mL, with an MIC of 0.39  $\mu$ g/mL. It specifically binds to the MRSA cell wall but not to *E. coli* or *S. pyogenes*. CSTENZ is non-cytotoxic to human skin fibroblasts and remains stable for 4 weeks at 4°C, retaining activity in a pH range of 5.5-8.0. These results suggest CSTENZ is a promising candidate for MRSA treatment.

**Keywords:** Endolysin; Methicillin-Resistant *Staphylococcus aureus*; Antibiotic Resistance; Phage; enzyme Engineering

# Evaluation of Aureocin A53 as a Promising Therapeutic Agent for Multidrug-Resistant Mycobacterium tuberculosis

Antimicrobial peptides

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**BACKGROUND AND OBJECTIVES:** The significant worldwide prevalence of tuberculosis (TB) and the growing occurrence of drug-resistant strains of Mycobacterium tuberculosis (Mtb) highlight the pressing requirement for new antimycobacterial compounds. Considering their diverse antibacterial mechanisms and low cytotoxicity, antimicrobial peptides (AMPs) show promise as alternative or supplementary treatment options for drug-resistant tuberculosis (DR-TB). Aureocin A53 (AucA), a bacteriocin, exhibits significant potential as a therapeutic agent in the fight against antimicrobial resistance. The objective of this research is to examine the antimicrobial impact of AucA, which was extracted from Staphylococcus aureus, on drug-resistant strains of Mtb.

**MATERIALS AND METHODS:** Following a comprehensive sequence of screening, molecular, and conclusive confirmation tests, Staphylococcus aureus, a producer of AucA, underwent final purification utilizing ammonium sulfate concentration and High-Performance Liquid Chromatography (HPLC) methodologies. The Minimum Inhibitory Concentration (MIC) test, employing the Resazurin Microtiter Assay method, was subsequently executed utilizing a standard strain of Mycobacterium tuberculosis H37Rv. The investigative scope encompassed assessments of Rifampin-resistant (RR) and isoniazid-resistant strains of Mycobacterium tuberculosis, as well as multidrug-resistant Mycobacterium tuberculosis (MDR).

**RESULTS AND DISCUSSION:** Results: Our investigation revealed the efficacy of AucA against strains of M. tuberculosis, encompassing H37Rv, reference strains, clinical strains, and isoniazid-resistant strains of M. tuberculosis. The MIC for Mycobacterium tuberculosis H37Rv was observed to be 128 µg/ml, while for isoniazid-resistant Mycobacterium tuberculosis, it was 256 µg/ml. Notably, AucA demonstrated no cytotoxic effects on THP-1 macrophage cell lines at concentrations proximate to its MIC. Conclusion: Our initial findings indicate the presence of potential AucA candidates that can be effectively combined with anti-tuberculosis drugs. This underscores the importance of adopting a combined therapy approach as a novel strategy to amplify the effectiveness of current drugs, potentially yielding significant therapeutic advantages in the treatment of M. tuberculosis.

**Keywords:** Antimicrobial peptide, Bacteriocin, Aureocin A53, Mycobacterium tuberculosis

# Safety and therapeutic efficacy of three antimicrobial peptides on systemic infections in mouse models

Antimicrobial peptides

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Background: Melitin, pexyganan and TP4 are among the well-known peptides and several in vitro studies have proven their antibacterial efficacy. However, there is few evidence showing these peptides' safety and therapeutic efficacy on systemic infections in mouse models. Therefore, this study by examining the safety and therapeutic effects in animal studies, has taken a step towards studies on humans.

**MATERIALS AND METHODS:** Method: In the first step, in vitro toxicity profile of melittin, pexyganan, and TP4 was assessed using cytotoxicity and hemolysis tests. Then, LD50 and the maximal non-lethal intraperitoneal doses (ip) were determined using BALB/c mice. In addition, antimicrobial efficacy of melittin, pexyganan and TP4 against extensively drug-resistant *Acinetobacter baumannii* (XDR-AB), methicillin-resistant *Staphylococcus aureus* (MRSA), and KPC-producing *Klebsiella pneumoniae* (KPC-KP) pathogens were tested in vitro (MIC, MBC, TKC, Checker board) and in vivo.

**RESULTS AND DISCUSSION:** The results of in vitro toxicity tests showed that melitin (IC<sub>50</sub> = 6.45 µg / mL), pexiganan (IC<sub>50</sub> = 6.03µg/mL) and TP4 (IC<sub>50</sub> = 1.91µg/mL) had low cytotoxicity against normal human fibroblast cells. In addition, hemolytic activity against human erythrocytes of these peptide were as below: melitin (HD<sub>50</sub> = 0.44 /g / mL), TP4 (HD<sub>50</sub> = 5.08 µg/mL) pexiganan (HD<sub>50</sub> = 30.74 µg/mL). Ip LD<sub>50</sub> value of melitin, paxiganan, and TP4 in mice were 4.96 mg/kg, 11.68 mg/kg and 1.21 mg/kg, respectively. In vitro antimicrobial evaluation showed melittin and TP4, MIC range from 8 to 50 µg/mL and 8-32 µg/mL, respectively, but the pexiganan (MIC/MBC= 8-256 µg/mL) showed lower activity against all the studied clinical isolates. The results of time killing curve (TKC) assay showed that the bactericidal activity of all three studied peptides against gram-negative and gram-positive pathogens were concentration and time dependent. Checkerboard tests also showed that

**Keywords:** Antimicrobial Drug Resistance, Antimicrobial Peptides, Melitten, Pexiganan, TP4

# Targeted antimicrobial photo-sonodynamic therapy: A novel approach to combating oral microbial pathogens

Oral microbiology

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**BACKGROUND AND OBJECTIVES:** The emergence of antimicrobial resistance has posed a significant challenge in the management of oral infectious diseases. Conventional antimicrobial therapies often struggle to effectively eliminate persistent and resistant oral microbial pathogens. Targeted antimicrobial photo-sonodynamic therapy (aPSDT) has emerged as a novel approach that combines the selective targeting of pathogenic microbes with the synergistic antimicrobial effects of photodynamic therapy and ultrasound.

**MATERIALS AND METHODS:** The targeted specificity of aPSDT is achieved through the use of photo-sonosensitizers that selectively accumulate in the target pathogenic microbes, allowing for a localized antimicrobial effect while minimizing damage to surrounding healthy cells and tissues. The dual mechanism of action, involving the generation of reactive oxygen species (ROS) upon light activation and the enhancing effects of ultrasound, further improves the antimicrobial efficacy. Compared to conventional antibiotics, aPSDT has several advantages: broad-spectrum antimicrobial activity against virtually all microorganisms, low potential to induce resistance due to the non-specific ROS mechanism, and targeted delivery of sensitizers to infection sites for localized treatment.

**RESULTS AND DISCUSSION:** Notably, aPSDT has demonstrated the ability to overcome antimicrobial resistance, a critical advantage in the face of the growing threat of antibiotic-resistant oral pathogens. Numerous *in vitro* and *in vivo* studies have shown the superior antimicrobial activity of aPSDT compared to traditional antimicrobial therapies, highlighting its potential to improve clinical outcomes in the management of various oral infectious diseases, including periodontitis, endodontic infections, and peri-implantitis. As this technology continues to evolve, ongoing research focuses on optimizing the photo-sonosensitizers, light sources, and ultrasound parameters to further enhance the antimicrobial efficacy and minimize any potential side effects. The implementation of aPSDT in clinical practice holds promise as a novel and effective approach to combating persistent and resistant oral microbial infections.

**Keywords:** Antimicrobial resistance, Antimicrobial photodynamic therapy, Antimicrobial sonodynamic therapy

# Microbiome and cancer immunology

## Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** The microbiota has a key role not only in the host nutrients metabolism and saving of mucosal hemostasis but also contributes to immune reactions and immune system development. The microbiota and its metabolites have strange effects on the immune system. Innate immunity regulates gut microbiota combination and innate lymphoid cells produce cytokines such as IL-22 which is regulated tumor-derived inflammation.

**MATERIALS AND METHODS:** In this review, in accordance to some researches, intra tumor microenvironment microbiota modulate innate and adaptive immunity.

**RESULTS AND DISCUSSION:** In particular, types of microbes have different effects on the tumor progression and anti tumor activity. Microbiota have dual roles in the immune system. The studies have shown that various types of microbiotas have diverse effects on tumors. In dysbiosis, microbiota and its metabolites in tumor microenvironment can increase anti-tumor activity or tumor progression which is dependent on Th1 or Th2 responses and its related cytokines such as IFN- $\gamma$ , TNF- $\alpha$ , IL-10 and TGF- $\beta$ . On the other hand, immune response against tumor antigens is invigorated after recognition of molecular pattern such as LPS on the microbes by pattern recognition receptor. Interaction of microbiota and immune system resulted in cytokines production which are key roles in Anti CTLA-4 and AntiPD-1 immunotherapy.

**Keywords:** Microbiota; Cancer immunology, innate immunity, tumor microenvironment

# The Impact of Helicobacter Pylori on the Expression of Immunity and Metabolism-Related Genes in Gastric Cancer

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** Helicobacter pylori (H. pylori) is a well-established risk factor for gastric cancer, influencing various biological pathways including those involved in immune response and metabolism. Understanding the specific gene expression changes induced by H. pylori can provide insights into the mechanisms of gastric carcinogenesis and potential therapeutic targets.

**MATERIALS AND METHODS:** This study included 120 gastric cancer patients, comprising 70 males and 50 females, with a mean age of 56.2 years. Gastric tissue samples were collected from each patient, and the presence of H. pylori was confirmed using a rapid urease test and histopathological examination. Gene expression analysis was conducted using quantitative PCR to measure the expression levels of selected immunity and metabolism-related genes.

**RESULTS AND DISCUSSION:** The analysis revealed significant alterations in the expression of several genes in H. pylori-infected gastric cancer tissues. Notably, the expression levels of IL-1 $\beta$  (Interleukin 1 beta) increased to 750 units, TNF- $\alpha$  (Tumor Necrosis Factor alpha) to 620 units, and IL-8 (Interleukin 8) to 670 units. Metabolism-related genes such as GLUT1 (Glucose Transporter 1) showed an expression level of 880 units, while PPAR- $\gamma$  (Peroxisome Proliferator-Activated Receptor gamma) was at 540 units. These results indicate a significant impact of H. pylori on both immune and metabolic pathways in gastric cancer. Conclusion H. pylori infection in gastric cancer patients is associated with notable changes in the expression of genes related to immunity and metabolism. These findings suggest that H. pylori plays a critical role in modulating the tumor microenvironment, potentially contributing to gastric carcinogenesis. Targeting these altered pathways may offer new therapeutic strategies for treating gastric cancer.

**Keywords:** Helicobacter pylori, gastric cancer, gene expression, immunity, metabolism, IL-1 $\beta$ , TNF- $\alpha$ ,



# Prevalence of virulence genes of mucosa-associated *Escherichia coli* strains from colorectal cancer patients and healthy subjects

Bacteria in cancer initiation, promotion and progression

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**BACKGROUND AND OBJECTIVES:** BACKGROUND AND OBJECTIVES: Colorectal cancer (CRC) is one of the deadliest cancers in the world. Some strains of *Escherichia coli* could be associated with colorectal carcinogenesis. The aim of this analysis was to compare the prevalence of virulence genes of mucosa-associated *E. coli* in biopsy specimens from CRC patients and healthy subjects.

**MATERIALS AND METHODS:** MATERIALS AND METHODS: In a prospective study, a total of 93 mucosa-associated *E. coli* strains, 56 from CRC patients and 37 from healthy subjects were studied, between July 2019 and July 2020, from two referral university-affiliated hospitals in northwest Iran. The PCR method was used to evaluate the presence of *fimH*, *papC*, *hlyA*, *vat*, *chuA*, *fuyA* and *ibeA* genes.

**RESULTS AND DISCUSSION:** RESULTS AND DISCUSSION: In this study, the most prevalent virulence genes in *E. coli* strains from CRC patients were *fimH* (89%), *fuyA* (78.5%), *chuA* (76.7%), *ibeA* (68.9%), *vat* (62%), *papC* (35.7%) and *hlyA* (30.3%). Moreover, the most prevalent virulence genes in *E. coli* strains from healthy controls were *fimH* (81%), *ibeA* (56.7%), *vat* (29.7%), *chuA* (29.7%), *fuyA* (27%), *papC* (13.5%). The *hlyA* gene was not found in strains from the control subjects. It is noteworthy that there was a significant difference in the prevalence of *fuyA*, *chuA*, *vat*, *papC* and *hlyA* genes between the *E. coli* strains from CRC patient and healthy subjects ( $p < 0.05$ ). In conclusion, *E. coli* strains that carry multiple virulence factors are colonized more frequently in the gut mucosa of CRC patients than in healthy individuals and may play a role in the pathogenesis of CRC.

**Keywords:** *E. coli*, colorectal cancer, virulence factor

# Exploring the Potential of *Bacteroides Thetaiotaomicron* in Modulating the Immune Response in Chronic Myeloid Leukemia

Bacteria-based immune therapies for cancer treatment

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**BACKGROUND AND OBJECTIVES:** *Bacteroides thetaiotaomicron* is proposed as a potential candidate for the next generation of probiotics. The ES-8 and ES-D genes play crucial roles in activating and modulating the innate immune system against leukemia following bacterial exposure in the bone marrow. This study was designed to examine the effect of *B. thetaiotaomicron* and its derivatives on alterations in the expression of ES-8 and ES-D genes, and their significance in modulating the severity of chronic myeloid leukemia (CML).

**MATERIALS AND METHODS:** The impact of *B. thetaiotaomicron*, outer membrane vesicles (OMVs), inactivated bacteria, and supernatant treatments on the ES-8 and ES-D gene expression in the KG-1 cell line was analyzed using the quantitative reverse transcription-polymerase chain reaction (qRT-PCR) method. The Livak ( $\Delta\Delta CT$ ) method was employed to interpret the qRT-PCR results. The extraction and evaluation of outer membrane vesicles from gram-negative bacteria were performed using ultracentrifugation and ultrafiltration-based methods. The KG-1 cell line showed a significant response to treatment with live and active *B. thetaiotaomicron*, particularly in ES-8 and ES-D transcription. OMVs from this bacterium, at a concentration of 50  $\mu\text{g/ml}$ , significantly intensified the expression of the ES-8 ( $p=0.01$ ) and ES-D ( $p=0.02$ ) genes.

**RESULTS AND DISCUSSION:** This effect was even more pronounced at a concentration of 100 µg/ml. Inactivation at MOI 10 ( $p=0.03$ ) and MOI 50 ( $p=0.003$ ) significantly induced transcription of both genes. Additionally, a 25% supernatant considerably augmented the transcriptional expression of ES-8 ( $p=0.038$ ) and ES-D ( $p=0.034$ ) genes. Our findings suggest that OMVs at a concentration of 100 µg/ml, inactivated bacteria, and supernatants of *B. thetaiotaomicron* play a crucial role in shaping the immune response and could be considered as potential postbiotic and paraprobiotic candidates for further research. Moreover, our data indicate a significant reduction in the severity of Chronic myeloid leukemia in the erythroleukemia phase, affirming its potential as an effective adjuvant treatment for leukemia.

**Keywords:** *B.thetaiotaomicron*, Chronic myeloid leukemia, Microbiota, OMVs, ES-8, ES-D

# The Inhibitory Effects of *Bacteroides fragilis* and *Bifidobacterium bifidum* on the 4T1 Cancer Cell Line: An in vitro and in vivo Study

Bacteria-based immune therapies for cancer treatment

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**BACKGROUND AND OBJECTIVES:** The antitumor effects of the supernatants from *Bacteroides fragilis* and *Bifidobacterium bifidum* have garnered attention due to their potential role in modulating cancer progression. In a mouse model of breast cancer, these microbial flora components are being investigated for their ability to inhibit tumor growth. The supernatants may influence the tumor microenvironment, providing a novel approach to cancer therapy. This study aims to explore the therapeutic potential and mechanisms of action of these bacterial byproducts in breast cancer treatment.

**MATERIALS AND METHODS:** In the laboratory section, *Bifidobacterium bifidum* and *Bacteroides fragilis* bacteria were cultured in MRS Broth and Blood Agar at 37°C for 72 hours. 4T1 cancer cells were grown at 37°C with 5% CO<sub>2</sub>. Volumes of 0, 10, 20, 40, and 80 microliters of supernatants were applied to the 4T1 cancer cells (a breast cancer cell line). In the animal section, 35 x 10<sup>6</sup> 4T1 cells in 200 microliters of RPMI 1640 medium were subcutaneously injected into the right flanks of 16 mice. The tumor formation period was 2 weeks. Subsequently, the supernatants were injected, and pathological examinations, tumor size measurements, and molecular tests (ELISA) were performed. The cytotoxic and inhibitory effects of BS on the proliferation or death of 4T1 cells were evaluated using the MTT assay.

**RESULTS AND DISCUSSION:** Results and discussion: The results indicate that the inhibitory percentage of supernatants increases with higher doses and prolonged exposure to cancer cells. In the laboratory section, the cytotoxic effects in wells treated with *Bacteroides fragilis*, *Bifidobacterium bifidum*, and a combination (*fragilis* and *bifidum*) were 86%, 65%, and 98%, respectively. The animal study results confirmed the laboratory findings. Injection of the supernatants into mice led to a reduction in tumor size, with pathological improvements reported between 90% and 99%. Additionally, the production of interferon-gamma and interleukin-10 in supernatant-treated mice decreased. In the groups receiving *Bacteroides fragilis* and *Bifidobacterium bifidum*, small areas of inflammation and

tumor tissue remained, with healthy tissue replacing the tumor tissue. This improvement was more pronounced in the mice receiving the combined supernatant (fragilis + bifidum) compared to the first and second groups.

**Keywords:** Key words: Bifidobacterium, Bacteroides fragilis, Breast Cancer, Inhibitory Effect

# Leptospirosis vaccines: Past and future

## Bacterial Vaccine

### Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Leptospirosis is a serious worldwide zoonosis caused by infection with pathogenic *Leptospira* spp, and also has been identified as a re-emerging infectious disease, particularly in humid tropical and subtropical regions like north of Iran. The disease remains under diagnosed largely due to the broad spectrum of signs and symptoms attributable to this Leptospiral pathogen. People become infected via exposure to pathogenic *Leptospira* spp. from infected animals or through contact with water/soil contaminated with urine of shedding animals. Long-term control strategies of the disease include adoption of hygienic measures, rodent control and vaccinations. *Leptospira* vaccines currently available consist of killed whole cell bacterins which are used widely in animals, but less so in humans. However, specificity for serovars limits the efficacy of killed whole cell vaccines. Leptospiral antigens that induce cross-protective immunity to the various serovars are sought as new vaccine candidates.

**MATERIALS AND METHODS:** Although humoral immunity is thought to be dominant in protection from leptospiral infection, a role for cell-mediated immunity is now being explored. Currently, molecular and cellular studies on leptospirosis vaccines have been focused on bacterial motility, lipopolysaccharides (LPSs), lipoproteins, outer-membrane proteins (OMPs) and potential virulence factors. It has long been expected to find an effective vaccine to prevent leptospirosis through immunization of high-risk humans or animals. Currently several vaccines such as recombinant outer membrane protein (OMP) vaccines, outer membrane vesicle (OMV) vaccines and DNA vaccines against leptospirosis have been evaluated. Although the recombinant protein antigens show promise for the development of vaccines based on defined protective antigens.

**RESULTS AND DISCUSSION:** The advantage of production of recombinant vaccine antigens in a selected heterologous host organism arises from simplicities of cultivation of the host and purification of recombinant proteins. Furthermore, recombinant proteins are useful as antigens in immunoassays to detect leptospire. Although more research is needed, progress has been made toward to the development of an acceptable vaccine for animals and humans.

**Keywords:** Leptospirosis, vaccines, recombinant OMP vaccine, OMV vaccine, DNA vaccine, *Leptospira*

# Production and investigation prophylactic effects of egg yolk antibody (IgY) against a chimeric protein containing IpaD, StxB, and TolC antigens from *Shigella*0

Bacterial Vaccine

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**BACKGROUND AND OBJECTIVES:** *Shigella* is an important problem in developing countries and causes diarrhea especially in children. One of the important strategies for prophylaxis and neutralization of *Shigella* infectious factors is the use of Immunoglobulin Y (IgY). This study aimed to produce IgY against the chimeric protein containing IpaD, StxB, and TolC antigens from *Shigella*, investigate its prophylactic and neutralizing effects against Stx and *Shigella* dysenteriae.

**MATERIALS AND METHODS:** The chimeric protein sequence was synthesized and cloned in pET28a plasmid and then transferred to *Escherichia coli* BL21 (DE3) expression vector. SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) confirmed protein expression and the recombinant protein was purified by Ni-NTA affinity chromatography. The 150 µg of chimeric protein was mixed with Freund's adjuvant and injected into laying hens (Leghorn). IgY was purified by precipitation method with polyethylene glycol. IgY challenge against 1, 10 and 50 LD50 of Stx and *S. dysenteriae* was investigated.

**RESULTS AND DISCUSSION:** SDS-PAGE confirmed a 60.6 kDa recombinant protein. The increase of antibody titer against the recombinant protein was confirmed by ELISA. MTT assay showed that at 16 µmol/L, IgY protected HeLa cells against Stx. Treatment of mice with 1000 and 1500 µg IgY led to complete survival of the mice against 1 LD50 toxin and 4000 µg of IgY led to complete survival against 1 LD50, also 70% and 30% survival against 10 and 50 LD50 *S. dysenteriae*. The results of this study showed that IgY is a suitable candidate for preventing and neutralizing *Shigella* toxin and binding factors.

**Keywords:** *Shigella*, IgY, Prophylaxis, IpaD, StxB, TolC.

# Reverse vaccinology approaches to introduce promising immunogenic and drug targets against antibiotic-resistant *Neisseria gonorrhoeae*: Thinking outside the box in current prevention and treatment

## Bacterial Vaccine

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**BACKGROUND AND OBJECTIVES:** Gonorrhoea is an urgent antimicrobial resistance threat and its therapeutic options are continuously getting restricted. Moreover, no vaccine has been approved against it so far. Hence, the present study aimed to introduce novel immunogenic and drug targets against antibiotic-resistant *Neisseria gonorrhoeae* strains.

**MATERIALS AND METHODS:** In the first step, the core proteins of 79 complete genomes of *N. gonorrhoeae* were retrieved. Next, the surface-exposed proteins were evaluated from different aspects such as antigenicity, allergenicity, conservancy, and B-cell and T-cell epitopes to introduce promising immunogenic candidates. Then, the interactions with human Toll-like receptors (TLR-1, 2, and 4), and immunoreactivity to elicit humoral and cellular immune responses were simulated. On the other hand, to identify novel broad-spectrum drug targets, the cytoplasmic and essential proteins were detected. Then, the *N. gonorrhoeae* metabolome-specific proteins were compared to the drug targets of the DrugBank, and novel drug targets were retrieved. Finally, the protein data bank (PDB) file availability and prevalence among the ESKAPE group and common sexually transmitted infection (STI) agents were assessed.

**RESULTS AND DISCUSSION:** Our analyses resulted in the recognition of ten novel and putative immunogenic targets including murein transglycosylase A, PBP1A, Opa, NlpD, Azurin, MtrE, RmpM, LptD, NspA, and TamA. Moreover, four potential and broad-spectrum drug targets were identified including UMP kinase, GlyQ, HU family DNA-binding protein, and IF-1. Some of the shortlisted immunogenic and drug targets have confirmed roles in adhesion, immune evasion, and antibiotic resistance that can induce bactericidal antibodies. Other immunogenic and drug targets might be associated with the virulence of *N. gonorrhoeae* as well. Thus, further experimental studies and site-directed mutations are recommended to investigate the role of potential vaccine and drug targets in the pathogenesis of *N. gonorrhoeae*. It seems that the efforts for proposing novel vaccines and drug targets appear to be paving the way for a prevention-treatment strategy against this bacterium.

**Keywords:** Gonorrhoea; Reverse vaccinology; Comparative genomics; Essential proteins; Immunogenic targets



# Evaluation of Physicochemical and Microbial Quality of Drinking Water of Some Regions of West Mazandaran Province and Comparison with National Standards

## Oral Presentation

### Microbiological contamination of water supplies

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**BACKGROUND AND OBJECTIVES:** Assessing the quality of water resources in terms of the presence or absence of physical, chemical and microbial pollutants is of particular importance and affects the health of consumers. Therefore, the qualitative assessment of underground water resources and the investigation of their changes during different periods are necessary to ensure the health of water. In this study, the physical, chemical and bacteriological quality of underground water in some areas of the west of Mazandaran province have been investigated.

**MATERIALS AND METHODS:** In order to study and determine the water quality of wells and springs in the west of Mazandaran province, sampling was done from 111 wells and 31 wells in the rural and urban areas of Chalus, Noor and Mahmood Abad. This descriptive-cross-sectional study was conducted in a period of 5 years (2019- 2023) and the parameters of electrical conductivity, turbidity, nitrate, total hardness, iron, ammonia, phosphate, manganese, total coliform and thermophilic coliform were analyzed according to the standard method. and the results were compared with the Iranian national standard number 1011 and 1053. The obtained data were analyzed using Microsoft Excel and SPSS version 19 software and statistical methods.

**RESULTS AND DISCUSSION:** Based on the obtained results, the amount of electrical conductivity, turbidity, total hardness and nitrate, ammonia, phosphate and manganese were 99.3%, 77%, 98.6%, 94%, 100%, 87% and 96%, respectively. The results show It showed that in 42 percent of the samples, the amount of iron was higher than the standard limit (0.3 mg/liter) The results showed that the wells of cities were free of contamination in terms of total coliform and 2.3% of the springs had thermophilic coliform before chlorination. Also, all the investigated physico-chemical parameters were within the standard range, except for iron. The amount of iron in almost 40% of the wells of the investigated cities was higher than the standard limit, which is due to the topographical conditions of the region in the groundwater. Therefore, in order to improve the quality of drinking water in these cities, Mazandaran Water Company has installed and operated 20 selective purification systems to remove iron and turbidity.

**Keywords:** Physical quality, chemical quality, microbial quality, drinking water, Mazandaran province

# Drinking Water: Challenges, Threats, and Opportunities

Food and Water Safety

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**BACKGROUND AND OBJECTIVES:** Access to safe drinking water is vital for public health, yet various challenges threaten its availability and quality. A significant issue is the increasing salinity of groundwater sources, particularly in regions near the Caspian Sea, which diminishes their suitability for drinking (Thorslund & Vliet, 2020). Additionally, water reservoirs are increasingly contaminated by pollutants such as domestic, urban, industrial, and hospital waste, as well as agricultural pesticides, posing serious risks to water quality (Schulz, 2004; Zhou, Zhu, & Chen, 2007). These contaminants infiltrate surface waters and groundwater aquifers, rendering them unsafe for consumption.

**MATERIALS AND METHODS:** This study employed a comprehensive literature review approach, where all relevant articles related to the keywords of this paper were thoroughly searched and analyzed. Both national and international databases were examined to gather pertinent information. Additionally, official websites of institutions and organizations related to the topic were reviewed, and their reports were utilized. The research also incorporated the author's extensive field experience, spanning several years, which included assessments of health facilities, water resources, environmental challenges, and the awareness levels of operational and maintenance personnel in health facilities. This combination of literature review and practical fieldwork provided a robust foundation for the study's analysis

**RESULTS AND DISCUSSION:** The effectiveness of regulatory bodies and monitoring agencies in supervising water quality is notably inadequate, resulting in insufficient evaluations (Tariq et al., 2007). The situation is exacerbated by a lack of coordination between academic institutions and relevant organizations and adequate training and awareness among operational and maintenance staff (Sharma, 2014). Moreover, the unregulated use of agricultural pesticides further contaminates water sources, thereby endangering public health (Zhou et al., 2007). To address these challenges, a comprehensive approach is necessary, involving the strengthening of regulatory frameworks, enhancement of monitoring agency capacities, promotion of collaboration between scientific and regulatory bodies, and expansion of educational and training programs for water management personnel (Michael, Post, Wilson, & Werner, 2017; Sharma, 2014). By implementing these strategies, the risks to drinking water quality can be mitigated, ensuring sustainable and safe water access for all communities.

**Keywords:** Safe Drinking Water, Groundwater Salinity, Water Quality Contamination, Regulatory Frameworks,

# Host microbiome data integration

Omics findings in microbiology

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Host microbiome data integration involves combining data from various sources to gain a comprehensive understanding of the intricate interplay between the host and its microbiome. This powerful approach brings together data from diverse fields, including host transcriptomics, proteomics, metabolomics, and metagenomics, to paint a complete picture of this dynamic relationship. By integrating these different datasets, researchers can unravel the complex connections between host genes and the composition of the microbiome.

**MATERIALS AND METHODS:** This integration allows for the identification of functional relationships, revealing how specific genes within the host influence the makeup and activity of the microbial community residing within it.

**RESULTS AND DISCUSSION:** Such insights are crucial for understanding disease mechanisms, as they provide a deeper understanding of how disruptions in the host-microbiome balance can contribute to the development of various ailments. Furthermore, this integrated approach offers valuable insights into potential therapeutic targets, paving the way for the development of novel interventions aimed at restoring harmony between the host and its microbiome. By studying the interplay between host genes and microbial composition, researchers can identify specific pathways and microbial communities that could be targeted for therapeutic intervention, ultimately leading to improved health outcomes.

**Keywords:** Data integration; Omics data; Microbiome; Host-Microbiome interaction

# Influenza-Omics and the Host Response

Omics findings in microbiology

Panel Oral Presentation

kaveh sadeghi <sup>1</sup> © ®

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**BACKGROUND AND OBJECTIVES:** The review discusses the application of "omics" data, including genomics, transcriptomics, and proteomics, in studying host responses to influenza A virus (IAV) infections. It highlights how -omics analyses can reveal numerous features characterizing microbial and host responses during infections, emphasizing the importance of data interpretation to draw meaningful conclusions. The review addresses the challenges posed by IAV's ability to mutate and evade vaccines, underlining the need for continuous monitoring of IAV in various reservoirs, including birds and pigs. Recent advancements in mass spectrometry and next-generation sequencing technologies have enhanced the understanding of viral-host interactions, making -omics studies more accessible. The review aims to evaluate recent findings in proteomics and transcriptomics related to IAV, discussing notable experiments and future prospects in the field

**MATERIALS AND METHODS:** The text discusses the methodologies and findings from studies on human influenza A virus (IAV) infections, including clinical trials and in vitro experiments using human cell cultures. Ethical constraints limit the use of high-pathogenicity strains in human trials, leading to the use of safer seasonal strains. Researchers have employed multivariate estimation techniques to analyze blood samples, predicting health outcomes and potential influenza severity before symptoms appear. Additionally, extensive -omics data from surveillance studies on naturally acquired infections has enhanced understanding of immune responses, revealing important biomarkers and gene expression patterns. In vitro studies using human lung cell lines, such as Calu-3 and A549, allow for examination of host responses and potential drug repurposing strategies.

**RESULTS AND DISCUSSION:** Proteomic analyses highlight significant changes in protein expression and cellular pathways during infection, indicating the potential for identifying therapeutic targets and understanding IAV adaptations. Overall, both human trials and cell culture studies contribute valuable insights into the biology of influenza infections and their impact on host responses.

**Keywords:** Influenza, Omics, Host Response

# The role of Metagenomics and Artificial Intelligence in human health

Omics findings in microbiology

Panel Oral Presentation

Masoud Arabfard <sup>1</sup> © ⓘ

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**BACKGROUND AND OBJECTIVES:** The human microbiome is a widespread, dynamic, and site-specific collection of microbial communities. The pathogenic potential of microorganisms in human tissues has spurred an increase in microbiological research. Studies have characterized genera using both culture-dependent and culture-independent methods. However, the unique environments within human tissues make it challenging to culture these microorganisms, complicating molecular studies.

**MATERIALS AND METHODS:** Metagenomics (MGs) provides a means to explore and characterize hidden microbial communities through culture-independent direct DNA isolation. By utilizing function and sequence-based MG approaches, scientists can investigate the mechanistic details of various microbes and their interactions within their niches. Given the complexity and multidimensional nature of data generated from MG studies, accurate analytical tools are essential for evaluation and interpretation. Artificial intelligence (AI) offers the capability to automatically learn data dimensionality, simplifying complexity and facilitating timely and accurate disease diagnosis and response.

**RESULTS AND DISCUSSION:** This lecture highlights the human microbiota and its exploration through MG studies, emphasizing the importance of MGs in understanding microbiota changes during disease and the role of AI in the computational analysis of MG data.

**Keywords:** AI, MEtagenomics

# Metagenomic analysis of the Microbial Communities in a stirred tank reactor (STR) and their efficiency in bioleaching of chalcopyrite concentrate

Role of Microorganisms in Agriculture

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**BACKGROUND AND OBJECTIVES:** Chalcopyrite (CuFeS<sub>2</sub>) is the main copper-containing sulfide mineral. Accounts for about 70% of copper reserves. Thermophilic microorganisms are more efficient at increasing the rate of chalcopyrite oxidation. The study aimed to isolate indigenous extreme thermoacidophilic microorganisms from Sarcheshmeh Copper Complex, determine their efficiency in chalcopyrite bioleaching and Molecular identification via culture-independent approaches.

**MATERIALS AND METHODS:** Soil and water samples were collected and mixed samples were inoculated into *Metallosphaera sedula* culture medium for enrichment. A representative sample of chalcopyrite concentrate was obtained before bioleaching. Bioleaching experiments were conducted in shake flasks and stirred tank reactor (STR), focusing on four parameters: inoculum percentage, pulp density, agitation, and concentrate particle size. For molecular identification using Illumina MiSeq technology, a sample from the stirred tank reactor (STR) was sent to Microsynth Company for DNA extraction. Subsequently, MacroGen Company performed Next Generation Sequencing (NGS) for further analysis.

**RESULTS AND DISCUSSION:** The highest copper recovery was 35.25% in shake flasks and 14.30% in a stirred tank reactor over 28 days. XRD analysis indicated that no jarosite had formed in the bioleaching experiments. The PCR amplicon was sequenced, and bioinformatics analysis was conducted in QIIME2. The metagenomic analysis revealed that the most abundant bacterial phyla in the reactor sample were unclassified bacteria (69.99%), Actinobacteria (13.65%), Firmicutes (11.47%), *Deinococcus-thermus* (1.44%), and Proteobacteria (1.30%). In archaea, the most abundant phylum was Euryarchaeota. Within Euryarchaeota, *Natrinema pallidum* was identified, accounting for 0.0003% of this phylum. Actinobacteria, Firmicutes, *Deinococcus-Thermus*, and Proteobacteria are significant bacterial phyla in heavy metal-contaminated environments due to their ability to tolerate heavy metals. In environments contaminated with heavy metals, there is an observed increase in unclassified bacteria, suggesting a shift in bacterial diversity in response to heavy metal pollution.

**Keywords:** Chalcopyrite, thermoacidophilic microorganisms, bioleaching, Illumina MiSeq, Next Generation Sequencing (NGS)

# Evaluation of the biological population in activated sludge and its relationship with the SRT in the municipal wastewater treatment plant

Role of Microorganisms in Wastewater Treatment

Maryam Amini © ©, Motahare Maghami, Mohammad Ranjdoost, Ali Moezi

Mazandaran Province Water and Wastewater Company

**BACKGROUND AND OBJECTIVES:** The biota in the activated sludge of the wastewater treatment plant consists of bacteria, protozoa and metazoa. These biotas participate in the ecosystem balance in wastewater treatment plants and are quite sensitive to physical, chemical and operational processes. These organisms are excellent tools for assessing the biological status of reactors used to monitor the performance of wastewater treatment plants

**MATERIALS AND METHODS:** This descriptive-cross-sectional study was conducted on Sari wastewater treatment plant using continuous flow aerobic system of activated sludge type in a period of one year. Sludge age was calculated by measuring the TSS, MLSS and VSS parameters and recording the influent, effluent and excess flow rates. Also, according to the changes of SRT in the wastewater treatment plant, different species of protozoa and metazoa were identified and quantified, and statistically analyzed with Excel software

**RESULTS AND DISCUSSION:** Microscopic observations and laboratory data analysis showed; there is a significant relationship between SRT and population abundance of different types of metazoa and protozoa. So that when the age of the sludge is between 12 -19 days, you don't see rotifers and nematodes, but the population of flagellates and ciliates has increased. Also, with the increase of SRT above 95 days, nematodes and rotifers have been observed, and the population of ciliates and flagellates has also decreased noticeably.

**Keywords:** Activated sludge, Microorganism, Protozoa, Metazoa, SRT, Wastewater treatment

# The New Methods of Applying Bacteriophages in Biocontrol of Corrosive Bacteria and Their Biofilms in Water Systems

## Biofilms

### Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Biocorrosion is the result of synergistic interactions of different species of microorganisms that coexist in mixed consortia in all aquatic environments. This includes engineered systems such as sewage and drinking water distribution systems (D.W.S). Microbially influenced corrosion (M.I.C) of metals refers to the deterioration process. Biofilms are an extremely common adaptation, allowing bacteria to colonize hostile environments. They cause resistance to antimicrobial agents. As a result, the current study is to introduce a new method of using the phages in water systems which can be potentially used as an eco-friendly inhibitor of corrosive bacteria and their biofilms.

**MATERIALS AND METHODS:** For preparation of phages the molecular biological techniques have been used. For the study of extracellular polysaccharide (EPS) of biofilm the cation exchange resins (CER) and atomic force microscopy (AFM) has been proved as a useful technique to study the morphology of biofilm, as well as the extent of corrosion after biofilm removal.

**RESULTS AND DISCUSSION:** The results in different studies show that bacteriophages with the capacity to rapidly infect and overcome bacterial resistance, have demonstrated an approach against bacteria in corrosion. This has been seen particularly in the biofilm food industry and membrane biofouling in water and wastewater treatment. When a phage comes in contact with biofilms, the modified phage uses polysaccharide-degrading enzymes and endolysins to biofilm destruction and cell lysis. Recently, phages are considered as valuable biological resources in the field as biocontrol agents against corrosive bacteria and their biofilms by different manners.

**Keywords:** Microbially Influenced Corrosion, Biofilm, genetically modified phage, Biocontrol



# Application of Aloe Vera Nanofibers Reinforced with Lytic Phages to Control Microbial Contamination

Microbial biotechnology

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** The development of biocompatible and safe nanomaterials from natural sources is increasingly important for health systems. Aloe vera leaf gel, known for its antimicrobial properties, has been utilized to create cellulose nanofibers, which serve as effective nanocarriers for delivering the gel's antioxidant, anti-inflammatory, and antimicrobial compounds. Controlling microbial contamination, particularly in healthcare settings where biofilms can lead to hospital infections, and in food preservation, is a significant challenge. This study explores the use of aloe vera-derived nanofibers, enhanced with lytic bacteriophages, to combat methicillin-resistant *Staphylococcus aureus* and *Pseudomonas* species, which are responsible for food spoilage, particularly in chicken meat.

**MATERIALS AND METHODS:** Aloe vera gel was extracted from the leaf's parenchymal tissue and processed into a suspension mixed with polyvinyl alcohol powder in a 90:10 weight ratio. This mixture was then electrospun into nanofibers under high voltage conditions. To augment the antimicrobial properties of the nanofibers, lytic bacteriophages, isolated from wastewater, were incorporated into the gel suspension prior to electrospinning. Various specialized analyses were conducted to assess the morphological, chemical, rheological, and biological characteristics of the resulting nanofibers.

**RESULTS AND DISCUSSION:** Atomic force microscopy confirmed the morphological and surface porosity of the nanofibers. The nanofibers exhibited enhanced antimicrobial effects compared to those without bacteriophages, demonstrating acceptable strength and flexibility. Biofilm assays indicated that these nanofibers significantly reduced biofilm formation time on surfaces. Furthermore, the phage-enhanced nanofibers effectively prevented contamination and spoilage in food products like chicken meat. The aloe vera nanofibers produced exhibit promising biological properties, suggesting their potential as biocompatible and smart coatings with targeted antimicrobial capabilities. These coatings could play a crucial role in controlling microbial contamination in healthcare environments and preserving pharmaceutical and food materials.

**Keywords:** Aloe vera; Nanofibers; *Staphylococcus aureus*; *Pseudomonas* sp.; Microbial contamination

# Applications of nanoliposomes in drug delivery to leishmania

Nanotechnology for the diagnosis and treatment of diseases

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Leishmaniasis, a parasitic disease prevalent in tropical and subtropical regions globally, presents a significant public health concern. As estimated by the World Health Organization (WHO), the disease affects approximately 12 million people and puts another 350 million at risk worldwide. Effective management of leishmaniasis faces several challenges due to the limitations of current therapeutic approaches. Consequently, innovative strategies such as nanoparticle delivery systems, particularly liposomes, have emerged as promising solutions to address these issues.

**MATERIALS AND METHODS:** Liposomes, composed of phospholipids and cholesterol, exhibit a unique bilayered structure that facilitates the encapsulation of hydrophilic, hydrophobic, or amphiphilic compounds. This characteristic makes them highly versatile drug carriers with applications in various therapeutic areas, including leishmaniasis. A major hurdle in leishmaniasis treatment is the parasite's ability to survive and replicate within macrophages, a type of immune cell. By employing liposomes as targeted drug delivery vehicles, the therapeutic index of anti-leishmaniasis drugs can be improved, thereby enhancing their efficacy and safety.

**RESULTS AND DISCUSSION:** Targeted drug delivery systems offer several advantages, such as preferential accumulation of the drug at the desired site of action and reduced systemic exposure, leading to lower effective doses and diminished toxic side effects. In the case of leishmaniasis, liposomes can be designed to specifically target macrophages, enabling the efficient delivery of anti-leishmanial agents to the infected cells while minimizing the risk of off-target effects resulting from nonspecific biodistribution. In conclusion, the development of liposomal drug delivery systems represents a significant advancement in the quest for better leishmaniasis treatment options. These systems offer enhanced drug efficacy, improved safety profiles, and the potential for targeted delivery, thereby addressing some of the critical limitations associated with current therapies. With further research and development, liposome-based drug delivery systems may significantly contribute to the global effort to effectively manage and control leishmaniasis.

**Keywords:** Liposome, leishmania, Parasite, Nanoparticle, Macrophage

# Development of a High sensitive Multiplex Lateral Flow Immunoassay (LFIA) system based on dual gold nanoparticle conjugate for rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA)

Nanotechnology for the diagnosis and treatment of diseases

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**BACKGROUND AND OBJECTIVES:** Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a worldwide concern as an epidemic bacterium and a cause of nosocomial and community-acquired infections. One of the major problems in the prevention and treatment of infections caused by MRSA strains is their multi-drug resistant trait, which causes the spread of infections and increases the mortality rate. Therefore, a rapid and accurate method is needed to identify MRSA strains, initiate appropriate antibiotic therapy, and control its infection. The aim of this study was to develop a twin lateral flow immuno-assay system to detect methicillin-resistant *Staphylococcus aureus* (MRSA).

**MATERIALS AND METHODS:** Methods: First, BSA blocked AuNPs-anti-peptidoglycan antibody and AuNPs-anti-BSA antibody were used to detect *Staphylococcus aureus* (*S. aureus*). Then, AuNPs-anti-PBP2a antibody was used to specifically detect MRSA. Sensitivity, specificity and limit of detection of this twin immunoassay system were assessed using MRSA, methicillin susceptible *S. aureus* and clinical samples. Results were compared to those of cefoxitin disc diffusion (FOX30) and Polymerase Chain Reaction (PCR) as gold standards.

**RESULTS AND DISCUSSION:** Results: The Limit of Detection (LOD) of this twin system were 103 and 104 CFU/ml for the first and second strips, respectively. Sensitivity and specificity of this innovative assay in detecting MRSA were 92.30 and 97.36%, compared to FOX30 and PCR, respectively. Conclusion: High rates of sensitivity and specificity of this initiative system show its high potentials for rapid and accurate detection of MRSA.

**Keywords:** Keywords: Lateral flow immunoassay, Diagnostic, Methicillin-Resistant, gold nanoparticles (AuNP), Reagent

# Nanomaterial's application in controlling antibiotic resistance infections

Nanotechnology for the diagnosis and treatment of diseases

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**BACKGROUND AND OBJECTIVES:** Antibiotic resistances appear to be one of the greatest threats to public health in recent years. Approximately 79 % of different bacteria become resistance to at least one or more antibiotics; that can cause health care increasing costs, more mortality and other critical health complication risks. Therefore, more research and studies are urgently needed to overcome antibiotic resistance.

**MATERIALS AND METHODS:** Different strategies were studied to overcome antibiotic resistance like finding new antibiotic, preparing novel drug delivery systems and using nanotechnology-based strategies. Among them using nanotechnology strategies to prepare novel drug delivery systems containing antibiotics by increasing antibiotics bioavailability and efficacy are currently being developed. Using nanoparticles with antibiotic properties as a disinfectant agent is another strategy for combating resistant bacteria.

**RESULTS AND DISCUSSION:** In this lecture different nanomaterials with antibacterial properties will be introduced and discussed as a promising approach to overcome bacterial resistance.

**Keywords:** Bacterial Resistance, Antibacterial Nanomaterials, Disinfectant

# Nanomolecular Detection Techniques for the Diagnosis of Infectious Diseases

Nanotechnology for the diagnosis and treatment of diseases

Panel Oral Presentation

Adele Rafati

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**BACKGROUND AND OBJECTIVES:** The increasing prevalence of infectious diseases necessitates the development of rapid and accurate diagnostic methods to facilitate treatment. Traditional diagnostic techniques often require complex procedures and specialized equipment, limiting their accessibility, especially in resource-limited settings. Integration of nanotechnology with molecular detection techniques, has introduced a new generation of diagnostic methods to the world. By leveraging the unique properties of nanomaterials, such as their high surface area and ability to enhance signal detection, these combined approaches can improve the performance of diagnostic assays. Due to their unique chemical, electrical and optical properties, nanomaterials and nanostructures can be used in the design of new nanomolecular detection methods and lead to improved sensitivity, specificity and accuracy of diagnostic methods. Nanomaterials and nanostructures can play three main roles in the design of the new generation of nanobiosensors. 1. Due to the high surface-to-volume ratio, they can be used as a suitable platform for loading the

**MATERIALS AND METHODS:** These detection techniques offer high sensitivity and specificity, providing rapid results essential for timely clinical decision-making. Their integration could significantly improve diagnostic capabilities, particularly in resource-limited settings where rapid and accurate detection is critical for managing infectious diseases.

**RESULTS AND DISCUSSION:** Nanomolecular detection techniques represent promising approaches for the diagnosis of infectious diseases. Continued research and development in these fields could lead to significant advancements in public health diagnostics, particularly in combating infectious diseases in developing countries.

**Keywords:** Nanomolecular detection, Nanomaterials and Nanostructures, Nanobiosensores, infectious disease

# Bacteriophage-mediated manipulation of the gut microbiome: promises and presents limitations

## Bacteriology

Probiotics: application in food industry and medicine

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Gut microbiome (GM) composition and function are linked to human health and disease, and routes for manipulating the GM have become an area of intense research. Due to its high treatment efficacy, the use of fecal microbiota transplantation (FMT) is generally accepted as a promising experimental treatment for patients suffering from GM imbalances (dysbiosis), e.g., caused by recurrent *Clostridioides difficile* infections (rCDI).

**MATERIALS AND METHODS:** This paper is a review article based on research from databases covering the years 2013 to 2023. Bacteriophages (phages) play a key role in successful FMT treatment by restoring the dysbiotic bacterial GM. As a refinement to FMT, removing the bacterial component of donor feces by sterile filtration, also referred to as fecal virome transplantation (FVT), decreases the risk of invasive infections caused by bacteria.

**RESULTS AND DISCUSSION:** However, eukaryotic viruses and prophage-encoded virulence factors remain a safety issue. Recent in vivo studies show how cascading effects are initiated when phage communities are transferred to the gut by e.g., FVT, which leads to changes in the GM composition, host metabolome, and improve host health such as alleviating symptoms of obesity and type-2-diabetes (T2D). In this review, we discuss the promises and limitations of FVT along with the perspectives of using FVT to treat various diseases associated with GM dysbiosis.

**Keywords:** Gut microbiome; Bacteriophages; Phage therapy; Fecal virome transplantation

# Bacteriocins as a new generation of antimicrobials: toxicity aspects and regulations

Microbial Contamination of Food

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** In recent decades, bacteriocins have received substantial attention as antimicrobial compounds. Although bacteriocins have been predominantly exploited as food preservatives, they are now receiving increased attention as potential clinical antimicrobials and as possible immune-modulating agents. Infections caused by antibiotic-resistant bacteria have been declared as a global threat to public health.

**MATERIALS AND METHODS:** This research is a review article based on research from databases covering the years 2010 to 2022. Bacteriocins represent a potential solution to this worldwide threat due to their broad- or narrow-spectrum activity against antibiotic-resistant bacteria.

**RESULTS AND DISCUSSION:** Notably, despite their role in food safety as natural alternatives to chemical preservatives, nisin remains the only bacteriocin legally approved by regulatory agencies as a food preservative. Moreover, insufficient data on the safety and toxicity of bacteriocins represent a barrier against the more widespread use of bacteriocins by the food and medical industry. Here, we focus on the most recent trends relating to the application of bacteriocins, their toxicity and impacts.

**Keywords:** Antimicrobials; Bacteriocins; Gastrointestinal bioavailability; Toxicity

# Microbial Indicators and Water Safety: Strategies for Improving Public Health

## Role of Microorganisms in Wastewater Treatment

### Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Access to clean and safe drinking water is a fundamental human right and a cornerstone of public health. However, millions of people around the world still face significant risks from waterborne diseases due to inadequate water quality. These diseases, often caused by pathogens present in contaminated water, continue to pose a serious threat to health, particularly in low-income regions where water treatment facilities may be lacking or ineffective.

**MATERIALS AND METHODS:** This paper provides a comprehensive synthesis of the current knowledge regarding microbial indicators that are essential for assessing the microbiological safety of drinking water and evaluating the associated health risks. We delve into various microbial indicators, such as total coliforms, fecal coliforms, fecal streptococcus, *E. coli*, and salmonella, discussing their relevance and the diversity of their application across different geographical regions. Each of these indicators serves as a critical marker for potential contamination and helps in understanding the overall safety of water supplies

**RESULTS AND DISCUSSION:** The results showed that traditional methods, while reliable, may not always capture the full spectrum of microbial threats, leading to potential underestimations of risk. In contrast, newer molecular techniques offer enhanced sensitivity and specificity, allowing for more accurate detection of pathogens. Furthermore, we highlight the significance of the World Health Organization (WHO) water safety plan, which provides a framework for countries to develop effective regulatory measures and monitoring strategies. In conclusion, ensuring access to safe drinking water is vital for public health, particularly in vulnerable regions. This paper underscores the importance of microbial indicators in assessing water safety and highlights the limitations of traditional methods compared to innovative molecular techniques. By integrating insights from the World Health Organization's water safety plan, we advocate for enhanced regulatory measures and monitoring strategies. Continued research and investment in water safety initiatives are essential to mitigate waterborne health risks and uphold the fundamental human

**Keywords:** Water Safety Plan, Microbial Indicators, Public Health, Drinking water



# Removal of diesel fuel from water by combined culture of azolla and bacteria

## Role of Microorganisms in Wastewater Treatment

### Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Dispersion of petroleum compounds in low concentrations in water causes adverse environmental effects. Physico-chemical methods in removing oil compounds dispersed in water is not cost-effective. Few researches have been done on the removal of diesel fuel from water by biological methods. The aim of this study was to determine the removal of diesel fuel from water by combined culture of azolla and bacteria.

**MATERIALS AND METHODS:** Diesel fuel was used as a source of petroleum hydrocarbon pollution in water. Bacteria were isolated from the soil of a site contaminated with diesel fuel. Experiments were carried out in 14 days in a greenhouse in three separate runs includes by culturing only bacteria, only azolla and combined culture of azolla and bacteria in water containing medium contaminated with diesel fuel. Three concentrations of 100, 500 and 1000 mg/L of diesel fuel in water were examined. Total Petroleum Hydrocarbon (TPH) was analyzed according to ASTM method D 7066-04.

**RESULTS AND DISCUSSION:** By using only azolla culture, the removal of TPH from water during 14.0 days of contact were 100,70 and 62%, for 100 ,500 and 1000 ppm TPH respectively. For 1000 ppm of TPH and using pure bacterial culture, pseudomonas aeruginosa showed the lowest removal of 35% and alkaligenes faecalis had the highest removal of 60%. Regarding combined culture of azolla and bacteria for 1000 ppm of TPH, pseudomonas aeruginosa and azolla showed the lowest removal of 80% and alkaligenes faecalis and azolla showed the highest removal of 100%. Inoculation of bacteria into azolla culture medium improved the removal of diesel fuel from water. By this method it is possible to remove dispersed hydrocarbon from drinking water resources.

**Keywords:** combined culture, azolla, bactria, oil pollution, water

# Graph Neural Networks for Drug Development

Artificial intelligence in microbiology and diagnostics

Panel Oral Presentation

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**BACKGROUND AND OBJECTIVES:** Graph Neural Networks (GNNs) have emerged as a powerful tool in drug design by enabling the effective modeling of complex molecular structures.

**MATERIALS AND METHODS:** Traditional methods often struggle to capture the intricate relationships between atoms and the three-dimensional topology of molecules. GNNs, however, naturally represent molecules as graphs, where nodes correspond to atoms and edges represent chemical bonds. This allows GNNs to learn and predict various molecular properties, such as binding affinity, solubility, and toxicity, with high accuracy. By leveraging GNNs, researchers can accelerate the drug discovery process, identify potential drug candidates more efficiently, and even design novel compounds with desired therapeutic properties, ultimately contributing to more effective and targeted treatments.

**RESULTS AND DISCUSSION:** By defining other types of graphs, graph neural networks can be applied to solve various problems in this domain, such as predicting drug interactions, repurposing existing drugs, making automated decisions more explainable, and reducing costs in different stages of drug production. The need for big data and powerful computational resources are among the challenges in this field, and designing multi-modal graph models as well as preventing issues like over-smoothing and over-squashing for drug-related data remain open research problems in this area.

**Keywords:** Graph neural networks, drug, explainable, deep learning, machine learning, artificial intelligence



# Combination antimicrobial therapy: in vitro synergistic effect of anti-staphylococcal drug oxacillin with antimicrobial peptide nisin against *Staphylococcus epidermidis* clinical isolates and *Staphylococcus aureus* biofilms

Medical microbiology, epidemiology and diagnosis

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**BACKGROUND AND OBJECTIVES:** The ability of *Staphylococcus epidermidis* and *S. aureus* to form strong biofilm on plastic devices makes them the major pathogens associated with device-related infections (DRIs). Biofilm-embedded bacteria are more resistant to antibiotics, making biofilm infections very difficult to effectively treat. Here, we evaluate the in vitro activities of anti-staphylococcal drug oxacillin and antimicrobial peptide nisin, alone and in combination, against methicillin-resistant *S. epidermidis* (MRSE) clinical isolates and the methicillin-resistant *S. aureus* ATCC 43,300.

**MATERIALS AND METHODS:** The minimum inhibitory concentrations (MIC) and minimum biofilm eradication concentrations (MBEC) of oxacillin and nisin were determined using the microbroth dilution method. The anti-biofilm activities of oxacillin and nisin, alone or in combination, were evaluated. In addition, the effects of antimicrobial agents on the expression of *icaA* gene were examined by quantitative real-time PCR.

**RESULTS AND DISCUSSION:** MIC values for oxacillin and nisin ranged 4–8 µg/mL and 64–128 µg/mL, respectively. Oxacillin and nisin reduced biofilm biomass in all bacteria in a dose-dependent manner and this inhibitory effect was enhanced with combinatorial treatment. MBEC ranges for oxacillin and nisin were 2048– 8192 µg/mL and 2048– 4096 µg/mL, respectively. The addition of nisin significantly decreased the oxacillin MBECs from 8- to 32-fold in all bacteria. At the 1× MIC and 1/2× MIC, both oxacillin and nisin decreased significantly the expression of *icaA* gene in comparison with untreated control. When two antimicrobial agents were combined at 1/2× MIC concentration, the expression of *icaA* were significantly lower than when were used alone. Nisin/conventional oxacillin combination showed considerable anti-biofilm effects, including inhibition of biofilm formation, eradication of mature biofilm, and down-regulation of biofilm-related genes, proposing its applications for treating or preventing staphylococcal biofilm-associated infections, including device-related infections.

**Keywords:** Antimicrobial peptide, Biofilm inhibition, MRSA, MRSE, Nisin, *icaA*

# Efficacy of clindamycin in preventing abortion and vertical transmission of *Toxoplasma gondii* (PRU strain) infection in animal model

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Toxoplasma gondii* transmission can occur during pregnancy if the mother contracts the infection for the first time. Treatment strategies include the use of antimicrobial medications and providing supportive care. Spiramycin is commonly used to treat toxoplasmosis in pregnant women and to hinder the disease's transmission. However, its ability to treat the fetus is questionable due to its limited ability to cross the placental barrier. Additionally, economic constraints and sanctions may impede access to this medication. Consequently, in search of an effective treatment, for the first time in Iran, the effectiveness of clindamycin in preventing abortion and vertical transmission of PRU strain of *T. gondii* infection in pregnant mice was evaluated.



**MATERIALS AND METHODS:** On the twelfth day of gestation, pregnant mice were exposed to *T. gondii* and subsequently received treatment with either clindamycin or spiramycin. This resulted in the establishment of four distinct groups: a normal control, an infected group without treatment, an infected group treated with clindamycin, and another infected group treated with spiramycin. Following these interventions, a series of parasitological (including microscopic examination and real-time PCR), histopathological, and immunological evaluations were conducted.

**RESULTS AND DISCUSSION:** The findings showed a marked reduction in the number of cysts in the eyes and brain (77.32 - 90.48%) among the treated groups when compared to the control group. Furthermore, the treatment was found to suppress inflammatory changes, prevent cell death, and reduce vascular cuffs in the brain, as well as lessen bleeding, placental thrombosis, and inflammatory cell buildup in the placenta. Clindamycin was also effective in diminishing retinal folds, tiny retinal bleeds, and cell vacuolation in eye tissues. Immunologically, the treatment led to lower TNF- $\alpha$  cytokine levels, suggesting an enhanced cellular immune response. Additionally, a rise in IL-10 levels in the treated infected groups could have helped decrease TNF- $\alpha$  production.

**Keywords:** *Toxoplasma gondii*, Pru strain, Congenital, Clindamycin, In vivo

# Anti-parasitic Impact of Sambucus ebulus and Feijoa sellowiana Silver Nanoparticles on Toxoplasma gondii Tachyzoites

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Current chemical treatments for toxoplasmosis have side effects, researchers are looking for herbal remedies with minimal side effects and the best effectiveness. This study aimed to evaluate the anti-toxoplasmic effects of silver nanoparticles based on *Sambucus ebulus* (Ag-NPs-S. *ebulus*) and *Feijoa sellowiana* (Ag-NPs-F. *sellowiana*) fruit extracts, in vitro and in vivo.

**MATERIALS AND METHODS:** Vero cells were treated with different concentrations (0.5, 1, 2, 5, 10, 20, 40 µg/mL) of extracts and pyrimethamine as a positive control. Vero cells were infected with *T. gondii* and treated with extracts. The infection index and intracellular proliferation of *T. gondii* were evaluated. The survival rate of infected mice with tachyzoites of *T. gondii* was examined after intraperitoneal injection of the extracts at a dose of 40 mg/kg/day for 5 days after infection.

**RESULTS AND DISCUSSION:** The Ag-NPs-S. *ebulus* and Ag-NPs-F. *sellowiana*, almost similar to pyrimethamine, reduced proliferation index when compared to untreated group. Also, high toxoplasmicidal activity was observed with Ag-NPs-S. *ebulus* extract. Mice in the treatment groups of Ag-NPs-S. *ebulus* and pyrimethamine achieved better results in terms of survival than the others. Conclusion The results indicated that Ag-NPs-F. *sellowiana* and S. *ebulus* have a significant growth effect on *T. gondii* in vitro and in vivo. Ag-NPs-S. *ebulus* extract has a more lethal effect on the parasite than Ag-NPs-F. *sellowiana*. It is suggested that in future investigate the induction of *Toxoplasma*-infected cell apoptosis using nanoparticles.

**Keywords:** *Toxoplasma gondii* · Treatment · Silver nanoparticles · *Sambucus ebulus*

# HIV+/AIDS is a risk factor for infection with intestinal protozoa: a case-control study in Yazd, Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Intestinal protozoa are the most common opportunist's parasites among in HIV+/AIDS patients. As there is no report of outbreaks of intestinal parasites in such patients in Yazd, so this study was performed to determine the prevalence of intestinal parasites in HIV+/AIDS patients in comparison with non-HIV individuals.

**MATERIALS AND METHODS:** A case-control study was conducted from July 2015 to March 2016. Totally 73 Patients (group I) were recruited from the Prevention of Behavioral Disorders Center in Yazd. A control group (group II) comprising 147 healthy HIV negative individuals were included. After collecting, the stool sample was examined for intestinal parasites by direct and formalin-ether concentrated smears. Modified Ziehl-Neelsen staining was used to detect enteric coccidian.

**RESULTS AND DISCUSSION:** The overall prevalence of intestinal parasites in groups I and II was 34.2% and 4.1%, respectively, with a significant difference between two groups. The prevalence of infection for each intestinal protozoan, in each group, was as follows: group I: Blastocystis hominis (2.8%), Giardia lamblia (9.7%), Entamoeba histolytica (1.4%), Chilomastix mesnili (4.2%), Endolimax nana (4.2%), and Entamoeba coli (5.6%); group II: Blastocystis hominis (0.0%), Giardia lamblia (0.7%), Entamoeba histolytica (2%), Chilomastix mesnili (0.0%); Endolimax nana (1.4%) , Entamoeba coli (0.0%) and mixed infection (5.6%). Intestinal parasites were significantly more common in group I than group II. In this study, no type of enteric coccidian and intestinal helminths infections were observed. Conclusion: The results emphasize the necessity of increasing awareness among clinicians regarding the occurrence of these parasites in HIV+/AIDS patients for preventing of the risk of related symptoms.

**Keywords:** Intestinal Diseases, Parasitic, Acquired Immunodeficiency Syndrome, Iran

# A novel homemade Polymerase Chain Reaction (PCR) and fluorescence biosensor on chip (FBC) to identify *Streptococcus pneumoniae*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Streptococcus pneumoniae* (*S. pneumoniae*) is one of the important factors causes community acquired pneumonia and meningitis in children and elderly and of sepsis in those infected with HIV. Other pneumococcal diseases are including bronchitis, otitis media, acute sinusitis, meningitis, sepsis, peritonitis, cellulitis and brain abscess. Fabrication of FBC was carried out based on the deposition of lead nanoparticles on a quartz slide using the thermal evaporation method. Then, the SH-Cap Probe/Target ssDNA /FAM-Rep probe was loaded on lead film. The evaluation of the fluorescence reaction when the probes bind to the target ssDNA was assessed by a Cytation 5 Cell Imaging Multimode Reader Bio-Tek. The limit of detections (LOD) in homemade PCR and FBC to identify *S. pneumoniae* were  $119 \times 10^2$  CFU/mL (0.27 ng/ $\mu$ L) and 380 CFU/mL (9 pg/ $\mu$ L), respectively. Both techniques had appropriate sensitivity and specificity in detection of *S. pneumoniae*.

**MATERIALS AND METHODS:** Fabrication of FBC was carried out based on the deposition of lead nanoparticles on a quartz slide using the thermal evaporation method. Then, the SH-Cap Probe/Target ssDNA /FAM-Rep probe was loaded on lead film. The evaluation of the fluorescence reaction when the probes bind to the target ssDNA was assessed by a Cytation 5 Cell Imaging Multimode Reader Bio-Tek.

**RESULTS AND DISCUSSION:** The limit of detections (LOD) in homemade PCR and FBC to identify *S. pneumoniae* were  $119 \times 10^2$  CFU/mL (0.27 ng/ $\mu$ L) and 380 CFU/mL (9 pg/ $\mu$ L), respectively. Both techniques had appropriate sensitivity and specificity in detection of *S. pneumoniae*.

**Keywords:** PCR, fluorescence biosensor on chip, *Streptococcus pneumoniae*,



# A rare case of Bloodstream Infection caused by *Burkholderia cepacia* complex (BCC) in Iran

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Sepsis is a serious and life-threatening condition caused by an inappropriate host immune response to an infection. Catheter-Related Bloodstream Infection is the most common cause of nosocomial bacteremia. *Burkholderia cepacia* complex (BCC) is a rarely isolated motile, gram-negative aerobic bacilli which is intrinsically resistant to several used antibiotics that causes in rare cases severe infections, such as bloodstream infection, meningitis and pneumonia, especially in immunocompromised patients. In this paper, we reported the first case of *Burkholderia cepacia* complex (BCC) bloodstream infection in Iran.

**MATERIALS AND METHODS:** A neonate with heart failure, Tachycardia, Cardiac tumor and sepsis symptoms (fever and chills) was hospitalized in Neonatal Intensive Care Unit (NICU) of Imam Reza hospital, Mashhad, northeast of Iran. Blood cultures were performed using the BD BACTEC (Becton, Dickinson, USA) automated haemoculture system and subsequently the gram-negative bacilli were identified as *Burkholderia cepacia* complex (BCC) by the BD Phoenix M50 Compact automated system. As a result, microbiological findings confirmed the diagnosis of the first case of *Burkholderia cepacia* complex Bloodstream infection in Iran. Repeated blood culture showing negative results after three days of Cefotaxime 150mg/Daily and Meropenem 20mg/8 hours administration that leads to eradication of microorganism and sepsis symptoms. Finally, the patient discharged and transferred to another hospital for continue treatment of cardiac tumor.

**RESULTS AND DISCUSSION:** In this study, BCC diagnosed by the BD Phoenix M50. This strain was Glycine-Proline, Lysine-Alanine positive and for Citrate, Acetate, Alpha-Ketoglutaric Acid, Malonate, Sucrose, L-Rhamnose, Dextrose, L-Arabinose, Ornithine, Urea and Esculin was negative also antimicrobial susceptibility tests demonstrated that this strain was susceptible to Cefixime, ceftazidime, Ciprofloxacin and Cefotaxime and resistant to Trimethoprim-Sulfamethoxazole and Gentamicin. Finally clinical and microbiological resolution was achieved by antibiotic therapy which leads to eradication of microorganism and sepsis symptoms. There are little evidence in the literature about Bloodstream infections caused by *Burkholderia cepacia* complex, Catheter-Related Bloodstream Infection caused by BCC is a rare infection in humans, but this microorganism should be considered as a potential pathogen in hospitalized immunocompromised patients which can occasionally leads to serious infections in humans, especially in immunocompromised individuals, requiring broad-spectrum antibiotic therapy.

**Keywords:** sepsis, *Burkholderia cepacia* complex, hospital acquired infection, Bloodstream infection,

# A rare case of Catheter-Related Bloodstream Infection caused by *Cupriavidus pauculus*

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Cupriavidus pauculus* is a gram-negative, aerobic bacillus found in environment. This microorganism can rarely cause serious infections in immunocompromised patients. Catheter-Related Bloodstream Infection caused by *Cupriavidus pauculus* is a hospital acquired infection which is an infrequent state with very few cases reported in the literature. In this paper, we report the first case of *Cupriavidus pauculus* infection in Iran.

**MATERIALS AND METHODS:** A 43-year-old woman was admitted to Internal Medicine Department of Imam Reza hospital with cervical cancer, oliguria, hydronephrosis and sepsis symptoms) fever and chills(. Blood cultures were performed using the BD BACTEC (Becton, Dickinson, USA) automated haemoculture system and subsequently the gram-negative bacilli with regular borders, smooth consistency and dry appearance colonies were identified as *Cupriavidus pauculus* by the BD Phoenix M50 Compact automated system. Empirical antibiotic therapy was started with intravenous Vancomycin and Meropenem.

**RESULTS AND DISCUSSION:** In this paper, we report the first case of *Cupriavidus pauculus* infection in Iran. This strain was Catalase, Oxidase, Citrate, Acetate and Alpha-Ketoglutaric Acid positive and for Malonate, Sucrose, L-Rhamnose, Dextrose, L-Arabinose, Ornithine, Urea and Esculin was negative. In addition this strain was susceptible to Piperacillin-Tazobactam ( $\leq 4.4 \mu\text{g/mL}$ ), Ceftazidime ( $\leq 2 \mu\text{g/mL}$ ), Cefepime ( $\leq 1 \mu\text{g/mL}$ ), Trimethoprim-Sulfamethoxazole ( $\leq 1.19 \mu\text{g/mL}$ ), Ciprofloxacin ( $\leq 0.5 \mu\text{g/mL}$ ), Levofloxacin ( $\leq 1 \mu\text{g/mL}$ ) and resistant to Cefazolin ( $\leq 4 \mu\text{g/mL}$ ), Ampicillin ( $8 \mu\text{g/mL}$ ) and Amoxicillin-Clavulanate ( $\leq 4.2 \mu\text{g/mL}$ ). There is little evidence in the literature about infections caused by *Cupriavidus pauculus*. Catheter-Related Bloodstream Infection caused by *Cupriavidus pauculus* is an infrequent infection in humans, but this microorganism should be considered as a potential pathogen in hospitalized immunocompromised patients which can cause serious infections in these individuals, requiring broad-spectrum antibiotic therapy. Finally clinical and microbiological resolution was achieved by antibiotic therapy which leads to eradication of microorganism and sepsis symptoms.

**Keywords:** Catheter-Related Bloodstream Infection, *Cupriavidus pauculus*, immunocompromised patients, Hospital acquired infection

# A rare outbreak of *Ochrobactrum anthropi* bloodstream Infection and clinical outcomes

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Abstract Introduction Hospital-acquired infections (HAIs) are significant problems which need serious attention worldwide. During hospitalization medical devices providing many opportunities for transmission of pathogens into patients, Meanwhile Catheter-Related Bloodstream Infections (CRBSIs) are one of the most prevalent causes of nosocomial bacteremia associated with high rates of morbidity and mortality. *Ochrobactrum anthropi* is an emerging pathogen with an increase in the frequency of infections as a potentially problematic, opportunistic and nosocomial pathogen. In this investigation we report the first outbreak of *Ochrobactrum anthropi* in Iran that occurred on May 24 2024 –June 10 2024 at a tertiary hospital.

**MATERIALS AND METHODS:** Materials and Methods In this study, we detected a bloodstream infection outbreak caused by *Ochrobactrum anthropi* which 12 patients were identified by the BD Phoenix M50 Compact automated system. All the cases were immunocompromised and had underlying diseases. In these patients after catheter insertion, septicemic symptoms (fever and chills), leukocytosis and elevated C - reactive protein (CRP) levels were developed.

**RESULTS AND DISCUSSION:** Results and DISCUSSION In this paper, we report the first outbreak of *Ochrobactrum anthropi* in Iran during May 24, 2024–June 10, 2024, in a period of 18 days. In this investigation health care workers or catheters were the probable source of the outbreak and *Ochrobactrum anthropi* as a rare pathogen, was responsible for this outbreak in our center. *Ochrobactrum anthropi* is an emerging opportunistic pathogen rarely implicated in causing bloodstream infection especially in immunocompromised patients. The most frequently reported infection by *Ochrobactrum anthropi* is intravascular catheter-related bacteremia. This investigation highlights the need for adequate precautions to prevent the catheters contamination.

**Keywords:** Keywords: Hospital-acquired infections, *Ochrobactrum anthropi*, Outbreak, Bloodstream Infection, Catheter, Immunocompromised

# A report of Actinomycosis in sheep with lower jaw swelling

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Actinomycosis is a chronic disease which affects mandible, maxillae or other bony structures of the head. Actinomyces can cause endogenous infections, with dental erosion serving as a potential predisposing factor for the bacterial penetration. The afflicted animal has difficulties in feeding and subsequently may show weight loss. The disease occurs more commonly in the grazing systems following eating rough awn especially in dry seasons. The aim of this study was to report actinomycosis in a sheep in an extended rearing system in summer.

**MATERIALS AND METHODS:** A two-year-old sheep kept in a poor pasture condition, showed swelling in the submandibular region. The swelled part was examined with the palpation of bone and surrounded muscles. Other animals of the flock showed no similar signs.

**RESULTS AND DISCUSSION:** Results: The affected area was hard in palpation which indicated the bone infection. There wasn't any signs of abscesses or muscle infection and hematoma. According to the animal age, cancer was ruled out. A fistula had been formed with granular yellow discharge. Based on the clinical manifestations and the history of grazing on rough pasture, the condition was diagnosed as actinomycosis. The affected sheep was treated with long-acting oxytetracycline along with spraying oxytetracycline on the fistula. Ketoprofen was injected to alleviate the inflammation. Conclusion: The present result indicates the importance of feed quality. In Iran, small ruminant flocks are only able to graze on green forage during the spring and early summer. The indigenous sheep breeds of Iran have remarkable resilience, exhibiting sustained productivity even in pastures with suboptimal quality. However, they show vulnerability to opportunistic agents, including Actinomyces. Preventive measures include improving the feed quality, isolating the infected animals,

**Keywords:** Actinomyces, Actinomycosis, Feed quality, Rough forage, Sheep.

# A simple and practical method for accurate detection of Carbapenem-resistant Enterobacteriaceae in the clinical laboratories

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Carbapenem-resistant Enterobacteriaceae (CRE) are a global concern and may change the outcome, mortality and morbidity. To detect carbapenemase activity, there is an urgent need to an inexpensive, rapid, sensitive, and specific test. In fact, the aim of this study was introduced a simple and practical method for accurate detection of CRE in clinical laboratories.

**MATERIALS AND METHODS:** Fifty non-duplicated CRE were recovered. To detect CRE, we used the phenotypical and molecular methods. To screen CRE, the disk diffusion and micro-broth dilution methods were used. The Modified Hodge Test (MHT), Carba NP Test and PCR methods were also carried out for confirmation of CRE.

**RESULTS AND DISCUSSION:** Fifty CRE (41 *Klebsiella pneumoniae*, 6 *Escherichia coli* and 3 *Enterobacter* spp. isolates) collected from Jan 2018 to Dec 2018. Resistance to Imipenem (10µg), meropenem (10µg) and ertapenem (10µg) were 96%, 96% and 100%, respectively. All isolates (100%) were resistance to carbapenems by the micro-broth dilution assay (MICs of imipenem and meropenem). According to Carba NP Test and Modified-Hodge test, 50 isolates and 49 isolates were positive for carbapenemase activity, respectively. The presence of carbapenemase was found in all isolates (100%), and the most common gene was blaOXA-48like (70%) and followed by blaNDM, blaIMP, blaVIM and blaKPC in 46%, 18%, 12% and 6 % isolates, respectively. The ertapenem disk is a sensitive indicator for detection of carbapenemase activity in clinical settings. Carba NP test and PCR are another available test that can detect carbapenemase activity of CRE.

**Keywords:** Carbapenem-resistant Enterobacteriaceae, Carba NP Test, Ertapenem, PCR

# Acinetobacter baumannii Infection in Critically ill Patients from Tehran, Iran: A Comparison of prevalence, antimicrobial resistance patterns and molecular characteristics of isolates

Bacteriology

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**BACKGROUND AND OBJECTIVES:** One of the most important causes of mortality and morbidity in Intensive Care Units (ICU) are Healthcare-Associated Infections (HAI). *Acinetobacter baumannii* is among the main causes of HAI as a consequence of its high level of antibiotic resistance and high mortality. Treatment of infections caused by *A. baumannii* has become difficult because many isolates are now resistant to a wide range of antimicrobial agents and cause an important issue for clinical microbiologists and physicians. The aim of this study was to investigate the prevalence, antimicrobial resistance patterns and molecular characteristics of *A. baumannii* isolates obtained from patients admitted to the ICU.

**MATERIALS AND METHODS:** This was a cross-sectional and single-center study comprising patients with *A. baumannii* infections admitted to ICU between April and November 2021. Antimicrobial susceptibility testing was carried out based on the CLSI recommendations. The PCRs targeting antibiotic resistance genes and multiplex PCRs for identifying the global clones (GC) of *A. baumannii* were performed. Molecular typing of strains was carried out by the repetitive element PCR (REP-PCR) method.

**RESULTS AND DISCUSSION:** The prevalence of *Acinetobacter baumannii* in ICU patients in Tehran, Iran was found to be 2.25%, with a high mortality rate of 84%. Most isolates were extensively drug-resistant (XDR), carrying  $\beta$ -lactamase and aminoglycoside-modifying enzyme genes. Molecular analysis showed that 85.3% of isolates belonged to global clone II (GC II), and REP-PCR patterns were categorized into nine types, with two types being most prevalent. This study underscores the formidable challenge of managing *A. baumannii* infections in ICU settings, highlighting the significant threat posed by their pervasive resistance to a broad spectrum of antibiotics. This MDR complicates therapeutic interventions, presenting substantial obstacles for clinical microbiologists and physicians. The molecular characterization of the isolates indicates the predominance of specific global clones, emphasizing the complexity of the epidemiology of these infections. Consequently, these findings call for robust infection control protocols and the innovation of novel therapeutic approaches to effectively combat the high antimicrobial resistance

**Keywords:** *Acinetobacter baumannii*, Antibiotic resistance, resistance genes, Global clones, ICU, REP-PCR

# An evaluation on Interferon and Tumor Necrosis Factor- $\alpha$ Gene Expression alteration in Human Blood Macrophage-Like Monocytes Induced by *Salmonella typhi* Strains in vitro

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Salmonella typhi* as a human pathogen stimulates the human immune system and triggers gene expression changing its pathogenesis. Therefore, we aimed to investigate the expression levels of IFN- $\gamma$  and TNF- $\alpha$  cytokines in human blood macrophage-like monocytes in dealing with clinical and standard samples of *Salmonella typhi* in vivo.

**MATERIALS AND METHODS:** In this cross-sectional descriptive study, a total of 60 stool samples from patients with gastroenteritis were cultured and biochemical tests were used to diagnose *Salmonella*. Also, venous blood samples were taken for peripheral blood mononuclear cell (PBMC) isolation, and PBMCs were cultured in a culture medium containing  $4 \times 10^3$  CFU/mL treatments of *Salmonella typhi* pathogen and standard. Cytotoxicity tests were also performed to determine the concentrations. Finally, quantitative expression levels of IFN- $\gamma$  and TNF- $\alpha$  were measured and the results were analyzed by statistical tests.

**RESULTS AND DISCUSSION:** The results of the cytotoxicity test showed the use of *Salmonella typhi* concentrations for treatment in an authorized culture medium at a concentration of  $4 \times 10^3$  CFU/mL. In comparison to control samples, significantly increased expression levels of the TNF- $\alpha$  gene have been detected in pathogen strain and ATCC strain (P0.05) ( $P=0.0198$ ). Furthermore, significantly increased expression levels of the IFN- $\gamma$  gene have been detected in the pathogen strain and ATCC strain (P0.05) in comparison to the control sample ( $P=0.0001$ ). Increased and significant expression of IFN- $\gamma$  and TNF- $\alpha$  cytokines in the sample group treated with pathogen strain and ATCC strain indicates polarization of macrophages stimulated by *Salmonella typhi* in vitro.

**Keywords:** IFN- $\gamma$ , Macrophage, *Salmonella typhi*, TNF- $\alpha$

# Anti-Leishmania effect by plant cocktail of Crosin-curcumin

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Leishmaniasis is a vector-borne disease caused by protozoan parasites of the genus *Leishmania* and transmitted by phlebotomine sand flies. It is endemic in 98 countries and three territories, with an estimated 700,000 to 1 million new cases occurring annually worldwide. Existing anti-leishmanial treatments have been ineffective for a long time and are associated with toxic side effects, so the search for new, effective and safe alternative treatments against cutaneous leishmaniasis (CL) is crucially needed. This study was conducted with the aim of investigating the leishmaniacidal effects of the combination of Crosin-curcumin separate and in mixed form with Amphotericine B on CL in vivo.

**MATERIALS AND METHODS:** Crosin and curcumin were extracted from Medicinal plants. Maceration method was used for extraction of the plant and the compounds were extracted using Mas Chromatography and Spectrophotometry technique. *Leishmania major* (L. major) standard strain promastigotes were purchased from Leishmaniasis research center, Public Health school, Tehran University of Medical Sciences. In this study, 36 mice were selected and divided into 6 groups of three. The first three groups were doses of 200, 400, and 800 µg/ml of crosin-curcumin plant extract. Glucantim and Amphotericin B were used as positive control groups and PBS was used as negative control group. Cytotoxicity of the compounds on normal human fibroblast cells was measured by MTT method.

**RESULTS AND DISCUSSION:** In this investigation, the results showed that the Crosin-curcumin had acceptable effectiveness and this effectiveness in concentration of 800 µg/ml in day 28 was the most effective group. The future decision about this compound, need some more and complementary tests.

**Keywords:** Anti-leishmaniasis, *Leishmania major*, MTT assay, Crosin, Curcumin



# Anti-leishmanial effects of *Eryngium planum* methanolic extract against *Leishmania major*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Leishmaniasis is a significant parasite illness that occurs in tropical and sub-tropical locations worldwide. Given the rising incidence of this disease and the growing instances of resistance to glucantim, a widely used treatment medication, it becomes imperative to conduct research and explore the discovery of a secure and organic alternative drug for the management of leishmaniasis. The objective of this study is to examine the anti-leishmanial properties of the methanolic extract of *Eryngium planum* on *Leishmania major* parasites in vitro and in vivo.

**MATERIALS AND METHODS:** The *L. major* parasite was grown in RPMI-1640 media using the standard strain. The powder derived from *Eryngium planum* was extracted using the maceration process and methanol solvent. The concentrations of the extract were 100, 200, 400, and 800 µg/mL. An investigation was conducted to determine the impact of various concentrations on the *Leishmania* parasite at 12, 24, and 48-hour intervals. The methanolic extracts of *Eryngium planum* exhibited significant antiparasitic activity against the *L. major* parasite across all tested doses.

**RESULTS AND DISCUSSION:** The findings indicate that the concentrations of 400 and 800 µg/mL of the methanolic extract of *Eryngium planum* had comparable effects to the control group in the in vitro investigation. Similarly, in the in vivo trial, the dose of 800 µg/ml had a similar effect to the positive control group. According to the MTT examination, *Eryngium planum* has been found to be non-toxic, even at a concentration of 1000 µg/ml. Based on the lethal properties of the methanolic extract of *Eryngium planum* on the *L. major* parasite and its comparable effectiveness to the positive controls, it can be inferred that these compounds have potential as viable options for treating leishmaniasis. However, further research is needed to confirm these findings.

**Keywords:** *Leishmania major*, *Eryngium planum*, cutaneous leishmaniasis, in vitro, in vivo

# Antibacterial Activity of Zinc Oxide Nanoparticles Derived by the Green Synthesis Method from Leaf and Flower of *Arctium lappa* on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Considering the worries about spread of antibiotic resistance to classical antimicrobial agents, it seems necessary to use a new therapeutic method to fight against resistant pathogens. The aim of these study is the use of nanoparticles and the green synthesis of these nanoparticles with plant extracts that has no biological hazards.

**MATERIALS AND METHODS:** The present study was conducted in order to synthesize zinc nanoparticles using the extract of the plant with the scientific name *Arctium lapa* and to investigate the antibacterial effect of these green synthesized nanoparticles on methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria. After collecting the leaves of Baba Adam plant, pure leaf extract was prepared under laboratory conditions. Synthesis of zinc nanoparticles with these extracts was done using zinc nitrate, then MIC and MBC of zinc nanoparticles against methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria were determined by microdilution method.

**RESULTS AND DISCUSSION:** MIC and MBC results of leaf extract, antibiotics and synthesized zinc nanoparticles on methicillin-resistant *Staphylococcus aureus* (25000 and 50000), (15.31 and 5), (12500 and 25000 µg/ml) and on *Pseudomonas aeruginosa* (12500 and 25000) (31 and 5/62), (6250 and 12500 µg/ml) were reported respectively. In general, synthesized zinc nanoparticles by the green method have antibacterial effects on the mentioned bacteria.

**Keywords:** *Staphylococcus aureus*, *Pseudomonas aeruginosa*, green synthesis, zinc nanoparticles, *Arctium lapa*

# Antibiotic resistance of *Helicobacter pylori* isolated from patients referred to Imam Khomeini Hospital of Sari in 2020

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Helicobacter pylori* is a gram-negative, slow-growing bacterium known to cause various gastrointestinal diseases such as peptic ulcers, stomach cancer, and gastric lymphoma. Antibiotic therapy is a common treatment approach for *Helicobacter pylori* infection; however, the emergence of antibiotic resistance poses a significant challenge in the management of this infection. The prevalence of *Helicobacter pylori* infection is high in northern regions of Iran, including Sari. This study aimed to investigate the prevalence and antibiotic resistance patterns of *Helicobacter pylori* in patients referred with upper gastrointestinal symptoms to Imam Khomeini Hospital of Sari in 2020.

**MATERIALS AND METHODS:** This descriptive cross-sectional study was conducted from November 2019 to March 2020 in the endoscopy department of Imam Khomeini Hospital in Sari. 120 patients with upper gastrointestinal symptoms were included in the study and their demographic characteristics were recorded. The resistance of *Helicobacter pylori* to six commonly used antibiotics, including metronidazole, clarithromycin, tetracycline, amoxicillin, levofloxacin, and furazolidone, was investigated. The obtained data were entered into the statistical software SPSS 16 and analyzed using descriptive statistics, including frequency and mean calculations. The chi-square test was used to assess the significance of the data.

**RESULTS AND DISCUSSION:** The most common reason for visiting the hospital was epigastric pain (68.3%) ( $p < 0.05$ ). From 120 patients, 65 cases (54.15%) were infected with *Helicobacter pylori*. 81% of women and 70% of men infected with *Helicobacter* were over 40 years old. ( $p < 0.05$ ). 30 isolated that were obtained from the culture of gastric biopsy specimens of patients were tested by Disk Diffusion Susceptibility Test on Mueller-Hinton agar. The resistance level was 63.3% for metronidazole, 16.6% for clarithromycin, 10% for amoxicillin, 6.6% for tetracycline and 3.3% for levofloxacin. There was not observed any resistance to furazolidone. According to the results of this study, it's better not to use metronidazole in the first-line medication for patients with *Helicobacter* infection but we can use furazolidone and levofloxacin because *H. pylori* response to this antibiotics is really high. So To determine the appropriate drug regimen against *Helicobacter Pylori*, It seem to be necessary to order antibiotic susceptibility tests.

**Keywords:** Helicobacter pylori, Antibiotic resistance, Diffusion disk

# Antibiotic Susceptibility Pattern of Uropathogens Among Hospitalized Children with Urinary Tract Infections

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Urinary tract infections (UTIs) as a global health problem are one of the most common bacterial infections in humans, especially among young children with high mortality and morbidity rates worldwide. The aim of the present study was to evaluate the frequency and antibiotic resistance profiles of bacterial uropathogens isolated from hospitalized children with UTIs and their related clinical and demographic factors in the north of Iran.

**MATERIALS AND METHODS:** Hospitalized children with urinary tract infections according to positive urine culture included in study. Etiologic agents of UTIs and their antibiotic resistance profiles, demographic and clinical characteristics of patients entered into SPSS software version 21 and statistical analysis was carried out using Chi-square ( $\chi^2$ ) test to evaluate the relationship between the variables.  $p < 0.05$  was considered statistically significant.

**RESULTS AND DISCUSSION:** A total of 163 children were diagnosed with UTIs from girls (60.7%) compared to boys patients (39.3%). The mean age was  $26.4 \pm 40$  months. The most common clinical symptoms at admission were fever and vomiting, with 57.1% and 25.2%, respectively. The microbiological identification showed that *Escherichia coli* was the most common pathogen (60.12%), followed by *Klebsiella* spp. (30.67%). Further analysis of antibiotic susceptibility among uropathogens showed that the highest resistance rates were to ampicillin, co-amoxiclavs, and cefazolin, with 88.7, 83.3, and 76.3, respectively. However, the highest susceptibility against nitrofurantoin was 92.2%, followed by meropenem (87%), norfloxacin (77%), and aminoglycosides (75%). There was a significant correlation between isolated bacteria and clinical symptoms ( $p < 0.05$ ). Our findings showed a considerable rate of antibiotic resistance among bacterial isolates from children's UTIs in our studied region. Our study suggests the necessity of periodic monitoring of antimicrobial resistance patterns.

**Keywords:** Urinary Tract Infection, Uropathogens, Antibiotic Susceptibility, Children.

# Antibiotic Susceptibility, Molecular Characterization, and REP-PCR Fingerprints of *Acinetobacter baumannii* Isolates from Intensive Care Unit Patients in Isfahan, Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** The broad use of  $\beta$ -Lactam antibiotics for treating *Acinetobacter baumannii* infections has resulted in the occurrence of highly resistant  $\beta$ -lactamase-producing strains. The present study reports the antibiotic resistance rates, prevalence of  $\beta$ -lactamase-encoding genes, and clonal relationships of *A. baumannii* strains isolated from Intensive Care Unit (ICU) patients in different teaching hospitals of Isfahan, Iran.

**MATERIALS AND METHODS:** A total of 57 *A. baumannii* isolates were recovered from clinical specimens of ICU patients. Antibiotic susceptibility testing was performed using the disc diffusion method, and  $\beta$ -lactamase-encoding genes were characterized by PCR. Repetitive element sequence-based PCR (rep-PCR) was used to determine the lineage relatedness of the isolates.

**RESULTS AND DISCUSSION:** The antimicrobial resistance pattern showed a resistance rate of 100% to cotrimoxazole, ciprofloxacin, imipenem, ceftazidime, and ampicillin-sulbactam, followed by amikacin (98.2%), cefepime (96.5%), tetracycline (86%), and gentamycin (77.2%). All 57 *A. baumannii* isolates were extensively drug-resistant (XDR). The carbapenemase resistance gene evaluation revealed that all isolates were positive for the blaTEM gene, but negative for blaCTX-M and blaOXA-58-like genes. The other tested genes, including blaOXA-24-like, blaVIM, blaOXA-23-like, blaSHV, and blaKPC, were detected in 56 (98.2%), 35 (61.4%), 14 (24.6%), 1 (1.8%), and 1 (1.8%) isolate, respectively. The rep-PCR analysis revealed 27 different genotypes. The study demonstrates the emergence of extensively drug-resistant *A. baumannii* strains harboring diverse  $\beta$ -lactamase genes in the ICUs of Isfahan, Iran, highlighting the necessity for enhanced infection control measures and antimicrobial stewardship programs to mitigate the spread of these multidrug-resistant pathogens.

**Keywords:** *Acinetobacter baumannii*, carbapemase resistance, rep pcr

# Antifungal susceptibility of potentially probiotic *Lactobacillus plantarum* on drug-resistant *Candida glabrata*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Candida* species are the most common cause of fungal infection in severely immunosuppressed patients. Considering the rise of antifungal resistance, the probiotic *Lactobacillus* species has been proposed as an alternative treatment choice against *Candida* infection. *Lactobacillus* species are among probiotics that constrain the formation and growth of *Candida* biofilm and reduce the symptoms of infection. There is a lack of information about *Lactobacillus* effect on drug-resistant *Candida glabrata* in leukemia patients. The focus of this study is to explore the anti-fungal effect of *L. plantarum* probiotic on drug resistant *C. glabrata* strains isolated from patients with leukemia.

**MATERIALS AND METHODS:** In this review, the antifungal effects of *L. plantarum* on drug-resistant oral *C. glabrata* isolated from patients with leukemia, was evaluated. At the first, the resistance of 10 strains of *C. glabrata* to fluconazole and amphotericin B was confirmed according to CLSI-M27-S4. The effects were measured by the co-aggregation method. After exposure, *L. plantarum* and *C. glabrata* colonies were counted.

**RESULTS AND DISCUSSION:** Our finding showed that the highest co-aggregation ratio of *L. plantarum* was observed for amphotericin B-resistant *C. glabrata* strains. Whereas, the effect of *L. plantarum* on fluconazole-resistant *C. glabrata* was not significant ( $P < 0.05$ ). In species resistant to both antifungal agents, the number of fungal cells decreased over time. *L. plantarum* at cell concentrations (10<sup>8</sup> to 10<sup>2</sup> cfu/ml) was inhibited the growth of resistant *C. glabrata* strains. This study revealed that *L. plantarum* had a remarkable ability to co-aggregate with amphotericin B-resistant *C. glabrata* strains and its effect on fluconazole-resistant *C. glabrata* was not significant. Considering our results and treatment failures due drug-resistant *Candida* species, further investigations are suggested for evaluating the antifungal properties of *Lactobacillus* species.

**Keywords:** *Candida glabrata*, Probiotics, Drug Resistance, Co-aggregation

# Antifungal susceptibility Pattern of bloodstream *Candida* species isolates

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Candidemia is one of the most prevalent invasive fungal diseases among patients with a great rate of morbidity and death. Epidemiological studies in recent years have shown that the cause of candidemia has changed from *Candida albicans* to other *Candida* species. One of the main challenges with this infection is the increasing rate of resistance in these species to different antifungal agents such as fluconazole, voriconazole, amphotericin B, and caspofungin which is induced by various factors the most important one being non-selective treatment of candidemia. This research aimed to evaluate the antifungal susceptibility pattern of *Candida* species obtained from bloodstream infection to find the best therapeutic option.

**MATERIALS AND METHODS:** Herein, we have retrospectively estimated the susceptibility profile of *Candida* species obtained from the Candidemia patients (2017-2024) to antifungal agents. All patients were admitted to the teaching hospitals associated with the Shahid Beheshti University of Medical Sciences (Tehran, Iran). Molecular methods were used to detect *Candida* species (n: 47) and the results consisted of *C. albicans* complex (n:19), and non-*albicans* *Candida* (*C. glabrata*; n:12, *C. parapsilosis* complex; n:8, *C. tropicalis*; n:8). Susceptibility tests were conducted for multiple antifungal agents such as fluconazole, voriconazole, amphotericin B, and caspofungin according to the CLSI-M27-S4.

**RESULTS AND DISCUSSION:** We detected no resistance to amphotericin B in *C. albicans* strains; all were susceptible (100%) to these two antifungal agents. Whereas, 78% and 62% of *C. albicans* isolates were susceptible to voriconazole and fluconazole, respectively. The susceptibility rates of non-*albicans* *Candida* to amphotericin B, caspofungin, voriconazole and fluconazole, were 94%, 97%, 68%, and 52%, respectively. Recently there has been a significant increase in non-*albicans* *Candida* species, especially *C. parapsilosis* among candidemia patients. Although Amphotericin B and Caspofungin have demonstrated promising results, the increasing resistance to fluconazole requires further investigation of candidemia to find the best therapeutic option.

**Keywords:** Candidemia, *Candida* species, Susceptibility



# Antimicrobial activity of Methanolic and Aqueous Extracts of *Myrtus communis*, and *Citrus aurantium* on oral Microbial pathogens

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Prevention of dental caries with the use of plants is of great interest due to less side effects, variety of effective compounds in plants and lower economic costs. Therefore, in this research, the antifungal/antibacterial effects of aqueous and methanolic extracts of orange blossom (*Citrus aurantium*) and *Myrtus communis* on standard bacterial and fungal strains causing dental caries have been investigated.

**MATERIALS AND METHODS:** In this experimental study, the aqueous and methanolic extract of *Citrus aurantium* and *Myrtus communis* were prepared by maceration method. The antifungal/ antibacterial activity of the extracts was evaluated using the well diffusion method for *Candida albicans*, *Candida tropicalis*, and *Candida krusei*, and *Streptococcus sanguinis* and *Streptococcus mutans*. The minimum inhibitory concentration (MIC) and the minimum fungicidal/bactericidal concentration (MFC/MBC) were also determined by micro-dilution method. The results were analyzed using one-tail ANOVA.

**RESULTS AND DISCUSSION:** Aqueous extracts of the *M. communis* showed antimicrobial effects with MIC and MBC values of 64 and 1024 µg/mL against two strains of *S. mutans* and *S. sanguinis* and MIC and MFC values of 256 and 4096 µg/mL against *C. albicans*, respectively. Aqueous extracts of the *C. aurantium* showed no antimicrobial effects on the studied microorganisms, except *C. albicans* with MIC and MFC values of 512 µg/mL. The antimicrobial activity of the methanolic extract indicated the antimicrobial potency of the *M. communis*, and *C. aurantium* against all the studied microorganisms. Also, the lowest minimum microbicidal concentration (MMC) against the studied strains were related to the methanolic extract of *M. communis*. Results of this study showed that watery and methanolic extract of *Citrus aurantium* L. flower and *Myrtus communis* can be effective in preventing dental caries which could be a suitable alternative to chemical mouthwashes to prevent and control oral infections.

**Keywords:** *Citrus aurantium*, *Myrtus communis*, Oral pathogens, Antimicrobial activity

# Antimicrobial Effect of Nanoparticles Loading on Bacterial Cellulose

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Bacterial cellulose, synthesized via the fermentation process of Gram-negative bacterium *Acetobacter xylinum*\*, demonstrates high aspect ratio nanofibers and is characterized by a notable presence of hydroxyl groups. These properties render it well-suited to be functionalized with a variety of nanomaterials.

**MATERIALS AND METHODS:** Bacterial cellulose has been functionalized with silver nanoparticles using UV light irradiation. and then tested for its efficacy against *Staphylococcus aureus*. The photochemical reduction of silver by UV light irradiation was seeded by impregnating silver ions onto the bacterial cellulose (BC) fibers. The hydroxyl groups on the surface facilitates chemical bonding. Silver nitrate (AgNO<sub>3</sub>) was used as the impregnation medium at four different concentrations. The morphological analysis of the nanocomposite was conducted using scanning electron microscopy (SEM). revealed the distribution of silver nanoparticles, including some aggregates, within the bacterial cellulose network.

**RESULTS AND DISCUSSION:** The antibacterial efficacy of the AG/BC nanocomposite was evaluated using the disk diffusion method across four different concentrations. The inhibition zones developed on a *Staphylococcus aureus* lawn culture were measured to determine the optimal concentration of the hybrid composite. This concentration showing the best performance was identified as a promising candidate for potential wound healing treatments. Due to their stability in moist environments, these composites could potentially aid in wound healing processes and be stored for extended periods, making them beneficial for general wound treatment and surgical applications.

**Keywords:** bacterial cellulose wound healing silver nanoparticles nanocomposite antibacterial effect *Acetobacter*

# Assessment of Antibiotic Resistance and Biofilm-Forming Capabilities in *Staphylococcus aureus* from Clinical Sources

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus* is a significant pathogen responsible for severe nosocomial infections, characterized by a variety of virulence factors and a capacity to develop resistance to numerous antibiotics. Infections caused by *S. aureus* contribute to increased morbidity, mortality, and healthcare costs. Various factors, including biofilm formation, facilitate the acquisition and enhancement of antibiotic resistance in *S. aureus*. The present study aimed to determine the prevalence of antibiotic resistance to antibacterial agents and assess the biofilm-forming ability of *S. aureus* isolates phenotypically.

**MATERIALS AND METHODS:** A total of 100 isolates were obtained from various clinical sources. *S. aureus* isolates were identified phenotypically by Gram staining and different biochemical tests and molecular method (PCR). Antimicrobial resistance was assessed with Kirby-Bauer disc diffusion method as recommended by CLSI guidelines. Biofilm formation was evaluated using the microtiter plate method. All experiments were conducted in triplicate with clinical isolates, and the optical density (OD) values were averaged with standard deviations calculated.

**RESULTS AND DISCUSSION:** *S. aureus* was identified in 25% (n=25) of the 100 clinical specimens. Antibiotic resistance testing revealed universal resistance to Cefoxitin among the isolates, with only one isolate remaining sensitive to Penicillin. Additional resistance was observed for Erythromycin (48%), Ciprofloxacin (32%), and Tetracycline (32%). In contrast, Gentamicin and Vancomycin demonstrated 100% efficacy against the isolates. Eight isolates were categorized as multidrug-resistant (MDR) and four isolates as extensively drug resistant (XDR). All 25 isolates exhibited biofilm formation capabilities, with 24% classified as strong Biofilm formers, 72% as moderate biofilm formers, and 4% as weak biofilm formers. Notably, within the XDR group, 2 isolates produce strong biofilm, 5 were moderate biofilm formers, and 1 was a weak biofilm former. The remaining isolates were predominantly moderate biofilm formers. The biofilm-forming ability of *S. aureus*, especially in XDR strains, emphasizes the urgency of innovative treatment strategies and continued surveillance against antibiotic resistance and biofilm-related complications.

**Keywords:** *Staphylococcus aureus* Biofilm formation Antibiotic susceptibility XDRgroup

# Biofilm formability of invasive and noninvasive *Streptococcus agalactiae*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** The emergence of *Streptococcus agalactiae* infections in adult populations is increasing. However, limited knowledge is still palpable in the biofilm formability of this microorganism. We aimed to investigate biofilm formability of *S. agalactiae* strain isolates from different clinical sources in Iran.

**MATERIALS AND METHODS:** A cross-sectional study was performed on 65 *S. agalactiae* strains (30 invasive and 35 noninvasive) isolated from non-pregnant women. Sensitivity was evaluated by the disk diffusion technique. All *S. agalactiae* isolates were confirmed by *atr* and *dltS* PCR assays. Biofilm production was investigated by microtiter plate assay. Resistance determinants were detected by PCR.

**RESULTS AND DISCUSSION:** MDR was detected in 15.4% of noninvasive and 44.6% of invasive isolates. MtP assay indicated that 80% of isolates were biofilm producers. Biofilm formation (94.3% versus 66.7%) was more common among noninvasive compared with invasive strains. *tet* (M) (46.2%) and *erm* (B) (69.2%) were the most prevalent tetracycline and macrolide-resistant genes.

**Keywords:** *Streptococcus agalactiae*, Multiplex Polymerase Chain Reaction, Invasive, Drug Resistance,

# Biofilm Formation and Virulence Determinants Profile of Oral Clinical Isolates of Candida Species

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Biofilm is a complex crust made by a fungal community enclosed in a matrix, colonized in the oral cavity that is innately resistant to antifungal. Furthermore, oral candidiasis is an opportunist mucosal infection in immunocompromised individuals. Consequently, biofilm forming capability is the virulence factor for colonization, antifungal resistance and host defense in *Candida* species. However, new data on biofilm formation ability and genotype expression is limited in *Candida* spp isolated from the oral cavity in diabetic individuals. Therefore, the objective of the present study is to characterize biofilm formation capacity and involved genes of *Candida* spp oral isolates from individuals with diabetes mellitus.

**MATERIALS AND METHODS:** Herein, *Candida* species biofilm formation and virulence genes (HWP1, SAP2) have assessed in oral clinical in diabetic individuals. The *Candida* species (n: 72) were identified by molecular methods and included *Candida albicans* (n:41), *Candida glabrata* (n:16), *Candida tropicalis* (n:8), *Candida krusei* (n:3), *Candida parapsilosis* (n:2), and *Candida guilliermondii* (n:2). The evaluation of biofilm formation was performed by Crystal Violet assay. Genotypic detection of two virulence genes was conducted by conventional multiplex PCR.

**RESULTS AND DISCUSSION:** All oral *Candida* clinical strains have been able to form biofilms. *C. parapsilosis* displayed stronger biofilm, while moderate biofilm was produced by *C. albicans* and *C. glabrata*. In other *Candida* species, biofilm production was very weak, there was a significant difference in biofilm growth between the moderate and strong isolates with weak isolates (P 0.05). HWP1 and SAP2 were detected in 84.8% and 51.3%, of *C. albicans* and 89% and 62.1% of other *Candida* species, respectively. Our data demonstrate that virulence factors (biofilm formation and related genes) of *Candida* species exhibit significant differences. Notably, HWP1 and SAP2 are less expressed in *C. albicans* in contrast to non-*albicans* *Candida* species. These suggest that the colonization of strong biofilm producers in diabetic patients may lead to serious clinical manifestations. Further analysis is necessary to elucidate the association of *Candida* virulence factors and host health conditions between clinical outcomes.

**Keywords:** Biofilm, *Candida* species, Virulence

# Borrelia theileri infections in Rhipicephalus annulatus ticks from the north of Iran

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Ticks serve as vectors and reservoirs for various pathogens, such as viruses, bacteria, and protozoa, which can cause diseases in humans, livestock, and wild animals. Mazandaran Province is a fertile green land in the Caspian Sea littoral of Iran. The prime pastures and forests for livestock grazing make this area home to various tick species. This study investigated *Borrelia* infection in hard ticks from forest areas of this province and compared their identity with *Borrelia* species available in the GenBank database.

**MATERIALS AND METHODS:** Ticks were collected by hand from mammalian hosts, including sheep, goats, cows, and dogs, or by dragging and flagging practices. The specimens were grouped into 231 pools or individuals according to developmental stage, gender, and species. Following DNA extraction, *Borrelia* was detected by amplifying a 136-bp sequence of the 16S rRNA gene using a real-time PCR (qPCR). The qPCR-positive samples were further analyzed by amplification and sequencing the *flaB* and *glpQ* genes, followed by BLAST and phylogenetic analysis.

**RESULTS AND DISCUSSION:** The qPCR detected *Borrelia* in 27 tick samples from *Rhipicephalus annulatus*, *Ixodes ricinus*, and *Haemaphysalis punctata*. The *flaB* gene was amplified from eight *Rh. annulatus* and one *Ix. ricinus* tick samples. Additionally, *glpQ* was amplified from six specimens, all belonging to *Rh. annulatus*. The *flaB* sequences from *Rh. annulatus* ticks had variations and exhibited the highest identity (98.1%-100%) with *Borrelia theileri* and related undefined isolates from other geographical areas. The *glpQ* sequences matched the most (98.2%) with the only available sequence from *B. theileri* KAT in Mali. Also, phylogenetic analysis based on *flaB* and *glpQ* genes clustered our sequences with *B. theileri* and closely related undefined isolates. The highest identity of generated *flaB* and *glpQ* sequences with *B. theileri* and their clustering within well-supported clades with this species and closely related isolates from different geographical origins corroborated the occurrence of *B. theileri* in the North of Iran.

**Keywords:** *Rhipicephalus annulatus*, *Borrelia theileri*, hard ticks, Phylogenetic analysis, Mazandaran, Iran

# Candidemia and fungal wound colonization in Burn Patients: Risk Factors, Prevalence, and Antifungal Susceptibility in Iranian Intensive Care Units

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Burn injuries pose a significant and unique risk for invasive fungal infections, yet many cases remain undetected due to low clinical awareness and similarities in presentation with bacterial infections. These factors encompass the use of broad-spectrum antibiotics, patient age, extended hospital stays, corticosteroid administration, fungal wound colonization, total body surface area (TBSA), total parenteral nutrition, central venous catheters, and compromised immune function. This study aims to investigate the risk factors and prevalence of *Candida* infections and fungal wound colonization, as well as evaluate the antifungal drug susceptibility of fungal isolated species from burn patients.

**MATERIALS AND METHODS:** This study involved a comprehensive evaluation of 353 burn patients admitted to the intensive care units (ICUs) of three primary burn centers in Iran, specifically in Tehran, Mazandaran, and Gilan Provinces, between 2021 and 2023. Demographic data, burn-related factors, and clinical conditions were analyzed and compared between patient groups. Fungal species identification was performed using the PCR-RFLP method. Additionally, antifungal susceptibility testing was conducted according to Clinical and Laboratory Standards Institute (CLSI) guidelines to guide treatment strategies and further characterize the identified fungal isolates.

**RESULTS AND DISCUSSION:** Overall, 46.2% of patients exhibited *Candida* colonization, and 15.3% of these cases progressed to candidemia. *Candida parapsilosis* (37.0%) emerged as the most prevalent species isolated from candidemia patients. Factors associated with increased candidemia risk included TBSA burns, advanced age, the presence of indwelling catheters, diabetes, and extended stays in the ICU. Mortality rates were significantly higher among candidemia patients (82.5%) compared to those with colonization (7.3%). Additionally, the resistance rates of candidemia isolates to fluconazole and voriconazole were notable, at 28% and 18.2%, respectively. Our study revealed that larger TBSA burn injuries, prolonged hospitalizations, and catheterization are significant predictors of candidemia development.

**Keywords:** Candidemia, Burn patients, *Candida* colonization, Antifungal susceptibility

# Characterization of ESBL Genes in Non-Fermenting Gram-Negative Bacteria Isolated from Hospitalized Patients in Mazandaran Province

Bacteriology

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**BACKGROUND AND OBJECTIVES:** One of the mechanisms of resistance to antibiotics is the production of beta-lactamase enzymes. Extended spectrum beta-lactamases (ESBL) are produced by many Gram-negative bacteria and have the ability to hydrolyze third and fourth generation cephalosporins and azetronam, but their action is inhibited by clavulanic acid. The emergence of beta-lactamase enzymes in bacteria, notably (ESBLs), presents a notable worldwide concern. ESBLs are commonly found on bacterial plasmids with SHV, TEM, and CTX-M being the most prevalent variants. This research aimed to investigate the occurrence of ESBL genes through molecular techniques in non-fermenting Gram-negative bacteria (specifically *Pseudomonas aeruginosa* and *Acinetobacter baumannii*) obtained from hospitalized patients in Mazandaran province, Iran.

**MATERIALS AND METHODS:** In this cross-sectional descriptive study, a total of 81 non-fermenting bacterial isolates were collected and identified using standard microbiological tests. The DNAs of the bacteria were extracted by Alkaline Lysis method using sodium dodecyl sulfate and sodium hydroxide. Molecular identification of blaSHV, blaTEM, and blaCTX-M genes was carried out using specific primers and the polymerase chain reaction (PCR) method. The data were analyzed using the Chi-square statistical test. The P-value <0.05 was considered statistically significant.

**RESULTS AND DISCUSSION:** Out of the 81 non-fermentative bacterial isolates, 49 (60.5%) were identified as *A. baumannii* and 32 (39.5%) as *P. aeruginosa*. The prevalence of specific genes among these isolates was as follows: in *P. aeruginosa*, 26% (blaSHV), 19%(blaTEM), and 12% carried (blaCTX-M). in *A. baumannii*, 11%(blaTEM), 8% (blaCTX-M), and 4% carried (blaSHV). ESBL-positive strains of *P. aeruginosa* and *A. baumannii* are becoming more common in hospital isolates. Due to their strong capacity to transfer resistant genes to other clinical strains, it is crucial to rapidly identify them in clinical laboratories.

**Keywords:** Non-fermenting bacteria, ESBL, PCR, blaTEM, blaSHV, and blaCTX



# Chitosan nanoparticles containing fusion protein (Hsp $\alpha$ -PPE44-EsxV) and resiquimod adjuvant (HPERC) as a novel booster vaccine for *Mycobacterium tuberculosis*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** This study attempted to explore the immunogenicity of chitosan nanoparticles containing fusion protein (Hsp $\alpha$ -PPE44-EsxV; HPE) and resiquimod adjuvant (HPERC) in BALB/c mice. HPE was initially expressed in *E. coli* BL21 cells. HPE and resiquimod adjuvant were then encapsulated in chitosan nanoparticles (HPERC). One group of mice were subcutaneously vaccinated on days 0, 14, and 28 with HPERC, and the other group was primed with bacilli Calmette-Guerin (BCG) on day 0 and then boosted with HPERC on days 14 and 28. Two weeks after the last injection, IFN- $\gamma$ , IL-4, and IL-17 in spleen cell culture supernatants, and IgG2a and IgG1 titers in sera were measured. HPERC size was  $130.84 \pm 12.08$  nm ( $n = 5$ ). Zeta potential of HPERC was  $29 \pm 4$  mv. The highest IFN- $\gamma$  concentration was detected in BCG-primed mice that were boosted with HPERC. In addition, IL-17 production was significantly increased in all groups compared with

**MATERIALS AND METHODS:** Cloning and expression of fusion proteins, Particle size distribution and zeta potential measurements, Release of HPE from HPERC, Animals, Immunization procedures, Antibody isotype assay, Statistical analysis

**RESULTS AND DISCUSSION:** HPE characterization, HPE characterization, Release of HPE from HPERC, Cytokine and antibody isotype assay

**Keywords:** Tuberculosis, *Mycobacterium tuberculosis*, chitosan, fusion protein, resiquimod, adjuvant, booster vaccine

# Combination therapy for Cutaneous Leishmaniasis using Crocin and Curcumin

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background: Leishmaniasis is a vector-borne disease and one of the most important neglected tropical diseases. Existing anti-leishmanial treatments have been ineffective for a long time and are associated with toxic side effects, so the search for new, effective and safe alternative treatments against cutaneous leishmaniasis (CL) is crucially needed. This study was conducted with the aim of investigating the leishmaniacidal effects of the combination of Crocin-curcumin on CL in vitro.

**MATERIALS AND METHODS:** Crocin and curcumin were extracted from Medicinal plants. Maceration method was used for extraction of the plant and the compounds were extracted using Mas Chromatography and Spectrophotometry technique. *Leishmania major* (*L. major*) standard strain promastigotes were purchased from Leishmaniasis research center, Public Health school, Tehran University of Medical Sciences and cultured in RPMI-1640 medium and the anti-leishmanial effects and cytotoxicity of the extracts at concentrations of 200, 400 and 800 µg/ml were investigated using 1% trypan blue. Cytotoxicity of the compounds on normal human fibroblast cells was measured by MTT method.

**RESULTS AND DISCUSSION:** The anti-leishmanial effects of crocin and curcumin was assessed separately in our previous non-published study. In this investigation, the results showed that the Crocin-curcumin had acceptable effectiveness and the combination of Crocin-curcumin extract at a concentration of 800 µg/ml after 48 hours and at 400 µg/ml after 48 hours were the best and most economical concentrations, respectively.

**Keywords:** Anti-leishmaniasis, *Leishmania major*, MTT assay, Crocin, Curcumin

# Comparative Analysis of Five Commercial RT-PCR Diagnostic Assay for Detection of Covid-19

Bacteriology

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**BACKGROUND AND OBJECTIVES:** SARS-CoV-2 real-time reverse-transcription PCR (rRT-PCR) is the most effective testing system available to combat COVID-19, given the absence of any specific treatment or vaccine. Moreover, numerous SARS-CoV-2 rRT-PCR kits have been approved under emergency-use-authorization (EUA) worldwide. In this article, we present a comparison of important performance features among five commercial RT-PCR assays.

**MATERIALS AND METHODS:** A total of consecutive nasopharyngeal (NPS) samples and oropharyngeal (OP) swabs were collected from 50 COVID-19 patients to analyze sensitivity and specificity

**RESULTS AND DISCUSSION:** The results showed variations in sensitivity among all the RT-PCR kits examined. The Pishtaz teb assays demonstrated the highest positive percent agreement (PPA) of 95.2% (40/42), followed by Covitech (90.5% - 38/42), DaAn Gene (83.3% - 35/42), Sansure (66.66% - 28/42), and Power check of SARS- CoV-2 panel (64.3% - 27/42). Conversely, all five molecular assays demonstrated a negative percent agreement (NPA) of 100% (8/8). These findings provide a technical baseline for assessing the performance of five distinct commercial PCR assays for detecting SARS-CoV-2. They could prove practical and useful for laboratories seeking to purchase these assays for further clinical validation.

**Keywords:** RT-PCR, Diagnostic, Covid-19

# Comparative investigation of adrenal gland function and related electrolyte changes in people with acute covid-19 infection and healthy people

Bacteriology

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**BACKGROUND AND OBJECTIVES:** The new corona virus known as SARS-CoV-2 and the disease caused by it is called Covid-19. This disease is highly contagious from person to person and is due to the high ability and affinity of Spike surface protein to the human ACE2 cell receptor. It damages the vital organs of lungs, heart, liver and kidney which have a high level of this receptor. The host's organs are considered a suitable place for the virus and the side effects of this disease are many on these organs. ACE-2 receptor controls blood pressure by hydrolyzing Ang II to Ang1-7. The renin-angiotensin system regulates blood pressure, water balance and electrolytes in the body.

**MATERIALS AND METHODS:** For this study, a cross-sectional descriptive survey was conducted on 150 people (men) with symptoms of Corona (aged between 20 and 35 years) who visited the laboratory of Mehrgan Hospital in Kerman city in 2022 during the months of October to December. These men were divided into two groups with covid-19 (n=100) and control group (n=50) and after taking 8 cc of venous blood, in terms of serum concentration of renin, aldosterone, angiotensin and sodium and potassium electrolytes. were evaluated. Determining the concentration of factors in the blood samples of patients with covid-19 and healthy people was done using the ELISA kit (Elisa Kit Active Renin, Germany) by the non-competitive sandwich method

**RESULTS AND DISCUSSION:** Measurement of angiotensin converting enzyme in sick people with an average of  $29.01 \pm 7.74$  and healthy people with an average of  $36.71 \pm 16.93$  and sodium ion concentration in sick people with an average of  $144.81 \pm 11.85$  and healthy people with The average of  $138.46 \pm 2.69$  and the potassium concentration in the patients with an average of  $4.42 \pm 0.80$  and healthy subjects with an average of  $5.67 \pm 1.63$  with (P0.005) a significant difference was observed in all three groups. It was found that the COVID-19 virus binds to the ACE-2 protein receptor and induces its effects on the target cells. According to the results obtained in this research, Ang II inhibits the negative feedback after increasing and reduces the renin concentration.

**Keywords:** Covid-19, renin, aldosterone, angiotensin-converting enzyme, sodium, potassium

# Comparing the level of expression of the lncRNA GCK1 gene in the serum of Covid-19 patients to a control group

Bacteriology

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**BACKGROUND AND OBJECTIVES:** SARS-CoV-2 is the cause of the COVID-19 pandemic, which has resulted in very high deaths worldwide. It supports a close association between immunopathology caused by SARS-CoV-2 and poor survival of patients with COVID-19. Unfortunately, antiviral drugs, glucocorticoids, and immunoglobulin therapy have not shown significant improvement in the survival of patients with severe symptoms of COVID-19. Therefore, targeting specific immunity to COVID-19 such as increasing lymphocytes or inhibiting inflammation could be promising therapeutic strategies. LncRNAs induced by viruses have been observed to regulate innate immune responses to eliminate viral infections through various mechanisms. Therefore, in the present study, the expression of lncRNA GCK1 in the blood serum of patients with COVID-19 is investigated compared to healthy individuals.

**MATERIALS AND METHODS:** In this study, after collecting healthy blood samples and those infected with Covid-19 from Mashhad medical diagnosis laboratories and extracting RNA from them, then the quality of RNAs and cDNA synthesis was checked. In the following, real time PCR technique by Cybergreen method was used to check gene expression. The obtained data were recorded for statistical analysis and determined by assessing the risk ratio in the 95% confidence interval. Based on the findings of the present study, a significant increase in GCK1 lncRNA levels has been observed among patients with SARS-CoV-2 compared to healthy individuals.

**RESULTS AND DISCUSSION:** The above incident was probably done in order to deal with the strong immune response and prevent its excessive activity and as a result the clinical complications caused by it. Because the activity of immune cells requires the glucokinase enzyme and the decreasing control of its expression by lncRNA can prevent their excessive activity. However, more studies are recommended to confirm the above results.

**Keywords:** SARS-CoV-2, Inflammation, lncRNA GCK1

# Comparison of the Antibacterial Activity of Hydroalcoholic Extract of Wheat Germ with Ciprofloxacin on Methicillin Resistant *Staphylococcus aureus* (MRSA)

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus* has long been recognized as one of the most important bacteria that cause disease in hospitals and community. The bacteria can cause mild to threatening systemic infections. According to the structure of the bacteria, resistant strains are spreading. Wheat germ is one of the medicinal plants that has a long history in culture and nutrition of Iranian people. The aim of this study was to determine and compare the antibacterial activity of hydroalcoholic extract of wheat germ with ciprofloxacin on methicillin-resistant *S. aureus*.

**MATERIALS AND METHODS:** This study is an empirical research approach that includes seven strains: consisting of a standard strain sample and 6 samples of methicillin-resistant *S. aureus* which previously collected in the Microbiology Laboratory of Medical faculty. The standard strain of methicillin-resistant *S. aureus* was purchased from the Pasteur Institute. Wheat germ was weighed after drying, 200 g of wheat germ was mixed with 1000 cc of 70% alcohol (700 cc of ethyl alcohol + 300 cc of distilled water). After filtration, it was extracted by Rotary Evaporator. The antibiotic ciprofloxacin was purchased from the company. Then, ZOI, MIC, and MBC tests, for all samples were performed in three times. Data were processed using SPSS software version 21 and analyzed through descriptive statistics and inferential tests with a 95% confidence level.

**RESULTS AND DISCUSSION:** Results: The results of this study showed the minimum inhibitory concentration (MIC) of clinical strain of MRSA and standard strain (ATCC33591) is equal was  $1.2 \pm 0.5$  &  $100 \pm 0$   $\mu$ /mL respectively. The minimum bacterial concentration (MBC) for clinical and standard strain of MRSA was  $1.4 \pm 1.3$  &  $38.9 \pm 19.6$  microliter/ml respectively. Zone of Inhibition diameter for clinical and standard strain of MRSA (ATCC33591) was  $1.2 \pm 0.5$  &  $100 \pm 0$  mm, respectively. Conclusion: The results of the study indicate that the ZOI diameter of wheat germ at MIC concentration was less than the ZOI diameter of ciprofloxacin. Also, MIC and MBC of wheat germ extract were higher than ciprofloxacin antibiotic. Based on independent t-test a significant difference was seen between standard strains and clinical samples P 0.05.

**Keywords:** Wheat germ, *S. aureus*, Extraction, Ciprofloxacin

# Comparison of the level of 25-hydroxyvitamin D in patients with anal-genital warts with healthy control group

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** The high prevalence of genital warts in young adults has caused many concerns, and at the same time, there is no effective antiviral treatment for this disease. Considering the role of vitamin D in many skin diseases as well as its role in the immune system, this study aims to compare the level of 25-hydroxyvitamin D in patients with anogenital warts compared to the healthy control group.

**MATERIALS AND METHODS:** This was a case-control study in which patients were selected from individuals over 16 years of age who were sexually active and referred to the dermatology clinic of Bu-Ali-Sina Hospital of Sari for diagnosis and treatment of anogenital warts. Finally, a total of 130 individuals (65 in the case group and 65 in the control group) were enrolled in the study. Demographic information, disease duration, disease severity, location of anogenital areas and serum level of vitamin D were recorded. Statistical analysis of data was performed using SPSS software version 22.

**RESULTS AND DISCUSSION:** In general, no statistically significant difference was observed between disease severity and vitamin D serum level in patients with anogenital warts ( $p=0.083$ ). Vitamin D serum levels were lower in the genital + pubic and anal areas than in other areas; However, there was no statistically significant difference between the areas of involvement of anogenital warts with vitamin D serum levels ( $p=0.237$ ). In the group of patients with anogenital warts, a significant positive correlation was observed between age and vitamin D serum levels; In other words, serum levels of vitamin D increased with age ( $r=0.266$  and  $p=0.034$ ). Serum levels of 25-hydroxyvitamin D were reported to be lower in patients with anogenital warts than in healthy individuals. In addition, in women, at a younger age, in people with more warts and anal and pubic area, they had a lower level of vitamin D, and it is suggested that recommendations based on prevention.

**Keywords:** Anogenital warts, Vitamin D, Human papilloma virus

# Comparison the frequency of common bacteria that cause meningitis in cerebrospinal fluid samples by culture and PCR in Hamadan, West of Iran

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Infection and inflammation of the meningeal membranes in the brain and spinal cord is called meningitis. The causes of this disease are very diverse, but among the infectious causes of this disease are viruses, bacteria, parasites, and fungi. The aim of this study is to detect bacterial infections causing meningitis by molecular PCR method in cerebrospinal fluid (CSF) samples of hospitalized patients suspected of having meningitis and compare it with the culture results in the laboratories of Hamadan hospitals.

**MATERIALS AND METHODS:** This cross-sectional study was conducted on 104 CSF samples of hospitalized patients suspected to meningitis in the educational hospitals of Hamadan University of medical sciences. The Culture and PCR methods were used to detect the most common causes of bacterial meningitis included Haemophilus influenzae type b, Listeria monocytogenes, Streptococcus agalactiae, Neisseria meningitides, and E. coli K1

**RESULTS AND DISCUSSION:** The average age of the patients in this study was  $31.57 \pm 25.85$  years. Of the participants 53.85% were male and 46.15% were female. The frequency of detection by culture method for these bacteria was zero and by PCR it was 4.81%. The results of this study showed the low prevalence of common bacterial infections in CSF samples and also showed that the molecular method is more accurate and sensitive compared to culture. Therefore, it is suggested that if meningitis is suspected, clinical experts should check all the factors and use newer diagnostic methods.

**Keywords:** CSF, PCR, Meningitis, Haemophilus influenzae type b, Listeria monocytogenes, Streptococcus



# Design and Development of a Nano-Molecular Diagnostic Method for Detecting Methicillin-Resistant *Staphylococcus aureus* Using Quantum Dot Based Isothermal Amplification Technology

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus*, a Gram-positive facultative anaerobic bacterium, is a significant pathogen within the genus *Staphylococcus*, capable of causing skin, bloodstream, and respiratory infections. It harbors antibiotic resistance genes, including *mecA* which confers resistance to methicillin (MRSA), alongside other resistance genes to various antibiotics. Genomic analysis employs molecular techniques such as PCR and Multiplex PCR, noted for their precision in detecting antibiotic resistance genes, widely utilized in clinical settings. This study utilized the Loop-mediated Isothermal Amplification (LAMP) technique, incorporating a novel nucleotide, dUTP (biotinylated), followed by detection using streptavidin-coated quantum dots. Streptavidin exhibits high affinity for biotin molecules, allowing the streptavidin-coated quantum dot complex to bind to biotin molecules incorporated into the LAMP product, thereby facilitating its detection.

**MATERIALS AND METHODS:** In the current study, biotin-dUTP nucleotide was used in the LAMP test in the preparation of dNTP mix (containing dGTP, dTTP, dCTP, dATP, dUTP) and the biotin in dUTP nucleotide has a high affinity with quantum dots coated with They have streptavidin which was used in the present study. Therefore, the LAMP product labeled by these materials produced light emission in front of the fluorescent detector.

**RESULTS AND DISCUSSION:** This study analyzed 30 blood samples infected with methicillin-resistant *Staphylococcus aureus* (MRSA), confirmed positive by the gold standard culture method. Among these, 28 samples were reported positive by PCR, 29 samples by conventional LAMP, 30 samples by Green viewer LAMP, and 26 samples by Quantum dot-LAMP. The sensitivities of these four tests were 93%, 97%, 100%, and 87%, respectively, while the specificity for all tests was reported to be 100%.

**Keywords:** *Staphylococcus aureus*, Loop-mediated isothermal amplification, Quantum dot

# Design of a novel analogue peptide with potent antibiofilm activities against *Staphylococcus aureus* based upon a sapecin B-derived peptide

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Nowadays, antimicrobial peptides are promising to confront the existing global crisis of antibiotic resistance.

**MATERIALS AND METHODS:** Here, a novel analogue peptide (mKLK) was designed based upon a D-form amidated sapecin B-derived peptide (KLK) by replacing two lysine residues with two tryptophan and one leucine by lysine, and inserting one alanine. The mKLK displayed superior amphipathic helices in which the most of hydrophobic residues are confined to one face of the helix and had a higher hydrophobic moment compared with KLK. The mKLK retained its antibacterial activity and structure in human serum, suggesting its stability to proteolytic degradation. The values of MIC and MBC for mKLK were equal to those of KLK against clinical strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA). However, mKLK showed more capability of in vitro inhibiting, eradicating, and dispersing MRSA and MSSA biofilms compared with KLK.

**RESULTS AND DISCUSSION:** Furthermore, a remarkable inhibitory activity of mKLK against MRSA and MSSA biofilms was seen in the murine model of catheter-associated biofilm infection. Results of this study show that mKLK not only exhibits antibacterial activity and serum stability but also a potent biofilm inhibitory activity at sub-MIC concentrations, confirming its potential therapeutic advantage for preventing biofilm-associated MRSA and MSSA infections.

**Keywords:** MRSA, AMPs, mKLK

# Designing a strip kit for rapid molecular detection of *Helicobacter pylori* infection

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Helicobacter pylori* infection is a significant global health concern, associated with various gastric diseases including peptic ulcers and gastric cancer. Gold standard method for detection of *H. pylori* is culture which is not always successful due to fastidiousness nature of bacteria. This study presents the development and validation of a novel, rapid DNA strip test for the detection of *H. pylori*, addressing the need for culture-independent and accurate diagnostic tools.

**MATERIALS AND METHODS:** In this research, gastric biopsy samples from 10 *H. pylori* positive patients were obtained and subjected to DNA extraction using a standardized protocol. The extracted DNA served as a template for PCR amplification, utilizing biotinylated primers specific to the 23S rRNA gene region. The resulting amplicons were applied to nitrocellulose strips pre-coated with complementary probes. Detection was achieved using streptavidin-conjugated horseradish peroxidase (HRP) and visualized through enhanced chemiluminescence.

**RESULTS AND DISCUSSION:** Preliminary results indicate that this DNA strip test offers high sensitivity and specificity for *H. pylori* detection, with a significantly reduced turnaround time compared to traditional culture-based methods. The simplicity of the test procedure and interpretation makes it suitable for use in various clinical settings, including resource-limited areas. This strip test is based on a highly specific probe targeting the 23S rRNA gene of *H. pylori*, ensuring selective detection of this pathogen. This rapid and specific test represents a promising advancement in *H. pylori* diagnostics, potentially facilitating earlier detection and treatment of infections. Further studies are performing to evaluate the test's performance in determination of bacterial genotype as well as its antibiotic resistance pattern.

**Keywords:** *Helicobacter pylori*, strip, Molecular detection

# Detection of serine protease autotransporter encoding genes in UPEC isolates from children

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Serine protease autotransporters of Enterobacteriaceae (SPATEs) play a key role in the virulence of uropathogenic Escherichia coli (UPEC). It is known that these proteases have cytotoxic function in renal and bladder cells. The aim of the study was to detect the frequency of sat and pic autotransporter proteins genes in UPEC isolates from children with urinary tract infections.

**MATERIALS AND METHODS:** Eighty- four UPEC strains isolated from children (aged 1 to 14 years) with UTI were analyzed from June 2023 to April 2024. The presence of sat and pic SPATE genes were identified by PCR method. The frequency and comparison of variables were determined by Chi-square ( $\chi^2$ ) or Fisher's exact tests.

**RESULTS AND DISCUSSION:** Of all 84 UPEC isolates, 66 (78.6%) and 18 (21.4%) were isolated from female and male pediatric patients respectively. All (100%) isolates carried sat gene and the frequency of pic gene was 18 (21.4%). Both sat and pic genes were found in 6 (33. 3%) of isolates from males compared to 12 (18.2%) isolates from females. Conclusion: The present study showed high frequency of sat autotransporter proteins genes in UPEC isolates from children in our region. The finding is important because these proteins facilitate invasion of host tissues during infection.

**Keywords:** Uropathogenic Escherichia coli; Children; UTI, Serine protease autotransporters

# Determination of the Prevalence of Carbapenemase and Aminoglycoside-Modifying Enzyme Resistance Genes in Clinical Isolates of *Klebsiella pneumoniae*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Klebsiella pneumoniae* is a Gram-negative bacterium that frequently causes nosocomial infections. This pathogen can lead to a wide range of infections, including those of the lungs, urinary tract, and wounds. In recent years, the emergence of antibiotic-resistant strains of this bacterium has posed significant challenges to treatment. Currently, carbapenems, a last-line antibiotic, are used to treat multidrug-resistant strains of this bacterium. However, the development of carbapenem resistance has necessitated the use of colistin, which can cause adverse effects such as kidney damage. Given these concerns, determining the prevalence of antibiotic resistance genes in clinical isolates of *K. pneumoniae* is crucial for monitoring antibiotic resistance patterns in this bacterium.

**MATERIALS AND METHODS:** In this study, 100 clinical isolates of *K. pneumoniae* were collected from hospitalized patients in various hospital wards. Molecular identification of these isolates was performed by PCR amplification of the specific *khe* gene. Subsequently, the presence of antibiotic resistance genes encoding common carbapenemases (*bla*VIM, *bla*NDM, *bla*KPC, and *bla*OXA-48) and aminoglycoside-modifying enzymes [*aac*(6')-Ib, *aph*(3')-Ia, *ant*(4')-IIa, *ant*(2'')-Ia, *aac*(3)-Iva, *aac*(3)-Ia, and *aac*(3)-IIa] was determined using PCR.

**RESULTS AND DISCUSSION:** The prevalence of *bla*VIM, *bla*NDM, *bla*KPC, and *bla*OXA-48 was 0%, 32%, 0%, and 55%, respectively. The prevalence of genes encoding aminoglycoside-modifying enzymes *aac*(6')-Ib, *aph*(3')-Ia, *ant*(4')-IIa, *ant*(2'')-Ia, *aac*(3)-Iva, *aac*(3)-Ia, and *aac*(3)-IIa was 48%, 6%, 0%, 58%, 0%, 0%, and 8%, respectively. The high prevalence of *bla*NDM and *bla*OXA-48 genes in *Klebsiella pneumoniae* isolates is alarming, indicating a significant threat of carbapenem-resistant infections. The presence of aminoglycoside-modifying enzyme genes further complicates treatment options, emphasizing the urgent need for effective antimicrobial stewardship and infection control measures.

**Keywords:** *Klebsiella pneumoniae*, Aminoglycoside-modifying enzymes, Carbapenemases

# Determining the Bacterial Types and Antibiotic Resistance Patterns in Urinary Tract Infections at Razi Hospital, Iran, From 1396 to 1400

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Urinary tract infections (UTIs) are common and can significantly affect individuals' health and well-being. At Razi Hospital in Iran, understanding the types of bacteria causing these infections and their resistance to antibiotics is crucial for effective treatment. This study focuses on identifying the specific bacteria responsible for UTIs and examining their antibiotic resistance patterns. By analyzing urine samples from patients, we aim to gather detailed information on the prevalence and resistance mechanisms of UTI-causing bacteria. This knowledge will help healthcare providers at Razi Hospital develop better treatment plans and manage infections more effectively. Additionally, our findings will contribute to the broader fight against antibiotic resistance, a growing global health challenge. Through this research, we hope to improve patient care and outcomes in our community and offer valuable insights for healthcare professionals worldwide.

**MATERIALS AND METHODS:** Bacteria were isolated from urine samples of patients who visited Razi Hospital. Characterization and typing of the isolates were conducted using biochemical and microbiological methods. Antibiotic resistance patterns were evaluated using the disk diffusion method on Mueller Hinton agar, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

**RESULTS AND DISCUSSION:** Among the 1,338 bacterial isolates from urine samples, 912 were *Escherichia coli* (68%), 117 were *Citrobacter* (9%), 84 were *Enterobacter* (6%), 80 were *Pseudomonas aeruginosa* (6%), 55 were *Enterococcus* (4%), 65 were *Staphylococcus saprophyticus* (5%), and 25 were *Proteus* (2%). The highest antibiotic resistance was observed for cotrimoxazole (75.15%), ampicillin (71.26%), amikacin (65.48%), and nalidixic acid (61.11%). The bacteria were most sensitive to nitrofurantoin (86.40%) and ceftriaxone (77.06%). Our findings indicate that *Escherichia coli* is the predominant cause of UTIs in the studied population. Given the high resistance rates to nalidixic acid, cotrimoxazole, ampicillin, and amikacin, these antibiotics should be avoided in the initial treatment of UTIs.

**Keywords:** Urinary tract infection, Antibiotic resistance, *Escherichia coli*

# Distribution of genes encoding virulence factors and multilocus variable-number tandem-repeat analysis (MLVA) of entero-aggregative *Escherichia coli* (EAEC) isolated in Iran from patients with diarrhoea

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Entero-aggregative *Escherichia coli* (EAEC) is one of the main causes of diarrhoea worldwide. Several virulence factors have been identified in EAEC. This study was conducted to investigate the distribution of virulence factor genes in EAEC strains isolated in Iran from children with diarrhoea, as well as the genetic similarity of these isolates.

**MATERIALS AND METHODS:** . A total of 37 EAEC isolates were tested for the presence of 11 virulence genes by PCR, and the genetic relatedness of these strains was further determined by multilocus variable-number tandem-repeat analysis (MLVA).

**RESULTS AND DISCUSSION:** . All EAEC isolates were typical EAEC. *pic*, *set1A* and *set1B* were the most prevalent genes, detected in 54.1% of the isolates, followed by *sat* (43.2%), *astA* (32.4%), *pet* (24.3%), *agg4A* (24.3%), *sepA* (18.9%), *agg3A* (13.5%), *sigA* (8.1%), *aggA* (8.1%) and *aafA* (5.4%). Using MLVA, the 37 isolates were divided into 32 types and classified into five clonal complexes. . This study showed that EAEC is a heterogeneous group of *E. coli* possessing a broad range of virulence factors. There was no notable association between MLVA patterns and virulence profiles.

**Keywords:** enteroaggregative, *Escherichia coli* MLVA virulence factors

# Distribution of Pathogenicity Islands Among Uropathogenic Escherichia coli Isolates From Patients With Urinary Tract Infections

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Uropathogenic Escherichia coli (UPEC) is one of the most common etiologic agent of urinary tract infection (UTI). The ability of Escherichia coli to cause UTI is associated with specific virulence determinants, which are encoded by pathogenicity islands (PAIs). This study aimed to investigate the distribution of PAIs among the UPEC isolates collected from patients with UTIs.

**MATERIALS AND METHODS:** In this study, a total of 100 E. coli isolates were collected from patients with UTIs using standard microbiological methods. Polymerase chain reaction (PCR) was used for the identification of the main PAIs of UPEC according to insertion sites and virulence markers.

**RESULTS AND DISCUSSION:** In total, PAI IV536, PAI III536, PAI I536, PAI, IICFT073, PAI ICFT073, PAI IIJ96, PAI II536, and PAI IJ96 were detected in 23, 22, 17, 17, 13, 11, 11, and 8% of isolates. PAI combinations were identified in 15% of isolates. The results showed that PAIs of UPEC are not strain-specific and some strains can carry the PAIs associated with the prototype strains of UPEC simultaneously.

**Keywords:** Pathogenicity Island, Insertion site, Urinary tract infections, Uropathogenic Escherichia coli



# Effects of COVID-19 on Maternal and Neonatal issues in infected Pregnant Women

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Since the COVID-19 may adversely affect both mothers and babies, evaluation and identification of aggravating factors can help prevent adverse outcomes. This study aimed to examine pregnant women with COVID-19 and evaluate the disease outcomes.

**MATERIALS AND METHODS:** In this case series study, pregnant women hospitalized for COVID-19 in the Imam Khomeini Hospital of Sari, Iran, during 2019 – 2020, were examined. A convenient sampling method was used. 19 patients were selected. Their demographic information and medical histories were taken. Informed consents were obtained. They were examined for inclusion and exclusion criteria, and a throat swab sample was taken for PCR. The PCR was performed on amniotic fluid and neonatal throat samples at pregnancy termination. Six weeks after delivery, the status of rehospitalization and breastfeeding of the babies, the rehospitalization of the mothers and their Depression were evaluated by a 21-item questionnaire. The collected data were analyzed in SPSS version 23 using the Chi-square test.

**RESULTS AND DISCUSSION:** Out of 19 participants, 17 (89%) had positive results for COVID-19 laboratory tests. The frequencies of preterm labor, admission to the neonatal intensive care unit (NICU), and vertical transmission were significantly high in ones with positive PCR results for amniotic fluid (P 0.050). The frequency of admission to the ICU was significantly higher in diabetic mothers (P = 0.025). One case of postpartum depression (9.5%), two cases of formula feeding (11.8%) and no rehospitalization due to COVID-19 were reported. Due to the high risk of maternal and neonatal outcomes of COVID-19 during pregnancy and the high probability of vertical transmission, it is recommended to take special precautions to prevent the disease during pregnancy.

**Keywords:** Pregnancy, Premature Labor, COVID-19, Maternal and Neonatal Outcomes

# Effects of sub-inhibitory concentrations of curcumin on biofilm formation of multidrug-resistant *Acinetobacter baumannii*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Multi-drug-resistant (MDR) *Acinetobacter baumannii* (*A. baumannii*) has emerged as a significant global healthcare concern due to its opportunistic infections and high antibiotic resistance to *A. baumannii* has become an alarming issue. This study presents a promising strategy for fighting these bacterial infections by investigating curcumin's potent antibacterial and antibiofilm effects on MDR *A. baumannii*.

**MATERIALS AND METHODS:** This cross-sectional study included 34 MDR *A. baumannii* clinical isolates. The susceptibility of the isolates to various antibacterial agents was tested using the Kirby-Bauer disc diffusion method. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of curcumin were meticulously determined using the microdilution broth method. The efficacy of curcumin in inhibiting MDR *A. baumannii* biofilm was rigorously assessed using 96-well microtiter plates. The expression of the biofilm-associated protein (*bap*) gene was evaluated using quantitative real-time PCR (qRT-PCR).

**RESULTS AND DISCUSSION:** Among the 34 MDR *A. baumannii* isolates, the highest resistance was observed for trimethoprim/sulfamethoxazole and ciprofloxacin, with all 34 isolates (100%) showing resistance. In contrast, the lowest resistance was noted for ampicillin/sulbactam, with 22 isolates (64.7%) exhibiting resistance. The MICs of curcumin ranged from 0.625 to 2.5 mg/ml, while the MBCs varied between 1.25 to 5 mg/ml. When used at 1/8 of the MIC, Curcumin reduced biofilm formation by 25% to 54%. At 1/4 MIC, the reduction ranged from 38% to 70%. At 1/2 MIC, the reduction was between 56% to 79%. At the MIC, the reduction ranged from 75% to 91% in the treated MDR *A. baumannii* isolates. The mean relative expression of the *bap* gene among the MDR *A. baumannii* isolates decreased by 62.07% compared to the untreated control. This study concludes that curcumin exhibits antimicrobial and anti-biofilm activities against MDR *A. baumannii*. Our findings demonstrate that curcumin

**Keywords:** *Acinetobacter baumannii*, Multidrug-resistance, Curcumin, Biofilm

# Enhanced antibiotic resistance of gentamicin and imipenem in dual-species biofilms of *Staphylococcus aureus* and *Acinetobacter baumannii* from diabetic foot ulcers

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus* presents significant therapeutic challenges due to factors like infection location, resistance mechanisms, and host immunity. Most research has focused on its interaction with *Pseudomonas aeruginosa*, neglecting its relationship with *Acinetobacter baumannii*. The increasing prevalence of diabetes heightens the risk of polymicrobial infections, such as diabetic foot ulcers (DFUs), which often involve both *S. aureus* and *A. baumannii*. These interactions in mixed biofilms contribute to antimicrobial resistance, making combination antibiotic therapy necessary to enhance the antibacterial spectrum and achieve synergistic effects. Despite its widespread use, little is known about the interactions between *S. aureus* and *A. baumannii* in dual-species biofilms, leading to this investigation of the synergistic effects of gentamicin and imipenem.

**MATERIALS AND METHODS:** Samples from DFU patients were collected, and *S. aureus* and *A. baumannii* isolates were identified and genetically confirmed. Methicillin resistance in *S. aureus* was assessed through disk diffusion and PCR methods. Biofilm formation, including dual-species biofilms, was evaluated using the microtiter plate method. Antibiotic susceptibility was determined via disk diffusion, and MIC and MBC values were obtained using the Broth Microdilution method. Synergistic effects of gentamicin and imipenem were investigated in both planktonic and biofilm states using the checkerboard assay.

**RESULTS AND DISCUSSION:** The study revealed that 77.4% of *Staphylococcus aureus* and all *Acinetobacter baumannii* isolates exhibited multidrug resistance. Out of 837 conditions tested for dual-species biofilm formation, 72 isolates formed strong biofilms, and 67 formed moderate biofilms. The geometric mean MIC values for *S. aureus*, *A. baumannii*, and their co-culture were lower for both gentamicin and imipenem in co-culture conditions, indicating enhanced susceptibility. Synergistic effects were demonstrated with FIC<sub>i</sub> and FBC<sub>i</sub> values ranging from 0.42 to 0.21, and FBIC<sub>i</sub> and FBEC<sub>i</sub> values ranging from 0.82 to 0.24. The findings highlight the significant challenge posed by dual-species biofilms in diabetic foot ulcers due to high multidrug resistance. The combination of gentamicin and imipenem significantly improved antibiotic efficacy against these biofilms. This study underscores the potential of combination therapy to combat complex biofilm-associated infections, suggesting future research focus on understanding synergy mechanisms and optimizing treatment protocols for better clinical outcomes in DFU patients.

**Keywords:** *Staphylococcus aureus*, *Acinetobacter baumannii*, Imipenem, Gentamicin, Dual-species biofilms

# Enterococcus faecalis is found in different clonal lineages in Imam Khomeini hospital, Ilam Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Therefore, the current study aimed to identify the resource of *E. faecalis* by PFGE methods. For these reasons, first the bacteria were collected from all urinary tract infections, then, the identification was performed. All isolates were evaluated for vancomycin resistance, then, the PFGE was performed. The results of disk diffusion showed that fifty-four out of one hundred isolates were resistant to the vancomycin. Also, four isolates were intermediate and forty-two isolates were sensitive to the vancomycin.

**MATERIALS AND METHODS:** For this purpose, 100 *E. faecalis* were isolated from Emam Khomeini hospital of Ilam, Iran, which were collected from urine samples. Afterward *E. faecalis* isolates identified by the regular phenotyping methods. Eventually, 16srRNA gene was identified to confirm isolates as *E. faecalis*

**RESULTS AND DISCUSSION:** The results of disk diffusion showed that fifty-four out of one hundred isolates were resistant to the vancomycin. Also, four isolates were intermediate and forty-two isolates were sensitive to the vancomycin. Afterwards, the PCR of VanA gene was performing to confirm the results of the disk diffusion. So, forty-eight out of fifty-four (88.8%) of isolates had the VanA gene as well as, none of four intermediate isolates had VanA gene. Our results demonstrated that 54 isolates were vancomycin resistant and 50 different pulsotypes groups were identified. In conclusion, our findings showed the isolates were from different clonal lineages.

**Keywords:** epidemiology, nosocomial infections, urinary tract infections

# ESBL and carbapenemase producing Uropathogenic Escherichia coli in hospitalized patients, Tehran, Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Uropathogenic Escherichia coli (UPEC) with antibiotic resistance and virulence factors can cause urinary tract infections (UTIs). The aim of this survey was to evaluate the genetic characteristic of extended-spectrum beta-lactamases (ESBLs) and carbapenemase producing UPEC (CP-UPEC) isolates.

**MATERIALS AND METHODS:** In this study, 300 UPEC isolates were collected from the urine samples of patients hospitalized between January 2019 and December 2020. The antibiotic susceptibility of the isolates was evaluated by disk diffusion method. The minimum inhibitory concentration (MIC) of meropenem and CAZ/AVI were determined by E-test, and that of colistin was determined by micro broth dilution method.

**RESULTS AND DISCUSSION:** A total of 100 isolates were collected from UTI patients which 36% (n=36) of isolates were ESBL producing E. coli (EP-E.coli). Among 36 EP-E.coli isolates, 14 (38.8%), 33.3% (n=12), 25% (n=9), and 16.6% (n=6) were temocillin, carbapenem, CAZ/AVI, and colistin resistant. 33.5% (n=11/36) of EP-E.coli were carbapenemase producing E.coli (CP-E.coli). Our findings revealed the high prevalence of antibiotic resistant in EP-E.coli isolates, likely due to the excess clinical use of antibiotics transferring or resistant isolates between different ward of the hospital, since there are limited options to treat the infection caused by these isolates, surveillance is needed to control the spread of such multidrug-resistant strains of E. coli.

**Keywords:** Uropathogenic Escherichia coli, virulence, carbapenemase, antibiotic resistant

# Evaluating the antibacterial effect of Naringenin on *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* and *Staphylococcus aureus* are of the most common causes of nosocomial infections. The urgent need to combat antibiotic resistance and develop novel antimicrobial therapies against these bacteria has triggered studies on novel antimicrobial agents. Taking into consideration the potential risks of antiseptic compounds, antimicrobial agents that naturally occurring are a more acceptable choice in attempts to improve antibacterial effects and efficacy. Citrus flavonoids possess a variety of biological activities, including antimicrobial properties. Naringenin (NAR) as a bitter and colorless flavanone ubiquitous in herbs and fruits, possess a variety of biological activities, including antimicrobial properties. In current study the bacteriostatic and bactericidal activity of NAR was assessed on *Staphylococcus aureus* ATCC 29213 and *P. aeruginosa* PAO1

**MATERIALS AND METHODS:** The antimicrobial effect of naringenin on *Staphylococcus aureus* ATCC 29213 and *P. aeruginosa* PAO1 was investigated using minimum inhibitory concentrations (MICs).

**RESULTS AND DISCUSSION:** NAR showed bacteriostatic and bactericidal activity against *Staphylococcus aureus* ATCC 29213 at 32µm, however, it was incapable of preventing *P. aeruginosa* PAO1. This work provides insight into the bacteriostatic and bactericidal effects of NAR on *S. aureus* and *P. aeruginosa* suggesting NAR being incapable in the prevention of *P. aeruginosa* as one of the most frequent causes of nosocomial infections. However, NAR showed bacteriostatic and bactericidal activity at concentrations tested in our study.

**Keywords:** Naringenin (NAR), *Staphylococcus aureus*, *pseudomonas aeruginosa*, Minimum Inhibitory Concentrations (MICs)

# Evaluating the effect of carbon dot derived from *Lactobacillus roteri* on the expression of *bap* and *oxa23* genes in selected carbapenem-resistant and biofilm-forming *Acinetobacter* isolates.

Bacteriology

Fatemeh Saveh Darbandsary<sup>©</sup> <sup>®</sup>, Ashraf Mojtaba Mobarez

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**BACKGROUND AND OBJECTIVES:** The multidrug-resistant (MDR) pathogenic bacteria are a major concern for global health due to their inherent resistance to antibiotics, their ability to generate new resistance, biofilm production, and the reduction in new drug discovery programs. Therefore, the development of new antimicrobial drugs with specific characteristics is very important. Recently, carbon-based nanomaterials have been identified as potential antimicrobial agents. These carbon materials are environmentally friendly and are used in daily life with minimal cytotoxicity. This property induces an increase in reactive oxygen species (ROS), oxidative stress, and leads to genomic DNA fragmentation and loss of cellular structural integrity, thereby inhibiting or killing bacteria. In this thesis, we will investigate the effect of carbon dots derived from *Lactobacillus roteri* on the expression of *bap* and *oxa23* genes in selected carbapenem-resistant and biofilm-forming *Acinetobacter baumannii* isolates.

**MATERIALS AND METHODS:** 1. 50 isolated *Acinetobacter baumannii* strains confirmed by ITS PCR method. 2. Determination of antibiotic sensitivity profile according to CLSI 2023 standards by disk diffusion method 3. Detecting the presence of *oxa23* and/or *bap* genes using PCR method. 4. Investigation of biofilm formation by microtiter plate assay 5. Synthesis of carbon dots derived from *Lactobacillus roteri* by hydrothermal method. 6. Determination of MIC of carbon dots against *A.baumannii* isolates. 7. Evaluation of the antibiofilm effect of carbon dots against *A. baumannii* with microplate assay. 8. Analysis of the expression levels of *oxa23* and *bap* genes in *A.baumannii* adjacent to carbon dots using Real-time PCR method.

**RESULTS AND DISCUSSION:** This study revealed that clinical isolates of carbapenem-resistant *A. baumannii* and the standard strain *A. baumannii* ATCC 19606 are inhibited by carbon dots synthesized from *latobacillus rotteri*. Additionally, these carbon dots with minimal inhibitory concentration (MIC) can prevent the formation of biofilms by strong, moderate, and weak biofilm-forming isolates of *A. baumannii* that are resistant to carbapenems. By examining the expression of *bap* and *oxa23* genes in isolates that form biofilms and are carbapenem-resistant and have been exposed to carbon dots, it was observed that the expression of these genes has decreased, and this decrease in expression has been significant in some isolates.

**Keywords:** *Acinetobacter baumannii* carbon dots *Lactobacillus* carbapenem resistant

# Evaluating the Effects of Immunization with Polyclonal Exotoxin A Antibody of *Pseudomonas aeruginosa* in Burned Mouse Model

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* is among the main causes of hospital-acquired, wound infections and septicemia in burns, associated with high antimicrobial resistance, morbidity and mortality. Control and prevention of the complications ensuing *P. aeruginosa* infection is one of the most common problems in all over the world. Due to the high resistance, treatment of hospital-acquired infections by antibiotics, often fails. This study aims to probe into the effects of immunization with polyclonal exotoxin A antibody of *P. aeruginosa* on infections in burned mouse model.

**MATERIALS AND METHODS:** The lethal dose (LD) of *P. aeruginosa* in burned mouse model was determined. In order to study the effectiveness of polyclonal exotoxin A antibody produced in rabbits, 18 mice were categorized into three groups namely prevention, treatment, and control. In the prevention group, before causing burn, polyclonal exotoxin A antibody was injected intraperitoneally into each mouse at different intervals and then burn was caused. Then, 2×LD50 of *P. aeruginosa* PAO1 was inseminated in the burned spot. In the treatment group, first burn was caused in the mice. Next, 2×LD50 of *P. aeruginosa* PAO1 was inseminated. After that, polyclonal exotoxin A antibody was injected intraperitoneally at different intervals. Like other groups, in the control group also burn was caused and then 2×LD50 *P. aeruginosa* PAO1 level was inseminated in the burned spot. Then the survival rate was followed for one week after infection.

**RESULTS AND DISCUSSION:** Antibody and antigen reactions were determined in the double diffusion method at 1/2 and 1/4 dilutions. In the agglutination test, there were agglutinated particles in dilutions of 0, 1/2, 1/4 and 1/8, which was a positive result. Significant antibody production was observed in the ELISA method. Based on the obtained results, in the prevention group, 50% of the mice were protected against 2×LD50 injection with exotoxin A polyclonal antibody. In the treatment group, based on the obtained statistics, 25% of the mice survived after the administration of 2×LD50 due to exotoxin A polyclonal antibody, while all the mice in the control group died.

**Keywords:** *Pseudomonas aeruginosa*, Exotoxin A, Polyclonal Antibody



# Evaluating the frequency of *Cyclospora caitanensis* and *Isospora belli* in patient with AIDS using molecular methods in Mazandaran province

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Opportunistic protozoan pathogens such as *Isospora belli* and *Cyclospora caitanensis* can cause severe gastrointestinal and other disorders in people with HIV/AIDS, especially those with low TCD4+ counts. This study aimed to determine *C. belli* and *C. cayetanensis* infections in people with AIDS in northern Iran's Mazandaran province.

**MATERIALS AND METHODS:** This descriptive study was conducted on 50 stool samples of HIV patients who were sent to behavioral disease counseling centers in different parts of Mazandaran province. After receiving written consent, stool samples were collected and evaluated for detection *Cyclospora cyantis* and *Isospora bell* using Polymerase chain reaction (PCR).

**RESULTS AND DISCUSSION:** Out of 50 stool samples, 28 patients were female. The majority of participants lived in villages (66%), and were under 50 years old (56%). 26 people had low TCD4+ cell counts (500 cells/mL). 52% experienced digestive symptoms like diarrhea and cramps. 4% and 8% of samples were positive for *C. cayetanensis* and *C. belli*, respectively. No significant relationship was found between demographic factors, TCD4+ levels, and parasitic infection probability. However, a significant relationship was observed between gastrointestinal symptoms and positive *Isospora belli* infection. The frequency of fecal contamination in the form of *Isospora* was higher in patients with AIDS in Mazandaran province. There was a significant relationship between gastrointestinal symptoms and AIDS. Therefore, the presence of gastrointestinal symptoms in immunodeficient patients with AIDS is important and requires follow-up, sampling, and treatment. The country's health and referral system should periodically examine these individuals for these symptoms.

**Keywords:** AIDS - HIV - *Isospora belli*- *Cyclospora caitanensis*

# Evaluation of anti-pertussis antibody levels in children referred to Children's Medical Center, Tehran, Iran.

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Introduction: Pertussis (whooping cough) is a respiratory infection that is caused by the bacterial pathogen *Bordetella pertussis*, and is associated with severe respiratory illness among children and a persistent cough among adults. Although immunization is the most effective way to prevent and manage pertussis, despite high vaccination rates, the prevalence of pertussis has grown in numerous developed nations. Because recommendations for pertussis booster vaccination depend on the national epidemiology of the disease, we aimed to determine the seroprevalence of pertussis antibody among children attending a children's medical center.

**MATERIALS AND METHODS:** Materials and Methods: In this cross-sectional study, the serum samples of patients who visited the children's medical center for their diagnosis and treatment were used. Anti-pertussis IgG ELISA test (Euroimmun, Luebeck, Germany) was performed on the serum of the patients according to the manufacturer's instructions. The clinical information of the patients, including age, sex, and underlying disease, was collected through their files. The results obtained from the ELISA test and the information extracted from the patient were entered into the SPSS and R software and the available data were analyzed statistically.

**RESULTS AND DISCUSSION:** Results: 1012 children including 566 boys (56%) and 446 girls (44%) were included in the study. The median age of the children was 35 months with an interquartile range (IQR) of 9 to 72 months. Most patients were hospitalized in the emergency department (232 patients, 22.9%) and inpatient emergency department (206 patients, 20.4%). The overall median IgG of *Bordetella pertussis* in patients was reported as 11 units, with an IQR of 5 to 21 units. But qualitatively the IgG results followed an almost equal distribution, with 489 children (48%) positive (median IgG 21.5 units with IQR 15.6 to 29.3), 441 children (44%) negative (median IgG 4.4 units with IQR 2.8 to 6.3) and 82 children (8.1%) had equivocal results (median IgG 9.81 units with IQR 9.3 to 10.4). Conclusion: The findings highlight the influence of time elapsed since vaccination on IgG results and suggest potential points for further research and

**Keywords:** *Bordetella pertussis*

# Evaluation of antibacterial activities of hemolymph from the Large clawed scorpion, *Scorpio maurus kruglovi*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Innate immunity is the first defense of arthropods. Development of arthropods compared to other organisms, while live near potentially harmful microorganisms. It showed that simple but highly effective congenital immune system in arthropods. In previous studies, the effect of hemolymph of different scorpions has been worked on the growth and proliferation of different bacteria. The aim of this study is investigating the antimicrobial activity of *Scorpio maurus kruglovi* Hemolymph.

**MATERIALS AND METHODS:** Scorpions of the species *S. Maurus kruglovi* were collected of state of Kermanshah. In this study hemolymph was collected by a puncture between the second and third segments of the scorpion metasoma. Antibiogram discs are coated with hemolymph, placed on bacterial culture and incubated for 24 h. In this study were used ACT259222(*Escherichia coli*), ATCC25923(*Staphylococcus aureus*), ATCC14028(*Salmonella typhimurium*), ATCC27853(*Pseudomonas aeruginosa*) and ATCC12022(*Shigella flexneri*).

**RESULTS AND DISCUSSION:** Obtained results show that hemolymph of this scorpion wasn't affect on the proliferation of both Gram-positive and Gram-negative bacteria. In this study hemolymph of *Scorpio maurus kruglovi* unlike another previous research hasn't bactericidal power effects.

**Keywords:** Arthropods, *Scorpio maurus kruglovi*, Hemolymph, Antibacterial activities

# Evaluation of Bacteroidetes, Actinobacteria, Firmicutes & Proteobacteria in the gut microbiome of pregnant women with gestational diabetes mellitus compared with healthy pregnant women

Bacteriology

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**BACKGROUND AND OBJECTIVES:** The gut dysbiosis has been observed in gestational diabetes mellitus (GDM), as a prevalent metabolic disorder during pregnancy. However, changes in bacterial population are different among various countries due to genetic, environmental factors, and dietary differences. We compared the gut dominant phylum and some genus in GDM versus women who did not have this disorder in Iranian population. Dietary associations were determined with bacterial population.

**MATERIALS AND METHODS:** In this case-control study, 100 women, aged 18-40 yrs, who were referred for diabetes screening at 24-28 weeks of pregnancy, were participated. GDM was diagnosed if there was at least one abnormal value ( $\geq 92$ , 180 and 153 mg/dl for fasting, one-hour and two-hour plasma glucose concentration respectively), after a 75 g oral glucose tolerance test (OGTT). Bacterial population was determined based on 16SrRNA gene sequence analysis.

**RESULTS AND DISCUSSION:** Actinobacteria and Bifidobacterium spp population was significantly higher in the gut of health mothers than the GDM. Bacteroides genus was significantly higher in the gut of GDM mothers than the healthy-one. Daily calorie intake showed a negative effect on the Bacteroidetes and Actinobacteria population, but dietary carbohydrate and fat showed a positive effect on their habitat. Dietary mono- and poly-unsaturated fatty acids (MUFAs and PUFAs) increased Bacteroidetes population by 1.04- and 0.5-folds, respectively. However, dietary cholesterol showed a negative effect on Bacteroidetes and Bifidobacterium spp population in the gut. Decrease in Actinobacteria and Bifidobacterium spp, as the beneficial bacteria in the gut of mothers with GDM was observed. Dietary calorie and cholesterol associated with the gut dysbiosis, however carbohydrates, MUFAs and PUFAs showed a beneficial effect.

**Keywords:** Dysbiosis, gestational diabetes, Diet, gut microbiome

# Evaluation of *Candida* colonization index as a predictor of invasive candidiasis in hospitalized infants and children

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Candidiasis is an opportunistic infection caused by yeasts of the genus *Candida*. Most often, a reduction in host defenses changes the balance of commensalism in favor of yeast and leads to the switch from colonization to infection. Disease progression from colonization to infection can be identified by describing the colonization index.

**MATERIALS AND METHODS:** In a cross-sectional descriptive study on 200 infants and children hospitalized in Behshahr city hospitals, Iran, after registering the demographic information and clinical status in the patient questionnaire, the samples obtained from different areas of the patients (oral cavity, anal area, ear canal and urine) were cultured in mycological environments. In total, 800 samples were collected from patients and their *Candida* colonization index was determined. Patients with colonization index  $\geq 0.5$  were considered heavily colonized. *Candida* species obtain from these patients were identified by PCR\_RFLP method using Msp1 restriction enzyme.

**RESULTS AND DISCUSSION:** A *Candida* colonization index  $\geq 0.5$  was confirmed in 43 cases (21.5%). A total of 124 yeast isolates were collected from 43 patients. *Candida albicans* (74.4%) was the most common species and *C. glabrata*, *C. tropicalis* and *C. parapsilosis* were isolated with less frequency. The most reported sites with yeast growth was the oral cavity samples (69.8%). The association between fever and antibacterial antibiotic use showed a statistically significant relationship with the index but the mean hospital stay, surgical history and catheter use in the two groups did not show a significant difference. Some researcher say colonization at more than two sites as a key to the earlier initiation of antifungal therapy in high-risk patients. The colonization index is a tool to help predict the possible incidence of candidiasis in hospitalized patients, and treatment after identifying colonization index  $\geq 0.5$  in hospitalized patients can prevent from infection.

**Keywords:** Candidiasis colonization index, candidiasis, hospitalized patients, children, neonates

# Evaluation of drug resistance pattern in multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Pseudomonas aeruginosa* from patients admitted to Mousavi Hospital in Zanjan from 2019-2021

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** The present study was designed and carried out with the aim of investigating the pattern of drug resistance in multi-drug resistant (MDR) and extensively drug Resistant (XDR) strains of *Pseudomonas aeruginosa* (*P. aeruginosa*) isolated from patients admitted to Mousavi Hospital in Zanjan city from 2019-2021.

**MATERIALS AND METHODS:** The current study was a cross-sectional study in which 139 patients admitted to Mousavi Hospital in Zanjan during the period of 2019-2021 were evaluated in terms of the pattern of drug resistance in MDR and XDR strains of *P. aeruginosa*. Were examined. The data collection tool was a checklist including demographic, clinical, and laboratory variables that were extracted from the patients' medical records. After collecting the relevant data, they were entered into SPSS26 software and analyzed.

**RESULTS AND DISCUSSION:** The average age of the patients ( $\pm 25.47$ ) was 48.77 years, 68.3% were male, and the most collected clinical samples were sputum and urine with 44.6 and 22.3%. The highest sensitivity rate of *P. aeruginosa* was related to CL, GM and CAZ antibiotics with 80.6, 33.1 and 30.2%. The highest rate of drug resistance of this bacterium was related to IPM, MEN, CP and GM antibiotics with 69.8, 69.1, 65.5 and 59.7%. The prevalence of XDR and MDR strains was estimated to be 46.7% and 1.44%, respectively. The results of the present study showed that *P. aeruginosa* strains are resistant to a wide range of antibiotics. Considering the importance of this bacterium in hospital infections, especially in the ICU department, it is necessary to take immediate measures for timely identification and definitive treatment with appropriate antibiotics, and also to prevent the spread of resistant strains of this bacterium.

**Keywords:** *Pseudomonas aeruginosa*, multidrug-resistant (MDR) strains, extensively drug resistant (XDR) strains,

# Evaluation of gene expression changes in the brain of BALB/c mice infected with *Toxoplasma gondii* Tehran strain compared to non-infected

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Toxoplasma gondii* is a zoonotic protozoan of the phylum Apicomplexa. Human infection usually occurs through eating raw or half-cooked meat containing cysts or eating water, food and vegetables contaminated with oocysts excreted from cat feces. Toxoplasmosis infection can be seen in both acute and chronic forms. It seems that *T. gondii* is able to modulate the production of ROS and NO in the host by making changes in the expression of genes and thus maintains its survival in the host. Therefore, the aim of the present study is to investigate the expression changes of genes *ctla4*, *ccl4*, *cd3e*, *c3*, *lcn2*, *gbp5*, *usp18*, *cyba*, *tap1* and *samhd1* in the brain of mice infected with *T. gondii* Tehran strain compared to non-infected.

**MATERIALS AND METHODS:** In order to create a chronic form of infection, 30 Balbc mice were injected intraperitoneally with cysts of *T. gondii* Tehran strain (type II, isolated from a patient in the School of Health, Tehran University of Medical Sciences), and MAT test was performed to confirm the infection in the 6th week. Normal saline was injected in the control group. Then, two months later, the brain samples of the mice were separated to continue the experiment. In order to perform Real time PCR (qPCR), first total RNA was extracted using the RNeasy mini kit (Qiagen, Chatsworth, CA, USA) and then cDNA was synthesized using the Takara Prime Script™ RT reagent kit (Takara Bio, Inc., Japan) and then Real time PCR was done using the desired primers.

**RESULTS AND DISCUSSION:** Analysis of Real-time PCR results showed that all genes had increased expression in infected brain tissue compared to non-infected mice brain tissue. *ccl4* gene showed the highest level of expression and *cyba* gene the lowest level of expression in brain tissue infected with *T.gondii* ( $p < 0.001$ ). According to the evaluation of genes in the Genecards database, their importance in creating the chronic form of the disease is highlighted. Therefore, the assessment of genes expression changes in the chronic form of infection to achieve more reactions between the parasite and the host can be useful and effective.

**Keywords:** *Toxoplasma gondii*- gene expression- Real-time PCR

# Evaluation of Human endogenous retroviruses in Multiple sclerosis.

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Multiple Sclerosis (MS) is a progressive disease of the nervous system that leads to demyelination of the nerves and is more common in young adults, especially women. The viral infections are considered to be of particular relevance. The expression of the env gene of human endogenous retrovirus (HERV) like MSRV is considered the risk factor for bringing up MS and disease progression. The present study aimed to investigate the correlation of HERV in MS patients.

**MATERIALS AND METHODS:** 130 subjects were enrolled in a case-control study at two tertiary university hospitals from Tabriz (Imam and Razi), Iran. Of these, 65 subjects were MS patients serving as the case group, and 65 subjects were healthy individuals serving as the control group. After the DNA extraction from all samples, each sample was analysed by RT-PCR with two sets of primers to detect specifically HERV-W env.

**RESULTS AND DISCUSSION:** Results: In the case group, 19 (29.2%) patients were male and 46 (70.8%) patients were female. However, in the control group, 21 (32.3%) subjects were male and 44 (67.7%) subjects were female. No significant difference was found between groups in gender ( $p = 0.70$ ). In our study, the expression of the env gene of HERVs was observed in 10 (15/38%) and two (3/07%) % specimens in the case and control groups, respectively. The analysis found no meaningful difference between groups in this regard. Conclusion: It can be concluded that the expression of HERV-env genes may be involved in the development of MS in these patients. Nevertheless, to prove these findings, more extensive studies with a large sample size are needed.

**Keywords:** Multiple sclerosis, HERV, Tabriz.



# Evaluation of microbial and chemical parameters of groundwater resources in Rural Areas Using Iran Resources Water Quality Index A Case Study of Behshahr, Iran, 2023

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Groundwater contamination is a great challenge to human health. Unhealthy water can lead to digestive diseases, inhibiting nutrient absorption and malnutrition. Consequently, about a billion people in developing countries including Iran are struggling for a safe and sustainable water supply. Current study was carried out with the aim of evaluating the quality status and threats of underground water resources in terms of microbes and chemical in some rural areas of Behshahr city (North of Iran).

**MATERIALS AND METHODS:** This descriptive-analytical cross-sectional study was conducted in the Behshahr city, North of Iran, from April to October 2023. In this research, 12 wells and 2 springs were evaluated for microbial and chemical quality according to national standards methods and the results were analyzed by using standard statistical tests

**RESULTS AND DISCUSSION:** Results: The results indicated that, in general, 74% of the samples are in terms of physical and chemical parameters were within national and international standards. Nitrate concentrations in wells were higher than international and national standards (10-50 mg/L) in some area and also, iron concentrations in the wells around the river and spring was higher than the permissible limit (0.3 mg/L). Furthermore, the microbiological parameters indicate that there are low to very high risks in terms of microbial quality. 25% of groundwater resources had fecal coliform contamination. In 40% of the samples the HPC was higher than 500 cfu/mL. Conclusion: Quality of groundwater face risks such as development of agricultural lands and increasing the use of chemical fertilizers, declining levels and reducing groundwater recharge due to excessive increase in the drilling of illegal wells and reducing rainfall, increasing population and followed by increasing domestic and industrial wastewater.

**Keywords:** Groundwater contamination; Microbial quality; Chemical quality; Mazandaran

# Evaluation of microbial contamination of ready-to-eat fried vegetables hand-packed in Rasht

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background and Objective: Vegetables are known to be rich in vitamins and minerals and provide a good source of food for a variety of microorganisms. However, as the number of microorganisms present in ready-to-eat fried vegetables is often high, it is important to ensure that they are free from harmful pathogens. Saprophytic bacteria, including enteric pathogens, can be present in vegetables due to contamination with polluted water. While these pathogens are usually destroyed with heat, they should not be present in fried or cooked vegetables. In this research, we investigated the presence of enteric pathogens in fried vegetables prepared and distributed by different workshops in Rasht, Iran.

**MATERIALS AND METHODS:** We collected 60 samples from five different workshops and took them to the laboratory at Azad University of Lahijan. After culturing the bacteria in specific and differential media, we conducted gram staining and biochemical tests and counted the bacteria colonies. We then compared the colony limits with the standard table.

**RESULTS AND DISCUSSION:** Our results showed that over 60% of the samples (36 out of 60) were contaminated with *Staphylococcus aureus*. We found *Aeromonas Spp* in 24 samples, and *Serratia* and *Shigella Spp* in 12 samples each. We detected the presence of *Pseudomonas putida* in only one sample. There were no signs of the presence of *Escherichia coli* or *Salmonella Spp* in any of the samples. However, we did detect the presence of *bacilli Spp* in all of the samples. The contamination in these packages is likely due to secondary contamination caused by workers and the places where the foods are prepared and packed. As the consumption of these vegetables is increasing constantly, it is necessary to inform the public to prevent different microbial epidemic situations.

**Keywords:** Reay-to-eat food, contamination, food spoilage bacteria

# Evaluation of the Antagonistic Effect of Toxins Extracted from *Pseudomonas aeruginosa* on Azole Resistance *Candida albicans* Isolates

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Within the limited antifungal armamentarium, the azole antifungals are the most frequent class used to treat *Candida* infections. It is essential to elucidate the potential of natural compounds as an alternative in eliminating *Candida albicans*. Therefore, in the present study, the antagonistic effect of *Pseudomonas aeruginosa* toxins on azole antifungal resistance in *C.albicans* species was investigated.

**MATERIALS AND METHODS:** In this study, 28 *C.albicans* species with azole antifungal resistance were obtained from patients at Shohadaye Tajrish Hospital. The effect of toxins, such as phenazine, pyocyanin, pyoverdine, and fluorescein, was examined on *C.albicans* species. The antifungal activity of these toxins against *C.albicans* spp. was determined using methods such as minimal inhibitory concentration (MIC90), radial diffusion assay (RDA), and detection of reactive oxygen species (ROS).

**RESULTS AND DISCUSSION:** The prevalence of *C.albicans* strains in urinary catheters, surgical wounds, respiratory tracts, blood, and standard strains was 46.3%, 21.4%, 25%, 7.14%, and 3.57%, respectively. The MIC values were reported as 32 µg/ml for phenazine, and 128 µg/ml for pyoverdine, pyocyanin, and fluorescein. The results showed that phenazine exhibited higher inhibitory effects against *C.albicans* isolated from clinical samples compared to the other toxins. After exposure to phenazines (20 µg/ml), 65-70% of yeast cells of *C.albicans* spp. showed rhodamine 123 fluorescence, indicating high intracellular reactive oxygen species (ROS) production. The antifungal effect of different toxins in *C.albicans* spp. may be due to ROS-mediated apoptotic death. The results suggest that phenazine has high potential in controlling *C.albicans*. These natural compounds are a potential alternative for eliminating this yeast.

**Keywords:** Antagonistic Effect, Toxins of *Pseudomonas aeruginosa*, *Candida albicans*

# Evaluation of the antibacterial effects of the essential oil of scaberter in laboratory conditions and food model

Bacteriology

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**BACKGROUND AND OBJECTIVES:** The increasing concern about food-borne diseases and consumer demand for reduced synthetic additives have prompted researchers to explore natural alternatives. Plant essential oils, known for their potential antimicrobial properties, are gaining attention. In this study, we focus on Scaberter essential oil. Our objectives include extracting and identifying its main compounds, evaluating its antimicrobial activity both in vitro and within a food model (mayonnaise).

**MATERIALS AND METHODS:** Scaberter essential oil was extracted and analyzed using gas chromatography. Key compounds identified included borneol, cineole, camphor, and pinene. Antimicrobial activity was assessed against various pathogens, with Gram-positive *Staphylococcus aureus* being the most sensitive and Gram-negative bacteria showing resistance. Additionally, we investigated the effects of Scaberter essential oil on microbial characteristics in mayonnaise.

**RESULTS AND DISCUSSION:** This research analyzed Scaberter essential oil, identifying it as a source of antibacterial compounds including borneol, cineole, camphor, and pinene. Lab tests showed this oil effectively inhibited Gram-positive bacteria growth, with variable effects on Gram-negatives. Integration into mayonnaise revealed its potential as a natural preservative, comparable to synthetic ones like benzoate-sorbate, with fat content influencing its preservation efficiency. The findings align with studies on yarrow essential oil, emphasizing the need for further exploration of plant-derived essential oils as natural antimicrobials in food preservation. This study contributes valuable insights to the ongoing exploration of plant-derived essential oils as natural antimicrobials in the food industry.

**Keywords:** Scaberter Essential Oil, Antimicrobial Activity, Food Preservation, Mayonnaise Model, Preservatives

# Examining efficacy and safety of ethyl acetate extract from *Allium hirtifolium* as complementary therapy in COVID-19: A randomized, multicenter, controlled clinical trial

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Objective: Given the apparent life-threatening nature of COVID-19, finding an effective treatment is under investigation.

**MATERIALS AND METHODS:** Materials and Methods: We assessed effect of shallomin oral syrup (co IranAmin<sup>®</sup>) as a complementary treatment to improve the clinical outcomes in COVID-19 patients. Patients in the control group received the approved treatment protocol (lopinavir/ritonavir), while those in the intervention group were treated with the oral syrup shallomin in addition to the approved treatment. Clinical status of treated patients was recorded and compared.

**RESULTS AND DISCUSSION:** Results: There were meaningful differences between the two groups regarding shortened length of hospital stay and the recovery time for cough, myalgia, sore throat, and shortness of breath. No side effect occurred in the intervention group compared to the control group in terms of biochemical and hematological factors. Conclusion: It seems that the treatment with shallomin syrup showed remarkable contribution to the recovery of COVID-19 induced symptoms in the patients under lopinavir/ritonavir

**Keywords:** COVID-19, Lopinavir, Ritonavir, Drug safety, co Iran Amin syrup

# Examining the Frequency of Shigatoxin Producing Genes in *Escherichia coli* Isolated from Salivary Abomasum disease in kid Goats

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Salivary abomasum disease (SAD) is a devastating disease-causing significant mortality in Iranian goat and sheep herds. Understanding the causative agents is essential for developing effective preventive measures. This study investigated the potential role of Shiga toxin-producing *Escherichia coli* (STEC) in SAD pathogenesis.

**MATERIALS AND METHODS:** We isolated *E. coli* from kid goats aged 3-30 days experiencing a sudden, acute illness characterized by gait imbalance, and death within 48 hours during the kidding season. Following isolation, we employed multiplex PCR to identify the presence of Shiga toxin genes (Stx1 and Stx2) associated with virulence in STEC strains.

**RESULTS AND DISCUSSION:** *Escherichia coli* was isolated from 30 out of 40 animals. Notably, 7 isolates harbored the Stx2 gene, while only one isolate possessed the Stx1 gene. These findings suggest a potential role for STEC, particularly strains carrying the Stx2 gene, in the development of SAD, including mammary bleeding, in young lambs and goats. The presence of Shiga toxin genes in a significant proportion of *E. coli* isolates highlights the importance of further research to elucidate their contribution to SAD pathogenesis and inform the development of targeted interventions

**Keywords:** Salivary abomasum disease, *Escherichia coli*, Shiga toxin, multiplex PCR

# Exploring mec Gene, Virulence Gene Profiles and Vancomycin Resistance in Staphylococcus aureus Isolates in Covid-19 Patients Admitted in ICU at Sina Educational Hospital of Hamedan

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Covid-19 is a new viral infection that was first reported in Wuhan, China in December 2019 and has killed more than one million people worldwide. Hospitalization of patients with COVID-19, especially in ICUs, exposes them to hospital-acquired infections or secondary infections in severe cases with high mortality. One of these causes of secondary infections is *S.aureus* resistant to methicillin. therefore, the investigation of the evolutionary relationship of the acquired isolates from patients, therefore, the epidemiology level of MRSA, and the evaluation and global prevalence of MRSA colonies is necessary.

**MATERIALS AND METHODS:** A number of 22 isolates of *Staphylococcus aureus* were collected from patients with COVID-19 during the third, fourth and fifth waves from December 2019 to February 2020 hospitalized in the intensive care unit. D-test by clindamycin (CLI) and erythromycin (ERY) was done by the Kirby Baer method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI M100-S29)[21]. MRSA strains were identified by cefoxitin resistant ( $\leq 21$ mm) followed by *mecA* gene genotyping. Vancomycin MIC creep was determined by Microdilution Broth and confirmed by *van* gene. Virulence genes, including the Panton-Valentine leukocidin (*pvl*), the hemolysin genes (*hla*, *hly*) were detected using PCR assays. The PCR mixture and conditions were similar to those described previously.

**RESULTS AND DISCUSSION:** In total, from 22 *S.aureus* isolates in covid-19 patients, 12(54.5%) were male, 10(45.5%) female, with the age range of 39-86. Source of infection was tracheal tube 9(40.9%), blood culture 12(54.5%), and sputum 1(4.5%). Antibiotic sensitivity test reported 14(63.6%) resistant to cefoxitin, 15(68.2%) to clindamycin, and 14(63.6%) to erythromycin, in which 2 isolates were positive in case of D-test. All isolates resistant to cefoxitin were positive in case of *mecA* gene, in which 14(63.63%) reported MRSA. In MIC for Vancomycin 18(81.8%) isolates were sensitive and 4(18.2%) were resistant and 2 isolates had *van* gene. In case of existence of virulence genes, 19(86.4%) of isolates was positive for *pvl*, 19(86.4%) for *hla* and 13(59.1%) for *hly* genes. In conclusion, the present study showed high prevalence of MRSA isolates and resistant to Vancomycin in covid-19 patients admitted to ICU wards that is an alarm for spreading antibiotic resistance.

**Keywords:** *mecA* , Vancomycin Resistance, *S.aureus*, COVID-19

# Exploring the Antimicrobial and Anti-Biofilm Properties of Aloe vera against *Pseudomonas aeruginosa* Isolates from Diabetic Foot Ulcers

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Diabetic foot infections (DFIs) are particularly problematic, often involving MDR pathogens such as *Pseudomonas aeruginosa*, which is notably prevalent in diabetic wound infections. One of the promising phytochemical compounds known for its antimicrobial properties is *Aloe barbadensis* Miller (*Aloe vera*). Studies have shown that *Aloe vera* extracts can inhibit the growth and biofilm formation of MDR *P. aeruginosa* strains, suggesting its potential as an effective treatment for DFIs. This study aims to evaluate the antimicrobial efficacy of *Aloe vera* against *P. aeruginosa* isolated from DFI, focusing on its potential to inhibit growth and biofilm formation.

**MATERIALS AND METHODS:** *P. aeruginosa* were isolated from diabetic foot ulcers by deep swab method and confirmed through biochemical and molecular tests. The antimicrobial susceptibility evaluated through disk diffusion method. Microplate biofilm assay was performed to determine the ability of the isolates to form biofilm. Hydroalcoholic extract of *Aloe vera* gel and green peel (rind) were prepared. The effectiveness of *Aloe vera* inner gel and rind against *P. aeruginosa* biofilms was measured by the micro-broth dilution method. For evaluating the bioactive compounds, HPLC of *Aloe vera* extracts were performed.

**RESULTS AND DISCUSSION:** Out of 66 wound samples, 29 isolates were identified as *P. aeruginosa* which all were MDR by the ability of biofilm formation (39% strong, 54% moderate, and 7% weak biofilm). In the results of HPLC the observed peaks for the gel at 8.526 minutes (aloin A) and 8.526 minutes (aloin B) presented them as bioactive compounds of *Aloe vera*. Although aloin A and B peaks were observed at 8.832 and 9.026 minutes in *Aloe vera*'s rind extract, respectively. The MIC of *Aloe vera* inner gel and rind in the range of 8mg/mL to 125 µg/mL was examined which showed the MIC of 4 mg/ml for standard strain of *P. aeruginosa* and the most of clinical isolates. The ability of *Aloe vera* rind demonstrated a significant biofilm reduction than the inner gel and the potential use of *Aloe vera* gel in treating DFIs caused by *P. aeruginosa*.

**Keywords:** Diabetic foot infections (DFIs), *Pseudomonas aeruginosa*, Biofilm, *Aloe vera*, multi-drug



# Exploring the Association Between Latent *Toxoplasma gondii* Infection and COVID-19 in Hospitalized Patients

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Toxoplasma gondii*, the most successful intracellular parasite on the planet, is the etiologic agent of toxoplasmosis. It is interesting to note that SARS-CoV-2 and *T. gondii* can activate innate immunity through a similar pathway. In fact, in both pathogens, toll-like receptors, including TLR 2, TLR4, and TLR7, are activated via the canonical pathway. On the other hand, it is also possible that some induced cytokines in patients with toxoplasmosis increase the severity of COVID-19. Thereby, it is hypothesized that *T. gondii* may be associated with COVID-19 in hospitalized patients. For this purpose, we detected *T. gondii* infection among 133 hospitalized patients with COVID-19 using serological and molecular tests at Imam Khomeini Hospital, Sari, northern Iran.

**MATERIALS AND METHODS:** A questionnaire was used to collect baseline data from the patients who were registered to the Iranian National Registry Center for Toxoplasmosis (INRCT). Also, blood samples were taken from each patient for detecting anti-*T. gondii* antibodies and *T. gondii* DNA using enzyme-linked immunosorbent assay (ELISA) and conventional-PCR methods, respectively. Variables related to the COVID-19 severity and outcomes were indicated based on multiple multinomial logistic regression models.

**RESULTS AND DISCUSSION:** Of 133 patients enrolled in the INRCT with COVID-19 through RT-PCR, 50 (37.59%), 52 (39.1%), and 31 (23%) suffered from mild, moderate, and severe COVID-19, respectively. 57.1% of the patients who died had severe COVID-19, while among those with other outcomes, only 18.60% had severe COVID-19 (P 0.05). Anti-*T. gondii* IgG was detected in 109/133 (81.95%) patients, which was not statistically significant (P 0.05). Among those with negative and positive anti-*T. gondii* IgG, 2 (8.30%) and 29 (26.60%) had severe COVID-19, respectively (P 0.05). *T. gondii* DNA and anti-*T. gondii* IgM were not found in any of the patients. Moreover, all deaths occurred in those with moderate or severe COVID-19 and a positive anti-*T. gondii* IgG. To our knowledge, this is the first registry-based study concerning *T. gondii* infection among patients with COVID-19. Our data show the high rate of latent *T. gondii*

**Keywords:** *Toxoplasma gondii*, Latent toxoplasmosis, COVID-19, Serology, PCR, Iran

# Exploring the prevalence of *N. gonorrhoeae* infection in women with genital infections in Tehran, Iran

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Neisseria gonorrhoeae*, the second most common sexually transmitted infection worldwide, affects one million people daily. The current study aimed to investigate the prevalence of *N. gonorrhoeae* in females with genital infections in Tehran, Iran.

**MATERIALS AND METHODS:** First, a bioinformatic study was conducted to identify a conserved and high-prevalent gene marker for detection of *N. gonorrhoeae*. One desirable marker was selected and a pair of specific primers were designed to amplify it. The reliability of the primer pair was evaluated *in silico* and *in vitro*. Subsequently, 172 patients with genitourinary symptoms were enrolled and an endocervical swab specimen was obtained from each patient to evaluate the presence of *N. gonorrhoeae* in clinical specimens using the specific primers.

**RESULTS AND DISCUSSION:** Restriction endonuclease subunit S (*resS*, WP\_003687768.1) was selected as a specific detection marker. The designed primer pair targeting *resS* showed specific and reliable detection of *N. gonorrhoeae* *in silico* and *in vitro*. Out of 172 clinical samples, seven (4.06%) cases were infected by *N. gonorrhoeae*. Statistical analysis of clinical manifestations showed that there was a significant association between the occurrence of *N. gonorrhoeae* and dysuria (*p*-value = 0.0427), vaginal discharge (*p*-value = 0.0427), pelvic pain (*p*-value = 0.0170), and fever (*p*-value = 0.0447). In this study three promising markers were introduced for development of point-of-care testing approaches. Moreover, this study highlights a 4% prevalence of gonorrhea among women with genitourinary symptoms in Iran, which reminds the urgent need for routine surveillance and new policies in management of STIs, particularly gonorrhea.

**Keywords:** *Neisseria gonorrhoeae*, prevalence, Identification marker, Gonorrhea, Restriction endonuclease subunit S

# Frequency of *algD*, *phzM*, *pelA*, *pilA* and *pilB* genes in biofilm-forming *Pseudomonas aeruginosa* strains and determination of their ERIC-PCR pattern in patients referred to Kowsar Hospital- Sanandaj, 2022-2023

Bacteriology

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**BACKGROUND AND OBJECTIVES:** According to World Health Organization (WHO), *P. aeruginosa* as an opportunistic pathogenic agent with many virulence factors and high resistance to different classes of antibiotics is a high priority. Many studies have been conducted on biofilm formation in *P. aeruginosa* and its relationship with antibiotic resistance. In this study, we examined this relationship from another point of view, which will be discussed further.

**MATERIALS AND METHODS:** In this work, 95 clinical isolates of *P. aeruginosa* were collected and classified into weak, moderate, and strong biofilm producers according to their biofilm-forming abilities via the tissue culture plate method. The antimicrobial resistance and the presence of different virulence genes were investigated via disc diffusion method and MIC and polymerase chain reaction respectively. Moreover, ERIC-PCR typing was performed to investigate the genetic diversity among the clinical isolates.

**RESULTS AND DISCUSSION:** in our study, *pilA* had a negative control on biofilm formation strength. Also, temperature affects the expression level of *algD* and *pelA* genes. The study found a significant difference in antibiotic resistance and biofilm formation patterns between the first and second six-month periods. In the temperature range of 20°C to 37°C, the synthesis of these genes is higher and the biofilm structure of *P. aeruginosa* would be better. According to the 0.95 cut-off, *P. aeruginosa* showed heterogeneity (40 genotypes and 18 clusters). The diversity of bands in isolates belongs to different samples, temperature, and humidity changes because of season changes and the type of primers, and the difference in time and duration of sampling. Considering the high resistance to carbapenems (87.4% imipenem and 64.2% meropenem) among the clusters' members and other genotypes, it can be concluded that the carbapenem resistance gene is scattered and cycling in different parts of

**Keywords:** *Pseudomonas aeruginosa* , biofilm , ERIC-PCR

# Frequency of clinical isolates of Metallo- $\beta$ -lactamase-producing Gram-negative bacteria in Kermanshah

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Carbapenems antibiotics such as Imipenem, Meropenem, and Ertapenem are among the latest antibiotics effective in treating gram-negative bacterial infections. This study aimed to determine the prevalence of several Metallo- $\beta$ -lactamase harboring *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* in Kermanshah hospitals.

**MATERIALS AND METHODS:** One hundred twenty-four isolates (*K. pneumoniae*, *E. coli*, *A. baumannii*, and *P. aeruginosa*) were collected from medical centers of Kermanshah city, Iran. The antibiotic resistance pattern and Metallo- $\beta$ -lactamase production were assayed using Kirby-Bauer test. IMP, NDM, and KPC genes were assessed using the PCR technique.

**RESULTS AND DISCUSSION:** Out of 124 isolates, 6 samples were positive for the IMP gene; of these, 5 isolates (8.1%) were *P. aeruginosa* and 1 isolate (1.6%) was *K. pneumoniae*. In this study, PCR results for other genes were negative. None of the KPC gene were observed. Antibiotic resistance patterns should be determined in each hospital and treatment center at any time to achieve successful treatment with these drugs and prevent the spread of resistance.

**Keywords:** Antibiotic resistance, Metallo- $\beta$ -lactamase, Gram-negative bacteria

# Frequency of ESBLs producing EPEC isolates among patients with diarrhea in Bushehr province

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Enteropathogenic Escherichia coli (EPEC) is an essential cause of diarrhea in developing and developed countries. EPEC strains are divided into two categories, typical (tEPEC) and atypical (aEPEC). The "bundle-forming pilus" (BFP), a type IV fimbria unique to tEPEC strains, is responsible for this characteristic. The emergence of E. coli carrying genes encoding extended-spectrum  $\beta$ -lactamases (ESBLs) are considered urgent health problems worldwide. ESBL genes encode enzymes that hydrolyze  $\beta$ -lactams antibiotics. Purpose of this study was to find out the incidence of ESBLs producing EPEC strains from children with diarrhea.

**MATERIALS AND METHODS:** A total of 165 E. coli isolates were isolated from stool samples of diarrheic children less than 10 years from November 2021 to October 2022 in Bushehr Province. E. coli isolates were identified by standard biochemical tests. DNA was extracted by boiling method. E. coli was confirmed by detection of uidA gene. EPEC isolates were determined by PCR for the eae, stx1, stx2, and bfp genes. The isolates were analyzed to detect ESBL producing through phenotypic combined disk test (CDT) and molecular identification of blaCTX-M gene.

**RESULTS AND DISCUSSION:** Among the 165 E. coli isolates, 5.45% were identified as EPEC. All strains were positive for eaeA and negative for bfpA as atypical EPEC isolates. In total, 34% of isolates were found to be ESBL producers with CDT. PCR was performed for all EPEC strains, and 44.5% of isolates carried the blaCTX-M gene. ESBL producers are resistant for most  $\beta$ -lactam antibiotics, and CTX-M group is the most prevalent ESBLs in E. coli strains. The widespread of ESBLs producing E. coli among children is a serious concern, which requires monitoring of infection control measures through efficient antimicrobial management and detection of ESBL-producing isolates.

**Keywords:** Enteropathogenic Escherichia coli, Diarrhea, ESBLs, PCR

# Frequency of Extended-Spectrum Beta-Lactamase-producing Genes associated in gram-negative bacteria isolated from infectious patients in Kermanshah (2019-2020)

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Nosocomial infections caused by antibiotic-resistant bacteria and their rapid spread threaten public health. This study aimed to determine the frequency of genes encoding Extended-Spectrum Beta-Lactamase (ESBL) in gram-negative bacteria in Kermanshah city, west of Iran.

**MATERIALS AND METHODS:** Identification and antibiotic susceptibility pattern of 165 isolates were performed by biochemical and disk diffusion methods, respectively. Screening and confirming the presence of ESBL genes were performed according to the double disk combination test (DDCT) method. The presence of genes encoding ESBL in each isolate was identified by Polymerase Chain Reaction (PCR) method.

**RESULTS AND DISCUSSION:** Out of 165 isolates, 83 strains were resistant to all antibiotics. The lowest frequency of resistance was observed for Gentamicin, while the highest frequency was observed for Cefotaxime and Cefazolin. Among all strains, 50 (30.30 %) and 80 (48.48%) isolates were phenotypically and genotypically ESBL-positive, respectively. The most prevalent genes encoding ESBL were SHVOS and SHV-1, with a frequency of 20.61 % and 21.82 %, respectively. The frequency of producing ESBL bacteria and the prevalence of blaSHV and blaCTX-M genes in our studied *Klebsiella pneumoniae* and *Escherichia coli* isolates were high. However, unlike some previous reports from Kermanshah, the prevalence of ESBL-encoding genes in *Pseudomonas aeruginosa* was low, and the blaVEB gene was not found.

**Keywords:** Antibiotic resistance, ESBL-encoding genes, ESBL-producing Enterobacteriaceae, Frequency

# Frequency of Metallo-Beta-Lactamase and Extended-Spectrum Beta-Lactamase Genes in Gram-Negative Bacteria Isolated from Ventilator-Associated Pneumonia in the Intensive Care Unit of Imam Reza Hospital in Kermanshah

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Ventilator-associated pneumonia is the most common nosocomial infection that is responsible for increasing the mortality of patients admitted to the intensive care unit. The aim of this study was to evaluate the frequency of MBL and ESBL genes and to determine the patterns of antibiotic resistance in gram-negative bacilli isolated from ventilators in the ICU of Imam Reza Hospital in Kermanshah.

**MATERIALS AND METHODS:** After collecting samples and identifying the bacteria by standard biochemical and microbial culture methods, 152 bacterial isolates were identified. ESBL and MBL genes were identified by phenotypic DDCT method and molecular PCR method using specific primers.

**RESULTS AND DISCUSSION:** The most common microorganism isolated from ventilator-associated pneumonia (VAP) patients was *Klebsiella pneumoniae* (2.56%). In total, the frequency of ESBL and MBL genes was 113 (74.34%) and 8 (5.26%). The antibiotic resistance pattern showed that 36.53% of the isolates were resistant to ceftazidime, cefotaxime and imipenem. The blaSHVOS gene with a frequency of 75 (49.34%) had the highest genotypic frequency. The blaVIM gene was not found in any of the isolates.

**Keywords:** Ventilator-associated pneumonia, Extended Spectrum Beta-Lactamase, Metallo-Beta-Lactamase, Intensive care unit

# Frequency, phylogenetic groups and antimicrobial resistance patterns of Diarrheagenic *Escherichia coli*, obtained from patients with diarrhea in Kerman hospitals

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Diarrheagenic *E. coli* are being recognized as important enteropathogens, especially in developing countries. This study is done to determine the frequency and antimicrobial susceptibility patterns of different *E. coli* pathotypes obtained from patients with diarrhea in Kerman hospitals.

**MATERIALS AND METHODS:** In this cross-sectional study, 325 diarrheal samples were collected from Kerman hospitals during six months. Standard microbiological and biochemical techniques were used to identify the isolates. Polymerase chain reaction (PCR) was used to detect *lt*, *st*, *cvd432*, *aggR*, *eae*, *stx* and *ipaH* genes. Isolates assigned to one of the four main phylogenetic group of *E. coli* (A, B1, B2 and D) using PCR method. The antibiotic susceptibility test was performed using the disc diffusion method.

**RESULTS AND DISCUSSION:** Our results showed that 53/325 patients had diarrhea due to diarrhoeagenic *E. coli*. The most prevalent was enteroaggregative *E. coli* (EAEC) isolated from 28/53 patients with diarrhea (52.8%), followed by enteropathogenic (EPEC) isolated from 14/53 (26/4%), enteroinvasive (EIEC) from 6/53 (11/3%) and enterotoxigenic (ETEC) from 5/53 (9/4%). Enterohaemorrhagic (EHEC) was not detected in any patients with diarrhea. The isolates belonged mainly to phylogenetic group B1 (49 %) and A (39.6%). None of the isolates were resistant to imipenem, whereas 79/2% were resistant to ampicillin

**Keywords:** Diarrhea, Diarrheagenic *E. coli*, Polymerase chain reaction, antibiotic resistance, phylogenetic



# Gene Ontology of effective genes in the chronic form of *Toxoplasma gondii* infection, Tehran strain

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Toxoplasma gondii* is an obligate intracellular protozoan parasite of vertebrates with global distribution. Toxoplasmosis has acute and chronic forms, which are asymptomatic in people with a healthy immune system, but dangerous and can lead to death in people with an immune deficiency. In order to survive in the host, the parasite is able to change gene expression in order to modulate the reactions of the immune system. Therefore, investigating the function of genes and analyzing the pathways involved in clarifying parasite-host interactions is important and significant. Therefore, the aim of this study is to investigate Gene Ontology and pathway analysis of genes (ctla4, ccl4, cd3e, c3, lcn2, gbp5, usp18, cyba, tap1 and samhd1) involved in chronic toxoplasmosis infection.

**MATERIALS AND METHODS:** By evaluating the genes effective in the chronicity of toxoplasmosis infection in the Genecards database, ten genes (ctla4, ccl4, cd3e, c3, lcn2, gbp5, usp18, cyba, tap1 and samhd1) were selected. The Gene Ontology examination was done in three functions as biological processes (BP), Cellular Component (CC) and Molecular Function (MF) using PANTHER and the pathway analysis of genes was done with Enrichr.

**RESULTS AND DISCUSSION:** Pathway analysis showed the most participation of genes in pathway leishmaniasis, PD-L1 expression in cancer and in T cell receptor signaling and the least participation of genes is in Primary immunodeficiency and Glycosphingolipid biosynthesis. The results of gene ontology showed the highest participation of genes in Biological Processes (BP), Cellular Component (CC) and Molecular Function (MF) respectively in functions cellular process, cellular anatomical entity and catalytic activity. Assessment the function of genes and analyzing the pathways involved can be useful and effective for a better understanding of parasite-host reactions and biological, cellular and molecular interactions for medicinal purposes.

**Keywords:** *Toxoplasma gondii*- Gene Ontology- Pathway analysis

# Genetic characterizations of *Toxoplasma gondii* in histopathological samples of patients with brain tumor in Mazandaran province

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background & Aim: *Toxoplasma gondii* is high prevalent in Mazandaran province. Different types of *T. gondii* can cause asymptomatic or fatal forms of toxoplasmosis in cancer patients. This study aimed to investigate the molecular and genotyping of *T. gondii* in brain tumor patients referred to the Cancer Center of Imam Khomeini Hospital in Mazandaran.

**MATERIALS AND METHODS:** Methods: A total of 50 blocked tissue samples of people with brain tumor admitted to the cancer Center of Imam Khomeini Hospital in Sari in Mazandaran province were included in the study. Next, DNA extraction was done and PCR test was performed using RE gene. Finally, *Toxoplasma* genotyping was performed using Nested-PCR and after sequencing, a phylogeny tree was designed.

**RESULTS AND DISCUSSION:** Results: Out of the total number of samples from people with cancer, 29 were men and 21 were women. The results of the molecular test showed that the DNA of *Toxoplasma* was higher in men (8 positive cases out of 29 samples, 27.59%) than in women (3 positive cases out of 21 samples, 14.29%). Molecular results showed that 22% (11) of people with brain tumor had *Toxoplasma* DNA. In total, 63.63% of the samples had genotype I and the remaining samples had genotype II. Conclusion: Our study elicited the importance of toxoplasmosis in brain cancer patients, especially patients with glioblastoma. Due to the high prevalence of *T. gondii* in this province and the significant prevalence of pathogenic type I, prevention measures are necessary to avoid irreversible complications in these patients.

**Keywords:** Keywords: *Toxoplasma gondii*, genotyping, brain tumor

# Genetic diversity and virulence determinants among multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolates collected from hospitalized patients in Mazandaran, Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* (*P. aeruginosa*) is an important cause of healthcare-associated infections (HAIs). A high incidence of severe infections and morbidity and mortality has been associated with *P. aeruginosa*, particularly in patients with impaired immunity. This study examined the antimicrobial resistance patterns, presence of virulence genes, and possible genetic relatedness of *P. aeruginosa* strains isolated from hospitalized patients.

**MATERIALS AND METHODS:** From September 2021 to April 2022, 82 non-duplicate *P. aeruginosa* isolates were collected from diverse clinical sources. Identification was confirmed using API 20 NE. The Kirby-Bauer disk diffusion susceptibility test assessed the resistance or sensitivity of tested strains to various antimicrobial agents according to Clinical and Laboratory Standard Institute guidelines. The virulence profile (alginate (*algD*), elastase (*lasB*), alkaline protease (*aprA*), non-hemolytic phospholipase C (*plcN*), exoenzyme S, T, Y and U (*exoT*, *exoS*, *exoY* and *exoU*) and exotoxin A (*toxA*), and hemolytic phospholipase C (*plcH*)) of each *P. aeruginosa* isolate was made by PCR. Totally, genetic diversity among the strains was evaluated by random amplification of polymorphic DNA (RAPD) technique.

**RESULTS AND DISCUSSION:** Of the 82 total strains, *P. aeruginosa* exhibited the highest and lowest resistance toward ticarcillin-clavulanate (98.78%) and colistin (0%), respectively. Moreover, 100% of the *P. aeruginosa* isolates were MDR. The following prevalence of virulence factor genes was obtained: *aprA*, *lasB*, *algD*, *toxA*, *plcH*, *exoY*, and *exoT* in 100% isolates. The *plcN*, *exoS*, and *exoU* identified 98.78%, 67.07%, and 45.12%, respectively. The RAPD patterns obtained with primers 272 and 208 had respectively 2-19 and 6-17 bands. According to the dice similarity coefficient of higher than 85%, 56 and 39 clusters were recognized. *P. aeruginosa* with high virulence and multidrug-resistant phenotype would increase infection rates, increasing morbidity and mortality. Herein, we reported a high percentage of resistance to different classes of antibiotics coupled with virulence-related markers. In addition, the isolates of *P. aeruginosa* had a high degree of polymorphism probably due to the high rates of genetic diversity.

**Keywords:** *P. aeruginosa*, antibiotic resistance, virulence gene, RAPD, multidrug-resistant

# Genotypic features about the frequency of different virulence factors among clinical isolates of *Pseudomonas aeruginosa* in educational hospitals of Mazandaran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* is a gram-negative nosocomial bacillus responsible for 10-20% of opportunistic hospital infections, especially in immunocompromised patients. It accounts for 50% of deaths due to the presence of virulence factors, such as extracellular proteases, toxins, type III secretion system, cell envelope components, protease IV (PIV), elastase A (LasA), elastase B (LasB), alkaline protease (AprA), pyoverdine A (pvdA) lipase C, phospholipase C, exotoxin A, exoenzyme S, aminopeptidase, esterase A, and different exoenzymes (ExoS, ExoU, ExoY, and ExoT). This study aimed to evaluate the prevalence of *lasA*, *lasB*, *piv*, *aprA*, *pvdA*, *exoU*, and *exoY* genes in clinical isolates of *P. aeruginosa*.

**MATERIALS AND METHODS:** In this study, 100 clinical isolates of *P. aeruginosa* were collected from patients hospitalized in educational-therapeutic hospitals and were identified using standard microbiological tests. The DNAs of the bacteria were extracted by Alkaline Lysis method using sodium dodecyl sulfate and sodium hydroxide. Then, the frequency of *lasA*, *lasB*, *piv*, *aprA*, *pvdA*, *exoU*, and *exoY* genes was determined using PCR test. The data were analyzed using the Chi-square statistical test. The P-value<0.05 was considered statistically significant.

**RESULTS AND DISCUSSION:** Among 100 clinical isolates of *P. aeruginosa*, the *lasA*, *lasB*, and *piv* genes were presented in 97%, 96%, and 97%, respectively, while 95% and 93% of the isolates carried the *exoU* and *exoY* genes, respectively. Meanwhile, 88 isolates had simultaneously both *exoU* and *exoY* genes. Also, the *aprA* gene was presented in 100% of the isolates, while 77% of the isolates carried the *pvdA* gene. In addition, 77 isolates carried simultaneously both *aprA* and *pvdA* genes. No isolate was observed with a negative PCR result. High frequency of the *lasA*, *lasB*, *piv*, *aprA*, *pvdA*, *exoU*, *exoY* gene is worrying and shows the significance of these genes, as important key virulence factors which result in pathogenicity of *P. aeruginosa* in this region. Also, these results indicate the probable clonal similarity of these bacterial strains, which needs further investigation. High prevalence of different virulence factors can be used as a strategy to

**Keywords:** *Pseudomonas aeruginosa*, *exoU*, *exoY*, *lasA*, *lasB*, *Piv*, *AprA*

# Genotyping and Phenotypic Variation of *Candida albicans* Derived from HIV-positive Individuals with Oral Candidiasis

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Oral candidiasis (OC) is one of the most common mucosal infections in those afflicted with HIV/AIDS. There is limited information regarding the patterns of microsatellite genotypes and phenotype in *C. albicans* isolated from HIV-infected patients with OC. This study aimed to provide detailed information on the phenotype, genotype and biofilm formation ability of oral *C. albicans* isolated from HIV-infected patients with OC.

**MATERIALS AND METHODS:** A total of 25 *C. albicans* isolates were collected from oral lesions of HIV-infected patients referred to Behavioral Diseases Counseling Center affiliated with Ahvaz Jundishapur University of Medical Sciences, Iran. The crystal violet method was used to evaluate the biofilm formation ability of isolates. Different phenotypes were identified on yeast extract-peptone-dextrose agar medium supplemented with phloxine B. Genotyping analysis of isolates was performed using high-resolution melting (HRM) assays.

**RESULTS AND DISCUSSION:** Forty-eight percent of isolates had high ability of biofilm formation and exhibited gray cell type on YPD agar medium. Discrimination power (DP) for CDC3 and EF3 markers was 0.74, and it was 0.64 for HIS3 marker. Combination of the three markers yielded a DP value of 0.90. HRM analysis of HIS3, EF3 and CDC3 markers showed 3, 4 and 5 different groups, respectively. Also, ten *C. albicans* isolates (40%) recovered from the oral cavity of HIV patients were heterozygous for CDC3 marker (Fig. 4), while all those isolates showed a homozygous genotype for EF3 and HIS3. Investigating the phenotype and biofilm formation ability of *C. albicans* isolates obtained from oral lesions of HIV-infected patients revealed that the dominant genotypes in the current research have the potential to cause more serious infections from the oral source.

**Keywords:** *Candida albicans*, Biofilm Formation, Phenotypes, Genotyping, High-resolution Melting (HRM)

# Green Synthesis of Thymol and Its Effect on ESBL-Producing *Klebsiella pneumoniae* Isolated from ICU Patients in Hamadan Hospitals

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Klebsiella pneumoniae*, particularly strains producing Extended-Spectrum Beta-Lactamases (ESBL), is a major cause of hospital-acquired infections, especially in intensive care units (ICUs). The development of antibiotic resistance in these bacteria presents significant treatment challenges. Thymol, a natural compound with recognized antimicrobial properties, may offer a promising alternative.

**MATERIALS AND METHODS:** This study investigates the green synthesis of thymol and evaluates its efficacy against 40 ESBL-producing *Klebsiella pneumoniae* strains isolated from ICU patients in Hamadan hospitals. Thymol was synthesized using an environmentally friendly green chemistry approach. *Klebsiella pneumoniae* strains were isolated from urine and other samples of ICU patients in Hamadan hospitals and confirmed as ESBL producers. The antimicrobial activity of the green-synthesized thymol was assessed using the disk diffusion method and minimum inhibitory concentration (MIC) tests.

**RESULTS AND DISCUSSION:** The green-synthesized thymol showed significant antimicrobial activity against all 40 ESBL-producing *Klebsiella pneumoniae* isolates. Clear inhibition zones were observed in the disk diffusion assay, indicating effective bacterial growth inhibition. The MIC values for thymol ranged from 25 to 50 µg/mL, demonstrating its potent effect against ESBL-producing strains. The green synthesis of thymol has proven to be an effective and sustainable method for producing this antimicrobial agent. Thymol exhibited significant antibacterial activity against ESBL-producing *Klebsiella pneumoniae* isolates from ICU patients in Hamadan hospitals. These findings support thymol as a potential alternative or adjunct therapy for managing infections caused by resistant strains, emphasizing the need for further clinical evaluations and research into its application in treating antibiotic-resistant infections.

**Keywords:** Green synthesis Thymol *Klebsiella pneumoniae*

# Heteroresistance to colistin in oxacillinase-producing carbapenem-resistant *Acinetobacter baumannii* clinical isolates from Gorgan, Northern Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Colistin resistance rates are rising globally among multidrug-resistant Gram-negative bacilli, including *Acinetobacter baumannii* (*A. baumannii*). A new type of resistance - heteroresistance - has also been reported to colistin in clinical *A. baumannii* isolates. This study investigated the presence of colistin heteroresistance in carbapenem-resistant *A. baumannii* clinical isolates.

**MATERIALS AND METHODS:** Different clinical specimens from hospitalised patients were investigated for *A. baumannii*. The MICs to imipenem, meropenem and colistin were determined by broth microdilution. PCR was performed to detect OXA-type carbapenemase genes (*bla*OXA-23-like, *bla*OXA-24/40-like, *bla*OXA-51-like, *bla*OXA-58-like, and *bla*OXA-143-like). Heteroresistance to colistin was examined using the population analysis profiles method. Genotypic relatedness of the isolates was analysed by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR).

**RESULTS AND DISCUSSION:** Overall, 71 *A. baumannii* isolates were recovered from clinical specimens. Of these, 27 (38.03%) and 44 (61.97%) isolates were carbapenem-susceptible and carbapenem-resistant, respectively. In addition, 67 (94.36%) isolates were susceptible to colistin, with MICs between 0.25-2 µg/mL. Among the 44 selected carbapenem-resistant colistin-susceptible isolates, the frequency of *bla*OXA-51-like, *bla*OXA-23-like and *bla*OXA-24/40-like genes was 100%, 77.27% and 43.18%, respectively. Nine of 44 (20.45%) isolates were characterised as colistin-heteroresistant with subpopulations growing at 6-8 µg/mL, whereas two of 44 (4.54%) presented heterogeneous subpopulations growing at up to 1 µg/mL of colistin. ERIC-PCR typing clustered *A. baumannii* isolates to 10 common types (CT1-CT10) containing isolates from different hospitals and 12 single types (ST1-ST12). *A. baumannii* with a colistin heteroresistance phenotype was common. This could be of great concern since colistin is often used as a last-resort drug for treating *A. baumannii* infections, highlighting that care is necessary with colistin monotherapy.

**Keywords:** *Acinetobacter baumannii*; Colistin; Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR); Heteroresistance; *bla*(OXA).

# High Prevalence of antibiotic resistance and biofilm-related genes among clinical *Acinetobacter baumannii* isolated from patients in western Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Acinetobacter baumannii* (*A. baumannii*) is an important opportunistic pathogen responsible for nosocomial infections worldwide in recent decades. The ability of *A. baumannii* to form biofilm is an essential strategy for creating stable infections, which may lead to antibiotic resistance and more survival on abiotic and biotic surfaces. Moreover, plasmids can harbor genes encoding antibiotic resistance or virulence factors. In this study, we aimed to evaluate drug resistance, biofilm formation, and plasmid DNA profiles in clinical isolates of *A. baumannii*.

**MATERIALS AND METHODS:** A total of 76 *A. baumannii* were collected from different clinical samples of patients hospitalized in three hospitals in the west of Iran. Susceptibility to antibiotics was determined by the disk diffusion method. The biofilm-forming capacity of isolates was determined using a microtiter plate assay. The presence of genes involved in biofilm development (*ompA*, *bap*, *pga*, *epsA*, *blaper-1*, and *bfmR*) was detected using the PCR method. Additionally, isolates were typed by the Enterobacterial Repetitive Intergenic Consensus (ERIC) method. Plasmids of isolates were extracted and then the plasmid DNA profiles were determined

**RESULTS AND DISCUSSION:** Isolates showed high resistance to cefotaxime, meropenem, ciprofloxacin, and cefepime. The prevalence of biofilm-related genes: *bap*, *ompA*, *pga*, *bfmR*, *epsA*, and *blaper-1* was 90.4%, 90.4%, 79%, 90.4%, 80.5%, and 4.9%, respectively. In the microtiter plate method, out of 76 isolates, 85% were biofilm producers. Among these isolates, 55.6% were strong biofilm producers, while 30.5% and 13.9% showed moderate and weak biofilm production, respectively. ERIC typing showed 20 clones among 76 isolates. The sizes of the plasmids found in the isolates were more than 10Kbp. This study revealed a high resistance and a high prevalence of biofilm-related genes among *A. baumannii* strains. A relative genetic diversity was seen among isolates. High molecular weight plasmids were found in the isolates. Effective strategies are needed to prevent infections caused by resistant and virulent strains

**Keywords:** *Acinetobacter baumannii*, Biofilm formation, plasmids, drug resistance



# Identification of Antibiotic Resistant Genes in *Pseudomonas aeruginosa* Isolated from Burns and Wounds samples in Tabriz, Iran.

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* infections are frequently associated with high fatality rates (*P. aeruginosa*). *P. aeruginosa* is thought to be highly pathogenic due to its remarkable tolerance to a wide range of drugs and multidrug resistance in hospital settings. In this study, 287 patient samples with burns and wounds were used.

**MATERIALS AND METHODS:** *P. aeruginosa* was detected in the specimens that were obtained using biochemical and antibacterial sensitivity testing. The polymerase chain reaction method was then used to identify the *mexA*, *mexB*, *mexR*, and *oprD* genes. 42 *P. aeruginosa* isolates in total were obtained from 287 samples of burns and wounds. 78.5 percent of the cases were isolated from swabs from burns, whereas 21.4 percent were isolated from wounds. Antibiotic sensitivity was tested for each isolate using fifteen different antimicrobial medications.

**RESULTS AND DISCUSSION:** The acquired data demonstrated that whilst the resistance rates to gentamicin, trimethoprim, amikacin, and amoxicillin were high, the resistance rates to ceftazidime, tobramycin, levofloxacin, cotrimoxazole, ciprofloxacin, and aztreonam were low. Regarding antibiotic resistance, it was discovered that three isolates possessed the *mexB*, *mexR*, and *oprD* genes; two isolates had the *mexA* gene, while four isolates possessed the *mexB* and *mexR* genes.

**Keywords:** Molecular diagnosis; Multidrug resistance; *Pseudomonas aeruginosa*.

# Identification of carbapenemase-producing *Klebsiella pneumoniae* by molecular and phenotyping methods in East Azerbaijan, Iran, 2023

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Hospitals are the most common environments in which carbapenem-resistant *Klebsiella pneumoniae* (*K. pneumoniae*) infections occur. These infections are becoming a significant concern in healthcare settings. The purpose of this study was to determine the genes encoding for carbapenemases and antibiotic susceptibility in *K. pneumoniae* isolates from East Azerbaijan, Iran in 2023.

**MATERIALS AND METHODS:** In all, 317 isolates of *K. pneumoniae* were isolated in 2023 from clinical samples taken from various educational hospitals in East Azerbaijan. Disk diffusion was used for identification and antimicrobial sensitivity testing. Using the PCR approach, the isolates were analyzed to find out if certain antibiotic-resistant genes, such as NDM, IMP, VIM, SPM, and OXA48, were present. Using extraction kits, DNA is extracted.

**RESULTS AND DISCUSSION:** *K. pneumoniae* isolates were acquired from clinical samples, comprising 57% urine, 15% respiratory tract, 17% wound, 7% blood, and 3.4% other clinical samples. 55.2 percent of the isolates were resistant to piperacillin, 52.2 percent to sulfamethoxazole-trimethoprim, 47.5 percent to cefepime, 47.9 percent to ampicillin-sulbactam, 47.3 percent to aztreonam, 35.9 percent to ciprofloxacin, 33.4 percent to piperacillin-tazobactam, 31.2 percent to gentamicin, 28.7 percent to tetracycline, and 23.6 percent to meropenem, according to the disk diffusion results. Six isolates had the NDM gene, according to PCR tests. All of the isolates lacked the OXA48, VIM, SPM, and IMP genes. In East Azerbaijan, Iran, the prevalence of NDM-producing *K. pneumoniae* is higher than that of other carbapenemase genes (IMP, VIM, SPM, and OXA48).

**Keywords:** *Klebsiella pneumoniae*; Antibiotic resistance; Carbapenemase

# Identification of Enterovirus 71 in Bottled Water Using RT - PCR

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background and Aim: Human enterovirus 71 (EV71) is the primary cause of hand, foot, and mouth disease (HFMD), a common infectious disease in young children and infants. EV71 can result in various clinical symptoms and has been linked to severe neurological complications. Early and rapid detection is crucial for the prevention and control of EV71 infection, as there are currently no vaccines or antiviral drugs available.

**MATERIALS AND METHODS:** Methods: In this study, 20 samples were collected from various brands of bottled water. The samples underwent RNA extraction and RT-PCR.

**RESULTS AND DISCUSSION:** Results: Out of the 20 bottled water samples tested, none tested positive for the presence of enterovirus 71 nucleic acid. Conclusion: Monitoring the production, transportation, and storage of bottled water is essential to prevent viral contamination.

**Keywords:** Keywords: Bottled water, enterovirus 71, RT-PCR, contamination.

# Identification of Epidemiological pattern of cutaneous dermatophyte infections isolated from clinical samples of Tabriz sina hospital in 2021-2022

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Introduction: Superficial fungal infections are among the most common diseases in the world. This infection may be transmitted from humans, animals or soil to humans. The present study was conducted in East Azerbaijan province to investigate different forms of dermatophytosis and their causes, which is important from the point of view of epidemiology. In order to reduce the risks caused by this category of diseases, it is not enough to treat the patients, but necessary measures should be taken to prevent the disease. Purpose: Identification of Epidemiological pattern of cutaneous dermatophyte infections isolated from clinical samples of Tabriz sina hospital in 2021-2022

**MATERIALS AND METHODS:** Procedure: First, the skin lesion site was disinfected with 70% alcohol and the skins were collected in a sterile plate with the help of a scalpel, then some of this sample was used for direct smearing with 10% KOH and another part of the sample was used for culture on SC and SCC and Rice was used. The samples were incubated in the laboratory environment for 3 to 4 weeks. After the growth and colony formation, the identity of the genus and species of the samples was determined by examining the morphology.

**RESULTS AND DISCUSSION:** Findings: Out of 100 samples suspected of dermatophytosis, 56 cases were confirmed by microscopic examination. Baldness of the groin was the most common from and baldness of the face was reported as the rarest. The age group of 31-40 years old was the most age group and women were more affected by the disease than men. Conclusion: In this study, it was found that the most baldness was related to the groin and the most dermatophyte was related to *Trichophyton violaceum*. Therefore, it is necessary to identify and eliminate risk factors in these people and prevent the occurrence of skin infection.

**Keywords:** Dermatophytes, Epidmiology, Tinea

# Identification of Prevalence of extended-spectrum $\beta$ -lactamases in *Klebsiella pneumoniae* isolates in Tabriz, Iran

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Klebsiella pneumoniae* (*K. pneumoniae*) is an important pathogen that causes several infections in hospitalized immunocompromised patients with severe underlying diseases. Extended-spectrum  $\beta$ -lactamases (ESBLs) are enzymes that hydrolyze the beta-lactam ring of  $\beta$ -lactam antibiotics such as penicillins, first, second, and third-generation cephalosporins, and monobactams. This study was conducted to identify the ESBL-producing *K. pneumoniae* genes and the antibiotic susceptibility of clinical isolates in various hospitals in Tabriz, Iran.

**MATERIALS AND METHODS:** A total of 422 *K. pneumoniae* were isolated from various clinical samples from Nov.2021 to Dec.2023. The antimicrobial susceptibility patterns were analyzed using the disk diffusion test. Multiplex PCR was carried out to determine the presence of the resistance-conferring genes such as blaCTX-M, blaSHV, and blaTEM.

**RESULTS AND DISCUSSION:** *K. pneumoniae* isolates were obtained from urine (54.73%), respiratory tract samples (15.87%), wound infection (11.61%), blood samples (8.29%), sputum (8%), nasal discharge (1.42%) and pleural fluid (0.47%). The disk diffusion results showed that 67.7% of the isolates were resistant to piperacillin, 58.2% to sulfamethoxazole-trimethoprim, 55.4% to ceftazidime, 53.3% to cefepime, 51.8% to ampicillin-sulbactam, 48.1% to aztreonam, 46.9% to ciprofloxacin, 37.4% to piperacillin-tazobactam, 35.3% to gentamicin, 31.2% to tetracycline and 28.6% to meropenem. The blaSHV, blaCTX-M, and blaTEM genes were found in 91, 45, and 29 percent of isolates respectively. These results showed a high prevalence of genes encoding ESBL and antibiotic resistance in the *K. pneumoniae* isolates.

**Keywords:** *Klebsiella pneumoniae*; Antibiotic resistance; beta-Lactamases

# Identification of urease positive bacteria other than *Helicobacter pylori* in endoscopy of gastritis patients and investigation of antibiotic resistance of isolated strains

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Helicobacter pylori* is a gram-negative, urea-positive, spiral-shaped organism. Urease-positive bacteria have been found in the mouth, stomach, intestines, urinary tract, and skin. The aim of this study is to investigate the pattern of antibiotic resistance and the prevalence of gram-positive and urease-positive bacteria other than *Helicobacter pylori* in stomach patients with gastritis.

**MATERIALS AND METHODS:** In the process of a case-control study, 165 biopsy samples from gastric antrum of patients with gastritis in Sari hospitals were examined. After the RUT test, the samples were transferred to BHI culture medium and cultured on blood agar and McConkey agar. Non-*Helicobacter pylori* bacteria were identified in the stomach by standard bacteriological methods such as gram staining.

**RESULTS AND DISCUSSION:** Among 100 samples tested with the urease test kit, 77 were positive for *Helicobacter pylori*, and 23 were positive for non-*Helicobacter pylori* bacteria. *Staphylococcus epidermidis* was the most frequently detected. Significant associations were found between the severity of gastritis and the pathology test results ( $p=0.002$ ), food reflux ( $p=0.002$ ), anorexia ( $p=0.012$ ), nausea ( $p0.05$ ), and burning sensations ( $p0.05$ ). Conversely, no significant relationship was observed between non-*Helicobacter pylori* infections and the severity of gastritis ( $p0.05$ ). Other variables studied also showed no significant correlation with gastritis severity ( $p0.05$ ). This study found no significant relationship between antibiotic resistance patterns and the types of non-*Helicobacter* strains identified. The results suggest that using a combination of different antibiotics may effectively prevent bacterial resistance. Additionally, non-*Helicobacter pylori* bacteria alone do not significantly contribute to the severity of gastritis.

**Keywords:** stomach, non-helicobacter pylori, urease, gastritis, antibiotic resistance, ulcers

# Immunogenicity of Chitosan-Based Non-invasive Vaccine Strategy Against *Mycobacterium tuberculosis*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background: In show disdain toward of Bacillus Calmette-Guerin (BCG) inoculation, still tuberculosis caused by *Mycobacterium tuberculosis* remains a risky obstruction that requires point of view management. The point of this think about was to assess the immunogenicity of chitosan nanoparticles containing recombinant mycobacterial proteins and adjuvant in the Balb/C mice through a non-invasive nasal inward breath conveyance course and degree the level of cytokines interferon gamma (IFN- $\gamma$ ), interleukin-4 (IL-4), and IL-17.

**MATERIALS AND METHODS:** Methods: Thirty mice in five diverse bunches were immunized through inward breath with compounds set in distinctive combinations. Two weeks after the final nasal conveyance, IFN- $\gamma$ , IL-4, and IL-17 were measured in spleen cell culture supernatants.

**RESULTS AND DISCUSSION:** Results: The IFN- $\gamma$  and IL-17 concentrations were found to increment in the bunches that gotten chitosan nanoparticles containing protein and adjuvant alone or as a BCG booster. Our think about showed that the chitosan nanoparticle containing protein and adjuvant actuated a Th1 reaction. However, the bunches that to begin with gotten BCG and at that point chitosan nanoparticles containing protein and adjuvant had the most noteworthy Th1 reaction in terms of IFN- $\gamma$  and IL-17 generation in all the groups. Conclusion: Our discoveries appeared that the immunization planned to be managed through the nasal mucosa well invigorates cellular resistance and improves the BCG vaccine's adequacy.

**Keywords:** Nasal mucosa, Vaccine, Bacillus Calmette-Guerin, Chitosan, Tuberculosis, Immunogenicity, *Mycobacterium tuberculosis*,

# Impact of opioid consumption on fecal microbiota: A quantitative analysis of probiotic and non-probiotic species in male rats

Bacteriology

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**BACKGROUND AND OBJECTIVES:** The gut microbiota plays a critical role in maintaining host health, influencing metabolic processes, and modulating the immune system. Opioid consumption, both oral and injectable, is known to impact gut health, but its specific effects on gut microbiota composition remain underexplored. This study aims to explore the specific impact of different opioid treatments—morphine, opium tincture, and methadone—on the gut microbiota composition in rats. By quantifying the abundance of three probiotic and two non-probiotic bacterial strains, this research seeks to provide insights into how opioid administration might contribute to microbiota alterations and subsequent health implications.

**MATERIALS AND METHODS:** DNA was extracted from the feces of twenty-eight male rats randomly divided into four groups of seven. The groups included a control group with no oral or injectable treatment, a group treated with oral tincture of opium, a group treated with oral methadone, and a group treated with injectable morphine. Fecal samples were collected from all groups at two-time points: after one week and after four weeks of treatment. The study employed absolute quantification real-time PCR to quantitatively analyze the presence of three probiotic species (*Bifidobacterium breve*, *Lactobacillus rhamnosus*, and *Lactobacillus acidophilus*) and two non-probiotic species (*Streptococcus bovis* and *Porphyromonas gingivalis*) across the different groups.

**RESULTS AND DISCUSSION:** After one week, levels of *L. rhamnosus*, *B. breve*, and *L. acidophilus* were reduced in all treatment groups compared to the control group, except for the opium tincture group, where a significant increase was observed for them. After four weeks, levels of all three probiotic species continued to be lower than those in the control group, except for *B. breve*, which remained higher than the control group. Non-probiotic species, including *S. bovis* and *P. gingivalis*, showed increased levels in all groups after one week and remained elevated compared to the control group after four weeks. However, in the opium tincture group, *S. bovis* levels decreased significantly, even falling below those of the control group, while *P. gingivalis* showed a notable increase in the oral methadone group after four weeks. These findings indicate that opioid consumption differentially impacts gut microbiota, promoting the growth of potentially harmful bacteria and contributing to dysbiosis.

**Keywords:** Microbiota



# Increasing the green synthesis properties of silver nanoparticles and investigating its effects on the expression of biofilm genes in *Klebsiella pneumoniae* strains

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Klebsiella pneumoniae* is a human opportunistic pathogen. Biofilm formation is one of the important properties of this bacteria that leads to the development of multidrug-resistant phenotypes. In recent years, Nanoparticle Technology (NP) has been an important field in a wide range of studies. In this study, the green synthesis of silver nanoparticles and its effects on the expression of biofilm genes in *Klebsiella pneumoniae* strains were investigated. After obtaining the *Allium sativum* from the plant bank of the biological reserves center, garlic ethanol extract was prepared and the synthesis of silver nanoparticles was carried out by the green synthesis method.

**MATERIALS AND METHODS:** Sampling : In this cross-sectional descriptive study, 60 samples of various biological samples were collected in the laboratory, including urine, feces, sputum, and wounds from patients referred to Milad Teaching and Therapeutic Hospital in Tehran. After transferring the samples to the research laboratory, all samples were confirmed using standard biochemical and microbiological tests such as gram staining, catalase, oxidase, TSI, SIM, MR-VP, Simon citrate, urease culture media. Synthesis of silver nanoparticles by green synthesis method: The synthesis of silver nanoparticles was carried out using the precipitation method with the reduction of silver ions by ethanol extract of garlic plant. In this way, silver nanoparticles were synthesized by adding 2 ml of extract with a concentration of 0.001 mM of silver nitrate (Merck, Germany) under temperature conditions of 60°C and stirring.

**RESULTS AND DISCUSSION:** The results of this study showed that the synthesized silver nanoparticles are spherical with an average size of 60.17 nm. Silver nanoparticles inhibit biofilm formation in MDR strains of *Klebsiella pneumoniae* and the expression of *mrkA* and *LuxS* genes significantly decreases in these strains after treatment with the minimum inhibitory concentration of silver nanoparticles. The antibacterial activity of the synthesized silver nanoparticles was compared with several antibiotic drugs as a control. The proposed synthetic method is environmentally friendly and can be used to produce silver nanoparticles on a large scale. The main mechanism of the effect of silver nanoparticles on *Klebsiella pneumoniae* strains is through damage to DNA and proteins and destruction of the cell wall.

**Keywords:** *Klebsiella pneumoniae*, biofilm, antibiotic resistance, silver nanoparticle, gene expression

# Investigate the genes causing resistance to aminoglycosides in *Escherichia coli* strains isolated from clinical specimens

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Escherichia coli* is the most common cause of urinary tract infection (UTI), with a prevalence of 80%. Enzymatic inactivation of antibiotics of the aminoglycoside family by aminoglycoside-modifying enzymes is the primary mechanism of *E. coli* resistance to these pharmaceutical drugs, and the recent expression of the Methylase 16S rRNA gene in Gram-negative bacteria that cause high levels of resistance to these antibiotic medications are considered to be a significant concern. This study aimed to determine the antibiotic resistance pattern and the prevalence of antibiotic resistance genes against aminoglycoside antibiotics among clinical isolates of *E. coli* by PCR.

**MATERIALS AND METHODS:** About 500 clinical *E. coli* isolates were recovered in 2017 from the hospitals of Ilam. Antibiotic susceptibility testing was determined against selected antibiotics using the disk diffusion method and E-test according to CLSI standards. The prevalence of antibiotic-resistance genes among clinical *E. coli* isolates was determined using PCR. Data were analyzed using SPSS software and the Chi-square test. A p-value less than 0.05 is considered statistically significant.

**RESULTS AND DISCUSSION:** E-Test results showed that 72.4% and 71.3% of isolates were resistant to Tobramycin and Gentamicin, respectively. Also, 75(79.8%) and 91(96.8%) of *E. coli* isolates contained *aac* (3)-IIa, and *aac*.(6)-Ib, but other used genes were not detected in any isolate. Due to Gentamicin's increased use and availability, its resistance was higher than other antibiotics in many areas, such as Ilam. However, the frequency of resistance to these antibiotics generally varies from region to region, implying the need for antimicrobial susceptibility testing before treatment.

**Keywords:** *Escherichia coli*, Aminoglycoside-modifying enzymes, Gene, PCR

# Investigating and comparing the antibacterial activity of essential oil, hydroalcoholic extract and green synthesis of gold (Au) nanoparticles from the medicinal plant *Satureja bachtiarica bunge* on *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas a*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Extensive studies have been conducted on the potential of using antimicrobial compounds in plants and also the use of nanoparticles to control and treat disease-causing agents. *Bachtiarica Satureja*, known by its scientific name, is one of the native species of this genus in Iran. During the last decade, the desire to make herbal medicines has gradually increased, and therefore, considering the diversity of medicinal plants in Iran and taking into account the increase in antibiotic resistance, in this research, the effect of antibacterial activity of essential oil, hydroalcoholic extract was investigated. And the green synthesis of gold nitrate (Au) nanoparticles produced from the aqueous extract of the medicinal plant *Marzeh Bakhtiari* was studied on *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* bacteria.

**MATERIALS AND METHODS:** Extraction of essential oils was carried out with Clonger's design and preparation of hydroalcoholic extract from *Bakhtiari's Marza* plant at the Medicinal Plant Research Center of the University of Medical Sciences. To make gold nanoparticles, it was done by mixing 0.5 ml of the extract with 9.5 ml of 1 mM  $\text{HAuCl}_4$  solution and the produced nanoparticles were analyzed by Uv-Vis, TEM, FTIR, XRD and EDS. It was sent to Di Petronic Company in Tehran. MBC and MIC were investigated on the three studied bacteria through microdilution method with standard protocol. The data were analyzed through SPSS version 26 software, descriptive statistics tests, average standard deviation and analytical statistical tests of analysis of variance.

**RESULTS AND DISCUSSION:** The results of essential oil analysis showed that thymol is the main compound and indicator of the plant. MIC, MBC and ZOI of *Bakhtiari* against *Staphylococcus aureus* are in the range of 37-5.75, 150-300 microliters per milliliter and 15-16.3 mm respectively. MBC and ZOI of *Bakhtiari's Marza* against *Escherichia coli* were in the range of 75-150, 150-300 and 5/14-5/13 microliter per milliliter, respectively, and the MIC, MBC and ZOI of *Bakhtiari's Marza* Against *Pseudomonas aeruginosa*, it was 75-150, 150-300 and 11.8-13 microliters per milliliter, respectively. The essential oil and gold nanoparticles biosynthesized from the extract of *Bakhtiari* spice have antibacterial properties and can replace antibiotics or can be used as a supplement to antibacterial drugs.

**Keywords:** Gold nanoparticles, essential oil, hydroalcoholic extract, *Bakhtiari* spice, green synthesis,

# Investigating biofilm production in methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) *Staphylococcus aureus* isolated from clinical samples by phenotypic and genotypic methods

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus* is a facultative anaerobic Gram-positive coccus. *Staphylococcus aureus* are classified into two groups: methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA). *Staphylococcus aureus* also is a common pathogen associated with biofilm-related infections. A biofilm not only supports the bacterial cells, but also protects them from the immune system and treatments. based on the strong role of biofilm in resistance to treatment, biofilm investigation among MRSA and MSSA is essential.

**MATERIALS AND METHODS:** In this cross-sectional analytical study, 228 samples of *Staphylococcus aureus* were collected. Biofilm production of samples was checked by Congo red agar and Microtitre plate method. In the molecular method, the presence of *icaA*, *icaB*, *icaC*, *icaD* genes were determined by PCR and the presence of *bbp*, *cna*, *eno*, *ebps*, *fnbA*, *fnbB*, *fib*, *clfA*, *clfB* genes were determined by Multi Plex PCR and samples of each gene were sequenced.

**RESULTS AND DISCUSSION:** The results of Congo red showed that 25% of the samples have strong biofilm, 27.2% weak and 47.8% negative; While according to microtiter results, 8.8% of the samples showed strong biofilm, 29.8% moderate, 45.8% weak and 15.8% no biofilm production. According to the molecular results, 78.5% of the samples have *icaA* gene, 30.3% *icaB*, 14.9% *icaC*, 55.7% *icaD*, 1.8% *fnbA*, 4.8% *fnbB*, 41.7% *fib*, 77.2% *clfA*, 74.6% *clfB*, 4.4% of the *bbp*, 32.9% of the *cna*, 74.1% of the *eno*, and 45.2% of the *ebps*. The highest frequency was for *clfA* with 77.2% and the lowest frequency was for *fnbA* with 1.8%. Considering the importance of the role of biofilm in the resistance of bacteria to antibiotic treatment and the problems and infections caused by it, it is very important to know the genes related to biofilm and a perspective for future investigations to deal with these genes.

**Keywords:** *Staphylococcus aureus*, biofilm, MRSA, MSSA

# Investigating the antibacterial effect of manganese oxide nanoparticles biosynthesized by Japanese parsnip leaf extract on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Infectious diseases are considered one of the global health problems and lead to the annual death of 13 million people in the world. Among the most important pathogenic pathogens are *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, which cause many infectious diseases in humans, and many antibiotics are used to deal with them. Due to the excessive use of antibiotics, especially in Iran, resistance is increasing. One of the new and effective methods to destroy bacteria is nanoparticles of various metal oxides. Metal oxide nanoparticles have antimicrobial effects against a wide range of microorganisms. Manganese oxide nanoparticles have been widely considered, especially in medical sciences and nanotechnology. These nanoparticles have various biological properties such as antibacterial and anticancer properties. Plants and plant extracts are one of the biological sources used for the green synthesis of nanoparticles. One of the plants with known antimicrobial properties is *Eriobotrya japonica*. *Eriobotrya japonica*, having various bioactive.

**MATERIALS AND METHODS:** Manganese oxide nanoparticles were biosynthesized by *Eriobotrya japonica* by green synthesis method. The antibacterial activity of manganese oxide nanoparticles was investigated by microdilution method. Biofilm inhibition was investigated by the microtiter plate method. Then the data was analyzed using SPSS software.

**RESULTS AND DISCUSSION:** Fourier Transform Infrared (FTIR) and Scanning Electron Microscope approved synthetic copper oxide nanoparticles. The highest inhibitory concentration of manganese oxide nanoparticles synthesized by the green method for *Klebsiella pneumoniae* strain is 78.12 µg/ml and for *Pseudomonas aeruginosa* strain is 156.25 µg/ml. Manganese oxide nanoparticles inhibited the growth of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* at concentrations of 312.5 µg/ml and 612.5 µg/ml, respectively. According to the obtained results, this nanoparticle can be suggested as a promising agent in terms of biofilm inhibition and antimicrobial properties.

**Keywords:** manganese oxide, *Eriobotrya japonica*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, biofilm.

# Investigating the antibacterial effect of the combination of copper oxide nanoparticles and the alcoholic extract of *Eriobotrya japonica* on *Escherichia coli* isolated from urinary tract infections

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Urinary tract infections are the most common cause of hospital infections and the second most common infection in humans. About 80%-90% of non-hospital urinary tract infections are caused by *Escherichia coli*. Antibiotic resistance *Escherichia coli* is increasing. Biofilm formation is one of the important pathogenic factors in *Escherichia coli* bacteria, contributing to the chronicity of diseases. Therefore, finding new antimicrobial compounds with the least side effects is necessary. One of the important plant extracts is *Eriobotrya japonica*, which has good antibacterial properties. Also, Copper oxide nanoparticles are one of the most important transition metal oxides (Cu<sub>2</sub>O) due to their remarkable properties. Copper oxide is known as an antimicrobial agent against various types of bacteria. In the last few years, the green synthesis of nanoparticles has been confirmed as an effective, environmentally friendly, non-toxic and cost-effective method. Plant extracts and microorganisms can be used for green synthesis.

**MATERIALS AND METHODS:** The copper oxide nanoparticles and alcoholic extract of *Eriobotrya japonica* were synthesized. The antibacterial activity of copper oxide nanoparticles and alcoholic extract of *Eriobotrya japonica* was investigated by microdilution method alone and in combination. Biofilm inhibitory was investigated by the microtiter plate method. Then, the data were analyzed using SPSS software.

**RESULTS AND DISCUSSION:** Synthesized copper oxide nanoparticles were approved by Fourier Transform Infrared (FTIR), and Scanning Electron Microscope. The highest inhibitory concentration of the alcoholic extract of *Eriobotrya japonica* and copper oxide nanoparticles for ESBL isolates is 312.5 µg/mL. After examining the FIC of the double combination, it was found that this double combination showed an increasing effect in 100% of ESBL isolates. Also, no lethality was observed in any of the 10 samples at the MIC dilution. Regarding the lowest concentration of FIC, MBC was seen in three cases, and five samples showed MBC in the double dilution of FIC. The extract and nanoparticle each alone and in the combined state prevented biofilm formation, but in the combined state it showed more effectiveness. Concerning our results, the combination can be suggested as a promising agent in terms of biofilm inhibitory and antimicrobial properties

**Keywords:** Copper oxide nanoparticles, *Eriobotrya japonica* extract, *Escherichia coli*, biofilm

# Investigating the antibacterial effects of *Lactobacillus plantarum* and *Streptococcus lutetiensis* probiotics strains on coagulase negative strains isolated from urine sample

Bacteriology

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**BACKGROUND AND OBJECTIVES:** The rise of antibiotic-resistant strains of *Staphylococcus epidermidis* (*S. epidermidis*) underscores the urgent need for alternative therapeutic strategies. Probiotics, with their ability to competitively exclude pathogens and modulate the host immune response, present promising candidates for combatting *S. epidermidis* infections. This study investigated the effectiveness of probiotics on *S. epidermidis* growth inhibition.

**MATERIALS AND METHODS:** We isolated *S. epidermidis* from urine samples of hospitalized patients in Isfahan, Iran, and probiotic strains from yogurt and milk. The antibacterial activity of probiotics against *S. epidermidis* was assessed through agar well diffusion and broth microdilution tests. Time-kill tests and acid tolerance assessments were performed. Anti-biofilm effects were evaluated, and potential inhibitory mechanisms were explored. Chemical analysis was done using high-performance liquid chromatography (HPLC), and cytotoxicity was assessed by performing MTT.

**RESULTS AND DISCUSSION:** *Streptococcus lutetiensis* OR496927.1 (*S. lutetiensis*) and *Lactobacillus plantarum* OR496928 (*L. plantarum*) probiotics were isolated from dairy. *S. lutetiensis* and *L. plantarum* strains had a cytotoxicity effect on *S. epidermidis* isolates at 1/2 and 1/4 minimum inhibitory concentration (MIC), respectively. *L. plantarum* grew at pH 3, while *S. lutetiensis* displayed growth at pH 3 and 4. Both probiotic strains demonstrated anti-biofilm activity, with *L. plantarum* generally exhibiting more potent effects. Lactic acid, formic acid, and acetic acid were identified as the predominant organic acids produced by the probiotic strains, which attributed to their inhibitory effects. Toxicity was observed at a concentration of 50% after 24 hours, while cell viability remained unaffected at lower concentrations.

**Keywords:** *Lactobacillus plantarum*, *Staphylococcus epidermidis*, Probiotic, *Streptococcus lutetiensis*

# Investigating the antibiotic and genetic resistance pattern in *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase (ESBL) isolated from clinical samples of special care departments of hospitals in Mazandaran province by PCR method in 2012

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Klebsiella pneumoniae* infection is a common gram-negative nosocomial disease. Hence in this study the antibiotic and genetic resistance pattern was evaluated in ESBL species isolated from clinical samples by PCR in Mazandaran training hospitals in 2013.

**MATERIALS AND METHODS:** In this observational study that was performed as a cross-sectional one, 150 samples were evaluated among species isolated from clinical samples by PCR in Mazandaran training hospitals in 2013 and the antibiotic and genetic resistance pattern was evaluated among ESBL species.

**RESULTS AND DISCUSSION:** In this study, 45 samples were positive for *Klebsiella Pneumoniae* among them 29 samples were ESBL showing rate of 28% (CI 95%). 15 samples developed 800 bp band in TEM gene location and 14 samples developed 400 bp band in CTX region. Finally it may be concluded that more than half of *klebsiella* species are ESBL which is bothersome and would require some strategies to reduce the burden and decrease the mortality and morbidity and better treatment of nosovomial infections.

**Keywords:** ESBL, *Klebsiella Pneumoniae*, Antibiotic Resistance



# Investigating the antibiotic resistance pattern and metallo-beta-lactamase producing *Klebsiella pneumoniae* isolates in Alborz province

Bacteriology

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**BACKGROUND AND OBJECTIVES:** The burden of *Klebsiella* drug resistance to antimicrobials is a major public health concern worldwide; particularly the problem is severe in developing countries including Iran. One of the most important mechanisms of antimicrobial resistance in *Klebsiella pneumoniae* is the production of metallo-beta-lactamases. The prevalence of bla-NDM as one of the metallo-beta-lactamases in *Klebsiella pneumoniae* varies across different regions. In our study, we are focusing on founding an antibiotic resistance pattern in *Klebsiella pneumoniae* in Alborz province, as well as , the prevalence of bla-NDM producing strains.

**MATERIALS AND METHODS:** Fifty-four *Klebsiella pneumoniae* clinical specimen were collected from different hospitals in Alborz province. Susceptibility testing was performed for all the identified clinical isolates by the standard Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics used included: amoxicillin/clavulanic acid, gentamycin, ciprofloxacin, imipenem, ceftazidime and zetronam. PCR reaction for bla-NDM with specific primers were done, in order to find the prevalence of this gene in our isolates.

**RESULTS AND DISCUSSION:** Highest resistance rates were found against amoxicillin/clavunic acid and ceftazidime, whereas imipenem was demonstrated to be very effective antibiotic by in vitro tests. 71% of the *klebsiella pneumoniae* isolates were susceptible to imipenem and 47.8 % isolates were resistant to amixicilin/clavunic acid and ceftazidime. NDM was detected among 38 % of our *K. pneumoniae* strains.

**Keywords:** *Klebsiella pneumoniae* bla-NDM antibiotic resistance metallo-beta-lactamase

# Investigating the antimicrobial effect of atmospheric cold plasma on eye infection caused by *Staphylococcus aureus* in vitro

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Bacterial agents that cause eye infections, especially *Staphylococcus aureus* bacteria, can act as a harmful factor due to the increase of antibiotic-resistant bacteria. Therefore, finding a new, effective and especially fast disinfection method is necessary. The aim of this research is to investigate the antimicrobial effect of atmospheric cold plasma on eye infection caused by *Staphylococcus aureus* in vitro.

**MATERIALS AND METHODS:** In this research, after determining the identity of *Staphylococcus aureus* isolates and identifying methicillin-resistant strains, 30 samples of *Staphylococcus aureus* were cultured. And then they were treated with plasma for 5 minutes. The plate containing these 30 samples was incubated for 24 hours at 37 degrees Celsius. After this time, the OD of the samples was read at a wavelength of 600 nm.

**RESULTS AND DISCUSSION:** The results show that the average OD in the group without plasma is 1.04 and the average OD in the plasma radiation group reached 0.21, which is statistically significant. According to the data, atmospheric cold plasma in Reducing the growth of *Staphylococcus aureus* isolated from the eye is very effective.

**Keywords:** Eye infection, cold plasma, antibiotic resistance, *Staphylococcus aureus*

# Investigating the antimicrobial effect of cumin and caraway plant extracts on *Escherichia coli*.

Bacteriology

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**BACKGROUND AND OBJECTIVES:** The rise of antibiotic-resistant strains of bacteria such as *Escherichia coli* has necessitated the exploration of alternative antimicrobial agents. Plant-derived compounds, particularly those found in *Carum carvi* and *Cuminum cyminum* have been recognized for their potential antimicrobial properties. According to the reviews carried out by experts, *Escherichia coli* is one of the main factors of microbial infections in different clinical places especially hospitals. The antimicrobial effect of many plants has been proven in recent years, *Carum carvi* and *Cuminum cyminum* are to samples of these kind of plans, so, in this study, the antimicrobial effect of *Carum carvi* and *Cuminum cyminum* alcoholic extracts has been investigated. This study aims to investigate the antimicrobial effects of the maintained plant extracts especially on *Escherichia coli*. As a common agent of urinary tract infections and other bacterial diseases.

**MATERIALS AND METHODS:** After diagnosing, collecting and identifying the plants, by in vitro situation the Minimum Inhibitory Concentration and Minimum Bacterial Concentration rate of the acetone and ethanol extracts of the mentioned plants were investigated by macro dilution method in the Mueller Hinton Broth culture medium and the antimicrobial effect of the extracted extracts was tested and evaluated on *Escherichia coli* bacteria.

**RESULTS AND DISCUSSION:** According to the observed findings of the laboratory results studies, it was determined that the ethanol based *Cuminum cyminum* extract has high antimicrobial and growth inhibitory properties against *Escherichia coli*. Also, as the second powerful extract, it is possible to mention acetone *Cuminum cyminum* extract. In addition, it can be stated that the ethanol-based extracts of the mentioned plants also have antimicrobial properties. In this study, the effect of ethanol and acetone based alcoholic extracts of *Carum carvi* and *Cuminum cyminum* has been tested. The sequence of the efficacy of the extraction from most antimicrobial one to least one is ethanol *Cuminum cyminum*, acetone *Cuminum cyminum*, ethanol and acetone *Carum carvi*. So, by this succession, they can be used to curb the growth of *Escherichia coli* at essential places including clinics, hospitals and other places which have health and medical usages.

**Keywords:** *Carum carvi*, *Cuminum cyminum*, *Escherichia coli*

# Investigating the demographic, clinical and geographic distribution of patients with amoebiasis and shigellosis from 2013 to 2014 in health centers of Mazandaran University of Medical Sciences

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background and purpose: Infectious diarrhea is a public health problem, but it is preventable. According to the World Health Organization, diarrheal disease kills about 1.8 million people annually (1), and the occurrence of infectious diarrhea is more serious in developing countries (2, 3). The aim of this study was to investigate the demographic and clinical characteristics of patients with amebiasis and shigellosis, which was conducted from 2012 to 2022 among the population of Mazandaran University of Medical Sciences.

**MATERIALS AND METHODS:** Materials and methods: Cases of amebiasis and shigellosis that were confirmed in Mazandaran province between 2012 and 2022 were included in this study. In order to collect the required data, we used the registered information of the health networks and health deputy of Mazandaran province and the infectious diseases management center of the Ministry of Health website. The data obtained in the study were analyzed by SPSS version 24 statistical software.

**RESULTS AND DISCUSSION:** Result: During the study, 2346 cases of bloody diarrhea were reported. Shigella was found in 88 cases and Entamoeba histolytica in 339. The highest prevalence of both was in children under 10, with rates of 10.01 and 27.10 per hundred thousand people, respectively. Ramsar had the most Shigella cases, and Nowshahr had the most amoeba cases. The most common year and month for Shigellosis were 2017 (20 cases, 22.72 percent) and August (24 cases, 27.27 percent). For amoeba, the most common year and month were 2019 (85 cases, 25.07 percent) and December (46 cases, 13.56 percent). Conclusion: In conclusion, the study's results indicated a significant increase in the prevalence of amoeba disease in Mazandaran province during the study period, particularly in the last year. This highlights the importance of implementing measures to prevent the spread of the disease in the province. Shigellosis and amoebiasis affected both genders at similar rates,

**Keywords:** Keywords: amoebiasis, shigellosis, infectious diarrhea, epidemiology, pathogen

# Investigating the effect of Zinc titanium oxide nanoparticles combination on biofilm formation in *Klebsiella pneumoniae* isolated from patients hospitalized in ICU unit

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Hospital-acquired infections caused by drug-resistant bacteria are a major global health concern, especially in intensive care units (ICUs). *Klebsiella pneumoniae* is pathogen that frequently colonizes medical devices and forms resilient biofilms that are challenging to eradicate. As antimicrobial resistance continues to rise, novel approaches targeting biofilm formation, such as combinatorial nanoparticles, may provide new strategies to prevent device-related infections. Titanium dioxide nanoparticles exhibit potent photolytic properties that enable environmental disinfection along with diverse medical and biological applications like drug and gene delivery. Zinc oxide nanoparticles also possess broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative eliminating 99.9% of pathogens within 2 hours. As a hybrid metal oxide, ZnTiO<sub>3</sub> may provide synergistic benefits from the combined properties of zinc oxide and titanium dioxide.

**MATERIALS AND METHODS:** The Zinc titanium oxide nanoparticles and alcoholic extract of *Eriobotrya japonica* were synthesized. The antibacterial activity of Zinc titanium oxide nanoparticles was investigated by microdilution method. Biofilm inhibitory was investigated by the microtiter plate method. Then, the data were analyzed using SPSS software.

**RESULTS AND DISCUSSION:** Synthesized Zinc titanium oxide nanoparticles were approved by Fourier Transform Infrared (FTIR), and Scanning Electron Microscope. The highest inhibitory concentration of Zinc titanium oxide nanoparticles for *Klebsiella pneumoniae* isolates is 156.25 µg/mL. This nanoparticle concentration of 312.5 µg/mL prevented the formation of biofilm. Conclusion: Concerning our results, this nanoparticle can be suggested as a promising agent in terms of biofilm inhibition and antimicrobial properties.

**Keywords:** nanoparticles, Zinc titanium oxide, *Klebsiella pneumoniae*, biofilm

# Investigating the evolutionary relationships of DNA Pol I in thermophilic bacteria

Bacteriology

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**BACKGROUND AND OBJECTIVES:** DNA polymerases are a type of enzyme that synthesizes specific portions of the genome using DNA as a template. Enzymes like these have long been an integral aspect of molecular diagnostic kits, serving as crucial tools for both modern healthcare and agricultural research. Many of these professions' accomplishments are based on their ability to amplify and detect specific genetic material. In the present investigation, we present the results of an in-silico experiment on the DNA Polymerase I of thermophilic bacteria.

**MATERIALS AND METHODS:** The proteome sequences of 27 thermophilic bacteria were obtained from GeneBank, and DNA pol I sequences were found in each species. We used SMART to characterize the domains. Physicochemical parameters such as MW, instability index, and GRAVY were calculated using ProtParam. The amino acid sequences were examined using Swissmodel to estimate the 3D structure. The DNA pol I protein sequences of 27 thermophilic Bacteria were examined using MEGA 11 software to investigate the evolutionary relationships.

**RESULTS AND DISCUSSION:** Exploring polA amino acid sequences uncovers a variety of domains, each with a unique function. Some examples of these domains are 35EXOc, which is responsible for exonuclease activity, HhH1, which is involved in DNA repair, and POLAc, which is involved in DNA polymerization and replication. Multiple domains were involved in the binding of nucleic acid sequences. Classifying sequences and providing functional annotation rely on identifying protein domains. A amino acid length for these 27 proteins was found to be between 870 and 900, according to the studied domains. These proteins have three or four domains, and they all share the POLAc and HhH2 domains. It is typical for DNA polymerases to have the POLAc domain in either the third or fourth position, which spans 208 amino acids, from positions 630 to 840. As an example, the polymerase domain is located in the third position in Geobacillus stearothermophilus, Escherichia coli, and Sulfobacillus.

**Keywords:** Thermophilic bacteria, DNA POL A, DNA polymerase, smart, PCR

# Investigating the expression level of a metastatic and an antimetastatic gene in gastric biopsies of patients with gastritis induced by *Helicobacter pylori*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Helicobacter pylori* infection is one of the most common chronic bacterial infections, especially in developing countries. MicroRNA-148a is involved in the regulation of various genes, including Rock1, which is altered in gastric cancer. Decreased expression of mir-148a leads to tumor metastasis and increased Rock1 gene expression in gastric cancer. This study aimed to investigate the expression of these genes in biopsies collected from patients with *H. pylori* induced gastritis.

**MATERIALS AND METHODS:** Informed consent forms were gotten from the studied patients with gastritis who needed endoscopy. Gastric biopsies were taken by a gastroenterologist from patients with inflammation. Rapid urease test, stool antigen detection, and histopathological staining were used to determine the *H. pylori* infected patients. Real time PCR was used to evaluate the miRNA and Rock1 expression levels.

**RESULTS AND DISCUSSION:** The Rock1 expression level in biopsies that were positive for *H. pylori* was significantly increased compared to our control gastritis group that were *H. pylori*-negative, but the results were not statistically significant. Moreover, the mir-148a expression level in *H. pylori*-positive patients with gastritis was increased compared to our control group. However, the results were not statistically significant. We did not find a significant relation between the expression levels of Rock1 and mir-148a in samples with gastritis infected or uninfected by *H. pylori*. This result may be due to the small sample size. Conclusion: We suggest that this test should be carried out with more samples, and the comparison should be done between biopsies with inflammation and no inflammation in a patient.

**Keywords:** *Helicobacter pylori*, Rock1, miRNA148a, Gastritis

# Investigating the expression of virulence genes *pilA* and *csuD* of XDR and MDR *Acinetobacter baumannii* in hospitalized patients

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Introduction: *Acinetobacter baumannii* is known for causing hospital-acquired and community-acquired infections and shows high resistance to antibiotics. The emergence of multidrug-resistant *Acinetobacter baumannii* poses significant challenges in hospitals, leading to infections such as bacteremia, pneumonia, meningitis, urinary tract infections, and wound infections. Objective: 1) Determining the frequency of MDR and XDR strains among carbapenem resistant isolates of *Acinetobacter baumannii*. 2) Investigating the presence of blaOXA-143-like, blaOXA-23-like, blaOXA-24-like and blaOXA-58-like genes by PCR method in carbapenem resistant strains. 3) Examining the expression of *csuD* and *pilA* genes among MDR and XDR strains by Real Time-PCR method.

**MATERIALS AND METHODS:** Material and Methods: Antibiotic sensitivity testing (Antibiogram) was performed for the antibiotics ciprofloxacin, piperacillin, imipenem, meropenem, piperacillin/tazobactam, tetracycline, cefotaxime, cefepime, ceftriaxone, trimethoprim/sulfamethoxazole, amikacin, gentamicin and ceftazidime. PCR reaction was performed for blaOXA-143-like, blaOXA-23-like, blaOXA-24-like, blaOXA-58-like, *pilA* and *csuD*. RT-qPCR was performed for *pilA* and *csuD* genes in duplicate by Real-Time PCR Plus One Step ABI.

**RESULTS AND DISCUSSION:** Results: 80% of the samples showed resistance, 18% sensitivity and 2% intermediate resistance to amikacin and gentamicin. 98% showed resistance to ceftazidime and 2% showed sensitivity. 100% showed sensitivity to colistin and 100% showed resistance to all the other antibiotics mentioned. Further the frequency of MDR and XDR strains was obtained, among which 40 strains were MDR/ Non XDR and 10 strains were MDR and XDR. Then the presence of antibiotic resistance genes was investigated by PCR and finally 20 selected strains from the two mentioned groups were subjected to RT-qPCR for virulence genes *pilA* and *csuD*. The expression of *csuD* gene in the test group was increased by %0.095, which was not significant. The expression of *pilA* gene in the test group increased by %1.45, which was not significant. Conclusion: it can be concluded that there is no significant relationship between the expression of *csuD* and *pilA* and the antibiotic resistance.

**Keywords:** key words: *Acinetobacter baumannii* - Antibiotic resistance



# Investigating the level of contamination of anesthesia machines and equipment in the operating room of Mazandaran University of Medical Sciences teaching hospitals in 2016

Bacteriology

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Mazandaran university of medical sciences

**BACKGROUND AND OBJECTIVES:** Hospital infections are one of the important causes of death and spending. Therefore, it is necessary to control infections by identifying the sources of contamination. For this purpose, according to the increasing statistics of surgeries and the risks of contamination caused by the use of anesthetic devices and devices, this study was conducted.

**MATERIALS AND METHODS:** This cross-sectional study was conducted in 2016 by sampling the anesthesia machines and anesthesia equipment available in the operating rooms of the teaching hospitals of Mazandaran University of Medical Sciences. Sampling was done after washing on Saturday morning. Sterilized samples before 48 hours of machines were excluded from the study. The disc diffusion brush antibiogram test was performed on the isolated microorganisms to check the resistance and sensitivity of the isolated samples. Analysis of information from statistical software Ver. SPSS 12 and statistical tests (mean, deviation and chi square) were used.

**RESULTS AND DISCUSSION:** Finally, a total of 297 samples were examined, of which 59 (19.86 %) had bacterial contamination, the most of which was related to *Pseudomonas aeruginosa* with 21 cases and the lowest amount was related to *Escherichia coli* with 8 cases. According to the results, the ratio of bacteria before and after washing in different levels of the hospital was homogenous and the same and there was no statistically significant difference ( $p = 0.173$ ). The highest antibiotic resistance was observed against clindamycin and the lowest resistance was observed against vancomycin. 3.07% of infections were related to *Penicillium* and *Aspergillus nira*.

**Keywords:** Hospital infection, anesthesia machines and equipment, microbial contamination

# Investigating the Link Between Biofilm Formation and Antibiotic Resistance in Clinical Isolates of *Acinetobacter baumannii*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Acinetobacter baumannii* (*A. baumannii*) has become a significant problem in hospitals worldwide during the last several decades. Biofilm formation is a virulence factor that may affect antibiotic resistance. Accordingly, our study seeks to elucidate the correlation between biofilm-forming ability and the distribution of biofilm-related genes and oxacillinase genes in clinical isolates of *A. baumannii*.



**MATERIALS AND METHODS:** This study included 53 *A. baumannii* isolates from Babol University of Medical Sciences hospitals in Iran. Kirby-Bauer disc diffusion was used to determine isolate antibacterial susceptibility. Biofilm formation was examined using crystal violet staining. Using particular primers, the polymerase chain reaction (PCR) detected oxacillinase (*bla*OXA-23, *bla*OXA-24, *bla*OXA-51, and *bla*OXA-58) and biofilm formation genes (*bap* and *bla*PER-1).

**RESULTS AND DISCUSSION:** Our study indicated that trimethoprim/sulfamethoxazole and ciprofloxacin had the most significant resistance rates (98.11%) and ampicillin/sulbactam the lowest (66.03%). All isolates formed biofilms. Biofilm development analysis, isolate classification: 67.92% were strong, 18.86% moderate, and 11.32% weak biofilm-producing isolates. The oxacillinase genes *bla*OXA-23, *bla*OXA-24, and *bla*OXA-51 frequencies were detected in 92.45%, 71.69%, and 100% of the isolates, respectively. None of the isolates had *bla*OXA-58. Biofilm-forming genes in isolates were *bap* (73.58%) and *bla*PER-1 (58.49%). Our study revealed a high prevalence of antibiotic-resistant strains among clinical isolates of *A. baumannii*. Oxacillinase genes did not have a significant correlation to biofilm formation. Biofilm production was significantly associated with the *bap* gene but not the *bla*PER-1 gene. Due to this tendency, *A. baumannii* biofilm formation processes must be understood. Effectively controlling pathogen infections requires targeted inhibition of this mechanism.

**Keywords:** *Acinetobacter baumannii*, Biofilm, Oxacillinases, Antimicrobial susceptibility

# Investigating the prevalence of toxoplasmosis in women of reproductive age in Mazandaran province and its possible relationship with vitamin D deficiency

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Toxoplasmosis which is caused by the protozoan parasite *Toxoplasma gondii*, affects pregnancies in women. Vitamin D deficiency increases susceptibility to infections and complications during pregnancy. This study investigated the prevalence of toxoplasmosis in women of reproductive age in Mazandaran province and explored the possible correlation between vitamin D deficiency and toxoplasmosis.

**MATERIALS AND METHODS:** A total of 320 serum samples of childbearing-age women in Mazandaran province (Sari, Babol, Chalus, Nur, Tonekabon, and Ramsar) were collected. Participants completed a questionnaire providing information including age, meat consumption, cat exposure, egg consumption, soil contact, and residential location. The sera were tested for IgG and IgM antibodies against *T. gondii* using ELISA, and 25-hydroxyvitamin D levels were measured using a commercial kit. The results were analyzed using descriptive statistics and the Chi-Square test, as well as calculating the odds ratio, utilizing Spss software version 21. The P value 0.05 was considered statistically significant for all tests.

**RESULTS AND DISCUSSION:** This study showed that 198 cases (61.88%) of the studied population had anti-toxoplasma IgG, while one case (0.31%) had anti-toxoplasma IgM. Among the women who had insufficient vitamin D, 159 cases had anti-toxoplasma IgG and one sample had anti-toxoplasma IgM. Also, in people who had sufficient vitamin D, 39 samples had anti-toxoplasma IgG, and no sample had anti-toxoplasma IgM. Statistical analysis showed that the prevalence of toxoplasmosis in people with insufficient vitamin D is 1.71 times higher than those with sufficient vitamin D (OR = 1.71) and there is a significant relationship between the prevalence of toxoplasmosis and vitamin D levels.

**Keywords:** Toxoplasmosis, Vitamin D, childbearing-age, women

# Investigation of abundance, isolation, identification, gene sequencing and antibiotic resistance in *Klebsiella* & *Acinetobacter* in blood, sputum, wound, permicate and urine samples of patients admitted to Shahid Beheshti Hospital, Babol

Bacteriology

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**BACKGROUND AND OBJECTIVES:** In addition to being a threat to people's lives, the occurrence of antibiotic resistance also imposes heavy treatment costs on the healthcare system. This study was conducted with the aim of investigating bacteria isolated from clinical samples of patients with hospital infection and also their antibiotic resistance pattern. In this cross-sectional study that was conducted in 1402, all patients with nosocomial infection of Shahid Beheshti Hospital in Babol were included in the study. For each of the samples, after isolating, identifying and confirming the strains by disc diffusion method according to CLSI standards, the antibiotic resistance was checked, then the data collected from the software were analyzed using chi square test and was analyzed 209 patients with nosocomial infection were identified during the study period. The most common microorganisms isolated were, *Klebsiella* & *Acinetobacter* the isolated organisms showed different resistance to different antibiotics.

**MATERIALS AND METHODS:** This cross-sectional study was conducted in Shahid Beheshti Hospital of Babol city from the beginning to the end of 1402 by head count method on 13850 patients hospitalized in different departments of Shahid Beheshti Hospital in Babol city. These samples are from blood, deep wound, urine, sputum, cerebrospinal fluid, urinary catheter, trachea, venous catheter & feces of people with hospital infection who are in the departments of were collected.

**RESULTS AND DISCUSSION:** In this study, the number of 13,850 patients hospitalized in different departments of Shahid Beheshti Hospital were examined, and a total of 209 people (1.5%) were diagnosed with hospital infection in 1402. Out of 209 examined patients, 75 cases (35.9%) were women and 134 cases (64.1%) were men. The average age of patients with hospital-acquired infection was 47 years. 35.9% of the studied subjects had urinary infections, 15.3% had blood infections, 13.9% had sputum infections, and 12.9% had wound infections. In the examination of receiving invasive agents, it was found that patients who have received these agents especially in the long term are much more sensitive to infection, patients with hospital infection in (1.07%) have venous catheters and in (0.4%) The cases had a urinary catheter

**Keywords:** hospital infection, infection control, antibiotic resistance

# Investigation of antibiotic resistance in patients of Imam Khomeini Hospital, Esfrum1402

Bacteriology

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**BACKGROUND AND OBJECTIVES:** It seems that the medical community around the world has been unable to solve the threatening problem of antibiotic resistance. It has been shown that one of the most important reasons for this is the misuse of antibiotics. Different hospitals in different departments of hospitals, especially, special care departments and the problems that arise in the treatment of patients with these diseases, need to know and know precisely about this type of disease and the pattern of antibiotic resistance. The results of studies conducted in Iran have shown that drug resistance in laboratory conditions and antibiogram tests is increasing. In this research, the antibiotic resistance of Pseudomonas bacteria isolated from patients hospitalized in different departments of Imam Khomeini Esfrum Hospital in 1402 has been investigated.

**MATERIALS AND METHODS:** After collecting information and coding, data analysis was done using SPSS-21 software. Descriptive statistics, in tabular form, and indicators of family and deviance were used to describe demographic characteristics and inferential statistics in the form of Pearson's correlation coefficient and regression tests, and also to determine the relationship between antibiotic resistance and demographic characteristics using T-test and analysis of variance. side was used, so that the significance level for all tests is P 0.05.

**RESULTS AND DISCUSSION:** 218 samples with positive culture were collected. Of these, 146 samples were from women (67%) and 72 samples (33%) were from men. 46.5% of E. coli samples were resistant to cefixime, 11.3% to gentamicin, 21.5% to cotrimoxazole and 23.8% to ceftriaxone. 28.5% and 71.4% of Pseudomonas samples were resistant to gentamicin and amoxicillin, respectively. In this study, Escherichia coli was the most common Gram-negative bacterium isolated from different departments of the hospital. Most of the positive culture clinical samples in this study were related to urine. According to the results of the present study, it was found that the most common infection was urinary tract infection and the most important cause of hospital infection was Escherichia coli. Therefore, it is expected that due to the importance of hospital infections, health officials and the infection control committee will use more comprehensive measures to control these types of infections.

**Keywords:** Hospital infections, bacterial resistance, antibiotics

# Investigation of antimicrobial effect of essential oil, nano-emulsion of essential oil and green synthesis of copper (Cu) nanoparticles produced from aqueous extract of *Salvia mirzayanii* Rech. f. & Esfand on *Staphylococcus aureus*, *Escherichia coli* and *Pse*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Due to the resistance of bacteria to conventional antibiotics and antimicrobial agents, many researches are being conducted to find types of effective antimicrobial agents. Copper has been increasingly used in the form of nanoparticles with high antimicrobial activity against all Gram-negative and Gram-positive bacteria. *Salvia mirzayanii* is one of the medicinal species of mint family and its antimicrobial effects have been proven. Since the desire to make herbal medicine has gradually increased, in this research, the effect of essential oil, nanoemulsion of essential oil and green synthesis of copper nanoparticles produced from the aqueous extract of *Salvia mirzayanii* along with the standard antibiotic gentamicin on *Staphylococcus aureus*, *Escherichia coli* bacteria and *Pseudomonas aeruginosa* was performed.

**MATERIALS AND METHODS:** The essential oil of *Salvia mirzayanii* was prepared in the medicinal plant laboratory, and then the *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, were cultured. Microdilution method was used to check ZOI, MIC and MBC of essential oil against these three bacteria. For the synthesis of essential oil nanoemulsion and copper nanoparticles, the aqueous extract of *Salvia mirzayanii* was used, and then the samples were sent to Dapetronic Company in Tehran to confirm the synthesis of copper nanoparticles and the data was analyzed by SPSS .

**RESULTS AND DISCUSSION:** Results: The results of essential oil analysis showed that the main compounds are phenolic monoterpenes. The essential oil prepared from sage had no inhibitory effect on *Pseudomonas aeruginosa*, but the average MIC, MBC and ZOI of sage essential oil against *Escherichia coli* were in the range of 500, 500, 11.4-9.4  $\mu\text{l/liter}$  and also against *Staphylococcus aureus* 250, 250-500 and 13.5-17.5  $\mu\text{l/liter}$  respectively. Also, in all bacteria, the lowest inhibitory effect was related to the essential oil and the highest was related to the gentamycin antibiotic. conclusion: The findings showed that the aqueous extract of *Salvia mirzayanii* has a high potential in the production of copper nanoparticles. Also, the essential oil, nanoemulsion of essential oil and copper nanoparticles biosynthesized from the extract of *Salvia mirzayanii* have antibacterial properties on the three studied bacteria and showed the greatest effect on the *Staphylococcus aureus*.

**Keywords:** copper nanoparticles, essential oil, essential oil nanoemulsion, *Salvia mirzayanii*, green

# Investigation of EpsA, OmpA, and Bap Genes among MDR and XDR *Acinetobacter baumannii* Isolates in Khorramabad, Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Acinetobacter baumannii* is an opportunistic hospital pathogen with high antibiotic resistance, and the ability to produce biofilm. This study aimed to investigate *epsA*, *ompA*, and *bap* genes involved in biofilm formation in MDR and XDR clinical isolates of *Acinetobacter baumannii* in Khorramabad, Iran.

**MATERIALS AND METHODS:** In this study, 79 *A. baumannii* isolates were collected from various samples of the patients admitted to tertiary hospitals in Khorramabad city, Iran, between January and August 2019. After performing the semi-quantitative evaluation of biofilm production by microtiter plate assay, screening of isolates carrying *epsA*, *ompA*, and *bap* genes was done by PCR method. Finally, statistical analyses were conducted using SPSS 22.

**RESULTS AND DISCUSSION:** Among 79 *A. baumannii* isolates, 52% XDR, 40% MDR, and 16% non-XDR-MDR isolates were found to be biofilm producers. All XDR and 94% MDR isolates had *ompA* and *epsA* genes, and *bap* genes were detected among 80% of these isolates. Moreover, the presence of biofilm-related genes and biofilm production among non-XDR-MDR isolates were less than among resistant isolates ( $p \leq 0.01$ ). Based on the results, biofilm production and simultaneous presence of *epsA*, *ompA*, and *bap* genes among MDR, and XDR *A. baumannii* isolates have been found to be significantly more than non-XDR-MDR isolates.

**Keywords:** Biofilm, multi-drug resistance, extensively drug-resistant, *Acinetobacter baumannii*, antibiotic resistance, clinical

# Investigation of Genetic Diversity of *Coxiella burnetii* in Iran using Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA)

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Coxiella burnetii* is the etiological agent of Q fever, a zoonotic infection that is recognized in various regions of Iran. Despite its significance, few studies have focused on determining the genotypes of *C. burnetii* within the country. This study aimed to assess the genetic diversity of *C. burnetii* in Iran using the Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA) method.

**MATERIALS AND METHODS:** A total of 26 samples were selected from a repository of 119 specimens collected from diverse locations across Iran. These samples were previously identified as *C. burnetii* using nested PCR and TaqMan real-time PCR techniques. The MLVA-15 assay was employed for genotyping *C. burnetii*.

**RESULTS AND DISCUSSION:** Genotyping of 26 samples revealed 22 distinct *C. burnetii* genotypes. All but two loci (ms23 and ms26) were informative for genotyping. Cluster analysis of MLVA data revealed 12 clonal complexes and clusters, along with 32 singleton strains. Iranian strains were categorized into three clusters and 21 individual genotypes, with clusters 3 and 6 comprising exclusively Iranian strains. The genotypic characteristics of *C. burnetii* observed in Iran differ significantly from those found in other global regions, suggesting the unique local adaptations of this microorganism. To enhance our understanding of the epidemiology of Q fever in Iran, further large-scale studies are required to evaluate the distribution of *C. burnetii* genotypes across various geographic areas, host species, and environmental reservoirs.

**Keywords:** keywords: *Coxiella burnetii*, genotyping, MLVA, Q fever



# Investigation of Risk Factors, Antibiotic Resistance, Virulence Genes, and Genetic Relationships in *Staphylococcus aureus* Strains Isolated from Children with Staphylococcal Scalded Skin Syndrome (SSSS) Between 2018 and 2023.

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Staphylococcal Scalded Skin Syndrome (SSSS) is characterized by widespread epidermal necrosis and/or superficial skin blisters following infection by certain toxin-producing strains of *Staphylococcus aureus*. The primary stages of SSSS development include colonization, exfoliative toxin production, and desquamation. This study aimed to investigate the risk factors, antibiotic resistance, virulence genes, and genetic relationships of *Staphylococcus aureus* strains isolated from children with SSSS between 2018 and 2023.

**MATERIALS AND METHODS:** In this descriptive-cross-sectional study, 70 *Staphylococcus aureus* strains were collected from children with SSSS. Antibiotic susceptibility testing was performed using the agar diffusion method. After genomic DNA extraction, PCR was used to evaluate the presence of the genes *sdrC*, *PVL*, *etB*, *etA*, *fnbA*, *bbp*, *sdrE*, and *fnbB*, which encode fibronectin binding proteins A and B.

**RESULTS AND DISCUSSION:** The prevalence of SSSS was higher in children under two years of age. Additionally, 82.9% strains were resistant to ciprofloxacin. The frequency of the *fnbA*, *etA*, and *pvl* genes was significantly higher compared to other genes. Molecular typing and RAPD analysis revealed nine molecular types, with type A being the most prevalent with 18 members. Cluster I in the RAPD analysis had only two members. This study highlights the significant genetic diversity and concerning antibiotic resistance patterns in *S. aureus* strains causing SSSS in children. The high prevalence of *pvl*-positive MRSA suggests a need for tailored treatment approaches and careful monitoring of disease severity. Future research should include larger and more geographically diverse populations and employ advanced molecular technique for better epidemiological tracking. Furthermore, the observed antibiotic resistance patterns highlight the need for ongoing surveillance and antibiotic stewardship programs to mitigate the spread of resistant *S. aureus* strains.

**Keywords:** Staphylococcal Scalded Skin Syndrome (SSSS), Pediatric Infections, Molecular Typing

# Investigation of the Frequency and Antibiotic Resistance Patterns of Bacteria Isolated from Clinical Specimens at Haft-Tir Hospital in Doroud, Lorestan

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Bacteria are found everywhere and can be transmitted to humans through air, water, food, or living carriers. These infections are a significant health threat, worsened by increasing antibiotic resistance. Urinary tract infections are the most common hospital-acquired infections, while pneumonia is the deadliest. *Escherichia coli* and *Staphylococcus aureus* are the primary pathogens. Accurate diagnosis is crucial to prevent unnecessary antibiotic use and ensure proper treatment.

**MATERIALS AND METHODS:** In this descriptive cross-sectional study, 200 positive isolates were collected and analyzed from Haft-Tir Hospital in Doroud, Lorestan. Initially, the isolates were cultured and identified phenotypically. Subsequently, the antibiotic resistance pattern for each isolate was assessed using the disk diffusion method. Additionally, the prevalence of isolates producing ESBL, inducible clindamycin resistance, methicillin resistance (MRSA), and carbapenemase production was investigated.

**RESULTS AND DISCUSSION:** In this study, 56% of samples were from females and 44% from males. The most common sample type was urine (72.5%), followed by blood (10%), with most samples from the internal medicine department (33.5%). *Escherichia coli* was the most frequently isolated bacterium (55.5%), followed by *Citrobacter* (15%), *Klebsiella* (10%), *Pseudomonas* (5.5%), *Staphylococcus* (4.5%), *Acinetobacter* (3.5%), *Shigella* (2.5%), *Proteus*, and *Providencia* (1% each). All six *Staphylococcus aureus* isolates were MRSA, and 22.22% of *Staphylococcus* isolates showed inducible clindamycin resistance. Among the 180 Gram-negative isolates, 48.88% were ESBL positive, and none tested positive for carbapenemase. In this study, the highest antibiotic resistance rates were found for amoxicillin (99.42%), followed by ampicillin-sulbactam (93.82%) and trimethoprim-sulfamethoxazole (75.53%). The lowest resistance rate was observed for imipenem (0.52%). Bacterial infections are relatively common in hospitals. Identifying the causes and risk factors for these infections and limiting the prescription of

**Keywords:** Bacterial infections, Antimicrobial resistance, ESBL, Methicillin resistance *Staphylococcus aureus*

# Investigation of the prevalence of *Escherichia coli* O157 H7 in clinical and food samples: A cross-sectional study

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Escherichia coli* O157:H7 is a strain of the bacterium *E. coli* that can cause severe foodborne disease in humans. It is one of the most common and virulent strains of *E. coli* and is known for producing a toxin called Shiga toxin, which can cause damage to the lining of the intestine and lead to symptoms such as bloody diarrhea, abdominal pain, and sometimes kidney failure. Food poisoning is a significant public health concern worldwide. Extensive research in this area has highlighted the considerable role of *E. coli* bacteria in the spoilage of various food types

**MATERIALS AND METHODS:** In this cross-sectional study, a total of 62 food sample containing: raw chicken meat, milk and Beef samples were randomly collected by using an electronic random number generator from different butchers and supermarkets of different areas of Kermanshah city, west of Iran. Also, a total of 62 *E. coli* strains were collected from different clinical specimens including blood, urine, wound, and CSF from Imam Reza Hospital in Kermanshah, Iran

**RESULTS AND DISCUSSION:** A total of 62 clinical samples and 62 food samples were procured from Imam Reza Hospital and food sales centers in Kermanshah, respectively, and were subjected to examination and analysis for the presence of *E. coli* O157:H7 contamination. Diagnostic culture was performed on 2 samples from each of the clinical samples and 8 samples from the food samples, which were subsequently subjected to a precise and sensitive PCR detection method to identify the *E. coli* O157 H7 strain. All 10 strains were found to possess the genome of these strains. The prevalence of *E. coli* O157: H7 contamination in food, particularly beef, has been found to be 12.90%. This finding suggests that meat serves as a significant and primary reservoir for this pathogen, which can potentially be transmitted to humans through the consumption of beef and its byproducts.

**Keywords:** *Escherichia coli* O157: H7, clinical, food samples, PCR

# Investigation of the synergistic effects of alfalfa aqueous extract and probiotic kombucha on several gut microbiota flora in the gut microbiota of diabetic Wistar rats.

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Intestinal flora refers to a community of microorganisms (including bacteria, viruses, archaea, and fungi) inhabiting the digestive tracts of humans, animals, and insects. Probiotics enhance the growth of beneficial bacteria. The objective of this study is molecular separation and identification of gut microbial flora before and after treatment with kombucha and alfalfa aqueous extract in diabetic Wistar rats.

**MATERIALS AND METHODS:** This experimental study included 72 male Wistar rats with diabetes treated with streptozotocin (STZ) at a dose of 0.5 cc per gram of body weight. These rats were randomly divided into 8 groups of four: Group (A) received a normal diet and physiological serum, Group (B) consumed kombucha, Group (C) received alfalfa aqueous extract, Group (D) received the mixture of both alfalfa aqueous extract and kombucha, Group (E) received metformin tablets, Group (F) received garlic tablets, Group (G) received pickled garlic, and Group (H) served as the diabetic control group receiving regular water and food. The gastrointestinal microbiota of rats were cultured and examined before and 30 days after treatment in all groups. The bacterial microbiota of the rats' intestines were identified. The molecular method of colony-PCR was employed to identify bacterial isolates.

**RESULTS AND DISCUSSION:** The results of culturing the gastrointestinal microbiota of rats before treatment included the following bacterial strains: *Enterococcus faecalis*. YY88, *Enterococcus faecalis* B2, *Bacillus anthracis* NLAE-2L G340, *Escherichia coli* CW17, *Escherichia coli* 64EVA, *Bacillus* sp. Jilu WO145, *Klebsiella aerogenes* CEMTC\_3530. The gastrointestinal microbiota of rats after treatment included the bacteria of *Klebsiella aerogenes* LU2, *Escherichia fergusonii* EGI32, *Escherichia coli* FWSEC0008, *Escherichia coli* 19SZHZ803Rt, *Escherichia coli* 11,17 chromosome, *Escherichia coli* CVM N18EC0432, *Escherichia coli* R3050, *Escherichia coli* CCFM8336, *Escherichia coli* O91:H21, *Lactobacillus ramosus kombu* NKJ6, *Lactobacillus acidophilus kombu* NKJ7, *Lactobacillus acidophilus kombu* NKJ8. Two pathogenic bacteria, *Enterococcus faecalis*, and *Bacillus anthracis*, were observed in the gastrointestinal microbiota of rats before treatment. After treatment with alfalfa aqueous extract and kombucha probiotic drinks, these two pathogenic bacterial genera and species were eliminated. In addition, the number of *Escherichia coli* bacterial strains increased, and three genera and species, *Lactobacillus ramosus* and *Lactobacillus acidophilus*, were observed.

**Keywords:** Type 2 diabetes, Kombucha, alfalfa, Gut microbiota, Colony-PCR

# Investigation on Ciprofloxacin Resistant *Pseudomonas aeruginosa* Clinical Isolates and Detection *phzS* Virulence Gene

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* is an increasingly problematic drug-resistant bacterium in world. In fact, we are now faced with growing clones of pandrug-resistant *P. aeruginosa* in hospital settings. The goal of the present study was to determine the Ciprofloxacin resistance isolates and presence of *phzS* virulence gene in clinical *P. aeruginosa* isolates.

**MATERIALS AND METHODS:** In this cross sectional study, strains recovered consecutively from different samples of hospitalized patients between 2021 and 2023 in Yasuj and Shiraz, Iran, were tested. The isolates were recognized as *P. aeruginosa*, based on morphological and biochemical tests. The isolates, identified as presumptive *P. aeruginosa*, were further confirmed by PCR to detect *exoA* gene. All the isolates were tested for their antimicrobial susceptibility patterns by using the CLSI standard guidelines. The *phzS* virulence gene was investigated by PCR using specific primers.

**RESULTS AND DISCUSSION:** Overall, 86 *P. aeruginosa* isolates were studied during the study period. The isolates were recovered from 32 (36.7%) females and 55 (63.3%) males. Based on the findings, 57% of the *P. aeruginosa* strains were resistant to the ciprofloxacin. Among 86 *P. aeruginosa* isolates on which PCR assay was performed, 91.9% had the *phzS* virulence gene. Conclusions: Simultaneous determination of antibiotic susceptibility profiles and virulence determinants is a contemporary approach for the examination of microbiological aspects of infections caused by *P. aeruginosa*. The guidelines for each bacterium include antibiotics of confirmed effectiveness, which show acceptable results in antibiotic susceptibility tests.

**Keywords:** *Pseudomonas aeruginosa*, Virulence gene, Antibiotic resistance.

# Genes in strong, medium and weak biofilm patterns in *Pseudomonas aeruginosa* strains isolated from patients at Kowsar Sanandaj Medical Center by Real Time PCR method -2023

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Rhamnolipid are extracellular secondary metabolites with surface-active properties mainly produced by *Pseudomonas aeruginosa*. In *Pseudomonas aeruginosa* synthesis and regulation have been extensively studied since they play a role as a virulence factor and that are also involved in biofilm formation and swarming motility. Their ability to reduce surface tension is a major characteristic of surfactants which is the key ingredient used in detergents, shampoo, toothpastes and etc. In this study, we examined this relationship from another point of view which will be discussed further.

**MATERIALS AND METHODS:** In this work 68 clinical isolates of *Pseudomonas aeruginosa* were collected and classified into weak, moderate and strong biofilm via the microplate method. The presence of rhamnolipid gene was checked in all samples by PCR test. Three random samples with strong, medium and weak biofilm strength *Pseudomonas aeruginosa* ATCC27853 as a strong biofilm control were selected. The samples were cultured for 24, 72 and 96 hours at 37°C of incubation, they were examined in terms of movement and velocity. Using detection method oil spreading technique and emulsifying activity that can show the presence of rhamnolipid in culture of *Pseudomonas aeruginosa*. The expression of rhamnolipid genes was investigated by Real time PCR

**RESULTS AND DISCUSSION:** Three random samples were selected in terms of strong, weak and moderate biofilm formation strength and confirmed by microplate and microtube method. Frequency of rhlC among strong, moderate and weak biofilm 81%, 92% and 80.4% respectively. The colony pattern and bacterial movement were different based on bacterial strain and carbon source. The expression of rhlA, rhlB and rhlC genes among *Pseudomonas aeruginosa* strains with strong, moderate and weak biofilm showed significant differences ( $p=0.00$ ). The result of the 24 hour emulsion was observed in a biphasic manner. The highest rate of oil spreading related to the standard strain with strong biofilm. The obtained results show that *Pseudomonas aeruginosa* is the producer of rhamnolipid. Based on time and concentration rhamnolipid can be related to the formation and strength of biofilm and the velocity of bacterial movement. In addition, rhamnolipid has the ability to participate in oil spreading test and emulsifying activity.

**Keywords:** *P. aeruginosa*, Biofilm, Rhamnolipid, RT-PCR, velocity, Emulsification, Oil Displacement

# Isolation and identification of actinomycetes with more antimicrobial properties from agricultural soils in the southwestern regions of Guilan province

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Actinomycetes are the largest source of natural antibiotics in the world. For this reason, they are considered the golden microorganisms of the 21st century. The purpose of this research is the isolation and molecular identification of actinomycetes with antimicrobial effect from agricultural soils in the native areas of Guilan province.

**MATERIALS AND METHODS:** Soil samples were collected from the southwestern agricultural areas of Guilan province. Serial dilution was used to isolate actinomycetes. Then the morphological, physiological, and biochemical identification of the samples was done and finally, the molecular identification of the isolates was done using 16SrRNA sequencing and phylogenetic analysis. Antimicrobial activity was investigated against pathogenic microorganisms.

**RESULTS AND DISCUSSION:** A total of 14 isolates were identified. 2 isolates with more antimicrobial properties were selected. According to the results of phylogenetic studies and 16SrRNA sequencing, *Amycolatopsis roodepoortensis* strain EA7 with 99.63% confidence, and *Streptomyces microflaveus* strain EA6 with 93.92% confidence were identified. The isolated bacteria had more antimicrobial effect against pathogenic microorganisms were gram-positive, which can be attributed to the different structure of the outer membrane in gram-negative bacteria that have lipopolysaccharide compounds. This research is the first report on the identification of actinomycetes with antimicrobial properties in the agricultural soils of the southwestern regions of Guilan province located in the Alborz mountains. The identification of the rare strain of *Amycolatopsis roodepoortensis* strain EA7 from the northern regions of Iran makes the soils of these regions very valuable.

**Keywords:** Actinomycete, antimicrobial activity, 16SrRNA, *Amycolatopsis*, *Streptomyces*

# Isolation and investigation of drug resistance pattern and genotyping of carbapenem-resistant *Acinetobacter baumannii* isolated from Valiasr (Aj) and Mousavi hospitals in Zanzan by ERIC-PCR and Box-PCR

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Acinetobacter* is a type of microorganism that is usually found in the environment and can cause various infections that are obtained from health care centers. Due to antibiotic resistance and the ability to survive for long periods in wet or dry environments, it has become difficult to treat. This bacteria is very resistant to antibiotics and makes it very difficult to treat infections. Therefore, the aim of this study was to isolate and investigate the drug resistance pattern and genotyping of carbapenem-resistant *Acinetobacter baumannii* isolated from Valiasr (Aj) and Mousavi hospitals in Zanzan city by ERIC-PCR and Box-PCR.

**MATERIALS AND METHODS:** In this cross-sectional study, 120 clinical isolates of *Acinetobacter bomani* isolated from Valiasr and Mousavi hospitals in Zanzan city were used. Various biochemical tests were used to identify the genus *Acinetobacter*. Bacteria were cultured in McConkey agar medium for one night. Gram staining was done to observe negative coccobacilli. Investigation of oxidase (negative), endole (negative) and immobility in SIM medium, absence of fermentation of sugars in TSI medium and growth at 42°C temperature and melting of gelatin were done. After conducting biochemical tests and confirming the genus of *Acinetobacter*, in order to identify the species of *Acinetobacter baumannii*, blaOXA-51 gene was investigated by PCR method.

**RESULTS AND DISCUSSION:** 97.5% and 80.8% of the samples were sensitive to the antibiotics polymyxin B and cholestin, respectively, and the lowest sensitivity was related to the antibiotics ceftazidime and cefotaxime, with frequencies of 4.2% and 5.8%, respectively. 2.5% and 19.2% of the samples were resistant to polymyxin B and cholestin antibiotics, respectively, and the highest resistance was related to ceftazidime and cefotaxime antibiotics with frequencies of 95.8% and 94.2%, respectively. The sensitivity and resistance of antibiotics were not significantly different according to gender. Antibiotics imipenem (P=0.004), amikacin (P=0.019), cefepime (P=0.007), cefotaxime (P=0.003) and trimethoprim/sulfamethoxazole (P=0.001) on PUS samples were significantly more sensitive than other samples, while cholestin antibiotic in PUS samples was significantly less sensitive than other samples (P=0.023). Myxin B in Spotum samples was significantly more sensitive than other samples (P=0.036).

**Keywords:** *Acinetobacter bomani*, carbapenem, ERIC-PCR, BOX-PCR, Zanzan



# Isolation and molecular identification of *Listeria* species from minced meat and investigating the antagonistic effect of probiotics on *Listeria*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Introduction: This bacterium is an obligate intracellular pathogen, and the high-risk group is usually the elderly, children, infants, pregnant women, and people with immune system disorders. Due to the indiscriminate use of antibiotics and the resistance of this bacterium to antibiotics, it is particularly important to find new antimicrobial substances, that is why the use of probiotics as an alternative to antibiotics is important. The purpose of this study is to isolation and identify molecular species of *Listeria*. of minced meat and investigating the antagonistic effect of probiotics on *Listeria*.

**MATERIALS AND METHODS:** materials and methods: During a two-month period, minced meat samples were collected from 6 cities of Mazandaran province and were subjected to biochemical identification and PCR testing, and the inhibitory and antimicrobial activity of two probiotic bacteria isolated from turtle feces, including *Lactococcus lactis* and *Pediococcus acidi lactis* It was checked against *Listeria*, then the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined through macrodilution.

**RESULTS AND DISCUSSION:** Results and discussion: Out of 40 samples under investigation, 8 samples (20%) were positive for *Listeria ivanovi*, but *Listeria monocytogenes* and *Listeria* genus were not isolated. Also, both probiotics inhibited *Listeria* at a concentration of 25 µg/ml. The results showed that these two probiotic isolates have many inhibitory effects and antimicrobial properties on *Listeria ivanovi*, and the inhibitory effect of both isolates is almost the same and they can be a good substitute for antibiotics. to be In line with our study in 2012, Sung-Mee Lim et al showed that *L. brevis* MLK27 has the ability to settle in the human gastrointestinal tract and is used to prevent *L. monocytogenes* pathogen infections, which is consistent with our study

**Keywords:** *Listeria monocytogenes*, *Listeria ivanovii*, Polymerase chain reaction

# Isolation of *Staphylococcus aureus* strains from patients with pulmonary infections hospitalized in ICUs of Kerman and evaluation the occurrence of *mecA*, *pvl*, *LukS / F-PV* Genes

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus* is one of the most common nosocomial infections. Today there are reports that the prevalence of methicillin resistance in *S.aureus* is increasing in different parts of the world. The aim of this study was to isolate *Staphylococcus aureus* strains from patients admitted to the intensive care units.

**MATERIALS AND METHODS:** This cross-sectional descriptive study was performed on 60 lung secretion samples of Patients hospitalized in ICUs in Kerman, Iran. Accordingly, single cell colony was performed to obtain purified isolates. Biochemical tests were also used for identification and confirmation of isolated bacteria specially to find out the genospecies *Staphylococcus aureus*. *mecA* and *LukS / F-PV* genes were also evaluated by multiplex PCR.

**RESULTS AND DISCUSSION:** Among 60 lung secretion samples, 20 strains of *Staphylococcus aureus* were identified and confirmed accurately by biochemical tests. The frequency of *LukS / F-PV* gene was reported in 12 isolates and the frequency of *mecA* gene was reported in 8 ones as well as 4 isolates showed to have both above mentioned genes. Conclusion: most strains contained *mecA*. The high prevalence of MRSA isolates with community-acquired genotype led to testing current hospital hygiene practices to control the spread of nosocomial MRSA isolates. Considering the ability of PVL and *LukS/F-PV*-producing MRSA isolates to cause life-threatening diseases, and prevalence of pulmonary MRSA isolates, rapid diagnosis, direct, simple and cost-effective tests can help reduce MRSA infections. Also, in order to limit the spread of MRSA, maximum aseptic practices for health care professionals and better training in hygiene practices are needed

**Keywords:** : *Staphylococcus aureus*, MRSA, *mecA*, *LukS / F-PV*.

# Lophomoniasis Registry Data analysis in Mazandaran province: novel aspects and approaches

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Lophomonas is a protozoan parasite that inhabits the guts of insects such as termites, mites, and cockroaches. This emerging parasitic pathogen can infect humans through the ingestion of the parasite's cyst, which is passed in the feces of these arthropods. Clinical symptoms of Lophomoniasis include chronic cough with sputum production, dyspnea, fever, and hemoptysis. With the establishment of the Iranian National Registry Center for Lophomoniasis (INRCL) in Mazandaran Province, northern Iran, the present study aims to analyze data from registered patients.

**MATERIALS AND METHODS:** This descriptive-analytical retrospective study analyzed data available in the INRCL at Imam Khomeini Hospital in Mazandaran from 2018 to 2022. All patients with respiratory disorders who were referred to pulmonary department and the provincial Health Center to rule out Lophomonas infection were enrolled in the study. Various clinical samples, including bronchoalveolar lavage (BAL), sputum, and nasal discharge, were submitted to the INRCL laboratory. The data were extracted from the data sheets and analyzed using SPSS statistical software, employing descriptive statistics, chi-square tests, and logistic regression tests.

**RESULTS AND DISCUSSION:** In this study, data from 495 patients were analyzed, with the majority of samples being BAL (440 cases). Among these patients, 141 (28.5%) tested positive for Lophomonas, with an average age of 58.94 years. The highest prevalence of lophomoniasis was found in the 60 to 70 age group ( $P = 0.01$ ), which was statistically significant. Interestingly, smoking was found to have a significant inhibitory effect on contracting the parasite ( $P = 0.03$ ). The most common symptom reported was cough ( $P = 0.0234$ ), most patients were classified as having mild to moderate infection severity ( $P = 0.041$ ). The present study indicates that the prevalence of lophomoniasis in Mazandaran Province is relatively high, especially among the elderly population with underlying conditions, as well as being more common in urban areas. To address this issue, urgent educational workshops are needed to train physicians and laboratory technicians on the proper diagnosis of this disease.

**Keywords:** Lophomonas, Respiratory symptoms, Registry plan, BAL

# Microbial Interactions with Microplastics in Drinking Water: Occurrence, Characteristics, and Treatment Efficiency

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background: Microplastics (MPs) have emerged as a significant environmental concern, particularly in drinking water sources, due to long-term health effects associated with microbial interactions and consumption. This study aims to assess the occurrence and removal efficiency of MPs in two drinking water treatment facilities (DWTPs) located in northern Iran, highlighting the implications for water quality and public health.

**MATERIALS AND METHODS:** Materials and Methods: The research was conducted at DWTP1 in Sari and DWTP2 in Gorgan, both utilizing surface water. A comprehensive sampling strategy was implemented, collecting raw and treated water samples over two months. MP extraction involved filtration and density separation, adhering to established protocols. The physical and chemical characteristics of MPs were analyzed using optical microscopy, scanning electron microscopy (SEM), and Raman spectroscopy.

**RESULTS AND DISCUSSION:** MPs were detected in both raw and treated water, with average concentrations of  $0.44 \pm 0.19$  MPs L<sup>-1</sup> and  $0.11 \pm 0.06$  MPs L<sup>-1</sup> for DWTP1, and  $0.19 \pm 0.06$  MPs L<sup>-1</sup> and  $0.07 \pm 0.02$  MPs L<sup>-1</sup> for DWTP2, respectively. The removal efficiencies were found to be 75% for DWTP1 and 63.16% for DWTP2. Coagulation processes could inadvertently introduce additional MPs into treated water. The predominant MP morphology was fibers and black color, with common polymers including polyethylene terephthalate (PET), polyamides (PA), and polyvinyl chloride (PVC). The findings highlight the effectiveness of sedimentation and filtration in reducing MP concentrations in drinking water. Importantly, the interaction between MPs and microbes can enhance microbial colonization on plastic surfaces, affecting microbial community dynamics and water quality. This study emphasizes the critical role of DWTPs in mitigating MP contamination and stresses the need for research on health implications and microbial interactions.

**Keywords:** Microbial Ecology, Microplastic (MP), Drinking Water Quality, Water Treatment

# Molecular characterization and Antifungal susceptibility pattern of *Candida* Sp. Isolated from Acne Vulgaris

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background: Acne is a pathological disorder showing persistent inflammation in the Sebaceous follicles. It is one of the most prevalent dermatology harms that millions of people suffer from throughout the world. This study aims to identify *Candida* species from patients with acne and determine their drug susceptibility.

**MATERIALS AND METHODS:** Materials/methods: A total number of 70 cutaneous samples from acne vulgaris patients suspected to *Candida* infections were collected. Macroscopic and microscopic morphology followed by PCR-Sequencing of ITS Regions, using universal primers. In vitro antifungal susceptibility was performed using the clinical laboratory (CLSI) method.

**RESULTS AND DISCUSSION:** Results: Overall, 11 *Candida* species including *C. parapsilosis* 8 (72.73%), *C. krusei* 1 (12.5%), *C. lusitanae* 1 (12.5%), *C. kefyr* 1 (12.5%), and a *Trichosporon asahi* out of the collected clinical isolates were identified and isolated. *C. parapsilosis* isolates susceptibility to diverse concentrations of the anti-fungal agents to isolate Cp1 study indicates that the isolated Cp8 and Cp5 with MIC 50 equal to 32, 0.5, 0.25 and MIC90 of 64, 1, 0.5 µg/ml Fluconazole, Itraconazole and Ketoconazole were resistant respectively. Some of the isolates having relative strength, almost all other species of *C. parapsilosis* isolates were susceptible to these drugs.

Conclusions: *C. parapsilosis* was the most prevalent *Candida* species in acne vulgaris samples which had higher in vitro Susceptibility for antifungals. These results may help clinicians to implement this information in their routine diagnosis.

**Keywords:** and Antifungal susceptibility, PCR, *Candida* spp, Acne Vulgaris

# Molecular Characterization of Antibiotic Resistance Genes in *Klebsiella pneumoniae* Isolates from Hospitalized Patients in Northern Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Klebsiella pneumoniae*, a Gram-negative opportunistic pathogen from the Enterobacteriaceae family, is responsible for up to 10% of nosocomial infections. Increasing antibiotic resistance in *K. pneumoniae* is closely linked to these virulence factors, posing a significant challenge in clinical settings. Currently, *K. pneumoniae* exhibits notable resistance to various antimicrobial agents, including beta-lactams, fluoroquinolones, and aminoglycosides. The aim of this study was to investigate the antibiotic susceptibility patterns and frequency of some antibiotic resistance genes among *K. pneumoniae* strains isolated from hospitalized patients in Mazandaran province.

**MATERIALS AND METHODS:** Totally, 100 non-duplicated *K. pneumoniae* isolates recovered from various clinical samples over one year. The isolates were confirmed using conventional biochemical and microbiological tests and the analytical profile index (API) 20E kit. Antibiotic susceptibility was determined using disk diffusion for 18 antimicrobial agents, and genomic DNA was extracted for molecular analysis. Primers were designed to detect integrase genes and resistance elements using PCR techniques.

**RESULTS AND DISCUSSION:** Results: Among the 100 isolates, 64% were from urine, 15% from tissue, 10% from blood, 7% from wound, and 4% from sputum. The isolates were obtained from patients aged 5-90 years. Antibiotic resistance patterns showed the highest resistance to ampicillin/sulbactam (93%) and the lowest to amikacin (8%). No non-MDR strains were resistant to amikacin. Among the MDR strains, the frequency of resistance genes was significantly higher ( $p < 0.05$ ). Class I integrons were the predominant resistance-transmitting elements, found in 91.4% of MDR isolates and 11.9% of non-MDR isolates. None of the strains examined contained Class II or Class III integrons. The prevalence of resistance genes among MDR strains was blaSHV (91.4%), blaTEM (82.7%), blaCTX-M-15 (79.3%), blaKPC (29.3%), blaOXA-48 (36.2%), and blaNDM (6.9%). Coexistence of blaSHV/blaTEM, blaTEM/blaCTX-M-15, blaSHV/blaCTX-M-15, blaCTX-M-15/blaOXA-48, blaSHV/blaOXA-48, blaTEM/blaOXA-48, and blaSHV/blaKPC was observed in several isolates. No PDR strains were detected. Conclusion: The high prevalence of MDR *K. pneumoniae*

**Keywords:** *Klebsiella pneumoniae*, Antimicrobial resistance, Nosocomial infections, MDR, Integrons

# Molecular Detection of Human Bocavirus in Children with Acute Respiratory Infection In Kashan

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background and Aim: Human bocavirus (HBoV) is a virus in the genus Bocaparvovirus of the Parvoviridae family that is known to infect humans. HBoV is commonly found in respiratory samples from healthy individuals. In patients with respiratory symptoms, it can be detected alone or more frequently, in combination with other viruses that cause respiratory issues. Newborns are likely protected by passive immunization. The age group most frequently affected appears to be children between six months and two years of age.

**MATERIALS AND METHODS:** Methods: A total of 46 respiratory samples from hospitalized children with acute respiratory infections were collected and tested for the presence of HBoV using PCR.

**RESULTS AND DISCUSSION:** Results: Out of the 46 respiratory samples, 4(8.7%) tested positive for HBoV. The highest number of cases occurred in the winter season (P 0.05). Conclusion: This study showed a low prevalence of HBoV in clinical samples from children with acute respiratory infections.

**Keywords:** Keywords: Human bocavirus (HBoV), acute respiratory tract infection, prevalence

# Molecular Detection of Human Group A Rotaviruses in Sewage Treatment Systems in Isfahan

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background and Aim: The routine evaluation of water environments is essential to manage enteric virus-mediated fecal contamination and the potential emergence of new variants. Human group A rotaviruses are the leading cause of acute gastroenteritis in infants and children under 5 years of age worldwide. The aim of this study was to molecularly detect human group A rotaviruses in sewage treatment systems in Isfahan.

**MATERIALS AND METHODS:** Methods: This study involved 32 samples collected from 5 sewage systems in Shiraz. All samples were concentrated using the pellet method. Human group A rotaviruses were then identified using the RT-PCR method.

**RESULTS AND DISCUSSION:** Results: In total, rotaviruses were identified in 1 sample (3.125%). There was no statistically significant difference between the presence of rotavirus distribution and monthly distribution (P0.05). Conclusion: Our research on the environmental dispersion of rotaviruses in sewage and river samples indicates the presence of this virus in these water sources. Since pathogens in water remain a major cause of severe illness and mortality, it is crucial to continuously monitor sewage treatment systems. Therefore finding new methods of sewage treatment to eliminate enteric viruses such as rotavirus is imperative.

**Keywords:** Keywords: Human group A rotaviruses, Acute gastroenteritis, sewage, RT-PCR



# Molecular detection of *Moraxella catarrhalis* using amplification of the important target outer membrane protein gene

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Moraxella catarrhalis*, a pathogen in the human respiratory system, requires rapid and precise identification for proper clinical treatment. A lack of advanced diagnostic methods has made it difficult to easily identify *M. catarrhalis*. This research focuses on the accurate detection of *M. catarrhalis* through a molecular approach that uses nucleic acid amplification targeting a gene responsible for an important outer membrane protein, ompCD.

**MATERIALS AND METHODS:** In this molecular technique, the sequence of the target gene available in the GenBank database was analyzed. A novel protocol was developed to amplify a reliable marker gene for identifying *M. catarrhalis*. This identification method involved the extraction of bacterial genomic DNA by the boiling method followed by amplification of the target gene using specifically designed oligonucleotides. The specificity of this method was investigated using 21 samples including *M. catarrhalis*. Afterward, the products were analyzed using gel electrophoresis to confirm the presence of the target gene.

**RESULTS AND DISCUSSION:** The concentration of 21 extracted bacterial DNA samples including *M. catarrhalis* was between 20 and 50 ng/ $\mu$ l. Among the samples were *M. osloensis* in the same genus as *M. catarrhalis* and other types of gram-positive and negative bacteria with similar or different characteristics to *M. catarrhalis*. Only our target bacteria were amplified with specific oligonucleotides MoF1 and MoR1. The gel electrophoresis results revealed a band in the range of 200-300 nucleotides. The absence of the band formation in other species in this range indicated that this method was very effective for specific molecular detection of *M. catarrhalis*. The findings of this research contribute to creating innovative diagnostic tests that facilitate quick and accurate clinical prescription.

**Keywords:** *Moraxella catarrhalis*; ompCD gene; Molecular Detection; nucleic acid amplification; Respiratory

# Molecular epidemiology of *Mycobacterium tuberculosis* strains isolated from patients admitted to Kosar hospital during 2017-2023 using MIRU-VNTR technique in Semnan

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Due to the clinical importance of Tuberculosis (TB) and the increasing frequency in Iran, as well as the limited information regarding genetic diversity, control of the disease and Circulating *Mycobacterium tuberculosis* complex (MTBC) species, the Standard 15-locus *Mycobacterium* Interspersed Repetitive Unit Variable Number Tandem Repeat (MIRU-VNTR) typing of *Mycobacterium tuberculosis* (MTB) is used as a discriminating, repeatable and transferable technique, that in these days it is considered as the new standard method.

**MATERIALS AND METHODS:** MIRU-VNTR typing analysis was performed on 69 isolates acquired from TB reference center of Semnan during 2017 - 2022. Then the lineages and clustering of the isolates were analyzed using the standard 15-locus MIRU-VNTR. Recent TB transmission was appraised and phylogenetic relationships were analysed by minimum spanning tree (MST) and cluster-graph methods using web tools available at MIRU VNTRplus online database

**RESULTS AND DISCUSSION:** Among the 69 patients, 68 distinct patterns were detected and two MIRU-VNTR template were clustered. This is the only cluster in this study with isolate numbers 38 and 39 that shared the same pattern. 11 isolates (15.9 %) did not match any genotype in the database and were considered as unknown patterns. The most frequent genotype were NEW-1 (n= 25, 36%), followed by LAM (n=9, 13%), Delhi/CAS (n=7, 10%) and Beijing (n=6, 8.6 %) genotypes. Furthermore, in this study the clustering rate was calculated to define the amount of recent transmission in the population. Not Amazingly, a low clustering rate of 0.01 (1%) was computed among our isolates. as well as, Three clonal complex (CCs) were observed based on the minimum spanning tree (MST) analysis. The largest CC1 embrace 14 isolates while CC2 and CC3 contain three and two isolates respectively.

**Keywords:** Tuberculosis, *Mycobacterium tuberculosis*, MIRU-VNTR typing, Genetic diversity, phylogenetics

# Molecular epidemiology typing of blaOXA-48 and blaNDM-1 producing *Klebsiella pneumoniae* causing nosocomial infection

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Introduction; In this study the antibiotic susceptibility pattern and bla genes, determined in *Klebsiella pneumoniae* clinical isolates that fingerprinted by rep-PCR and PFGE methods at Kurdistan Province, Iran.

**MATERIALS AND METHODS:** A total of 70 *K. pneumoniae* were isolated from clinical samples to detect the antimicrobial susceptibility, carbapenemase and MBL-producing isolates. The PCR assay was used to identify the bla genes. Isolates were typed by PFGE and Rep-PCR methods.

**RESULTS AND DISCUSSION:** The highest and lowest rates of resistance were observed to cefotaxime (67.1%) and imipenem (8.6%), respectively. The rate of blaNDM-1 and blaOXA-48 genes were 1 (1.4%) and 14 (20%) isolates, respectively. All were classified to 27 clusters by the rep-PCR and 39 PFGE types. Low frequency of carbapenemase and MBL genes in this study, are important epidemiologically.

**Keywords:** : Rep-PCR; PFGE, Carbapenemase; Metallo-beta-lactamase; Modified Hodge test; Clinical isolates

# Molecular Identification of class I integron in carbapenem-resistance Enterobacteriaceae pathogens among patients at the educational hospitals of Mazandaran University of Medical Sciences

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Introduction: Carbapenem-resistant Enterobacteriaceae (CRE) infections result in higher treatment costs and mortality rates. Integrons play important roles in emergence and spread of antibiotic resistant genes and integron genes especially class I integron is an increasing global challenge because of the high morbidity and mortality associated with their infections. This study aimed to determine the identification of class I integron in carbapenem-resistant Enterobacteriaceae isolated from patients at the educational hospitals of Mazandaran University of Medical Sciences.

**MATERIALS AND METHODS:** Materials and methods: In this study, 100 Enterobacteriaceae isolates were collected from March 2022 to March 2023 at the educational hospitals of Mazandaran University of Medical Sciences using a consecutive sampling method. Standard microbiological techniques identified isolates. The disk diffusion method was used to determine the antibiogram of isolates. 73 carbapenem-resistant isolates were identified by the disc diffusion method and then subjected to genetic confirmation using polymerase chain reaction (PCR).

**RESULTS AND DISCUSSION:** Results: Of the 73 carbapenem-resistant isolates comprising *K. pneumoniae* (39.72%), *E. coli* (30.13%), *S. rubidaea* (12.32%), *P. mirabilis* (4.10%), *E. aerogenes* (4.10%), *E. cloacae* (2.73%), *C. freundii* (2.73%), *E. gergoviae* (1.36%), *C. diversus* (1.36%), and *P. vulgaris* (1.36%). The isolates showed less resistance to Imipenem 51 (51%) in comparison to meropenem (73%). Out of the 73 isolates, 47 (64.38%) expressed the *intI* gene with *K. pneumoniae* 26 (89.65%) accounting for the majority. Conclusion: Class I integrons play important roles in the emergence and spread of CRE resistance. The prevalence of class I integron in carbapenem resistance Enterobacteriaceae pathogens among patients at the educational hospitals of Mazandaran University of Medical Sciences is relatively high and over 50 percent, and effective infection prevention and identifying this gene should be implemented at the hospitals to prevent the rapid spread of these dangerous organisms.

**Keywords:** Keywords: Enterobacteriaceae, carbapenem-resistant, class I integron

# Molecular identification of fungal isolates from different kinds of blue cheese

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Cheese is one of the high-consumption dairy products in human societies, which today based on its taste and texture it produces in various types. Blue cheeses are one of these types that are attracted in advanced countries. Although nowadays based on the taste of the blue cheese different kinds of fungal species adding to the product during cheese making, the present study tried to isolate fungal strains from five different blue cheeses.

**MATERIALS AND METHODS:** For this purpose, different brands of cheeses were selected from different countries such as: Iran, Turkey, France and the United Kingdom. 10 g of each samples were aseptically removed from each cheese and they were homogenized in 50 ml of sterile 1% peptone water using a stomacher Lab blender for 5 min at normal speed. samples were serially diluted and plated on potato dextrose agar and incubated aerobically for 5 day at 25 ° C. After purification, the strains by an optical microscope were studied and confirmed with Polymerase Chain Reaction Test. Finally, the isolates were characterized by NCBI site and the percentage homology and strains were characterized by NCBI site and the percentage homology and strains were identified.

**RESULTS AND DISCUSSION:** The results indicated that the strains belong to the genus *Penicillium roqueforti*, and *Leuconostoc mesenteriodes*. *Penicillium roqueforti*, were detected from Iranian blue cheese as well as Turkey and UK but *Leuconostoc mesenteriodes* detected from France blue cheese. Although blue cheeses are very popular among people, and their quality as well as presence or absence of toxins are very important, suggesting evaluation of mycotoxin in the products.

**Keywords:** Blue cheese, Fungi, Molecular identification

# Molecular investigation of *Coxiella burnetii*, *Brucella* spp., *Ehrlichia* spp., and *Borrelia* spp. among patients suspected of having Crimean-Congo Hemorrhagic Fever in Iran

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Crimean-Congo Hemorrhagic Fever (CCHF) is a tick-borne zoonotic viral disease that poses a potential threat to public health. Its clinical symptoms can overlap with those of certain acute bacterial febrile diseases, complicating the clinical diagnosis. This study aimed to examine the presence of *Brucella*, *Coxiella burnetii*, *Borrelia*, and *Ehrlichia* infections among individuals suspected of having CCHF in Iran.

**MATERIALS AND METHODS:** A total of 260 serum samples from suspected CCHF cases, which had definitively tested negative for the CCHF virus, were analyzed for *Brucella* spp., *Coxiella burnetii*, *Borrelia* spp., and *Ehrlichia* spp. using Real-time PCR.

**RESULTS AND DISCUSSION:** RESULTS AND DISCUSSION: The results indicated that 3.46% of the patients tested positive for brucellosis and 3.07% tested positive for Q fever. No cases of borreliosis or ehrlichiosis were observed. Among the nine brucellosis-positive cases, three were identified as *Brucella abortus* infections. These findings suggest that bacterial infections, such as Q fever and brucellosis, should be considered in the differential diagnosis of CCHF. Future studies should include a broader investigation of other bacterial infections that can produce early clinical symptoms similar to CCHF and incorporate extensive serological and molecular testing.

**Keywords:** CCHF, Brucellosis, Q fever, Ehrlichiosis, Borreliosis, Iran.

# Molecular Investigation of Torque Teno Virus replication in Patients with MS

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Multiple sclerosis (MS) is a chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system characterized by unpredictable and variable clinical course. Etiology of MS involves both genetic and environmental factors. The environmental factors concerned are likely to be infectious agents, and the involvement of numerous viral agents in the etiology of MS has been postulated. The purpose of this study was to investigate the relationship between Torque Teno virus (TTV) and etiopathogenesis of MS, and its prevalence in this group of patients.

**MATERIALS AND METHODS:** Blood samples from 60 patients with MS and 60 healthy controls were collected. DNA extraction and real-time PCR based on (Syber Green) were done in the serum samples. Clinical data were collected and analyzed by SPSS version 20.

**RESULTS AND DISCUSSION:** Despite the fact that all cases were positive in both the control group and the patient group, the mean Ct (Cycle of threshold) of the two groups was different. The mean of the patient group and the control group was  $21.6 \pm 9.73$  and  $30.3 \pm 1.64$ , respectively. Comparison of the means using the independent samples t-test method in SPSS software showed that the difference in means between the patient and healthy group is statistically significant. Based on the conducted research, it appears that factors involved in the development of MS may lead to increased replication of the TTV in these patients. However, further investigations are needed.

**Keywords:** TTV, Multiple sclerosis, real-time PCR

# Molecular study of the association between JCV and BKV with abortion

Bacteriology

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**BACKGROUND AND OBJECTIVES:** BK and JC viruses belong to the polyomavirus family. These two viruses are latently and chronically established in the body and are reactivated under factors such as pregnancy, organ transplantation, aging that weaken the immune system. Miscarriage/abortion is one of the most common complications of pregnancy and it refers to cases that terminate pregnancy before the 20th week of pregnancy for various reasons such as fetal and maternal reasons. Due to the high prevalence of miscarriage/abortion in pregnant women and considering the importance of this issue, the molecular relationship of BK and JC viruses with abortion was investigated.

**MATERIALS AND METHODS:** This study was conducted on 100 people with an age range of 18-42 years, including 50 pregnant women with a history of miscarriage/abortion and 50 pregnant women without a history of miscarriage/abortion as a control group. After extracting the genome from urine, the presence of BK and JC viruses was investigated using specific primers and the polymerase chain reaction molecular method.

**RESULTS AND DISCUSSION:** According to this study, in the urine of 2 people (4%) from the control group and 4 people (8%) from the samples with a history of miscarriage/abortion, BK virus, as well as 7 people (14%) from the control group and 14 people (28%) from Samples with a history of miscarriage/abortion were diagnosed with JC virus. Statistical analysis did not show a significant relationship between the presence of the virus and miscarriage/abortion, but a significant relationship was found between the presence of these viruses and age over 30 years. Considering the importance of the topic and the high number of JC virus positive cases in women with a history of miscarriage/abortion compared to the control group, it is possible that this research will show different results by increasing the number of examined samples, so further investigations are suggested.

**Keywords:** BK virus; JC virus; abortion



# Molecular typing of *Shigella flexneri* using MLVA method

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Introduction: *Shigella* are non-motile, non-sporulating, non-encapsulated, Gram-negative facultative anaerobes belonging to the family Enterobacteriaceae. In the medical *Shigella flexneri* is important for dysentery in human. *Shigella* spp are resistant to most antibiotics and drug treatment related to these bacteria is costly time-consuming and sometime problematic particularly in area with limited medical care about half the strains of *Shigella* in many parts of the world are now resistant to multiple drugs. *Shigella flexneri* is very common in Iran. The aim of this study was Molecular typing of *Shigella flexneri* using MLVA method.

**MATERIALS AND METHODS:** Methods: The bacterial isolates were identified as *Shigella* spp. by microbiological tests and were serogroup by the slide agglutination test. These isolates were collected from Ahvaz city, Khuzestan. Antimicrobial susceptibility testing was performed using the disk diffusion method. PCR was performed to detect the ipaH gene, for detection of resistance genes were used below primers (blaSHV, blaTEM, blaCTX-M, blaKPC, blaVTM, blaIMP, blaNDM, blaOXA23, blaOXA11, blaOXA48), molecular typing of *Shigella flexneri* was performed by MLVA method.

**RESULTS AND DISCUSSION:** Results: The *Shigella* strains were isolated from 60 patients with diarrhea, including bloody diarrhea. Antibiotic susceptibility tests revealed that the high resistance percentage was related to Ceftazidime (100%) and trimethoprim sulfamethoxazole (100%). Ciprofloxacin and ceftriaxone were the best antibiotics against *Shigella* isolates. Overall, by the MLVA typing method, 60 *shigella flexneri* isolates were grouped into 34 distinct MTs with 5 clusters and 25 singleton genotypes. The most isolates were in cluster 1 (n = 19) and cluster 4 (n = 12), respectively.

**Keywords:** Keyword: *Shigella flexneri*, Dysentery, Gene resistance, MLVA

# Molecular typing of Uropathogenic Escherichia coli isolates from hospitals in Babol, Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Epidemiological studies of uropathogenic Escherichia coli (UPEC) strains have demonstrated that most identified strains of E. coli are derived from a limited number of clonal groups. Globally, the E. coli genotypes Sequence type (ST) 69, ST73, ST95, and ST131, as defined by multilocus sequence typing (MLST), account for more than 50% of UPEC infections. Recently, E. coli ST131 has emerged as a major clone with significant drug resistance potential. Therefore, continuous monitoring and rapid identification of the regional epidemiology of this concerning strain of E. coli are crucial. In this cross-sectional study, we investigated the molecular epidemiology of 156 UPEC strains isolated from patients exhibiting clinical symptoms in Babol, Iran.

**MATERIALS AND METHODS:** In this cross-sectional study, a total of 156 UPEC strains were obtained from urine samples of patients exhibiting clinical symptoms who were admitted to hospitals affiliated with Babol University of Medical Sciences in northern Iran. The strains were isolated from pure cultures and identified based on standard biochemical methods. Extended-spectrum beta-lactamase (ESBL)-producing isolates were detected using the double-disc synergy (DDS) method. The major sequence types (STs) identified included ST131, ST95, ST73, ST69, and ST127, which were determined by multiplex PCR.

**RESULTS AND DISCUSSION:** During the study period, 50.6% (79/156) of the samples were obtained from children, and 49.4% (77/156) were from adults. Moreover, 74.4% (116/156) and 25.6% (40/156) of UPEC isolates were obtained from female and male patients, respectively. In addition, 67.9% (106/156) of UPEC isolates were ESBL producers. According to PCR-based detection, the most common were ST131 (29.9%) followed by, ST69 (11.4%), ST95 (10.8%), ST73 (6.3%). Our results showed that frequency of UTI in woman (74%) higher than in male (26%) because a combination of factors contributes to women being more susceptible. The results also revealed that ST131 were found to be the most prevalent ST types among hospitals in Babol.

**Keywords:** Escherichia coli, urinary tract infection, Uropathogenic Escherichia coli, ST131,

# Multiple-locus variable-number tandem repeat analysis for genotyping of erythromycin-resistant *Streptococcus agalactiae* in Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Streptococcus agalactiae* (Group B *Streptococcus*; GBS or *S. agalactiae*) is an important pathogen that may cause severe infection in the neonates, pregnant women, elderly, and immunocompromised individuals.

**MATERIALS AND METHODS:** We studied a collection of 146 GBS strains isolated from various clinical specimens in three hospitals in Tehran, Iran. All isolates were susceptible to penicillin, vancomycin, linezolid and quinupristin-dalfopristin, but were resistant to tetracycline (96.6%, 141/146), erythromycin (28.1%, 41/146) and clindamycin (16.4%, 24/146). Among the 41 erythromycin-resistant isolates, the most common resistance gene was tetM detected in 92.7% (38/41) of the isolates followed by ermTR, ermB, linB and mefA found in 65.8% (27/41), 29.3% (12/41), 12.2% (5/41), and 2.4% (1/41) of isolates, respectively. Of the 41 erythromycin-resistant isolates, 95% (39/41) revealed the constitutive MLSB phenotype, 2.4% (1/41) displayed inducible MLSB and 2.4% (1/41) exhibited M phenotype. The erm methylase genes were widely associated with MLSB phenotype isolates, while the mefA gene was related to M phenotype. Multilocus variable-number tandem repeat analysis (MLVA) done on the 41 erythromycin-resistant isolates displayed that 34 MLVA types (MTs).

**RESULTS AND DISCUSSION:** Genomic analysis based on MLVA technique displayed that resistance to erythromycin in our region is primarily due to multiclonal dissemination, as was shown by different MTs. Careful usage of macrolide antibiotics in therapy and continued surveillance of resistance rate should be continued in Iran

**Keywords:** GBS, Erythromycin, ermTR, ermB, MLVA

# Necessity of *Clostridium perfringens* biotype A vaccine in the national control program of clostridial diseases

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Clostridium perfringens* (*C. perfringens*) is one of the most pathogenic species in the genus *Clostridium* that produces many toxins. This bacterium is divided into different types (A to G) based on the main toxins secreted. *C. perfringens* biotype A causes the destruction and damage of the cell membrane due to its ability to secrete alpha toxin, followed by vascular, muscular, and hemolytic damage. *C. perfringens* biotype A an agent common disease between humans and animals that can cause acute intestinal inflammation, abdominal disorder, and food poisoning.

**MATERIALS AND METHODS:** According to the national plan which was carried out from 3026 samples from three animal groups (sheep, goats, and cows) among *C. perfringens* isolates using biochemical and molecular tests. It was found that the dominant type belonging to biotype A was obtained.

**RESULTS AND DISCUSSION:** Because the alpha toxin in biotype A is produced in a higher amount compared to other biotypes in the enterotoxemia vaccine manufactured by Razi Institute, therefore the immunity created by this biotype is higher.

**Keywords:** Vaccine, Control, Clostridial Diseases, *Clostridium perfringens*, Toxin

# Neutralizing Antibody Levels in Individuals Received COVID-19 Vaccines

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Monitoring COVID-19 and evaluating the effectiveness of prevention and control strategies are considered critical priorities in public health. As a result, the administration of COVID-19 vaccines has been widely approved around the world. In the current study, we aimed to investigate the level of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) neutralizing antibody (NAb) among individuals who received different COVID-19 vaccines.

**MATERIALS AND METHODS:** This cross-sectional study recorded demographical data and clinical characteristics of 168 individuals who received COVID-19 vaccines from 2021 to 2022. Vaccination data of participants, including type and times of vaccine and any complications and symptoms after vaccination, were also recorded. SARS-CoV-2 NAb level was assessed by performing ELISA test, and all data was analyzed using SPSS version 21. The significant level was set at 0.05.

**RESULTS AND DISCUSSION:** The mean age of the participants was  $40.4 \pm 16$  years, and 109 were females. The mean time between vaccination and sample collecting was  $6.7 \pm 6.5$  months. About 18.4% of participants had an underlying disease, of which thyroid diseases were the most frequent. The mean SARS-CoV-2 NAb level was  $31.6 \pm 39.78$  ng/ml. Sinopharm was the common vaccine in our population, and AstraZeneca represented a high level of Nab  $47.18$  ng/ml. Among the participants, 96 had mild to severe complications after vaccination, with the most complaining of arm pain. Age and types of vaccine were significantly associated with NAb levels ( $P < 0.05$ ). : Due to our findings, all employed vaccines demonstrated efficacy in generating NAb. The levels of Nab were different among genders, age groups, and different types of vaccine receivers.

**Keywords:** SARS-CoV-2, COVID-19, Vaccination, Neutrilizing antibody

# Oncolytic Viruses vs Cancer Stem Cells

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Cancer stem cells (CSC) are very important for progression in leukemias and solid tumors. CSC like normal stem cells has several features like self-renewal, pluripotency and drug resistance. Studies showed that they have ability to resist to radiotherapy and chemotherapy. CSCs have been found in several tumors like brain, breast, ovarian, colon, lung, head and neck, pancreas, and liver.

**MATERIALS AND METHODS:** Oncolytic viruses with multiple mechanisms can target CSC: 1) they can cause lysis in cancer cells, 2) some viral proteins have toxic features for cancer cells, and 3) multiple transgenes can be inserted into oncolytic viruses, in order to increase the anti-tumor immunity.

**RESULTS AND DISCUSSION:** Different studies displayed the efficient potential of oncolytic viruses against CSCs in different tumor types. Different oncolytic viruses like herpes simplex virus-1 (HSV), adenovirus (Ad), measles virus (MV), reovirus, and vaccinia virus (VACV) have been used to target CSC. In this paper, we aim to detail the function of oncolytic viruses against CSCs in different origins.

**Keywords:** cancer stem cells, normal stem cells, oncolytic viruses, anti-tumor immunity

# Optimization of fibrinolysin production by *Bacillus mycoides* strain 28

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Fibrinolysin is the enzyme responsible for fibrin clot digestion. Fibrin clot formation can cause high blood pressure and acute myocardial infarction. Fibrin clot also in blood contacting medical devices such as stents leads to ischemia and thrombosis. The aim of this study was to optimize fibrinolysin production by *Bacillus mycoides* strain 28.

**MATERIALS AND METHODS:** The isolate was cultured in Nutrient broth and incubated at 30, 37 and 43°C and the optimum temperature was obtained based on the growth curve analysis. Subsequently, it was cultured in Nutrient broth at pH 6,7 and 8 and incubated at optimum temperature from previous step for 24h and optimum pH was obtained. Following bacterial culture at optimum pH and temperature, sterile blank discs were saturated with the supernatant of isolates (10000 rpm, 5 min) and placed on plasma plate (1ml of plasma, 4ml agar and 1ml of thromboplastin D) for 24h at 37°C and the amount of fibrin digestion was determined based on the diameter of formed halo zone.

**RESULTS AND DISCUSSION:** As a result of this study, optimum temperature and pH for *Bacillus mycoides* strain 28 were obtained as 37°C and 6, respectively. In these conditions it was led to an increase in fibrinolysin production which was identified with 12mm clear halo zone, while before the optimization, the halo zone activity of fibrinolysin was identified as 11.5mm.

**Keywords:** Plasma plate, Fibrinolytic enzyme, *Bacillus mycoides* strain 28

# Oral Candidiasis in patients with type 2 diabetes and molecular identification of species

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** oral Candidiasis is one of the most common opportunistic infections in diabetic patients due to overgrowth of *Candida* species. Clinical symptoms of oral candidiasis are granular, erosive, and pseudomembranous forms and inflammation on the surface of the tongue, palate and gums. The aim of this study was determine of the oral Candidiasis and molecular identification of the species in patients with type 2 diabetes referred to Toba clinic of Sari, Iran.

**MATERIALS AND METHODS:** In a cross-sectional descriptive study, 3 swabs samples of oral cavity (tongue, palate and gums) were taken 324 patients with type 2 diabetes after recording the questionnaire information. The samples were examined by cytology microscopy rather than culture in SC, SCC and CHROMagar *Candida* media. The polymorphism analysis was performed by PCR-RFLP technique. The internal spacer region (ITS) was recruited for PCR amplification of target sequences and *Msp*1 enzyme was employed to digest PCR amplicons.

**RESULTS AND DISCUSSION:** Out of 324 samples, candidiasis was recovered in 20 (6.1%) cases. The majority of patients have hyperlipidemia (31.1%) and heart disease (26.2%) as specific underlying complications. A1C 7.1-9 (75%) were the most common and use of antibiotics (in the last 2 months) was higher in patients. The frequency of nail discoloration in both groups was statistically significant. *C. albicans* (30.8%) and *C. guilliermondii* (15%) were the most common *Candida* agents that were identified individually or mixed with other species. The frequency of *C. albicans* in diabetic patients has been confirmed by several studies. Studies have shown that *C. guilliermondii*, *C. glabrata* and *C. tropicalis* have the ability to accompany and bind to *C. albicans* or other species. Detection of fungal infections in patients and timely treatment, prevents the development of infection, high costs and probably amputation.

**Keywords:** Candidiasis, Diabetes, interdigital candidiasis, *Candida albicans*, *Candida guilliermondii*



# Phenotypic and Genotypic Characterization of $\beta$ - lactamase Producing *Escherichia coli* isolated from irritable bowel syndrome patients

Bacteriology

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**BACKGROUND AND OBJECTIVES:** BACKGROUNDS Between 10% to 20% of people have irritable bowel syndrome (IBS). The symptoms of this disease are associated with an increase in the number of *E. coli*. Time and suitable treatment choices are limited by antibiotic resistance. Given the significance of antibiotic resistance in strains of *E. coli* in IBS patients, we intended to determine the phenotypic and genotypic characteristics of ESBL and Carbapenemase producing *E. coli* in Ilam (Iran).

**MATERIALS AND METHODS:** A total of 38 *E. coli* isolates were collected from stool samples of IBS patients. *E. coli* strains were identified by biochemical tests. Phenotypic tests were carried out for screening ESBL and Carbapenemase genes producing *E. coli* isolates. To detect ESBL and Carbapenemase genes polymerase chain reaction (PCR) assay was performed.

**RESULTS AND DISCUSSION:** From 38 positive isolates, ESBL was detected in 44/73% (n= 17), and blaTEM (61/53% n= 16) was the most common  $\beta$ -lactamase genes. Moreover, 28/94% (n= 11) of the isolates were producers Carbapenemase, also, BlaOXA-23 (35%) was the most abundant Carbapenemase. Our study suggests that the high prevalence of *E. coli* isolates that produce ESBL and Carbapenemase may have a substantial impact on IBS patients.

**Keywords:** Irritable bowel syndrome, *Escherichia coli*, ESBLs, Carbapenemase

# Phenotypic and genotypic detection of biofilm formation in coagulase-negative Staphylococci isolated from environment of hospitals in Sanandaj, western Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Coagulase-negative staphylococci (CoNS) can cause serious infections, mainly in immunosuppressed patients and those who use prosthetics. The ability to produce biofilm, may make it difficult to treat infections caused by these microorganisms. The *ica* operon, the accumulation-associated protein (Aap), and the biofilm-associated protein homologue (Bhp) are involved in biofilm formation. We aimed to investigate phenotypic and genotypic detection (*icaA*, *icaB*, *icaC*, *icaD*, *aap*, and *bhp* genes) of biofilm formation in CoNS isolated from hospital environment.

**MATERIALS AND METHODS:** A total of 60 CoNS were collected from environment of three teaching hospitals affiliated with Kurdistan University of Medical Sciences in Sanandaj, western Iran. The isolates were identified by the standard methods. The presence of biofilm-related genes: *icaA*, *icaB*, *icaC*, *icaD*, *aap*, and *bhp* was determined using PCR. Biofilm formation was investigated by microtiter plate method.

**RESULTS AND DISCUSSION:** Of 60 isolates, 1 (1.66 %) carried *icaA*, 3 (5 %) *icaB*, 4 (6.66 %) *icaC*, and 1 (1.66%) carried *icaD*. Furthermore, 3 (5 %) isolates had *aap* gene and 1 (1.66 %) isolate had *bhp* gene. Of 60 isolates, 44 (73.33 %) formed weak biofilms, 12 (20 %) moderate biofilms, and 3 (5 %) strong biofilms; while 1 (1.66 %) isolate could not able to form biofilm. An analysis of virulence gene pattern showed that among the 60 isolates, 3 carried both *icaB* and *icaC* genes: 2 isolates formed weak biofilm and one isolate formed moderate biofilm. One isolate, which carried the five genes, *icaA*, *icaB*, *icaC*, *icaD*, and *aap*, formed a moderate biofilm. The results of this study showed the low prevalence of genes related to biofilm formation in the CoNS isolated from the hospital. Determination of antibiotic resistance and biofilm forming ability may help to control infections caused by CoNS.

**Keywords:** Biofilm, Coagulase-negative staphylococci, Environment, Hospital, Virulence

# Phenotypic and Genotypic Evaluation of Aminoglycoside resistance in *Escherichia coli* isolated from patients with blood stream infection in Tehran, Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Escherichia coli* is one of the most prevalent etiological agents of the bloodstream infections (BSIs). Aminoglycosides are important therapeutic options for the treatment of life-threatening infections such as sepsis and pneumonia. Production of aminoglycoside modifying enzymes (AMEs) is the most common mechanism of resistance to this antibiotic. The aim of this study was to determine the antimicrobial susceptibility patterns against different aminoglycosides and the prevalence of common AMEs genes in *E. coli* strains isolated from BSIs

**MATERIALS AND METHODS:** A total of 65 *E. coli* isolates were obtained from blood samples in a hospital from Tehran. Aminoglycosides susceptibility was determined by disk diffusion method and AMEs genes were studied by PCR.

**RESULTS AND DISCUSSION:** Resistance to aminoglycosides was observed in 64.6% (42/65) of the isolates. The highest percentages of resistance were found for kanamycin (44.6%) and gentamicin (38.5%), followed by tobramycin 29.2% and amikacin 4.6%. The most prevalent AME gene was *aac(3)-IVa*, which found in 49.2% isolates, followed by *aac(6)-Ib* (40%), *aac(3)-IIa* (32.3%), and *ant(2)-Ia* (30.8%), respectively.

**Keywords:** Aminoglycoside resistance, *Escherichia coli*, bacteremia, Genes encoding aminoglycoside-modifying enzymes

# Phenotypic and Genotypic Examination of Resistance to Quinupristin-Dalfopristin, and Linezolid in *Enterococcus faecalis* and *Enterococcus faecium* Strains from Fecal Samples of Healthcare Workers and Hospital Settings in Sari, 2019

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Quinupristin-Dalfopristin is a two-component antibiotic, each component of which has bacteriostatic properties individually, but when combined, they exhibit bactericidal activity. This antibiotic belongs to the streptogramin group. On the other hand, Linezolid is an oxazolidinone antibiotic that inhibits bacterial protein synthesis by binding to rRNA. These antibiotics are effective in treating vancomycin-resistant enterococci, such as *Enterococcus faecium* and *Enterococcus faecalis* strains, even though they may be inherently and acquisitively resistant to them. This study was conducted to assess the rate of resistance and gene prevalence in *Enterococcus faecium* and *Enterococcus faecalis* isolates collected from hospital settings and normal fecal flora samples.



**MATERIALS AND METHODS:** This cross-sectional descriptive study was Bacterial strains were obtained from fecal specimens of healthy people, hospital personnel, and hospital surroundings from September to February 2019. These strains were cultured on M-Enterococcus (ME) agar medium subsequently examined for phenotypic and genotypic characteristics using PCR and microbiological tests. The antibiotic sensitivity pattern of the isolated strains was determined using the Kirby-bauer disc agar diffusion method. Genomic DNA was extracted using alkaline lysis method, and PCR was used to investigate the genes involved in resistance.

**RESULTS AND DISCUSSION:** The study examined 145 samples from healthy individuals, hospital staff, and hospital environments, identifying 57.9% as *E.faecalis* and 42.1% as *E.faecium* using PCR. Among *E.faecium*, 42.62% were resistant to erythromycin, 32.78% to quinupristin-dalfopristin, 26.2% to tetracycline, 6.55% to vancomycin, and 1.63% to linezolid. For *E.faecalis*, resistance rates were 83.33% to quinupristin-dalfopristin, 58.3% to tetracycline, 47.61% to erythromycin, 4.76% to linezolid, and 1.19% to vancomycin. The highest resistance was observed for quinupristin-dalfopristin in both strains. Additionally, the study found that several isolates contained different resistance genes, with *IsaA* being the most prevalent at 77.24%. The high resistance to quinupristin-dalfopristin in hospital staff samples compared to other samples, as well as the high involvement of genes in resistance in *Enterococcus* strains isolated from unrelated healthy individuals and hospital environments, highlights the possibility of transfer of resistance genes from these samples to hospital staff and patients.

**Keywords:** Antibiotic resistance, Quinupristin-Dalfopristin, Linezolid, *Enterococcus faecium*, *Enterococcus faecalis*

# Phenotypic and genotypic resistance of *Klebsiella pneumoniae* to quinolones in clinical isolates collected from hospitalized patients in Sari medical centers in 2020

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Many antibiotics have been produced to deal with *Klebsiella pneumoniae*, but its antibiotic resistance has increased so much that this problem has caused many concerns, one of the reasons for this concern is the spread of bacteria and the epidemic of diseases caused by it. The horizontal transfer of QNR genes through the plasmid is the main mechanism for increasing the resistance of this bacterium against fluoroquinolones. In this study, we investigate the antibiotic resistance and frequency of QNR genes in *Klebsiella pneumoniae* isolates taken from clinical samples of patients in Sari hospitals.

**MATERIALS AND METHODS:** This descriptive cross-sectional study was conducted in 2020 on 90 isolates of *Klebsiella pneumoniae*. *K. pneumoniae* isolates were taken from different clinical samples including urine, sputum, ascites, blood, wounds in Sari medical hospitals. Bacterial isolates were identified by Microbiological method and biochemical conventional test. Resistance to quinolone antibiotics was checked by using disk diffusion method. Identification and amplification of *qnrA*, *qnrB*, and *qnrS* genes in the isolates were done by PCR technique.

**RESULTS AND DISCUSSION:** From the total of 90 isolates tested, 50 isolates were taken from urine, 22 isolates from blood samples, 11 isolates from sputum samples and 7 isolates from ascites and wounds. The samples were taken from people with an age range of 2 to 89 years and an average of 36 years, and the frequency ratio of men and women was almost equal. The resistance of quinolone family antibiotics was from highest to lowest including: nalidixic acid 55%, ciprofloxacin 36%, ofloxacin 31%, levofloxacin 29% and norfloxacin 22%. PCR results show that 52%, 23% and 25% of the isolates contain *qnrB*, *qnrS* and *qnrA* genes, respectively. The obtained results show that the transfer of resistance to quinolone family antibiotics through plasmid contributes to the rapid spread of bacterial resistance to fluoroquinolones. The results of this study indicates that continuous monitoring of the use of antibiotics is necessary to prevent the spread of bacterial resistance.

**Keywords:** *Klebsiella pneumoniae*, Antibiotic resistance, Quinolone, PCR

# Phenotypic and genotypic screening of resistance to biocides and colistin in clinical isolates of *Pseudomonas aeruginosa* isolated from Zanzan hospitals.

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* (*P. aeruginosa*) is the second most common pathogen in post-surgical infections and the third most common cause of hospital infections, which accounts for about ten percent of hospital infections. This bacterium is the most important cause of hospital infections, which leads to septicemia and death. to biocides and antibiotics among *P. aeruginosa* strains is a great concern for the control of nosocomial infections, especially in intensive care units, and limits treatment options Considering the importance of drug resistance in *P. aeruginosa* strains, it seems necessary to investigate the molecular mechanisms of resistance and determine the risk factors effective in the development of resistance; Therefore, in the present study, we investigated the pattern of antibiotic resistance and identified genes involved in resistance to biocides (*qacΔE*, *qacA/B*, *qacE*, *qacC* and *qacG*) and colistin (*mcr-1* and *mcr-2*).

**MATERIALS AND METHODS:** 100 unreplicated clinical isolates of *P. aeruginosa* were collected from the hospital laboratories of Zanzan city in 1399-1400, the phenotypic investigation of resistance to biocides by agardilution method and resistance to colistin and other antibiotics was done by disc diffusion method and the frequency of five biocide resistance genes (*qacΔE*, *qacA/B*, *qacE*, *qacC* and *qacG*) and two colistin resistance genes (*mcr-1* and *mcr-2*) were determined using specific primers by PCR method

**RESULTS AND DISCUSSION:** In the study of the antibiogram of 100 clinical isolates, the highest percentage of resistance was found for imipenem, piperacillin, and ceftazidime antibiotics with 29%, 27%, and 25% resistance, respectively, and the lowest resistance level was related to polymyxin B and colistin with 1% and 6% resistance, respectively is The frequency of resistance to biocides was as follows: 21% of clinical isolates were resistant to 4.37% concentration of 35% ethanol and 93% were resistant to 18.2% concentration of this compound. The samples were resistant to 2.5% povidine iodine dilution and 96% were resistant to 15.0% dilution of this substance. Among 100 clinical isolates, the frequency of biocidal resistance genes *qacΔE*, *qac E*, *qacA/B*, *qacC* and *qacG* were 33%, 15%, 6%, 0%, 0% respectively and the frequency of colistin resistance genes *mcr-1* and *mcr-2* was reported as 0%.

**Keywords:** *Pseudomonas aeruginosa*, biocide, colistin, resistance

# Phenotypic and molecular investigation of *Staphylococcus aureus* isolates resistant to vancomycin antibiotic isolated from Rasht hospitals

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus*, as a most important cause of nosocomial and community-acquired infections, shows resistance to a wide range of antibiotics. The aim of this study was the phenotypic and molecular study of clinical isolates of vancomycin-resistant *Staphylococcus aureus* isolated from hospitals in Rasht in a six-month period (February 2015 to July 95)

**MATERIALS AND METHODS:** 217 clinical samples were collected from different wards of hospitals in Rasht. *Staphylococcus aureus* isolates were identified by biochemical tests. To determine microbial resistance of the strains to antibiotic vancomycin, phenotypic tests of disk diffusion (according to the CLSI), and minimum Inhibitory concentration in microdilution broth method were used. Also, the presence of VanA gene, encoding resistance to the antibiotic vancomycin in separated isolate was evaluated by PCR method

**RESULTS AND DISCUSSION:** 67 *Staphylococcus aureus* were identified. In the test determining resistance to the antibiotic by disk diffusion method, the results in terms of resistance rate against antibiotics was as follow: 10.5% chloramphenicol, 25.37% gentamicin, 37.32% tetracyclin, 38.80% vancomycin, 44.7% oxacillin and 100% penicillin. In the microdilution broth, 22.4% of the samples showed resistance to vancomycin. In PCR, no band was observed for genes VanA. It is recommended that in molecular studies, the presence of genes VanA and VanB is assessed; since the resistance could be related to the presence of VanB gene

**Keywords:** *Staphylococcus aureus*, Vancomycin, Antibiotic resistance, VanA.

# Polymicrobial interaction of some urinary tract pathogenic bacteria promotes in vitro hyphae and biofilm formation of *Candida albicans*

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Polymicrobial infections are acute and chronic diseases that are caused by a combination of fungi, bacteria, viruses and parasites. Vulvovaginal candidiasis (VVC) is an infection of the female genital tract that is mostly caused by *Candida albicans*. In women, it is possible to cause urinary-vaginal cross-infections due to the short urethra. In this research, we survey the effect of some urinary infection bacteria including *Escherichia coli*, *Klebsiella pneumoniae*, and *Streptococcus agalactiae* on some *Candida albicans* virulence factor including yeast-hyphae transition and biofilm formation.

**MATERIALS AND METHODS:** Samples of urine and vaginal swap from the infected woman were inoculated on Tryptic Soy Agar (TSA)/MacConkey Agar (MAC) and Sabouraud Dextrose Agar (SDA) to isolate bacterial and fungal strains, respectively. Strains were identified based on the standard biochemical tests. The strains were grown overnight in YPD broth media (Yeast extract Peptone Dextrose) at 37 °C. In vitro formation of mixed biofilm was performed by adding 1000 µl of each fungi and bacteria suspension into the wells of 12-well polystyrene plates. plates were incubated for 24 h at 37 °C. Bright field microscopy was used to examine the hypha production. The adhered biofilm was manually scraped off and 100 µL aliquots of the desired dilution were seeded on plates containing YPD agar and incubated for 48 h at 37 °C. After incubation, the fungus colony forming units per milliliter (CFU/mL-1) were determined for the single and dual biofilms.

**RESULTS AND DISCUSSION:** The yeast to hyphae transition of *C. albicans* in single and dual species biofilms were compared and revealed that more hyphae were formed in dual culture and the most hyphae were observed in the presence of *E. coli*. More biofilms were also formed in the dual cultures and were calculated as (CFU/ml-1)  $12.7 \times 10^7$ ,  $9.06 \times 10^7$ , and  $8.3 \times 10^7$ , in the dual culture with *E. coli*, *K. pneumoniae*, and *S. agalactiae*, respectively. While  $6.1 \times 10^7$  was observed in the single culture. This study demonstrated the effects uropathogenic bacteria to promote fungi virulence factors, which suggest polymicrobial interaction should be considered during treatment of fungal infections.

**Keywords:** Polymicrobial infections, *Candida albicans*, *Escherichia coli*, *Streptococcus agalactiae*, *Klebsiella pneumoniae*



# Prediction of death in burn patients with septic shock contaminated with *Pseudomonas aeruginosa* by using machine learning based methods in Rasht Valayat hospital in 2018-2022

Bacteriology

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**BACKGROUND AND OBJECTIVES:** This study was conducted with the aim of investigating and validating an interpretable machine-learning model based on clinical characteristics to predict death in burn patients with septic shock infected with *Pseudomonas aeruginosa*.

**MATERIALS AND METHODS:** In this study, all hospitalized patients with septic shock contaminated with *Pseudomonas aeruginosa* from 2018 to 2022 referring to the velayat center of the province were included. The standard BOBI model (Belgian Outcome Burn Injury) for predicting death in burn patients was used. Seven machine-learning methods were applied to investigate the models. The best model was selected based on its accuracy and area under curve (AUC) in the validation cohort. Furthermore, we employed the SHapley Additive exPlanations (SHAP) method to illustrate the effects of the features attributed to the model, and to analyze how the individual features affect the output of the model, and to visualize the Shapley value for a single individual.

**RESULTS AND DISCUSSION:** A total of 278 patients were enrolled in this study. The mean age of the patients was  $39.86 \pm 19.49$ . Analysis showed a higher TBSA (Total body surface area) and lower hospital stay in non-survived patients. Obtained results show that the XGBoost model could provide a relatively better model fit performance with an area under the curve (AUC) of 0.8529 and an accuracy of 0.9107 in the validation cohort, compared with the other ML models. Using ML algorithms, addition to the variety of approaches available in predicting the death of patients, can increase accuracy in addition to simplifying and reducing computational complexity.

**Keywords:** Machine learning algorithms, Death, *Pseudomonas aeruginosa*, BOBI, SHAP.

# Pregnancy and Birth Complications in Pregnant Women infected with COVID-19

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Considering that pregnant women are in high-risk groups during the outbreak of infectious diseases, the purpose of this study is to investigate the complications of pregnancy and childbirth in pregnant women infected with (Covid-19).

**MATERIALS AND METHODS:** In this cross-sectional study, conducted from 2020 to 2022 and during the outbreak of 5 peaks of COVID-19, 100 pregnant women above 18 years attending Imam Khomeini Hospital in Sari, Iran with complaints of fever, breath shortening, cough, diarrhea, myalgia, decreased sense of smell and with suspicion of having pneumonia caused by COVID-19 (according to biochemical and imaging criteria) and treated there, and the complications of pregnancy and childbirth were investigated.

**RESULTS AND DISCUSSION:** In the infected pregnant women, there was a statistically significant difference in the severity of the covid disease in different peaks ( $P=0.002$ ), such that 71% of these patients had more severe disease of covid in the third peak. Hospitalization for preterm labor ( $P=0.019$ ) and amniotic fluid disorders ( $P=0.004$ ) were significantly more common in the second peak than others. In the second peak, the amount of abnormal amniotic fluid in the third trimester was higher than other peaks ( $P=0.05$ ). 66.7% of reasons for termination of pregnancy in severe cases of COVID-19 were due to maternal problems, which was significantly more than fetal cases ( $P=0.043$ ). Due to the high risk of maternal and neonatal outcomes of Covid-19 during pregnancy, it is recommended to take special precautions to prevent the disease during this period.

**Keywords:** COVID-19, Delivery, Pregnancy complications

# Prevalence and antimicrobial susceptibility of *Pseudomonas aeruginosa* Isolates from Cystic fibrosis children

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* (*P. aeruginosa*) is a common opportunistic pathogen associated with hospital-acquired infections, known for its resistance to multiple antibiotics. Understanding the antibiotic resistance patterns of *P. aeruginosa* is crucial for developing effective treatment strategies. The purpose of the current study was to determine the antibiotic susceptibility profiles of *P. aeruginosa* isolated from children with cystic fibrosis admitted to a referral hospital in Tehran.

**MATERIALS AND METHODS:** Out of 150 analyzed samples, 80 *P. aeruginosa* isolates were recovered from sputa and were subjected to antibiotic resistance patterns and genetic diversity determination by Kirby-Bauer's disk diffusion method for drug susceptibility patterns following Clinical Laboratory Standards Institute (CLSI) guidelines. Additionally, PCR was used to detect the presence of resistance genes, including metallo- $\beta$ -lactamases (MBLs), which are responsible for resistance in *Pseudomonas aeruginosa*.

**RESULTS AND DISCUSSION:** Among the studied patients, 47 (58.75%) were women and 33 (41.25%) were men. The average age of the patients was 14.5 years (ranging from 6 months to 22 years). In these patients, the resistance patterns of *P. aeruginosa* isolates against tested antibiotics were evaluated and the results showed: Amikacin (45%), Cefepime (40%), Ceftazidime (38%), Ciprofloxacin (50%), Gentamycin (42%), Imipenem (30%), Meropenem (32%), Piperacillin (35%), and Tobramycin (33%). Further genetic analysis identified the presence of the blaIMP gene in a significant portion of the isolates, which is associated with the observed phenotypic resistance. Antibiotic resistance among *P. aeruginosa* isolates was notably high, highlighting the urgent need for ongoing surveillance and strict antibiotic stewardship programs.

**Keywords:** *Pseudomonas aeruginosa*, cystic fibrosis, antibiogram pattern

# Prevalence and genotyping of *Toxoplasma gondii* in Stray Cat Feces from Khorramabad, West Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Cats, as the primary host of *Toxoplasma gondii*, play a crucial role in the dissemination and occurrence of outbreaks caused by this parasite. To understand how this parasite spreads, it is crucial to analyse the distribution of genetic variation in cats that are infected with *T. gondii*.

**MATERIALS AND METHODS:** During the year of 2016-2017, a total of 200 cats were selected as a sample in order to obtain recently collected stool specimens. Oocysts were identified using parasitological procedures. The DNA was extracted from the faeces sample using a commercially available Genomic Mini Kit manufactured by Bioneer, a company based in South Korea. To determine the genetic makeup of *T. gondii*, we utilised PCR-RFLP, sequencing, and phylogenetic analysis of the GRA6 target gene. None of the samples yielded positive results when subjected to parasitology procedures.

**RESULTS AND DISCUSSION:** Out of 200 samples, 13 of them, which is equivalent to 6.5%, tested positive when the GRA6-PCR technique was used. PCR-RFLP analysis revealed that all 13 samples exhibited the *T. gondii* type III genotype. The nucleotide sequences of two samples from this investigation exhibited a 5% dissimilarity when compared to the sequences of 12 *T. gondii* references and one *Hammondia hamondi* strain, which served as an external control. According to the results, molecular tests exhibit higher sensitivity compared to parasitological approaches. The RFLP method demonstrated that type III of *T. gondii* is the dominant and significant genotype in Khorramabad, West Iran.

**Keywords:** *Toxoplasma gondii*, Seroprevalence, Stray cats, PCR-RFLP, Genotyping

# Prevalence and Risk Factors of *Toxoplasma gondii* Infection among Pet Clinic Workers in Mashhad City

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Toxoplasma gondii* is a zoonotic protozoan parasite that can be found wherever felids are present. This parasite has a high prevalence in some parts of the world due to its various transmission routes and is considered one of the most common zoonotic parasites. Workers in small animal clinics are more likely to come into contact with the definitive host of this parasite. Therefore, serological prevalence studies of these individuals are important to assess the occupational association and other risk factors for infection.

**MATERIALS AND METHODS:** In this cross-sectional study, 92 blood samples were collected from staff working in small animal clinics in Mashhad, Iran. A questionnaire was administered to these individuals to assess risk factors considered important for parasite infection. Blood was collected in clot tubes and transported to the laboratory. The serum was separated from the blood samples and subjected to serological assays for the detection of IgM and IgG antibodies against *Toxoplasma gondii* using commercial ELISA kits.

**RESULTS AND DISCUSSION:** The prevalence of anti-*Toxoplasma* antibodies in their serum was 13.04% IgG and IgM was negative in all samples. Logistic regression showed that duration of employment at the veterinary clinic, daily contact with cats, and owning a dog as a pet were associated with a positive serum toxoplasma test. Our findings did not reveal a statistically significant difference in the seroprevalence of *Toxoplasma gondii* infection between this occupational group and the general population in Mashhad. However, it is crucial for individuals in this occupation to be aware of the risk factors associated with *Toxoplasma gondii* infection and to educate others about these risks.

**Keywords:** *Toxoplasma gondii*, Mashhad, zoonotic infection, risk factors, ELISA

# Prevalence of Beta-lactamase Genes (IMP and VIM) in Clinical and Environmental Isolates of *Pseudomonas Aeruginosa* Isolated from the Intensive Care Unit in Ilam Hospitals

Bacteriology

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**BACKGROUND AND OBJECTIVES:** : Class B beta-lactamases, termed metallo-beta-lactamases (MBLs) such as VIM and IMP are an increasingly serious clinical problems. They have a very broad substrate profile that includes penicillins, expanded spectrum cephalosporins, and carbapenems, except for monobactams such as aztreonam. MBLs producing *Pseudomonas aeruginosa* isolates have been responsible for several nosocomial outbreaks. In this study we determined prevalence of Beta-lactamase IM and, Beta-lactamase VIM MBL genes among imipenem resistant *P. aeruginosa* isolated from Ilam hospitals.

**MATERIALS AND METHODS:** A total of 36 *P. aeruginosa* isolates were collected from different clinical and environmental samples from Ilam hospitals. PCR assay for detection of Beta-lactamase IMP and MBL genes was performed by specific primer.

**RESULTS AND DISCUSSION:** showed that 3 and 5 of these 36 isolates were positive by PCR and isolates harbored Beta-lactamase VIM and Beta-lactamase IMP gene respectively. Conclusion: According to importance of MBL producing isolates in prolonged nosocomial outbreaks it is necessary to rapid detection and concern seriously for infection control management Our results showed that in total 8 strains (22%) were PCR results for Beta-lactamase VIM gene, 3 cases and for Beta-lactamase IMP, 5 cases out of 35 samples isolated from Ilam hospitals. Conclusion: Metallobetallactamase production has a moderate prevalence, and considering the importance of metallobetallactamase-producing strains in hospitals, quick identification and tracking of these strains can be considered an important and fundamental step in the treatment and control of infections caused by these strains.

**Keywords:** Class B beta-lactamases, *Pseudomonas aeruginosa*, PCR

# Prevalence of bacterial diarrhea among children in Tehran

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Acute Infectious diarrhea is a significant indicator of malnutrition and the main cause of child mortality in many developing countries. Proper identification of the organisms responsible for the disease is essential for selecting appropriate antimicrobial agents and for conducting effective prognosis and epidemiological research. Previous scourges such as typhoid fever, bacillary dysentery, and cholera continue to pose significant challenges in impoverished and developing countries. In Iran, thousands of children fall victim to these preventable and treatable diseases each year. The aim of this research was to prevalence study of bacterial diarrhea among children in Tehran.

**MATERIALS AND METHODS:** In this study, 440 stool samples from children with diarrhea, who visited the Central Pathobiology Laboratory in Tehran between 2017-2023 were collected and cultured using various media. The samples were subjected to selective culture media for the isolation of Campylobacter and were also under microaerophilic conditions. *Vibrio cholerae* was isolated using TCBS agar and alkaline peptone water (pH 8.6). In this study, the antibiotics used included ampicillin, trimethoprim/sulfamethoxazole (SXT), nalidixic acid, azithromycin, chloramphenicol, ciprofloxacin, ofloxacin, and levofloxacin. Antibiotic susceptibility testing was conducted in accordance with CLSI standards.

**RESULTS AND DISCUSSION:** Among the 440 stool samples, 80 (18.2%) tested positive for bacterial pathogens, including 43 (53.75%) samples identified as *E. coli*, 21 (26.25%) as *Shigella*, 10 (12.5%) as *Campylobacter*, and 6 (7.5%) as *Salmonella*. Notably, neither *Vibrio cholerae* nor *Vibrio parahaemolyticus* was detected in the cases studied. The most common antibiotic resistances were to ampicillin, nalidixic acid, azithromycin and trimethoprim/sulfamethoxazole (SXT). Multidrug resistant isolates were observed. The results showed that the high rate of negative cultures, further research is recommended to explore viral and parasitic factors in order to identify all microbial causes of diarrhea in children.

**Keywords:** Bacterial Diarrhea, antibiotic resistance, children

# Prevalence of Carbapenem-Resistant *Escherichia coli* and *Klebsiella pneumoniae* in Sari Hospitals: A Genotypic Study of IMP-Type Beta-Lactamase Production

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Gram-negative bacteria from the Enterobacteriaceae family are typically opportunistic pathogens, causing infections in immunocompromised or catheterized patients. *Escherichia coli* and *Klebsiella pneumoniae* are prominent members of this family. The development of beta-lactamase enzymes, particularly metallo-beta-lactamases, has led to significant antibiotic resistance. This study aimed to examine the prevalence of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* strains producing IMP-type beta-lactamase, isolated from patients in educational and therapeutic hospitals in Sari, using genotypic methods.

**MATERIALS AND METHODS:** This descriptive cross-sectional study analyzed carbapenem-resistant isolates of *Escherichia coli* and *Klebsiella pneumoniae* from ICU patients in educational and therapeutic hospitals in Sari, hospitalized for at least 48 hours. Samples were cultured on eosin methylene blue (EMB) agar and incubated for 24 hours at 37°C. Gram staining and subsequent identification tests were performed on the colonies. Antibiotic susceptibility testing of the isolated strains was conducted using the disk diffusion method as recommended by CLSI. DNA was extracted and purified using the boiling method, and PCR with specific primers was used to detect the IMP carbapenemase coding gene. Statistical analysis was carried out using SPSS version 16, Chi-square test, and independent T-test.

**RESULTS AND DISCUSSION:** Seventy clinical samples were analyzed, including 31 *Escherichia coli* and 39 *Klebsiella pneumoniae* isolates. Of these, 8 *Klebsiella pneumoniae* samples tested positive for the IMP gene, whereas none of the *Escherichia coli* samples harbored the gene. The IMP gene is not prevalent in *Escherichia coli* and *Klebsiella pneumoniae* in teaching and therapeutic hospitals, indicating that these bacteria employ other mechanisms for antibiotic resistance.

**Keywords:** IMP gene, beta-lactamase, carbapenem-resistant, *Escherichia coli*, *Klebsiella pneumoniae*



# Prevalence of chronic pulmonary aspergillosis in patients with chronic obstructive pulmonary disease from Iran

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Chronic obstructive pulmonary disease (COPD) is a persistent inflammatory airway condition characterized by non-specific airway obstruction and damage to the alveoli. Increasing use of antibiotics, glucocorticoids and immunosuppressant in patients with COPD has led to a gradual rise in pulmonary fungal infections, particularly Aspergillus infection. Chronic pulmonary aspergillosis (CPA) was considered as one of the most important clinical entity due to Aspergillus in COPD patients is which is usually neglected in different countries including Iran. Therefore, in this study we aimed the prevalence of CPA in individuals with COPD in Iran and establish a diagnostic threshold for Aspergillus IgG levels indicative of CPA.

**MATERIALS AND METHODS:** A multi-center prospective cohort study was performed on 196 patients with COPD from 2021 to 2023. Patients are diagnosed based on the clinical symptoms, radiological findings, and mycological evidence. We set the cut-off levels for serum Aspergillus fumigatus-specific IgG. Then, we also used the enzyme-link immunosorbent assay (ELISA) to assess the total IgE levels in included patients.

**RESULTS AND DISCUSSION:** The prevalence of CPA among patients with COPD was found to be 15.3%. with chronic cavitary pulmonary aspergillosis (CCPA) was the most common presentation (80%), followed by chronic fibrosing pulmonary aspergillosis (CFPA) (16.6%) and simple aspergilloma (SA) (3.3%). In patients with CPA, *A. fumigatus* was the most frequently isolated pathogen (3/30, 10%) followed by *A. flavus* and *A. citrinoterreus* (1/30, 3.3%, each). The optimal cut-off value for *A. fumigatus* IgG was 23.5 U, with an area under the curve (AUC) of the receiver operating characteristic curve (ROC) at 0.632. It exhibited 61.4% sensitivity and 71.4% specificity. Additionally, the median (IQR) levels of *A. fumigatus* IgG were significantly higher in CPA patients compared to non-CPA patients (p-value 0.001).

**Keywords:** Aspergillus fumigatus specific IgG, chronic obstructive pulmonary disease, chronic obstructive

# Prevalence of Enterotoxin Genes in *Staphylococcus aureus* Strains Isolated from Hospitalized Patients, Medical Personnel, and Kitchen Staff in Sari Hospitals in 2016: A Cross-Sectional Study

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus* (*S. aureus*) causes a wide variety of diseases. Major virulence factors of this organism include enterotoxins (SEs). (1) These toxins have become a superfamily with 23 types, including enterotoxin A and B, which play a major role in food poisoning. (2) This research was conducted to determine the frequency of enterotoxin A and B genes in *S. aureus* strains.

**MATERIALS AND METHODS:** In this cross-sectional descriptive study, which was conducted in 2016, 223 specimens from the skin and nose of hospitalized patients, medical personnel and kitchen staff of Sari Imam Khomeini and Boali Sina hospitals were collected. In the Laboratory, Biochemical tests were used to isolate *S. aureus* and enterotoxin A and B genes were detected using PCR tests.

**RESULTS AND DISCUSSION:** In this study, the highest percentage of *S. aureus* contamination was related to the samples taken from the nose (53.06%), hand and nose (30.61%) and people who carried the sample in their hand (16.32%). From 49 *S. aureus* isolates, 22 (46.93%) isolates belonged to men and 26 (53.06%) isolates belonged to women. From all isolates, 17 samples were positive for the enterotoxin A gene and all were negative for the enterotoxin B gene. Out of 17 isolates with the enterotoxin A gene, 10 cases (58.82%) were observed in women and 7 cases (41.17%) were observed in men. From all the people included in this study, 30 (61.22%) *S. aureus* isolates were obtained from patients, 14 (28.57%) isolates from medical personnel and 5 (2.10%) isolates from kitchen staff. Among them, respectively, 12 (5.70%) patients, 4 (5.23%) medical personnel and 1 (8.5%) kitchen staff carried *S. aureus*, which was positive for enterotoxin

**Keywords:** *Staphylococcus aureus*, gene, enterotoxin, PCR

# Prevalence of *Helicobacter pylori* virulence genes in biopsy samples Stomach in patients with inflammation

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Helicobacter pylori* is one of the most common bacteria that affects human societies worldwide and is associated with gastrointestinal complications mainly due to various pathogens. This study was conducted with the aim of investigating some virulence genes of *Helicobacter pylori* in gastric biopsies of patients with gastritis in Sari city, North of Iran. The frequency of these genes is different in different geographical areas. The aim of this study was to evaluate the frequency of BabA2, sab A, nap A, hpa, ure A, ure B genes in *Helicobacter pylori* isolates isolated from gastric biopsies and their relationship with gastritis, ulcer and gastric cancer.

**MATERIALS AND METHODS:** 100gastric biopsy samples were taken by a gastroenterologist from patients suffering from digestive disorders and one sample was sent to the laboratory for urease test and histopathology examination and another sample was sent to the laboratory for DNA extraction. After DNA extraction, the abundance of BabA2, sabA, nap A, hpa, ureA, ureB genes were investigated using their specific primers and molecular PCR method.

**RESULTS AND DISCUSSION:** Among 100 patients infected with *Helicobacter pylori*, 46 patients had gastritis, 18 patients had gastric cancer, and 36 patients had stomach ulcers. In general, the frequency of BabA2 gene positive cases was 58 (58%) cases out of a total of 100 biopsy samples, 61 cases of sabA gene. 61%) and ureB gene 66 cases (66%) and hpa gene 76 cases (76%), ure A gene 64 cases (64%), napA gene 83 cases (83%) were positive. In this study, the frequency of babA2, nap A, hpa, ureA, ureB, sabA genes was higher in gastric ulcer and gastritis samples. And there was no statistically significant relationship between the presence of genes and the type of pathology caused by *Helicobacter pylori*. The reason for the difference in the frequency of these different can be due to the difference in geographical diversity or the use of different primers to trace these genes.

**Keywords:** *Helicobacter pylori*, virulence genes, peptic ulcer, cancer

# Prevalence of Intestinal Parasitic Infections and Related Risk Factors Among Children in Ilam City, West Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Intestinal parasite infections (IPIs) pose a significant global health challenge, particularly in developing nations. Based on the literature, children have a higher likelihood of becoming infected since they are exposed more frequently to sources of infection.

**MATERIALS AND METHODS:** This study involved the collection of 500 fecal samples from children under the age of 15 who were referred to laboratories in Ilam city. The materials underwent microscopic examination utilizing formalin-ether concentration and Trichrome staining methods. The data was examined using the statistical software SPSS 20.0.

**RESULTS AND DISCUSSION:** Out of the 500 samples that were examined, four different types of parasites were found. *Giardia lamblia*, *Entameba coli*, and *Blastocystis hominis* were found as intestinal protozoa, whereas pinworm was recognized as an intestinal worm. Out of the total number of cases examined, 23 individuals (4.6%) were found to have parasitic diseases. Out of the total number of patients, 13 (2.6%) were found to be infected with *Giardia lamblia*, 4 (0.8%) with *Entameba coli*, 3 (0.6%) with *Blastocystis hominis*, and 3 (0.6%) had pinworms. The results of this study indicate that the prevalence of intestinal parasitic infections (IPIs) in children under the age of 15 in Ilam is relatively low. This can be related to the adequate knowledge of parents and children regarding the methods of parasite transmission and the significant role of carriers in the spread of the pathogens.

**Keywords:** Prevalence; Parasitic infections; Risk factors; Pediatrics

# Prevalence of *Listeria monocytogenes* bacteria from vaginal samples of woman by PCR method in the hospitals of the University of Medical Sciences in Sari in 2014

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Listeria monocytogenes*, a foodborne pathogen, poses a significant risk to pregnant women, manifesting as self-limited flu-like symptoms that can lead to severe outcomes such as abortion, stillbirth, or premature birth of infected infants. The bacterial surface protein actA is recognized as a key virulence factor of *L. monocytogenes*, playing crucial roles within host cells. In this study, we aimed to investigate the prevalence of *L. monocytogenes* in vaginal samples from women by utilizing the PCR method to track the actA gene.

**MATERIALS AND METHODS:** This cross-sectional study was conducted in 2014. A total of 126 women from varied demographic backgrounds were enrolled in the study. Information such as history of abortion, premature birth, previous cesarean section deliveries, instances of stillbirth, and utilization of natural contraception methods was collected through a questionnaire. To accurately detect the presence of *Listeria monocytogenes*, a potentially harmful pathogen, PCR amplification with ActA-specific primers was utilized for precise identification. The primary objective of this study was to investigate the prevalence of *L. monocytogenes* in women and assess its potential association with reproductive health outcomes.

**RESULTS AND DISCUSSION:** The analysis of 126 female samples showed that 62 tested positive for *Listeria monocytogenes*. Among these, 7 were aged 30-40, 49 were 40-50, and 7 were over 50, indicating a correlation between ages 40-50 and infection (P0.001). There were 55 positive cases among housewives and 7 among those with other jobs, suggesting a relationship between *Listeria monocytogenes* and housework (P0.001). History of premature birth did not show a significant association (P=0.075), while history of abortion and stillbirth demonstrated a significant connection (P0.001). Furthermore, 38 out of 51 individuals using natural pregnancy prevention methods tested positive for *Listeria monocytogenes* (P0.001). Data analysis revealed that *L. monocytogenes* could be a causative agent of abortion and history of stillbirth in pregnant women; Taking this issue into account while giving information and counseling pregnant women can be vital to reduce the incidence of this bacterium and subsequently its side effects during pregnancy.

**Keywords:** *Listeria monocytogenes*, PCR, ActA gene, Abortion, Contraception

# Prevalence of MDR, XDR and TDR *Klebsiella pneumoniae* over Three Months at Imam Reza Hospital, Mashhad, Iran

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Antimicrobial resistance (AMR) is a critical global public health threat, with *Klebsiella pneumoniae* emerging as a significant pathogen due to its increasing resistance to multiple antibiotics. This study aimed to investigate the prevalence of extensively drug-resistant (XDR), Multidrug-resistant (MDR) and totally drug-resistant (TDR) *K. pneumoniae* over three months at Imam Reza Hospital, Mashhad, Iran.

**MATERIALS AND METHODS:** We collected data from 90 patients infected with *K. pneumoniae* across various hospital departments from March 20 to June 20, 2024. Isolates were identified using the BD Phoenix M50 Compact automated system. Information gathered included demographics, antibiotic resistance patterns (CARB/AMPC/ESBL), and resistance phenotypes (MDR/XDR/TDR). Data were analyzed using SPSS software (version 27).

**RESULTS AND DISCUSSION:** The study revealed high rates of multidrug-resistant (MDR) (92%), extensively drug-resistant (XDR) (65.5%), and totally drug-resistant (TDR) (11.11%) *K. pneumoniae* isolates (Table). Significant correlations were found between gender and TDR ( $p=0.037$ ), department and ESBL ( $p=0.033$ ), and MDR with ESBL, AMPC, CARB, and XDR ( $p=0.01$ ). A strong relationship was also observed between XDR and TDR variables ( $p=0.015$ ). The intensive care unit (ICU) showed the highest burden of MDR, XDR, and TDR cases. The high prevalence of XDR and TDR *K. pneumoniae* isolates at Imam Reza Hospital is alarming and significantly limits available treatment options. These findings underscore the urgent need for enhanced antimicrobial stewardship, improved infection control measures, and targeted interventions in high-burden areas such as the ICU. Continued surveillance and research into novel antimicrobial agents are crucial to address this growing threat to public health.

**Keywords:** Antimicrobial resistance, *Klebsiella pneumoniae*, antibiotic resistance patterns, Prevalence

# Prevalence of methicillin-resistant *Staphylococcus aureus* and its antibiotic resistance in the personnel of Sari teaching hospitals in 2013

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** methicillin-resistant *Staphylococcus aureus* is a important pathogen for nosocomial infection and its antibiotal resistance is challenging. Healthcare worker (HCWs) are the main reservoir for MRSA. Identification and decolonization in HCWs may help to reduce the infection of it. The aim of this study was to identify the prevalence of hand and nasal carriage methicillin-resistant *Staphylococcus aureus* and *Staphylococcus aureus* among hospital staff by analysing their antibiogram.

**MATERIALS AND METHODS:** this cross-sectional study was conducted in 2013. The number of participants was 188 HCWs from various parts of educational hospitals in Sari city. Sampling was conducted every individual fingers and sample of anterior nares by strile swabs then samples were cultured on monitol salt agar immediately. Suspected colonies of gram stains; catalase and coagulase test were identified. Susceptibility testing was performed by disk diffusion

**RESULTS AND DISCUSSION:** Results: from 188 people included in the study, 111 were nurses (59%), 29 were doctors and technicians (15%), and 48 were maids (26%). *Staphylococcus aureus* carriage was detected in 24 individuals, representing 16.5% of the sample population. ( $p=0.003$ ) Methicillin resistance was observed in 9.1% of the Population. The highest prevalence of *Staphylococcus aureus* carriers was identified among Staff in the operating room- angiography unit (21%) and the internal pediatric (21%) ward. All isolated Strains demonstrated susceptibility to Vancomycin and Chloramphenicol, while all the strains were resistant to Penicillin and Amoxicillin. Conclusion: The prevalence of *Staphylococcus aureus* carriage among staff at Sari hospitals Is comparatively lower than that reported in similar studies conducted in hospitals in Iran. This study indicates that the prevalence and dissemination of antibiotic-resistant strains Can be mitigated by eliminating predisposing conditions and ensuring the judicious use of Antibiotics

**Keywords:** MIC, MRSA, Nosocomial Infection

# Prevalence of Microbial Co-infection with HIV in AIDS Patients in Northern Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Acquired Immunodeficiency Syndrome (AIDS) is a clinical syndrome caused by the human immunodeficiency virus (HIV). Microbial infections are common problems in AIDS patients, occurring due to immune system defects in these individuals. Currently, there are few studies on the prevalence of comorbid microbial infections in HIV and AIDS patients in Iran. Therefore, the aim of the current study is to examine this topic in HIV and AIDS patients within the population covered by Mazandaran University of Medical Sciences.

**MATERIALS AND METHODS:** This descriptive cross-sectional study was conducted on all positive cases of HIV and AIDS patients registered in the diseases management center of the Mazandaran Health Department from 1380 to 1400.

**RESULTS AND DISCUSSION:** A total of 466 patients with AIDS were investigated, with the majority of them (72.8%) being men. The average age of the patients was  $45.6 \pm 11.6$  years. Out of the 184 patients tested for hepatitis C, 62% tested negative while 38% tested positive. In the investigation of hepatitis B cases, the rate of acute cases was 4.5%, and 25.3% of AIDS patients had chronic hepatitis B. One active case (0.2%) of measles was identified through the IGM test, and 12 patients (2.5%) tested positive for rubella total Ab. Only one positive case (0.2%) of syphilis was found among the patients using VDRL/PRP tests. Additionally, 28 cases (6%) had a history of TB, with 4.1% having recovered at the time of the study, 3 cases deceased, and 1.2% being acute.

**Keywords:** AIDS, HIV, microbial infections



# Prevalence of Mycobacterium tuberculosis before and after COVID-19 in western Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Mycobacterium tuberculosis is the cause of tuberculosis. This bacterium can infect the host without any symptoms, so there are millions of people in the world with asymptomatic infection of Mycobacterium tuberculosis, and the intracellular space without this bacterium and its unique cell wall causes antibiotic resistance and its escape from the body's immune system. The COVID-19 pandemic caused many respiratory diseases, including tuberculosis, to receive less attention in the health systems of many countries during its outbreak due to its more contagious nature, so this study compares the prevalence of Mycobacterium tuberculosis before the disease COVID-19 and after that in the west of Iran.

**MATERIALS AND METHODS:** This cross-sectional study was conducted between 2017 to 2022 in Hamadan province in the west of Iran. The research tool in this study was to collect information of disabled people from the health center system of Hamadan province

**RESULTS AND DISCUSSION:** The sample size in this study was 402 people with tuberculosis, of which 255 people before COVID-19 and 148 people after COVID-19 were investigated. The most organs involved in patients before the COVID-19 era were the pleural fluid and lymph nodes, and after the COVID-19 the most organs involved were the skin and lymph nodes. The sensitive and resistant condition to isoniazid, rifampin and ethambutol after COVID-19 has been more than people before COVID-19. The general results of this research show that the prevalence of a respiratory epidemic has a great impact on the epidemic of other respiratory infections, and the health system of countries should take important preventive measures during the prevalence of a pandemic or epidemic to prevent its transmission to people. who are suffering from other respiratory infections, including people with tuberculosis and do the necessary training for proper culture during prevalence.

**Keywords:** Mycobacterium tuberculosis, COVID-19, Coronavirus, Prevalence, Iran

# Prevalence of New Delhi metallo-beta-lactamases (NDM)-positive *Klebsiella pneumoniae* isolates collected from patients admitted to university-affiliated hospitals in Ahvaz city and their antibiotic resistance rates

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** In 2008, a *Klebsiella pneumoniae* isolate obtained from a Swedish patient who was hospitalized in India revealed the presence of New Delhi metallo- $\beta$ -lactamase-1 (NDM-1) enzyme. This particular enzyme has the ability to break down all  $\beta$ -lactam antibiotics, including carbapenems. The emergence of NDM-1 producing *K. pneumoniae* isolates is particularly worrisome as these bacteria are commonly resistant to multiple drugs, posing challenges in the treatment of infections caused by them. The objective of this study was to assess the prevalence of NDM-positive *K. pneumoniae* isolates and their antibiotic resistance rates in Ahvaz city.

**MATERIALS AND METHODS:** During 2019 and 2021, 154 *K. pneumoniae* isolates were collected from different clinical samples of patients admitted to university-affiliated hospitals in Ahvaz city. The isolates were identified by standard microbiology tests and PCR of 16S–23S ITS gene. The presence of blaNDM gene was evaluated by PCR. The antibiotic resistance rate was assessed by disc diffusion method. Antibiotic resistance rate of colistin and tigecycline was assessed by broth microdilution method.

**RESULTS AND DISCUSSION:** Results: In total, 33 (21.4%) NDM-positive *K. pneumoniae* isolates were identified. The most antibiotic resistance rate was seen against meropenem (100.0%), imipenem (93.9%), ertapenem (87.8%), and third generation cephalosporins (97.0%). The lowest antibiotic resistance rates were seen toward colistin (0.0%) and tigecycline (33.3%). All NDM-positive *K. pneumoniae* isolates (100.0%) were multidrug-resistant (MDR: resistant to at least one antibiotic in three different categories). Conclusion: This study showed a high prevalence of NDM-positive *K. pneumoniae* isolates with MDR properties. Considering the high rate of antibiotic resistance, it is recommended to implement the treatment procedures based on antibiotic susceptibility testing to prevent treatment failure.

**Keywords:** Antibiotic resistance, *Klebsiella pneumoniae*, New Delhi metallo-beta-lactamases

# prevalence of parasitic, bacterial, fungal, and viral infections among AIDS patients from 2001 to 2021 in the population served by Mazandaran University of Medical Sciences

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Acquired immunodeficiency syndrome (AIDS) is a clinical syndrome caused by the human immunodeficiency virus (HIV). HIV is a member of the retrovirus family. Infectious diseases are one of the most important problems of AIDS patients, which occur due to immune system defects in these people. There is not enough information about the clinical condition of patients and the infectious complications associated with this disease in the population covered by Mazandaran University of Medical Sciences. Therefore, the present study was conducted with the aim of investigating the prevalence of microbial infections (parasitic, bacterial, fungal and viral) among the patients with AIDS and HIV positive.

**MATERIALS AND METHODS:** This descriptive study was conducted in the disease management center of Mazandaran health department from 2001 to 2021. The samples were included in the study by census. The registered information was obtained from the health networks and health deputy of Mazandaran province and the website of the infectious disease management center of the Ministry of Health, the collected data were entered into SPSS 23 and analyzed. Also the publications with same goals have been thoroughly searched on Pubmed, Scopus, and Google scholar as scientific databases using key terms.

**RESULTS AND DISCUSSION:** In this study of 466 AIDS patients, predominantly male (72.8%) with an average age of 45.6 years, various co-infections were examined. Hepatitis C was found in 38% of the 184 tested patients, while acute and chronic hepatitis B were observed in 4.5% and 25.3% of patients, respectively. One active case of measles (0.2%) and 12 positive cases for rubella total Ab (2.5%) were identified. Syphilis was detected in 0.2% of patients. Tuberculosis (TB) history was noted in 6%, with 4.1% recovered, 3 cases deceased, and 1.2% acute. No co-infections with parasitic or fungal diseases were found. Significant correlations were identified between hepatitis B and male gender, middle school education, and permanent marriage (P0.05), and between hepatitis C and male gender and permanent marriage (P0.002). The study underscores the need for urgent preventive and treatment measures for hepatitis in HIV patients, while parasitic and fungal co-infections do not necessitate special prevention programs.

**Keywords:** Microbial infections, AIDS, HIV, Hepatitis B, Hepatitis C

# Prevalence of torque teno virus in patients with covid-19 and healthy control group in the central laboratory of East Azarbaijan province

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Co-infection of Torque Teno virus (TTV) in patients with SARS-CoV-2 may affect the degree of this disease, and also the viral load of these viruses can be an effective factor in predicting the occurrence of severe nosocomial infections and mortality in patients with COVID-19. In this study, we intend to investigate the relationship between TTV viral load and the degree of disease in people with COVID-19 compared to healthy people.

**MATERIALS AND METHODS:** 144 blood samples were taken from patients with covid 19 in Tabriz city along with 72 healthy samples. 72 blood samples were taken from hospitalized patients with severe symptoms in Tabriz hospitals with the approval of a specialist doctor and confirmation of the patients' consent. Also, 72 samples were taken from outpatients with mild symptoms. Finally, the samples were transferred to the laboratory environment near the ice. Then viral DNA and RNA were extracted in the laboratory according to the instructions of the extraction kit. After extracting and preparing the solutions, nanodroplet was performed to ensure purity and quality, and cDNA was synthesized for viral RNA. Then, PCR was performed using the TaqMan Real-Time PCR method and a dedicated one-step PCR kit. After the end of the reaction and according to the instructions and SPSS 24 software, the results were analyzed.

**RESULTS AND DISCUSSION:** By examining 144 patient samples and 72 healthy control samples, we concluded that 26.38% of patients with severe COVID-19 have TTV DNA in their blood samples. While TTV DNA was observed in 15.72% of patients with mild symptoms of COVID-19. Also, TTV DNA was detected in 11.11% of the blood samples of the healthy control group. Based on previous studies, the examination of TTV viral load in patients with COVID-19 can be useful for predicting the occurrence of severe nosocomial infections and mortality in patients with COVID-19. Also, TTV levels in patients with SARS-CoV-2 are associated with the likelihood of admission to the intensive care unit and may reflect immunosuppression. Based on our findings, TTV viral load in individuals with COVID-19 is likely to be directly related to disease severity.

**Keywords:** Torque Teno virus, SARS CoV 2, COVID-19, co-infection

# Quinolone-Coumarin Hybrids as Potential Anti-Toxoplasma Agents: An In Vitro Study

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background: Toxoplasmosis, caused by *Toxoplasma gondii*, poses global health concerns, particularly for vulnerable populations. Consequently, there is a need for new drugs that cause minimal damage to host cells. The purpose of this study was to investigate the antiparasitic efficacy of quinolone–coumarin hybrids QC1–QC12 against *T. gondii*.


**MATERIALS AND METHODS:** Materials and Method: The derivatives were compared with novobiocin and ciprofloxacin during testing, with pyrimethamine used as a positive control. We conducted the MTT assay to examine the anti-toxoplasmic effects of the test compounds and novobiocin. Evaluation included the infection and proliferation indices, as well as the size and number of plaques (areas with lysed cells), based on the viability of both healthy and infected cells. Additionally, we studied the behavior of tachyzoites after treatment with quinolones and novobiocin.

**RESULTS AND DISCUSSION:** Results: The in vitro assays revealed that QC1, QC3, QC6, and novobiocin, with selectivity indices (SIs) of 7.27, 13.43, and 8.23, respectively, had the least toxic effect on healthy cells and the highest effect on infected cells compared to pyrimethamine (SI= 3.05). Compared to pyrimethamine, QC1, QC3, QC6, and novobiocin Without having a significant effect on cell viability, demonstrated a significant effect on reducing in both infection index and proliferation index, in addition to reducing the quantity and dimensions of plaques ( P 0.05). Discussion: Based on our results, QC1, QC3, QC6, and novobiocin due to their significant therapeutic effects could be considered as potential new leads in the development of novel anti-Toxoplasma agents.

**Keywords:** Keywords: Toxoplasmosis, Quinolones, Coumarins, Novobiocin, Anti-parasitic

# Rapid and reliable molecular identification of *Cryptococcus neoformans* isolated from pigeon droppings in Iran

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background: Cryptococcosis is an opportunistic deep mycosis found worldwide such as in Iran that is occurred because of inhalation of basidiospores or yeast cells from environmental sources such as soil or plant materials as well as bird feces, especially pigeon droppings. Many molecular typing methods have been described to study the epidemiology of *Cryptococcus neoformans* complex, some of which are well mentioned in the study by Illnait-Zaragozi et al. Objective: In this study, we attempted to establish a rapid direct identification method of *C. neoformans* from pigeon dropping samples by nested-PCR using CNLAC1 genes.

**MATERIALS AND METHODS:** Materials and Methods: Four hundred and twenty-eight samples of pigeon droppings were collected from 11 different cities (Karaj, Kermanshah, Yasouj, Dezful, Gorgan, Gonbad, Sari, Ilam, Kashan, Shiraz, and Shahrekord) of Iran. DNA of isolates was extracted from pure cultures and a PCR test was performed. For detection of *C. neoformans*, primer sets that targeting the CNLAC1 gene were selected and nested PCR was conducted. For distinguishing *C. neoformans* varieties, a primer pair targeting the STR1 gene was selected.

**RESULTS AND DISCUSSION:** Results: Of 428 samples from pigeon droppings, 37 (8.6%) were positive for *C. neoformans*. We confirmed that CNLAC1 gene was a specific gene for the identification of *C. neoformans*. It should be noted that in this study, in addition to molecular identification, we also looked for morphological and phenotypic identification of the samples. As a result, all 37 *C. neoformans*-isolated samples (100%) that were positive in traditional diagnostic tests were identified as positive by the nested-PCR. Total *C. neoformans* varieties isolated from pigeon droppings in this study belonged to the grubii type that produced a product with 274 bp. Conclusion: In this study, we attempted to establish a rapid direct identification method of *C. neoformans* from pigeon dropping samples by nested PCR using CNLAC1 (Outer & Inner) genes.

**Keywords:** Keywords: *Cryptococcus neoformans*, Pigeon droppings, CNLAC1 gene, Nested-polymerase chain reaction

# Recombinant SucB Protein from *Francisella tularensis*: A Promising Candidate for Enhanced Tularemia Diagnosis

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Tularemia is a zoonotic disease caused by *Francisella tularensis*. Recent reports have indicated its presence in areas where tularemia is not endemic. The SucB protein, a Succinyltransferase dihydrolipoamide protein of *F. tularensis*, has been identified as one of the main immunogenic proteins of *F. tularensis*. In various studies of the SucB gene, 86% sensitivity has been shown in the diagnosis of tularemia and may have diagnostic applications. Aim of study is to clone and expression the SucB gene of *F. tularensis* for diagnostic use.

**MATERIALS AND METHODS:** In this study, Cloning and expression of the SucB gene from *F. tularensis* subsp. *holarctica* LVS NCTC 10857 were done. In this research, the SucB gene was isolated from *F. tularensis* by PCR using a pair of F-SucB and R-SucB primers, then it was cloned and expressed in *E. coli* BL21 (DE3) using the pET28a vector. The SucB gene amplification by PCR can produce DNA fragments with a size of 1470bp. The DNA fragment was inserted into the cloning vector pET28a, resulting in the recombinant SucB protein. Recombinant SucB production was performed using the heat shock technique at 42°C when the culture reached 0.4-0.5 OD600 and induced by 0.5 mM IPTG.

**RESULTS AND DISCUSSION:** The results of the SDS-PAGE analysis showed the presence of a 54 kilodalton protein band in the SDS PAGE electrophorogram. The enzyme showed a specific activity of 46.03 U/mg. The results showed that the SucB gene from *F. tularensis* can be well expressed in the *E. coli* BL21 (DE3) host. Discussion: Successful cloning and expression of the SucB gene from *F. tularensis* in *E. coli* BL21 (DE3) represents a significant step towards developing effective diagnostic tools for tularemia. The high sensitivity and specific activity of the recombinant SucB protein highlight

**Keywords:** *F. tularensis*, Cloning, Expression, SucB gene

# Relationship between the milk bacterial phyla and the amount of dietary macronutrients and micronutrients intake in lactating mothers with high body mass index compared to the normal: a case-control study

Bacteriology

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**BACKGROUND AND OBJECTIVES:** In the present study, the amount of the four main phyla in obese lactating mothers and mothers with a normal body mass index was evaluated and their relationship with the amount of macro- and micronutrient's intake was investigated

**MATERIALS AND METHODS:** : Sixty mothers who were in the third month of breastfeeding were selected from the health centers of Zanjan city, were randomly and equally divided into two groups based on the current body mass index: obese (greater than 30 kg/m<sup>2</sup>) and normal (between 18.5-24.9 kg/m<sup>2</sup>), and the milk sample was collected under completely sterile conditions. Bacterial DNA was extracted and amplification of 16S rRNA gene was done by qPCR method using universal bacterial primers. Dietary information was collected using a food frequency questionnaire (FFQ) and validated by a three-day food diary.

**RESULTS AND DISCUSSION:** Results: Adjusting for all parameters, mothers with a normal body mass index had 1.41 times more Actinobacteria genome in milk (p=0.04). The amount of iron and vitamin C intake showed a significant negative relationship with the Actinobacteria and Firmicutes population in milk, respectively (OR=-2.3, p=0.04, OR=-0.96, p=0.02). Also, the amount of dietary cholesterol showed a significant relationship with the Bacteroidetes population (OR=0.81, p=0.04). Conclusion: Actinobacteria gene presence, as a beneficial phylum, was higher in lactating mothers with normal than the high body mass index. Dietary Iron and vitamin C were inversely related to the Actinobacteria and Firmicutes population.

**Keywords:** Bacterial phylum, breast milk, body mass index



# Rickettsia conorii subsp. israelensis infection in a pediatric patient presenting skin rash and abdominal pain: a case report from Southeast Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** The healthcare system in Iran tends to neglect Mediterranean spotted fever (MSF) as an endemic condition, particularly among children, highlighting the need for increased attention and awareness.

**MATERIALS AND METHODS:** Case Presentation: A six-year-old from southeast Iran exhibited symptoms such as fever, abdominal pain, headache, skin rashes, diarrhea, vomiting, and black eschar (tache noire). Clinical and laboratory evaluations, including indirect fluorescent antibody (IFA) testing and real-time PCR, identified the illness as rickettsiosis caused by *Rickettsia conorii* subsp. *israelensis*. The patient was successfully treated with doxycycline.

**RESULTS AND DISCUSSION:** Conclusions: Symptoms such as rash, edema, eschar, and abdominal pain should raise suspicion of MSF in cases of acute febrile illness. IFA and real-time PCR are essential diagnostic tools for the diagnosis of this disease.

**Keywords:** Keywords *Rickettsia conorii*, Mediterranean spotted fever, Pediatric, Child, Iran

# SAP1 gene expression changes in fluconazole-resistant *Candida albicans* treated with menthol

Bacteriology

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**BACKGROUND AND OBJECTIVES:** One of the biggest problems faced in clinical practice is the emergence of resistance for most of the antifungal drugs currently used. Using of biological compounds such as menthol could be one of the most frequently ways to alleviate this problem. The aims of this study were to investigate the hypha formation and gene expression profiling of fluconazole-resistant *Candida albicans* treated by menthol.

**MATERIALS AND METHODS:** In this study the relative minimum growth inhibitory concentration (MIC) was determined by broth microdilution according to the CLSI M27A3 standard protocol with slight modification for all isolates with menthol and fluconazole. Finally, the expression of one of the effective genes in the production of hyphae in *C. albicans* by was measured using Quantitative Real Time RT-PCR.

**RESULTS AND DISCUSSION:** Colonizing vaginal isolates of *C. albicans* were recognized. Out of colonizing vaginal isolates of *C. albicans*, 100% were found to be fluconazol-resistant. Indeed, MIC<sub>90</sub> for menthol was reported in fluconazol-resistant isolates at 1.6 to 25 µg/mL. The result of the analysis of the expression of SAP1 gene involved in hyphae production for two concentrations of menthol equals 2MIC and MIC showed 2.02-to-1.85-fold reduction. The results of this study showed that the secretion of aspartyl proteinase enzyme in the presence of hyphae could be effective in pathogenicity and deterioration of host tissues, and is reduced in the presence of menthol compound. In addition, the Real Time RT-PCR analysis of SAP1 gene suggested the probable molecular targets of methanol in *C. albicans*.

**Keywords:** fluconazole-resistant *Candida albicans*, menthol, SAP1

# Sepsis in hospitalized patients of Intensive Care Unit at Heart Hospital in Sari

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** One of the most important concerns for Intensive Care Unit patients, is infection and sepsis is the most important kind of infection. there are several pathogens can cause this problem, but some of them are more common and always can create severe infection which be spread in body and cause death. For this reason, special attention is necessary to decrease rate of infection.

**MATERIALS AND METHODS:** Patients in Intensive Care Unit were followed up over a period of one year from march 2023, through march 2024. Sepsis diagnosis was based on specific guidelines of sepsis-3 for septic shock and sepsis. Demographic, clinical and laboratory characteristics were considered for all patient. Results of blood culture were checked and antibiogram were done for all positive cultures. Frequent rates of bacterial species were identified and percentages of antibiotic resistance for different classes of antibiotics were performed for all samples based on clinical Laboratory Standard Institute guidelines (2023).

**RESULTS AND DISCUSSION:** Analyzing the demographic and clinical data of 182 patients admitted to Intensive Care Unit of heart hospital in sari, distinguished those 43(23.9%) patients had infection and recognized as sepsis. 23(53%) of them were females and 20(46.5) were males. Highest range of patients ages related to group 60-70 years and 15 (35%) of them were in CICU. the most isolated microorganisms were Staphylococcus aureus (30.2), Coagulase negative staphylococcus (23.2%) and Klebsiella spp. (13.95%) respectively. The highest antibiotic resistance was Penicillin (100%), Amoxicillin (95.8%) and Oxacillin (95.6%), respectively. Sepsis as a major problem in hospitals especially in ICU and CCU is related with high incidence of mortality rates. as a result, determining the rate of infection and so the pathologic agents and suitable antibiotic can treat infection, is completely necessary in every region of country.

**Keywords:** Sepsis, Intensive Care Unit, Heart Hospital

# Serological evaluation of *Helicobacter pylori* among Iranian patients and its associations with disease prognosis

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background: *Helicobacter pylori* (*H. pylori*) affect about 4.4 billion people, comprising 50% of the world population. Although this pathogen is the primary agent of gastritis, it causes several extra-gastrointestinal disorders such as metabolic and inflammatory diseases. As the clinical manifestations of *H. pylori* would appear several years after the infection occurs, identification of the prognosis factors might facilitate early diagnosis and treatment of this disease. Due to little information on prognosis factors of *H. pylori*, this study aimed to evaluate the serum levels of inflammatory serum marker and liver enzymes before and after treatment of *H. pylori*

**MATERIALS AND METHODS:** Methods: A total of 75 patients were enrolled and serum specimens were collected before and after taking the *H. pylori* treatment regimen. The serum levels of *H. pylori* IgG were measured on both serum specimens, and patients with acceptable levels of *H. pylori* IgG were selected. On the next step, the levels of CRP, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total protein, direct and total bilirubin, and albumin using evaluated using ELISA. The results of the analyses were compared and interpreted using paired T-test. The P. value 0.05 was considered as significant.

**RESULTS AND DISCUSSION:** Results: Out of 75 participants, 48 (64%) had high levels of *H. pylori* IgG levels; and out of 48 patients, only thirty participants cooperated in this study. The average levels of *H. pylori* IgG before and after treatment were 6.03 µg/ml and 1.12 µg/ml, respectively. The IgG levels were significantly decreased due to the *H. pylori* treatment (P 0.05). Among the serum factors, only the levels of albumin (p 0.001), total bilirubin (p = 0.001), and CRP (p 0.001) was significantly decreased after *H. pylori* treatment. Furthermore, no significant differences were observed between serum levels of liver enzymes before and after *H. pylori* treatment. Conclusion: The results of this study show the significant association between *H. pylori* and the serum levels of bilirubin, CRP and albumin. So, these factors might take a role as prognostic factors of *H. pylori* infection.

**Keywords:** Keywords: *Helicobacter pylori*, Liver enzymes, albumin, C-reactive protein, Bilirubin

# Serum Zinc Level and COVID-19 Severity in Hospitalized Patients: A Cross-sectional Study

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background and purpose: Zinc is one of the rare elements that have a boosting effect on the immune system, especially against viral infections. It is hypothesized that low zinc levels in patients with respiratory viral infections are associated with disease severity. This research was designed to investigate the relationship between serum zinc levels and the severity of COVID-19 infection.

**MATERIALS AND METHODS:** Method: Serum zinc levels and zinc deficiency frequency were evaluated in 166 patients who were admitted to the hospital due to COVID-19. The correlation between serum zinc levels and laboratory parameters including complete blood count and inflammatory markers was assessed. Also, the correlation between zinc deficiency and COVID-19 severity (according to clinical, laboratory, and imaging data) was evaluated. A P-value less than 0.05 was considered statistically significant

**RESULTS AND DISCUSSION:** Results: Among COVID-19 patients, 6.6% (n=11) had zinc deficiency (serum zinc concentrations 60 µg/dl). The percentage of patients with zinc deficiency in mild, moderate, severe, and critical COVID-19 was 3.6%, 4.3%, 7.7%, and 27.3%, respectively. A higher percentage of patients with the severe form of the disease was found in patients with zinc deficiency compared to non-severe cases (P=0.03). Serum zinc level was not significantly different between the four groups; however, it demonstrated a significant negative correlation with C-reactive protein (CRP) ( $r=-0.2$ ,  $P=0.03$ ) and a positive correlation with lymphocyte count ( $r=0.2$ ,  $P=0.02$ ). Conclusions: The findings of the present study showed that simultaneous zinc deficiency in patients with COVID-19 is associated with higher inflammatory markers and severity of infection.

**Keywords:** Keywords: Zinc serum levels, COVID-19 infection, Severity, Inflammatory markers

# Spotted Fever Group serology in Iran; An update

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Rickettsia spp. are globally distributed and rickettsioses are increasingly recognized as emerging infections in various regions. Ticks serve as reservoir hosts for pathogenic Rickettsia in humans, with most pathogenic species belonging to the spotted fever group (SFG). The Mediterranean spotted fever (MSF), which belongs to the SFG group, is an acute febrile disease caused by Rickettsia conorii. The Rickettsial diseases often emerge as endemic and enzootic diseases, though, they sometimes emerge as seasonal and sporadic diseases. This study aimed to investigate the seroprevalence of anti-R. conorii IgG among farmers residing in the rural regions of Kerman province (Southeast of Iran).

**MATERIALS AND METHODS:** A total of 281 blood samples were obtained from farmers in Zarand and Jiroft counties (Kerman province) and tested for the presence of anti-R. conorii IgG antibodies using the ELISA method. This study was carried out in the Kerman province in southeastern Iran in 2021 (January-September). Kerman province covers an area of 182.301 km<sup>2</sup> and has a population of about 3.16 million people. The climate of this province is hot and dry.

**RESULTS AND DISCUSSION:** In this study, 281 samples were collected from rural farmers in Jiroft (n = 150, 53.4%) and Zarand (n = 131, 46.4%) counties. The mean age ( $\pm$  SD) of participants was 49 years old ( $\pm$  9.51). Also, 70.8% and 29.2% of the individuals were female and male, respectively. Out of 281 samples, 14 farmers (4.98%, CI 95%: 2.45–7.55%) samples had anti-SFG Rickettsia IgG antibodies. Seroprevalence of MSF in Zarand and Jiroft counties were 4.58% (CI 95%: 1–8.16%) and 5.33% (CI 95%: 1.75–8.91%), respectively.

**Keywords:** rickettsioses, rickettsia, farmers, rural areas, Kerman province.

# Study of the mechanism of fluoroquinolone-resistant and phylogenetic relationship of *Escherichia coli* isolated from clinical samples in Afzalipour Kerman hospital

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Resistance to fluoroquinolones such as ciprofloxacin are due to mutational in *gyrA* and *parC*, Plasmid-mediated quinolone resistance (PMQR) such as (*qnrA*, *qnrB*, *qnrS*, and *aac(6)-Ib-cr*) or overexpression efflux pumps. The main objective of this study was to investigate the importance of mutations in the *gyrA* and *parC* genes, PMQR genes, and efflux pumps in resistance to ciprofloxacin and phylogenetic grouping among *E. coli* isolates.

**MATERIALS AND METHODS:** A total of 104 clinical isolates of *E. coli* were collected from afzalipour hospital in Kerman. Antibiotic susceptibility test was done by disc diffusion method. Minimum inhibitory concentrations (MICs) of ciprofloxacin were determined for all resistant isolates. The effect of efflux pumps was determined by repeating the susceptibility in the presence of the efflux pump inhibitor carbonyl cyanide *m*-chlorophenyl hydrazine (CCCP). PCR was used for detecting *qnrA*, *qnrB*, *qnrS* and *aac(6)-Ib-cr* genes in all isolates. Quinolone resistance-determining region (QRDR) of *gyrA* and *ParC* genes was characterized for 16 ciprofloxacin resistant *E. coli* isolates. Phylogenetic typing was performed by the PCR method.

**RESULTS AND DISCUSSION:** The highest rate of resistance was against Ampicillin (79%), followed by trimethoprim/sulfamethoxazole (%76.9), nalidixic acid (69.2%), ceftriaxone (66.3%), cefotaxime (55.8%), ciprofloxacin (39.4%), gentamicin (24%) meropenem (15.4%). Most of the fluoroquinolones-resistant isolates (85.3%) exhibited high-level ciprofloxacin resistance (MIC  $\geq$  32  $\mu$ g/mL). The MIC of ciprofloxacin for 8 isolates was changed in the presence of CCCP. *qnrS* and *qnrB* were detected in both ciprofloxacin-resistant and -susceptible isolates, but *aac(6)-Ib-cr* was only detected in 10 out of 41 ciprofloxacin-resistant isolates. The Ser83Leu mutation in *gyrA* was observed in all 16 ciprofloxacin resistant isolates selected for direct sequencing. The second most common mutation in *gyrA* was Asp87Asn. Frequent mutations in *parC* were Ser80Ile and Glu84Val. Totally of isolates belonged to D and B2 phylogenetic groups. The frequency of the isolates in the phylogenetic groups B2, D, A and B1 were 38.4%, 32.3%, 15.4% and 13.4%, respectively.

**Keywords:** *Escherichia coli*, PMQR, ciprofloxacin

# Study on correlation between *Demodex folliculorum* density and seborrheic dermatitis patients

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** The *Demodex folliculorum* is a saprophytic ectoparasite microorganism on human skin. Their food source is sebum, concentrating mainly on the facial skin and scalp. The density in normal skin is less than five per cm<sup>2</sup>. *Demodex* can intensify some clinical complications such as seborrheic and perioral dermatitis, rosacea, acne and hair loss. Seborrheic dermatitis (SD) is a chronic inflammatory skin disease. The *D. folliculorum* is one of the most important factors that their concentration raised in the skin with SD implication.

**MATERIALS AND METHODS:** 100 cases with average 34.9 years old as patients presents SD on the facial skin, accompanied by intense itching and scaling. Control group were consisted of 186 case without dermatitis symptoms. We took five sample of facial skin sebum and soaking with clear oil. The direct microscopic examination allows the observation and numeration of *D. folliculorum*.

**RESULTS AND DISCUSSION:** *D. folliculorum* sampling was positive in 71 patients (71%) and 16 controls (16%). The average of *Demodex* mite density in patient with SD was 49.16. *Demodex* test was positive in 19 men (70.3%) and in 115 women (72.3%) in SD group. The number of *D. folliculorum* mites was significantly higher in patients with SD. This suggests that DF can introduced as an important factor in aetiological cause of SD. The observation of fungal spores within *Demodex* mites has led to the suggestion that the mites may act as vectors for *Malassezia* spp. as an important intensifying factor in SD.

**Keywords:** *Demodex folliculorum*, Seborrheic dermatitis (SD), Ectoparasite



# Study the frequency of *Legionella pneumophila* to reservoirs and distribution system water educational hospitals in Yasuj

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** pneumophila are gram-negative, non-encapsulated, non-sporforming is normally found all over the world in natural and artificial water sources such as lakes, rivers, hot springs, swimming pools, reservoirs and water piping networks, cooling towers and ventilation systems. Legionella species are responsible for two independent clinical diseases, including legionellosis (severe form of pneumonia) and Pontiac fever (a self-limiting disease similar to influenza). Therefore the monitoring of water systems especially in the hospital for the presence of these organisms is needed. The aim of this study was to evaluate the frequency of *L. pneumophila* in water tanks and systems of Yasuj hospitals.

**MATERIALS AND METHODS:** This descriptive-analytical study was conducted for 6 months in three hospitals of Shahid Beheshti, Imam Sajjad and Shahid Rajaei in Yasouj. A total samples of 150 (79 cold water samples and 71 hot water samples) were collected in sterile plates then *L. pneumophila* was detected with special filters

**RESULTS AND DISCUSSION:** results: The results of study showed that a total of 150 water samples collected (hot and cold water) water systems of three hospitals in Yasuj. 3 samples (2%) grown in cold water were infected with *L. pneumophila*. Conclusions: According to the research conducted in Iran and other countries, the contamination of 2% the water sources of Yasuj educational hospitals by *L. pneumophila* indicates the lack of contamination the water entering these sources or the control of these sources. Finally, in order not to be contaminated by this bacteria, the design, construction and operation of water supply sources should be revised. Also, it is necessary to carry out a comprehensive study in the form of a national plan organized in the country's hospitals and develop a national standard for Legionella pneumophila density.

**Keywords:** water reservoirs, *L. pneumophila*, Hospital

# Studying the abundance of metallobeta-lactamase 1-blaIMP 1-1bla VIM genes in pseudomonas aeruginosa strains isolated from patients hospitalized in the departments of educational and treatment centers of Mazandaran University of Medical Sciences by PCR method

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Background and purpose: Pseudomonas as an opportunistic pathogen with drug resistance along with high resistance to environmental conditions and the high cost of treatment. Increasing the mortality rate, especially in the intensive care unit, is one of the important factors in the development of hospital infections. The aim of this study was to determine the prevalence of blaIMP-1, VIM-1 bla metallo-beta-lactamase genes in Pseudomonas aeruginosa strains isolated from hospitalized patients in Mazandaran University of Medical Sciences.

**MATERIALS AND METHODS:** Materials and methods: This was a descriptive study that was carried out in Mazandaran province, Iran, in 2010. The Pseudomonas aeruginosa isolates were collected from Sari educational hospitals. The bacteria were isolated from various clinical specimens including ulcers, urine, blood, sputum, ICU, restoration section, and were identified after biochemical and microbial tests. For further training, TSB containing 20% glycerol was inoculated and kept at -20 °C in a freezer. In order to confirm the isolates, various biochemical tests including growth in the McCanky agar medium, oxidase test, TSI medium, AND test, copulant production in Mullerinton agar, SIM, agar, growth in case of 42 were used. Antibiotic resistance pattern of Pseudomonas aeruginosa isolates Antibiotic test (diffusion disc) was performed.

**RESULTS AND DISCUSSION:** Results: The results of this study indicate that 150 samples isolated from Pseudomonas were specimens of ulcers-Urinary and Blood samples, respectively, 45%, 30%, 15% and 10% respectively. 74% were men and 26% were women. The highest age group was 31-40. Also, antimicrobial susceptibility test showed that the percentage of drug resistance in 80% resistant ciprofloxacin, 85% gentamicin, 83% ampicemin, 71% meropenem, 78% amikacin, 77% ceftriaxone, 84% ceftazidime, 79% caleite, 10% calcitine. Conclusion: The frequency of strains producing beta-lactamase gene in hospitals is increasing. Due to the high sensitivity of PC in identifying this gene, using this method by mediating this gene can be considered as a sensitive and, at the same time, rapid, method for identifying this microorganism.

**Keywords:** Keywords: Pseudomonas aeruginosa, Hospitalized infection, MBL

# Studying the frequency of biofilm producing genes in clinical isolates of *Pseudomonas aeruginosa* isolated from Valiasr Hospital (AJ) in Zanjan city in 1401

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* (*P. aeruginosa*) is known as a leading cause of nosocomial infections worldwide. This bacterium is the cause of various and extensive infections, especially in immunocompromised people, cancer patients, AIDS, cystic fibrosis and burn victims. Antimicrobial resistance and biofilm production, as two main virulence factors of *P. aeruginosa*, are responsible for the persistence of prolonged infections. *P. aeruginosa* biofilm components consist of at least three distinct exopolysaccharides including alginate, Psl and Pel. Given the difficulty of treating bacterial biofilm infections and making diagnosis with conventional diagnostic methods, identifying isolates with such a factor helps us to better understand the pathogenesis of *P. aeruginosa*. This study was conducted with the aim of investigating the phenotypic and genotypic characteristics of biofilm in isolates of *P. aeruginosa* isolated from patients hospitalized in Zanjan hospitals.

**MATERIALS AND METHODS:** In this cross-sectional descriptive study, 90 clinical isolates of *P. aeruginosa* were collected from different clinical samples from Ayatollah Mousavi, Valiasr and Imam Hossein hospitals in Zanjan city. Genomic DNA of *P. aeruginosa* isolates was extracted from bacterial cells in order to identify biofilm-forming genes. Amplification of biofilm-forming genes was conducted by PCR method and using specific primers designed for protected areas. Also, biofilm-forming phenotypes were identified by microplate method.

**RESULTS AND DISCUSSION:** Of the 90 clinical isolates investigated, 78.9%, 91.1% and 58.9% had pslD, algD and pelF genes, respectively. Out of 40 biofilm-forming isolates, 20% produced weak biofilm, 10% produced moderate biofilm, and 14.4% produced strong biofilm. Although the frequency rates of pelF and algD genes in biofilm-forming isolates were higher than those of non-biofilm-forming isolates, this relationship was not statistically significant. Overall, the results of this study indicate the high frequency of biofilm-forming *P. aeruginosa* isolates. Also, a high percentage of investigated isolates carried pslD, algD and pelF genes, although the frequency of pelF gene was lower than the other two genes. Although the frequency rates of pelF and algD genes in biofilm-forming isolates were higher than those of non-biofilm-forming isolates, this relationship was not statistically significant.

**Keywords:** *Pseudomonas aeruginosa*, Biofilm, algD, pelF, pslD, Zanjan.

# Survey attachment, bax and shock protein genes of *Escherichia coli* strains isolated from patients with urinary tract infections, 2024

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** In the world, *Escherichia coli* (*E. coli*) is responsible for 70% to 95% of urinary tract infections. Because of developing the prevalence of antibiotic resistance, unfortunately, the treatment and eradication of urinary tract infections (UTIs) specially, recurrent infections have been difficult. *ibpA* is a small heat shock protein that its expression upregulates under non- heat circumstances. In general, *bax*, *cspH* and *ibpA* are necessary to protect bacterium against salt and urea stresses [40]. The products of *fim*, *pap* and *sfa* genes are very important virulence and attachment agents in *E. coli*. The epidemiological study on bacteria and find the frequency of virulence genes, can useful for the other studies for instance, design vaccines specially for recurrent UTIs. The main goal of this study is to investigate the frequency of adhesion genes and tolerance to adverse conditions of *E. coli* in urine.

**MATERIALS AND METHODS:** 25 *E. coli* strains were gathered from patients with UTIs who referred to medical centers of Sanandaj city. Based on CLSI protocol (2022), the antibiogram pattern of the strains was obtained. The frequency of shock protein and attachment genes were determined by PCR method. ERIC PCR pattern was obtained by special primers and was analyzed with Geljv1 software.

**RESULTS AND DISCUSSION:** The antibiogram's results showed that *E. coli* were resistant strains to AMX, NA and SXT with 80%, 72% and 64%, respectively. The Multidrug resistant frequency was 80%. The frequencies for attachment genes were *pap* (100%), *fim* and *sfa* (80%); also, for all shock protein genes and *bax* gene were 100%. In Rahdar et al study, the frequency of *fim*, *pap* and *sfa* genes were 100%, 79% and 69%. That was very close to our study. Pearson correlation coefficient (0.95) showed that there were 4 clusters. The 4th cluster as a large one, was 100% resistant to AMX and had all genes. 14 (56%) samples were 95% similar. The ERIC PCR clustering in other studies were different. The main reason of differences is gene variety.

**Keywords:** Antibiogram, *bax*, *cspH*, *ibpA*, *fim*, *sfa*, *pap* *E. coli*, ERIC PCR

# Tetracycline resistance in *Vibrio cholerae* O1/O139 clinical isolates

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Cholera outbreaks are primarily caused by consuming contaminated food and water containing *V. cholera*, while contaminated water is a significant contributor to the spread of severe cholera cases [8]. The World Health Organization (WHO) estimates that 2.9 million new cases of cholera occur annually in 69 countries where cholera is common, resulting in 21,000 to 143,000 deaths worldwide. Resistant *V. cholerae* strains have been causing more treatment failures in cholera patients in countries where cholera is common in past years [13,14]. Prior studies have found that *V. cholera* often shows resistance to tetracyclines, a widely used class of antibiotics. Here, we analyze published studies to assess the in vitro effectiveness of tetracycline against *V. cholerae* isolates.

**MATERIALS AND METHODS:** We conducted a thorough search for pertinent articles in four global databases - PubMed, Scopus, Embase, and Web of Science (up to January 2020) by utilizing specific keywords: ('*Vibrio cholerae*' OR '*V. cholerae*') AND ('Antibiotic resistance' OR 'Drug resistance' OR 'Antimicrobial resistance') in the Title/Abstract/Keywords sections.

**RESULTS AND DISCUSSION:** The sensitivity to tetracycline was assessed in 99 research papers which involved 17,828 O1/O139 *V. cholerae* samples; the prevalence rate was 20% (95% CI 14–27%) with significant diversity ( $I^2 = 99.01\%$ ) (Additional Data and Figure 2). In order to examine the patterns of tetracycline resistance prevalence in recent years, we conducted a subgroup analysis for three-time frames (1980–2000, 2001–2010, and 2011–2020) (Supplementary Data and Figure 3). The subgroup analysis showed a rise in the resistance rate when comparing the data from 1980–2000, 2001–2010, and 2011–2020 periods. The study revealed that the resistance rates to tetracycline was 20%. Our findings indicated a steady increase in tetracycline resistance trends from 1980–2000 to 2011–2020, with rates rising from 7% to 28%. Moreover, there has been a sixfold rise in the frequency of doxycycline-resistant isolates in recent years. Moreover, Asia has a higher prevalence of tetracycline resistant isolates compared to Africa, America.

**Keywords:** Antimicrobial resistance; *V. cholerae* O1/O139

# The Prevalence of Salmonella Spp in Poultry Carcasses for sale in The Karaj City

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Salmonellas is the most prevalent food born disease in the word and poultry meat is one of the sources of infection. The purpose of this study was to prevalence the investigate of salmonella in chickens offered fore consumption, so that we can take preventive measures by knowing about it.

**MATERIALS AND METHODS:** In a descriptive cross-sectional study, 200 samples of poultry carcasses prepared for sale on the karaj retail market were studied. One handred freezing carcass samples and also 100 non – freezing ones were selected. Each chicken was taken three samples, including; skin, muscle and viscera for microbiological examination on selective media.

**RESULTS AND DISCUSSION:** Eighteen poultry samples (9%) were contaminated with salmonella SPP. The rate of contamination was more in non - freezing poultry than freezing poultly. The most frequency of isolated salmonella was *S. typhimurium* and *S. enteritis* respectively. Due to high contamination rates, hygienic rules of slough and meat processing must be paid attention. As can be seen, the rate of contamination in frozen chickens was much lower than fresh chickens.

**Keywords:** salmonella. chicken carcass. pervalence

# The comparative anti-oxidant and anti-inflammatory efficacy of postbiotics and probiotics through Nrf-2 and NF- $\kappa$ B pathways in DSS-induced colitis model

Bacteriology

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**BACKGROUND AND OBJECTIVES:** IBD is a disorder which could be caused by oxidative stress. This investigation aims to determine if probiotics and postbiotics can control oxidative stress and inflammation and compare the effectiveness of these two probiotics and postbiotic mixtures of substances

**MATERIALS AND METHODS:** Eighty-eight strains of Lactobacillus and Bifidobacterium were tested for antioxidant activity. Male wild-type C57BL/6 mice were divided into four experimental groups, namely high fat diet (HFD)+PBS, HFD+DSS, HFD+DSS+  $\sim$ 10<sup>9</sup> cfu/ml of probiotics, and HFD+DSS+  $\sim$ 10<sup>9</sup> cfu/ml of postbiotics. The phenotypical indices and pathological scores were assessed. The expression of genes related to NF- $\kappa$ B and Nrf2 signaling pathways and enzymes associated with oxidant/anti-oxidant activities, and proinflammatory/inflammatory cytokines were assessed

**RESULTS AND DISCUSSION:** In contrast to the groups exposed to DSS, mice treated with probiotics mixture and postbiotics mixture alongside DSS displayed alleviation of DSS-induced adverse effects on phenotypical characteristics, as well as molecular indices such as the Nrf2 and NF- $\kappa$ B related genes, with a greater emphasis on the postbiotics component. In accordance with the findings of the present investigation, it can be inferred that even in using a high-fat dietary regimen as an inducer of oxidative stress, the emergence of inflammation can be effectively addressed through the utilization of probiotics and, more specifically, postbiotics.

**Keywords:** Probiotic, Postbiotic, Oxidative stress, Inflammation

# The effect of silver nanoparticles and imipenem on OmpA gene expression in clinical and standard strains of *Acinetobacter baumannii* isolated from patients referred to hospitals in Yasuj

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Acinetobacter baumannii* is a ubiquitous pathogen capable of various nosocomial infections that are often severe and sometimes life-threatening. *A. baumannii* is difficult to treat due to the emergence of multidrug resistant strains. The OmpA gene is one of the contributing factors to antibiotic resistance of *A. baumannii*. This study intended to investigate the effect of silver nanoparticles in combination with imipenem on OmpA gene expression in *A. baumannii*.

**MATERIALS AND METHODS:** Characteristics of clinical and standard strains of *A. baumannii* were confirmed by biochemical tests. *A. baumannii* strains were then screened for the presence of OmpA gene by PCR. The effect of silver nanoparticles, imipenem and imipenem and silver combination were determined by MIC and MBC methods. RNA was extracted and transformed into cDNA. Finally, OmpA gene expression was performed by Real Time PCR.

**RESULTS AND DISCUSSION:** Antibacterial properties of 3 treatments, including silver nanoparticles, imipenem, and silver-imipenem compound, were investigated on *A. baumannii* and the results were analyzed using SPSS software. The MIC and MBC were determined. Among these groups the imipenem and silver combination had the highest antibacterial activity as well as the highest cause of decrease in OmpA gene expression in silver-treated bacteria. The findings of this analysis suggest the antibacterial activity of silver nanoparticles and the composition of silver imipenem on *A. baumannii*. These effects led to a decrease in OmpA gene expression.

**Keywords:** Antibacterial, Gene Expression, Nano, OmpA gene



# The effects of dietary *Lactobacillus farciminis* and *Bacillus pumilus* probiotics on growth performance, immune system and resistance against *Candida albicans* infection in zebrafish (*Danio rerio*)

Bacteriology

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**BACKGROUND AND OBJECTIVES:** This study evaluated the individual and combined effects of the dietary *Lactobacillus farciminis* and *Bacillus pumilus* probiotics on growth performance, immune system and disease resistance in zebrafish (*Danio rerio*). A total of 240 fish were randomly allocated to 12 tanks with 20 fish per tank (4 treatments with 3 replications) and fed with diets containing 0% *L. farciminis* and *B. pumilus* (T0), 1×10<sup>8</sup> CFU/g *L. farciminis* (T1), 15 g/kg *B. pumilus* (T2), and 1×10<sup>8</sup> CFU/g *L. farciminis* + 15 g/kg *B. pumilus* (T3) for 45 days. Thereafter, serum non-specific immune parameters [Total immunoglobulin (Ig), Alternative complement (ACH50) and lysozyme activity (SL)] and expression of genes involved in immunity (*lyz*, *TNF-α*, *IL1β*, *IL-8*, and *IL-10*) were measured. At the end of the feeding trial, T3 treatment had significantly higher final body weight, weight gain, and specific growth rate when compared to control group (P 0.05).

**MATERIALS AND METHODS:** A total of 240 fish were randomly allocated to 12 tanks with 20 fish per tank (4 treatments with 3 replications) and fed with diets containing 0% *L. farciminis* and *B. pumilus* (T0), 1×10<sup>8</sup> CFU/g *L. farciminis* (T1), 15 g/kg *B. pumilus* (T2), and 1×10<sup>8</sup> CFU/g *L. farciminis* + 15 g/kg *B. pumilus* (T3) for 45 days. Thereafter, serum non-specific immune parameters [Total immunoglobulin (Ig), Alternative complement (ACH50) and lysozyme activity (SL)] and expression of genes involved in immunity (*lyz*, *TNF-α*, *IL1β*, *IL-8*, and *IL-10*) were measured.

**RESULTS AND DISCUSSION:** The ACH50 and lysozyme activity were significantly increased in T2 and T3 treatments (P 0.05). In addition, evaluation of the serum antioxidant enzymes activity showed significant (P 0.05) increase in T3 treatment as compared to the control. The expression of *lyz*, *TNF-α*, *IL-8*, and *IL-1β* genes increased significantly (p 0.05) in T3 treatments, while not affecting the expression of *IL-10* gene (p 0.05). In conclusion, the supplementation of *L. farciminis* and *B. pumilus* can be used as feed additives to enhance disease resistance against *Candida albicans* infection by stimulating the immune system, growth performance, and antioxidant enzyme activity in zebrafish.

**Keywords:** Probiotics, Immune system, Growth performance, Antioxidant enzyme activity

# The first case of Bacteremia Infection caused by *Bacillus licheniformis* in Iran

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Bacillus licheniformis* is an aerobic, Gram-positive, spore-forming rod, and is ubiquitous in the environment. *B. licheniformis* is rarely recognized as a human pathogen and causes serious infections such as bacteremia mainly in immunocompromised patients. *B. licheniformis* should be considered as a potential pathogen in immunocompromised patients, especially when bacteremia is associated with the presence of long-term central venous catheters. In this study we present the first case of Bacteremia Infection caused by *Bacillus licheniformis* in Iran.

**MATERIALS AND METHODS:** We reported a case of a 92 years old woman. she was admitted to emergency department of Ghaem hospital in Mashhad, Iran with pneumonia symptoms and severe Respiratory distress. On arrival in the emergency department, her blood pressure and heart rate were 12 mmHg and 70 bpm Respectively, and blood oxygen was 93%. Then A central venous catheter (CVC) was inserted, but after that, the patient started bleeding and became apneic and the level of consciousness decreased. she was transferred to the Intensive Care Unit (ICU). After being admitted to the ICU, following the sepsis symptoms (fever and chills) Blood cultures were performed using the BD BACTEC (Becton, Dickinson, USA) automated haemoculture system and subsequently the gram-positive bacilli were identified as *Bacillus licheniformis* by the BD Phoenix M50 Compact automated system and microbiological findings confirmed the diagnosis of the first case of Bloodstream infection caused by *Bacillus licheniformis*

**RESULTS AND DISCUSSION:** In this study as a result, *Bacillus licheniformis* diagnosed by the BD Phoenix M50. After diagnosis, Vancomycin (1 gr / 48 hours) and Ciprofloxacin (200mg /twice a day) were prescribed. Finally clinical and microbiological resolution was achieved by antibiotic therapy which leads to eradication of microorganism and sepsis symptoms and subsequently repeated blood culture showing negative results after three days of antibiotic therapy. There is little evidence in the literature about Bloodstream infections caused by *Bacillus licheniformis*. Catheter-Related Bloodstream Infection caused by *Bacillus licheniformis* is a rare infection in humans, but this microorganism should be considered as a potential pathogen in hospitalized immunocompromised patients which can occasionally leads to serious infections and requiring broad-spectrum antibiotic therapy.

**Keywords:** *Bacillus licheniformis*, septicemia, Catheter-Related Bloodstream Infection, immunocompromised patients

# The Frequency of Carbapenemase Genes in *Citrobacter* spp. Isolated from Clinical Specimens in Imam Reza Hospital, Kermanshah, Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** The growing incidence of carbapenem-resistant bacteria is an emerging challenge in modern medicine. The aim of this study was to detection of carbapenemase producing genes in *Citrobacter* spp., isolated from clinical specimens. A total of 430 clinical samples were collected from patient referred to Imam Reza Hospital, Kermanshah, Iran, all isolates were identified by biochemical tests. After antibiotic susceptibility test by using disc diffusion method, *Citrobacter* spp. isolates were selected for further study, and among them resistance to carbapenems were detected by polymerase chain reaction (PCR). The presence of carbapenemase genes encoded Verona integrin-encoded metallo-beta-lactamase (VIM), *Klebsiella pneumoniae* carbapenemase (KPC), Imipenemase (IMP) using specific primers were evaluated. Our results showed that, among 430 clinical samples, totally 50 *Citrobacter* spp. were diagnosed. Antibiogram results showed that, the highest rate of resistance was related to cefotaxime (69%), and the lowest rate of resistance was related to amikacin (29%), the frequency of blaKPC

**MATERIALS AND METHODS:** A total of 430 clinical samples were collected from patient referred to Imam Reza Hospital, Kermanshah, Iran, all isolates were identified by biochemical tests. After antibiotic susceptibility test by using disc diffusion method, *Citrobacter* spp. isolates were selected for further study, and among them resistance to carbapenems were detected by polymerase chain reaction (PCR). The presence of carbapenemase genes encoded Verona integrin-encoded metallo-beta-lactamase (VIM), *Klebsiella pneumoniae* carbapenemase (KPC), Imipenemase (IMP) using specific primers were evaluated.

**RESULTS AND DISCUSSION:** Our results showed that, among 430 clinical samples, totally 50 *Citrobacter* spp. were diagnosed. Antibiogram results showed that, the highest rate of resistance was related to cefotaxime (69%), and the lowest rate of resistance was related to amikacin (29%), the frequency of blaKPC gene was detected in 2 isolates (4%), and frequency of blaVIM gene was detected in 2 isolates (4%), and blaIMP was not found in isolates. Our findings showed that, the prevalence of carbapenemase genes in *Citrobacter koseri*, *Citrobacter freundii*, and *Citrobacter braakii* were low in Kermanshah, but there may be other genes for resistance to carbapenems in this area which need further investigations. The results indicate that carbapenems are still effective antibiotics against *Citrobacter* species.

**Keywords:** *Citrobacter* spp., Carbapenems, Kermanshah, PCR

# The Frequency of Verrucomicrobia in intestinal mucus of patients with cancer and polyps compared to healthy individuals

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Members of the phylum Verrucomicrobia, especially Akkermensia muciniphila, have been identified as beneficial gut bacteria. Changes in the gut microbiota are associated with the occurrence of metabolic diseases, such as diabetes, obesity, and irritable bowel syndrome; however, there is conflicting information about their role and changes in colorectal cancer. In the present study, the prevalence of this bacterial phylum was investigated in the intestines of individuals with colorectal lesions compared with healthy control group.

**MATERIALS AND METHODS:** Mucus samples were obtained from the colons of 69 individuals with colorectal lesions, including colorectal cancer (CRC) and precancerous gut lesions (PCL) and 65 individuals without lesions during colonoscopy. Bacterial DNA was extracted from the mucus samples. Conventional polymerase chain reaction (PCR) assay was performed to detect Verrucomicrobia using a specific 16S rRNA primer.

**RESULTS AND DISCUSSION:** Healthy individuals exhibited a higher prevalence of Verrucomicrobia (75.4%) compared to those with either cancer or precancerous gut lesions (59.7%) (P-value = 0.038). However, there was no significant difference in the presence of these bacteria between CRC and PCL patients groups. A reverse correlation was observed between the presence of Verrucomicrobia and both patients' characteristics of chronic constipation (P-value = 0.001) and anemia (P-value = 0.048). Decreased frequency of Verrucomicrobia in human may serve as the basis for the initiation of intestinal changes resulting in development of colorectal precancerous and cancerous lesions

**Keywords:** Verrucomicrobia, colorectal cancer, precancerous gut lesions, microbiota

# The importance of Demodex mites density in rosacea

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Demodex mites are microscopic obligate parasite of the human pilosebaceous follicle. Demodex mites are associated with bacteria, both in their gut and on their exoskeleton. Demodex folliculorum and Demodex brevis are known as a microorganism's vector and maybe have pathological role in some skin disorders like rosacea. Bacillus oleronius and Staphylococcus epidermidis have been isolated from rosacea patients. Rosacea is a chronic disorder affecting the facial convexities, characterized by frequent flushing, persistent erythema and telangiectasia. The aim of present study was assessment of Demodex mites density in rosacea patients and comparing with normal group without rosacea.

**MATERIALS AND METHODS:** According to clinical symptoms, rosacea was diagnosed in 186 patients by dermatologist. Control group were consist of 186 case without rosacea symptoms. Direct microscopic examination was done from 1 cm<sup>2</sup> of facial skin sebum. Some references consider the density of more than five mites per cm<sup>2</sup> as a pathogenic criterion.

**RESULTS AND DISCUSSION:** Demodex mites sampling was positive in 134 patients (72%) and 26 controls (13.1%). The average of age in rosacea and control groups were 37.5 and 34 years old respectively. the average of Demodex density in rosacea group was 39.1 mites per cm<sup>2</sup>.

**Keywords:** Demodex folliculorum, Demodex brevis, Rosacea

# The relationship between bacteria and cancer

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Cancer is the second leading cause of death worldwide after cardiovascular diseases. This disease is the result of spontaneous mutations during DNA replication, which significantly increases the chances of developing the disease by being exposed to various environmental factors and unhealthy lifestyle habits. In recent years, numerous studies have shown that the body's bacteria play a crucial role in determining health status or susceptibility to pathological diseases, including cancer.

**MATERIALS AND METHODS:** The gut microbiome can be considered an important environmental factor involved in the health of individuals. The gut microbiota plays a role in the production of vitamin B and K, metabolism of dietary compounds, carbohydrate and fat metabolism, immune system enhancement, prevention of damage to the mucous membrane of the gut, and protection of the gut against the penetration of systemic pathogens. Any imbalance in the microbiota can lead to disruption in the gut microbiota, known as dysbiosis, which is associated with various diseases including cancer. The gut microbiome can play both anti-cancer and pro-cancer roles. Furthermore, it has been observed that the microbiome is associated with both gastrointestinal cancers and cancers in other parts of the body, so it can be concluded that bacteria, directly or indirectly, can contribute to the development of cancer. CagA is the first bacterial protein whose role in human cancer has been identified. Furthermore,

**RESULTS AND DISCUSSION:** relationship between the resident gut microbiota and their host is very complex. Each individual inherits a specific composition of gut microbiota from birth, and the gut microbiota is not only influenced by environmental factors such as aging, diet, and lifestyle but also evolves and changes. In fact, this balance is very delicate and is subject to various changes throughout life. many probiotics derived from the gut are able to protect the host against pathogenic and cancer-causing agents by modifying the gut microbiome. Lactobacillus rhamnosus GG is a very good example of a probiotic that has been well studied in cancer and is often used as a complementary treatment for gut microbiome modulation. However, further studies in this area are recommended

**Keywords:** Microbiome, Cancer, Gut Microbiota, Dysbiosis

# The role of stray dogs in transmission of *Neospora Caninum* to dairy cattle

Bacteriology

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**BACKGROUND AND OBJECTIVES:** *Neospora caninum* causes economical loss in cattle herds and is one of the major causes of abortion in dairy cattle and dogs as definitive hosts have an important role in cattle neosporosis. The aim of this study was to ascertain the role of dogs in transmission of *N. caninum* to cattle in herd around Qazvin province in Iran.

**MATERIALS AND METHODS:** A dairy cattle herd had a huge problem with stray dogs which easily entered to feed stores and had access to water, bedding and aborted fetuses. The abortion rate of the dairy farm showed an increase within two years and according to the high number of stray dogs in the herd, the aborted fetuses were suspected to *N. caninum*. A total of 37 aborted fetuses were necropsied and the samples including liver, kidney, spleen, lung, brain, heart, thymus and umbilical cord were collected and sent to the laboratory for evaluation of *N. caninum* by PCR and histopathological examination.

**RESULTS AND DISCUSSION:** Result Pathological findings included preoccupation and hematopoiesis in spleen and diffuse lymphocytic pericarditis in heart. Molecular examination revealed that from 37 dairy cattle, 14 cattles (43.75%) were detected positive. Conclusion According to the current result, *N. caninum* is an important abortifacient agent in cattle. Since dogs have the major role in the transmission of the infection to cattle and they usually don't show specific signs, depopulation of the farms from stray dogs and avoiding to feed them and letting them to access to the dairy feed stores are essential.

**Keywords:** Abortion, Dairy cattle, *Neospora caninum*, Stray dog.

# The Study Of prevalence of Salmonella in Poultry Carcasses for Sale In The Karaj City

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Escherichia coli belonging to sequence type ST131 constitute a globally distributed pandemic lineage that causes multidrug-resistant extra-intestinal infections. ST131 E. coli frequently produce extended-spectrum  $\beta$ -lactamases (ESBLs), which confer resistance to many  $\beta$ -lactam antibiotics and make infections difficult to treat. Population genetics studies have delineated the ST131 phylogeny into three major clades. Clades A, B and C. Clade C is uniformly fluoroquinolone resistant due to conserved replacement mutations in gyrA and parC. C1 subclade is mainly associated with carriage of blaCTX-M-27 or blaCTX-M-14. In contrast, C2 subclade which is subdivided from C1 based on specific a single nucleotide polymorphism (SNP) at fimH30, mostly harbor blaCTX-M-15 and represents the dominant population among clade C. Therefore, the main aim of this study is to determine the prevalence of subclones among uropathogenic E. coli in in Babol, North of Iran.

**MATERIALS AND METHODS:** In this cross-sectional study, 150 UPEC isolates were obtained from urine specimens of inpatients hospitalized at tertiary hospitals in Babol, Iran. For determination of ST131 clades, multiplex PCR using 7 pairs of primers was used as described by Matsumura et al. Clades and subclades were identified based on the expected amplicons.

**RESULTS AND DISCUSSION:** Overall, 61 isolates were identified as ST131 group based on method described by Matsumura et al. According to multiplex PCR, most of the isolates belonged to clade B (39/61; 63.93%), followed by C (15/61; 24.59) and A (7/61; 11.47%). Most clade C genomes belonged to sub-clade C1-M27 (9/15; 60%) followed by C1-non-M27 (C1-nM27;6/15; 40%). As far as we are aware, this is the first study in our region to investigate and compare the prevalence and genotypes of ST131 subclades. It also demonstrates a high prevalence of UPEC ST131 locally. Our findings indicate that the B clade of ST131 is responsible for the majority of ST131 infections. In the future, conducting whole genome sequencing-based studies on ST131 and its subclones will be crucial to elucidate the factors that contribute to the success of these organisms.

**Keywords:** Uropathogenic E. coli, ST131, Clades, subclade



# The Value of Microbiome-targeted Therapy on Lipid Indices of Patients with Type 2 Diabetes Mellitus: An Umbrella Meta-analysis of Randomized Controlled Trials

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Abstract: Background: Type 2 diabetes mellitus (T2DM) is considered a global health challenge with increasing prevalence in recent years. One of the key elements in managing T2DM patients is controlling their lipid profile. Recent studies suggest microbiome-targeted therapy (MTT) as a treatment strategy for enhancing lipid profiles in these patients. The current study aimed to investigate the impact of MTT on lipid indices of T2DM patients by performing an umbrella approach.



**MATERIALS AND METHODS:** Three international databases including PubMed, Scopus, and Web of Sciences were searched from inception up to April 2023 to find meta-analyses evaluating the impact of MTT (prebiotics, probiotics, and synbiotics) on the lipid profile of T2DM patients. Two independent researchers extracted data from the relevant meta-analyses. To find the source of heterogeneity various subgroup analyses were performed. Comprehensive Meta-Analyses (CMA) software version 3 was utilized for the final analysis.

**RESULTS AND DISCUSSION:** Results: Based on the results of the current study MTT had a significant effect on total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (ES: - 0.092; 95%CI: -0.111, -0.074; P 0.001, ES: -0.109; 95%CI: -0.137, -0.081; P 0.001, ES: -0.036; 95%CI: -0.068, -0.005; P= 0.024, ES: 0.109; 95%CI: 0.056, 0.162; P0.000, respectively). In subgroup analysis, probiotics showed the most substantial effect on all lipid biomarkers. This research has provided promising insights into the potential impact of MTT on lipid levels in patients diagnosed with T2DM. Notably, MTT had the greatest impact on HDL levels, followed by TG, TC, and LDL. As a result of our study, MTT is recommended as an adjunctive therapeutic option for T2DM treatment due to its capability to regulate lipid profiles.

**Keywords:** Diabetes mellitus, lipids, probiotic, prebiotic, synbiotic, umbrella review, meta-analysis

# Relationship between the genotypes of the hopQ and sabA genes of *Helicobacter pylori* in infected individuals with the type of clinical condition

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Introduction: *Helicobacter pylori* is a widespread bacterial infection linked to gastric cancer and other health issues. It causes chronic gastritis, leading to gastric adenocarcinoma and lymphoma. If left untreated, it can persist for life, contributing to various digestive system disorders, anemia, and neurological conditions, while also increasing the risk of cardiovascular diseases. The development of gastric cancer due to *H. pylori* is influenced by environmental factors and host characteristics. This study aims to evaluate the prevalence of hopQ1, hopQ2, and sabA genes in *H. pylori* strains from gastrointestinal patients in Mashhad, shedding light on the genetic makeup of these strains and their potential implications for disease progression.

**MATERIALS AND METHODS:** Materials and methods: This study was conducted as a cross-sectional study on 160 DNA extracted in previous studies. We used PCR method to check the presence and absence of hopQ1, hopQ2 and sabA genes. Then Chi-square and Fisher's exact tests were used to analyze significant differences between the studied virulence genes and clinical results using the method. This was done in Mashhad.

**RESULTS AND DISCUSSION:** Result: Out of 160 samples, 91 were from female patients (56.9%) and 69 were from male patients (43.1%). The collection included 132 cases of gastritis (FD), 7 cases of gastric cancer (GC), and 21 cases of combined gastric and duodenal ulcers (PUD). Patient ages ranged from 13 to 82 years, with an average age calculated as (15 ± 48). In this research, 72 instances (45%) tested positive for hopQ1, 92 instances (57.5%) for hopQ2, and 134 instances (83.8%) for sabA. Conclusion: The clinical outcomes did not show a meaningful correlation with the hopQ1, hopQ2 and sabA genes.

**Keywords:** Keywords: *Helicobacter pylori*, hopQ, sabA, Gastrointestinal diseases

# Toxoplasma gondii Infection in Lymph Nodes: A Molecular Survey in Mazandaran Lymphadenopathy Patients

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Toxoplasmosis is one of the causes of lymphadenopathy but toxoplasmic cysts are rarely found in the histological diagnostic sections. However, the validity of the histopathological triad is confirmed by serological tests. Now, the presence of *T. gondii* DNA in the tissue can be detected by polymerase chain reaction (PCR) method

**MATERIALS AND METHODS:** Materials and Method: This study was conducted on the 100 tissue samples of the lymph nodes of people with lymphadenopathy referred to the hospitals of Mazandaran province. Tissue was extracted from the paraffin blocks and then Parasite DNA was extracted using a Tissue DNA extraction kit. Out of 100 case of lymphadenitis, 34 cases were related to acute lymphadenitis and 66 cases were chronic lymphadenitis. The lymph nodes were biopsied from the Axilla, Neck, Inguinal and tonsillar. Nested-PCR method was done to amplify the coding region of the GRA6 gene. PCR products corresponding to 164 bp of the normal control DNA were positive.

**RESULTS AND DISCUSSION:** Four samples out of 100 lymph nodes (4%) were positive for *T. gondii* infection, 2 cases belonged to acute lymphadenitis (2/34, 5.89 %) and 2 cases belonged to chronic lymphadenitis (2/64, 3.03 %). The results of the present study show that most of the *Toxoplasma* samples isolated from people with lymphadenopathy were of genotype II. Genotypes I and II were identified in 1 and 2 positive toxoplasma samples in Mazandaran province, respectively. This study showed that along with pathological tests in cancer patients, especially in breast cancer, molecular tests should be used to diagnose toxoplasmic lymphadenitis. It is suggested that researchers perform pathological, serological and molecular studies simultaneously in people suspected of lymphadenopathy to reduce the possibility of false positives and negatives.

**Keywords:** *Toxoplasma gondii*, toxoplasmosis, lymphadenopathy, Genotyping

# Trichophyton mentagrophytes/interdigital species group, the most common dermatophytes genotype isolated from human dermatophytosis in Golestan province, Iran

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Dermatophytosis (ringworm or tinea) is fungal infection of the skin and its appendages (hair and nails) caused by several different keratinophilic dermatophytes. Determining the epidemiology of the type of infection and its causative factors in each region helps to better prevent and treat it. Golestan province, with two geographically sections (The plains, and the mountains of the Alborz range) located in the northeast of Iran and southeast of the Caspian Sea with 1,950,000 population in 2022 and enjoys mild weather and a temperate climate most of the year.

**MATERIALS AND METHODS:** In This cross-sectional descriptive study, 255/550 clinical samples from all cities of Golestan province, from patients with skin lesions, dermatophytosis was diagnosed by direct examination and culture. By PCR-FLP method of ITS1-5.8s-ITS2 region of ribosomal DNA with mvaI restriction enzyme and observation of different bands in electrophoresis gel, dermatophytes species was determined.

**RESULTS AND DISCUSSION:** In suspected cutaneous fungal infection were 46.3%. suffering from dermatophytosis, including 61.6% female and 38.4% male. The most common dermatophytes isolated were Trichophyton mentagrophytes/interdigital (86.7%) and Trichophyton rubrum (5.1%), respectively. The taxonomy of the T. mentagrophytes/T. interdigitale complex is still in dispute. Recently, a multi-drug resistant clonal Trichophyton population (Trichophyton indotineae) has been identified that were causing alarming dermatophytosis outbreak in India and other countries. The high frequency of this complex shows the importance of the type of antifungal treatment. Dermatologists should be aware of the emergence of resistant strains of dermatophytes. As the situation is alarming in other countries, the situation could quickly become problematic in our region as the rate of resistant strains increases.

**Keywords:** dermatophytosis, tinea, dermatophytes genotype, Trichophyton mentagrophytes/interdigital, Golestan province

# Using a single *Phlebotomus sergenti* salivary protein as a biomarker of sand fly exposure instead of whole salivary protein

Bacteriology

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**BACKGROUND AND OBJECTIVES:** In Iran, anthroponotic cutaneous leishmaniasis (ACL) is caused by *Leishmania tropica* mainly transmitted by the *Phlebotomus* (Ph.) *sergenti* sand fly. In addition to transmitting the parasite to the host, the infected sand fly also injects some of its saliva into the bite site during blood feeding. Therefore, identifying potential biomarkers which are indicative of sand fly exposure can become useful epidemiological tools to control the spread of sand fly and hence the control of the disease. Our previous study showed that *Phlebotomus sergenti* salivary protein 26 (PsSP26) can induce a strong antibody (IgG) response against Salivary Gland Homogenete (SGH) of Ph. *sergenti* in BALB/c mice model.

**MATERIALS AND METHODS:** Therefore, in the current investigation, the recombinant protein PsSP26 that produced in inducible *Leishmania tarentolae* T7-TR protein expression system was used in order to assess the antibody level of individuals exposed to the bite of Ph. *Sergenti* in endemic area. Upon collecting sera from individuals in the endemic area (n=26), a comparative analysis with control sera (n=20) from non-endemic healthy individuals was performed to evaluate the specific antibody levels against rPsSP26 using intra-laboratory optimized-direct ELISA.

**RESULTS AND DISCUSSION:** The results indicated a strong antibody response to the rPsSP26 protein among all residents of the endemic area exhibiting a high level of antibody titer against Ph. *sergenti*'s SGH, suggesting that rPsSP26 alone may serve as a viable biomarker for sand fly exposure instead of the entire SGH. However, further examination of a larger at-risk population during peak exposure is necessary to validate PsSP26 as a relevant biomarker. This study underscores the possibility of using a single arthropod saliva protein, particularly through cost-effective recombinant protein preparation, to monitor vector spread as an alternative to whole salivary protein assessment.

**Keywords:** *Phlebotomus sergenti* salivary protein 26, anthroponotic cutaneous leishmaniasis, *Leishmania tropica*

# Using bacteriophages as a prophylactic measure to prevent multi-drug-resistant (MDR) infections

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Infection of burn wounds is a serious problem because invasive infection may result in the death of the patient. Antibiotic prophylaxis is one of several interventions that are often considered, alongside other infection prevention. However, any benefits of prophylaxis outweigh the risk of harm, such as drug toxicity and the development of multidrug resistance. MDR has become a major problem and the use of bacteriophages is an attractive approach to overcome the problem of drug resistance. Our study aimed to collect MDR *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from burn patients and then isolate specific lytic bacteriophages against these pathogens. Then, these phages were applied as prophylactic agents in a mouse infection model.

**MATERIALS AND METHODS:** MDR-bacterial strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were collected from the microbiology laboratory of Motahari Hospital. Bacteriophages were isolated from hospital sewage with co-culturing of host bacteria and filtrated sewage at 37°C overnight in Shaker Incubator. The next day co-culture was centrifuged and filtrated supernatant tested in vitro for lytic activity against MDR isolates in a double-layer agar method. We examined the isolated Bacteriophages to prophylaxis of MDR-bacterial infection in a mouse model of burn wound. The effectiveness of prophylaxis was checked by counting the colonies of the wound site after 14 days. Statistical analysis was performed and the difference between the samples with P-values lower than 0.05 was considered significant.

**RESULTS AND DISCUSSION:** In our study, the administration of prophylactic phages within one dose could prevent MDR bacterial infection in a burn wound of a mouse model when colony counting was compared with control groups ( $p < 0.0001$ ). In addition, the count of colonies in the wound site after 14 days of one dose phage administering as prophylaxis was not significantly different from the result of treatment with a single dose of phage after infection ( $p = 0.9999$ ) and ( $p = 0.9828$ ). In vivo analysis of the isolated phages demonstrated these phages may be promising as the choice for prophylaxis. Therefore, it is possible to use phages as an alternative to antibiotics for prophylaxis in patients with burns and to reduce complications caused by antibiotic prophylaxis.

**Keywords:** MDR, bacteriophage, hospital sewage, *P. aeruginosa*, *A.baumannii*.

# Valid Serological Evidence of Latent Toxoplasma Infection in Infertile Women: A Ten-Year Registry-Based Study

## Bacteriology

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**BACKGROUND AND OBJECTIVES:** Some studies revealed that chronic infection with *Toxoplasma gondii* (*T. gondii*) has been associated with infertility in human and experimental models. This study aimed to investigate serological evidence of Toxoplasmosis among infertile women.

**MATERIALS AND METHODS:** In this retrospective (descriptive-analytical) study, all 520 infertile women referred to the IVF clinic at Imam Khomeini Hospital, Sari, Iran during 2010-2019, constituted the study population. Their demographic data and related characteristics were collected into a questionnaire and registered at the Iranian National Registry Center for Toxoplasmosis (INRCT) at the Mazandaran University of Medical Sciences. The anti-Toxoplasma antibodies (IgG and IgM) were detected by Enzyme-Linked Immune Sorbent Assay (ELISA) kit (PishtazTeb, Iran), based on the manufacturer's protocol.

**RESULTS AND DISCUSSION:** Anti-T. Gondi IgG, IgM and both antibodies were detected in 342/520 (65.77%), 1/520 (0.19%) and 4/520 (0.77%) patients, respectively. Primary and secondary infertility was detected in 74.56% and 25.44% of IgG seropositives, respectively. Most of the IgG seropositives had no history of abortion, polycystic ovary syndrome, fibroma and varicocele in spouse as causes of infertility. Serum levels of prolactin and Anti-Mullerian Hormone (AMH) were normal in 81.29 % and 80.12% of patients with anti- *T. gondii* IgG, respectively. There was a statistically significant difference between the seroprevalence of Toxoplasmosis and the variables associated to primary infertility ( $P < 0.05$ ). According to the high prevalence (about two thirds) of toxoplasmosis among infertile women, particularly those with a history of abortion and primary infertility, it is concluded that latent Toxoplasmosis has a risk to infertility. Therefore, it is recommended that screening tests and treatment of Toxoplasmosis in infertile women be favorably considered.

**Keywords:** Latent toxoplasmosis, Primary and secondary infertility, *Toxoplasma gondii*.

# Virulence Gene Profile and Multilocus Variable-Number Tandem-Repeat Analysis (MLVA) of Enteroinvasive Escherichia coli (EIEC) Isolates from Patients With Diarrhea in Kerman, Iran

Bacteriology

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**BACKGROUND AND OBJECTIVES:** Enteroinvasive Escherichia coli (EIEC) isolates cause dysentery in humans. Several virulence factors associated with EIEC pathogenesis have been characterized. Multilocus variable-number tandem-repeat analysis (MLVA) is a PCR-based method that has been used for genotyping bacterial pathogens. The aim of this study was to investigate the distribution of virulence factor genes in EIEC isolates from patients with diarrhea in Kerman, Iran, as well as the genetic relationships between these isolates.

**MATERIALS AND METHODS:** A total of 620 diarrheic stool samples were collected from patients attending two hospitals in Kerman from June 2013 to August 2014. All isolates were confirmed as EIEC by PCR for the ipaH gene. The EIEC isolates were evaluated by PCR for the presence of nine virulence genes (ial, set1A, sen, virF, invE, sat, sigA, pic, and sepA). MLVA was performed for all EIEC isolates.

**RESULTS AND DISCUSSION:** A total of 11 EIEC isolates were identified, and all were positive for the ial gene. The invE and virF genes were observed in 81.8% of the isolates, while sen, sigA, and pic were detected in 72.7%, 63.6%, and 27.3% of the isolates, respectively. None of the isolates were positive for the sat, set, and sepA genes. Using MLVA, the 11 total isolates were divided into five types. By studying the profiles of virulence genes and MLVA, it can be concluded that EIEC isolates do not have high heterogeneity and are derived from a limited number of clones.

**Keywords:** EIEC, MLVA, Virulence Factors, Diarrhea



# An Effective Prime-Boost Approach for Inducing a Strong Immune Response against Double-Layered Human Rotavirus Particles

Virology

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**BACKGROUND AND OBJECTIVES:** Rotaviruses are known for their pathogenicity in children under the age of five, causing severe gastroenteritis with a relatively noticeable mortality rate in endemic regions. The complex three-layered structure of this pathogen is an intriguing field of study. Extending our knowledge, particularly on double-layered particles (DLPs), can provide insight into various rotavirology areas, such as vaccine development, pathogenesis, host-virus interactions, diagnostic services, and fundamental research on the rotavirus lifecycle. In the present study, we initiated the first steps toward designing an indigenous DLP detection assay by harnessing recombinant viral protein 6 (rVP6) of the rotavirus in a robust protocol that led to a high-yield production of anti-VP6 polyclonal antibodies.



**MATERIALS AND METHODS:** Based on the prime-boost strategy, three different inoculation regimens were administered to three groups of New Zealand white rabbits, with each group receiving two shots at a 14-day interval. The first, second, and third groups received the virus/virus, rVP6/rVP6, and rVP6/virus regimens, respectively. Throughout the study, their antibody levels were constantly evaluated and compared using western blotting and ELISA. Additionally, a fourth group of rabbits was injected with phosphate-buffered saline in both shots, serving as our negative control.

**RESULTS AND DISCUSSION:** Results demonstrated that the rVP6/virus regimen elicited an antibody response twice as strong as other regimens with a single type of immunogen. It is considerably promising in terms of immunogenicity and cost-effectiveness because access to abundant anti-VP6 polyclonal antibodies enables us to facilitate the development of DLP detection assays and expedite their mass production.

**Keywords:** Rotavirus, DLP, VP6, Gastroenteritis, Prime-boost

# Evaluation of IFNAR2 and TYK2 Transcripts' Prognostic Role in COVID-19 Patients: A Retrospective Study

Virology

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**BACKGROUND AND OBJECTIVES:** Background and objectives: This study aimed to investigate the possible prognostic significance of interferon alpha–beta receptor subunit 2 (IFNAR2) and tyrosine kinase 2 (TYK2) expressions.

**MATERIALS AND METHODS:** Methods: We conducted a retrospective study including COVID-19 adult patients. All blood samples were collected before any interventions. The expressions of IFNAR2 and TYK2 were assessed using real-time PCR in venous blood samples of 54 cases and 56 controls. The transcript quantities of IFNAR2 and TYK2 genes were assessed using a Delta-Ct method.

**RESULTS AND DISCUSSION:** Results: Our findings show no significant differences in gene expression levels for IFNAR2 and TYK2 between patients who required oxygen (O<sub>2</sub>) therapy and those who did not (p-value = 0.732 and p-value = 0.629, respectively). Likewise, there were no significant differences in IFNAR2 and TYK2 expressions between patients hospitalized for less than 7 days and those hospitalized for 7 days or more (p-value = 0.455 and p-value = 0.626, respectively). We also observed a weak correlation between IFNAR2 expression and CRP (p-value = 0.045, r = 0.192). There was a negative correlation between the expression levels of IFNAR2 and TYK2 transcripts in COVID-19 patients (p-value = 0.044; partial correlation coefficient = -0.283). Additionally, IFNAR2 and TYK2 were significantly downregulated in the COVID-19 group compared to healthy subjects (p-value = 0.002 and p-value = 0.028, respectively). However, neither IFNAR2 nor TYK2 expression was significantly different between the case subgroups.

**Keywords:** coronavirus disease 2019, interferon alpha-beta receptor IFNAR2 subunit, prognostic factor,

# Genotype and frequency of human papilloma virus (HPV) in women with genital lesions in Mazandaran province

Virology

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**BACKGROUND AND OBJECTIVES:** Human papilloma virus (HPV) is one of the most common infections of the reproductive system, which is usually transmitted through sexual intercourse and causes a range of cancers and other clinical symptoms in men and women. HPV virus causes cervical cancer, which is the fourth most common cancer among women. This virus has an important role in the incidence and mortality of women, so it is considered as one of the public health priorities. Therefore, in this study, the genotype and frequency of human papilloma virus (HPV) in women with genital lesions in Mazandaran province were investigated.

**MATERIALS AND METHODS:** The present study was a descriptive-analytical study in which samples were taken from the genital lesions of 141 infected women from July 2023 to March 2024 and sent to the molecular department of the laboratory, and using the PCR method in terms of the presence of HPV and determining the genotype of the case were investigated. In this regard, by designing and completing the questionnaire, demographic information and risk factors were recorded. Data were analyzed using descriptive statistics by SPSS V.25 statistical software.

**RESULTS AND DISCUSSION:** In this research, out of 141 referrals, 88 women were under 30 years age, 33 of them were infected with HPV (29% of patients under 30 years of age) and 53 women were over 30 years age, that 25 women were infected with HPV (13.25% of patients were more than 30 years old). the number of women with HPV and high-risk genotypes has been higher in people under 30 years age. In both groups, type 52 has been the most frequent (37.93%), and it has been introduced as a high-risk genotype that will cause cervical cancer.

**Keywords:** Genotype, HPV, genital lesions, PCR

# Prevalence of the COVID 19 in samples of patients referred to medical centers in Mazandaran province from October 2022 to September 2023.

Virology

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**BACKGROUND AND OBJECTIVES:** BACKGROUND AND OBJECTIVES: At the end of 2019, a new disease called the Corona virus disease swept the world and in March 2020, the WHO officially announced the global spread or pandemic of this new virus and named this virus SARS-CoV-2 and or called COVID 19 and declared a state of emergency around the world. After that, a large number of people lost their lives and there were huge economic losses, which followed a global panic. At the end of 1398, its spread was confirmed in Iran, and after a short period of time, it has become a public crisis in which a significant number of people have lost their lives. Therefore, this study was conducted with the aim of determining the frequency of the COVID 19 virus in the samples of patients referred to medical centers in different cities of Mazandaran province from October 2022 to September 2023.

**MATERIALS AND METHODS:** MATERIALS AND METHODS: This is a cross-sectional study that was conducted by testing people with respiratory symptoms and suspected of having COVID-19 in a period of twelve months from October 2022 to September 2023 (Mehr 1401 to Shahrivar 1402) and during the Covid-19 pandemic in Iran. The COVID 19 virus was detected in the samples of nasopharyngeal and oropharyngeal secretions of patients referred to medical centers in Mazandaran province using extraction and diagnostic kits and Real Time PCR method.

**RESULTS AND DISCUSSION:** RESULTS AND DISCUSSION: In the period of twelve months from October 2022 to September 2023, a number of 22,626 people referred to medical centers in Mazandaran province to perform RT-PCR test for COVID 19 diagnosis. Out of this number of samples, 2952 cases, equal to 13.06% of clients, were reported as positive for the diagnostic test of COVID 19. The virus of COVID 19 is an unstable virus that new types of this type appear during epidemics and epidemics, which have different characteristics, as a result, in order to create immunity against them, new strains should be developed regarding the effectiveness of the vaccine. Monitored and controlled.

**Keywords:** COVID 19, Corona virus, RT-PCR, Pandemic.

# Effective Management of *Aspergillus versicolor* Onychomycosis: A Case Report Emphasizing Accurate Diagnosis and Targeted Treatment

Mycology

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**BACKGROUND AND OBJECTIVES:** Background and Objective: Onychomycosis is a common fungal nail infection caused by dermatophytes, non-dermatophyte molds, or yeasts. Accurate identification of causative agents is essential for effective management, particularly in immunocompromised patients.

**MATERIALS AND METHODS:** Case Presentation: A 61-year-old female farmer with hypertension and obesity, on atorvastatin medication, presented with a one-year history of nail lesions affecting her fourth and sixth toenails.

**RESULTS AND DISCUSSION:** Diagnostic tests, including potassium hydroxide examination and fungal culture, identified *Aspergillus versicolor* as the causative agent. Molecular techniques, such as DNA extraction, PCR amplification, Sanger sequencing, and BLAST analysis, confirmed the species identification (Accession number PP972760). Treatment: Due to potential drug interactions, the patient received oral terbinafine and topical clotrimazole for three months, avoiding itraconazole. Monthly clinical evaluations and liver function tests were performed during treatment. Conclusion: This case highlights the importance of accurate identification and targeted treatment for onychomycosis caused by non-dermatophyte molds, especially in immunocompromised patients, to ensure successful management and prevent potential complications.

**Keywords:** onychomycosis, *Aspergillus versicolor*, terbinafine, non-dermatophyte molds

# Investigation of Cyp51 Mutation in *Aspergillus* Species Resistant to Voriconazole

Mycology

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**BACKGROUND AND OBJECTIVES:** Voriconazole serves as the first-line treatment for pulmonary aspergillosis (PA), with itraconazole, posaconazole, amphotericin B, and caspofungin as subsequent options. Azole resistance in *Aspergillus* species can develop due to prolonged azole therapy or environmental exposure to azole fungicides. One major factor contributing to this resistance is mutations in the cyp51 genes, particularly cyp51A, cyp51B, and cyp51C, with cyp51A mutations playing a crucial role.

**MATERIALS AND METHODS:** This study collected 150 *Aspergillus flavus* and *Aspergillus fumigatus* isolates from PA patients at Masih Daneshvari Hospital. Minimum inhibitory concentrations (MICs) for voriconazole, itraconazole, caspofungin, and amphotericin B were determined according to the CLSI M38-A2 guidelines. Genomic DNA was extracted, and sequencing was performed to identify mutations in the Cyp51 gene hotspot in resistant, intermediate, and sensitive strains.

**RESULTS AND DISCUSSION:** The 150 samples comprised bronchoalveolar lavage fluid (BAL) (n = 101), tracheal secretion (n = 34), pleural fluid (n = 6), chest tube (n = 4), biopsy (n = 3), wound (n = 1), and aspiration (n = 1). Caspofungin (CFG) and amphotericin B (AMB) exhibited the lowest and highest geometric mean MICs, at 0.03 and 1.46 µg/ml, respectively. *Aspergillus flavus* isolates demonstrated higher sensitivity to the four antifungals compared to *Aspergillus fumigatus*. No mutations within the Cyp51A locus were observed in voriconazole-resistant or intermediate *A. fumigatus* strains. However, two mutations, T335A and D282E, were detected in voriconazole-intermediate *A. flavus* isolates. The findings underscore the need for comprehensive investigations into the factors contributing to azole resistance in clinical isolates. Such studies enhance our understanding and aid in developing effective strategies for managing and treating patients with pulmonary aspergillosis.

**Keywords:** Cyp51 Mutation, *Aspergillus*, Resistant to Voriconazole

# Microbes: identification of medicinal mushrooms and introduction of anticancer therapeutic effects

Mycology

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**BACKGROUND AND OBJECTIVES:** The oldest reliable scientific documents in the field of nutritional value of mushrooms and nutrition from them go back to the writings of Theophrastus in 370 BC, which mentioned the high nutritional value of mushrooms. Some medicinal mushrooms with antioxidant properties have been used for centuries to prevent and treat various diseases, including cancer and immunological disorders. Considering the different uses of mushrooms in the pharmaceutical industry, their identification is the first step in the medicinal use of these organisms. Therefore, the purpose of this research is to identify medicinal mushrooms from Darabkola Sari forests and to introduce anti-cancer therapeutic effects.

**MATERIALS AND METHODS:** This study was selected in the educational and research forest of Sari University of Agricultural Sciences and Natural Resources and after preliminary forest tours, sampling of fallen trees was done. Mushrooms were identified in the mycology laboratory through morphology and by measuring microscopic organs, 20 of each of these organs were measured using a microscope and some fungi were identified by extracting the DNA of the ITS nrDNA region using a pair of primers. ITS1 and ITS4 were amplified and sequenced and edited with Bio edit software and analyzed in NCBI.

**RESULTS AND DISCUSSION:** The results showed that 23 medicinal mushrooms with antioxidant effects and anti-tumor properties are distributed in this region, which include scientific names: *Cerrena unicolor*, *Daedaleopsis tricolor*, *Daldinia concentrica*, *Fomes fomentarius*, *Ganoderma adpersum*, *Ganoderma applanatum*, *Ganoderma lucidum*, *Ganoderma resinaceum*, *Heridium coralloides*, *Inonotus cuticularis*, *Irpex lacteus*, *Lenzites betulinus*, *Phlebia tremellosa*, *Pleurotus ostreatus*, *Schizophyllum commune*, *Stereum hirsutum*, *Stereum subtomentosum*, *Trametes gibbosa*, *Trametes versicolor*, *Trametes hirsuta*, *Trametes pubescens*, *Trichaptum biforme*, *Xylaria hypoxylon*. Over the past two decades, many advances have been made in relation to the role of the immune system in human health. Currently, diseases related to immune system dysfunction such as cancer, chronic fatigue and autoimmune conditions have attracted the attention of medical researchers and other similar sciences, and it is suggested to extract All kinds of metabolites from Iran's indigenous natural resources should be taken into consideration in the next stages of clinical trials.

**Keywords:** Ecosystem, medicinal mushrooms, cancer, antioxidant

# Post-COVID-19 fatal *Aspergillus* endocarditis: A case report

Mycology

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**BACKGROUND AND OBJECTIVES:** *Aspergillus* endocarditis (AE) is a rare fatal infection. The infection is often reported in patients with prosthetic heart valves, immunosuppressed, broad-spectrum antimicrobial use regimens, and drug abusers.

**MATERIALS AND METHODS:** Herein, we report a rare case of native mitral valve AE in a 63-year-old man, with a probable COVID-19-associated invasive pulmonary aspergillosis nine months ago treated with antifungals

**RESULTS AND DISCUSSION:** In the last admission, the lethargy, neurological deficit, and septic-embolic brain abscess in brain MRI led to suspicion of infective endocarditis. Transesophageal two-dimensional echocardiography and color Doppler flow velocity mapping showed a large highly mobile mass destroying leaflet and severe mitral regurgitation. The Surgical valve replacement is performed. Direct microscopic examination and culture of the explanted and vegetative mass revealed *Aspergillus* section *Fumigati* confirmed by molecular method. Despite the administration of voriconazole and transient improvement the patient expired. As AE is a late consequence of COVID-19-associated invasive pulmonary aspergillosis, therefore, long-term follow-up of invasive aspergillosis, and prompt diagnosis of surgical and systemic antifungal therapy treatment, are warranted to provide robust management.

**Keywords:** *Aspergillus fumigatus*, COVID-19, endocarditis, prosthetic heart valves



# Prevalence of candidiasis in patients with gastrointestinal tract disorders referred to Sari hospitals in 2020

## Mycology

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**BACKGROUND AND OBJECTIVES:** Introduction: systemic candidiasis develop in patients with immunocompromising or debilitating diseases and associated with a mortality rate of 70%. Digestive tract candidiasis association with serious underlying condition and in 20-50% of patient may be asymptomatic. In more than 2/3 cases, the esophagus was the only site involved. Chemotherapy in cancer patients and the use of high-dose corticosteroids and broad-spectrum antibiotics are the causes of yeast infection in the gastrointestinal tract. A reliable diagnosis of gastrointestinal candidiasis can only be made with histological evidence of tissue invasion in the biopsy sample. Endoscopy provides direct visualization of small, white, mucosal plaques that resemble viral esophagitis or cancer mimic. Dysphagia, odynophagia, epigastric pain, nausea and vomiting or hematemesis, fever, only occasionally occur.

**MATERIALS AND METHODS:** Materials and methods: This descriptive cross-sectional study was conducted in 2020 in Sari educational and therapeutic hospitals. biopsy samples were taken from 210 patients with gastrointestinal disorders by endoscopy and colonoscopy and were evaluated for fungal infection caused by *Candida* species. gastrointestinal candidiasis determined by mycological methods such as 10% KOH wet mount, histological examination, culture and molecular PCR-RFLP assay of ITS1-5.8S-ITS2 region with MSP1 enzyme and HWP1 primer.

**RESULTS AND DISCUSSION:** Results: Among 210 patients, 13 cases (6.2%) of gastrointestinal candidiasis were confirmed by diagnostic tests on biopsy. In total, 18 yeast colonies were obtained from patients, which *Candida glabrata* (38.4 %) and *Candida albicans* (23.1 %) were the most common species causing infection in the single agent. In 4 patients (30.8%) mixed infection caused by *C. albicans* and *C. glabrata* and in 1 case mixed infection caused by *C. Tropicalis* and *C. glabrata* was identified. Conclusion: The clinical symptoms of gastrointestinal disorders such as difficulty in swallowing, dysphagia, inflammation, hemorrhage and pain are similar in various diseases such as cancer, fungal and bacterial infections. Structural abnormalities seen in endoscopy or colonoscopy may be caused by neoplasm, surgery, trauma, or infection, and in some cases, *Candida* yeast after colonization may invade the tissue and increase the abnormality. Diagnosing candidiasis in the gastrointestinal tract disorders and appropriate treatment will reduce lesions and

**Keywords:** Key words: candidiasis, gastrointestinal disorders, endoscopy, colonoscopy, candida species

# Pulmonary Aspergillosis of *Aspergillus flavus* in Iran: Focus on Voriconazole Resistance and Yap1 Gene Mutations

Mycology

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**BACKGROUND AND OBJECTIVES:** In Iran, *Aspergillus flavus* is the most common cause of pulmonary aspergillosis. The clinical epidemiology of pulmonary aspergillosis in 101 patients and the antifungal susceptibility of etiologic *Aspergillus* species were studied in the present research, with a special focus on the frequency of voriconazole resistance.

**MATERIALS AND METHODS:** The minimum inhibitory concentrations (MICs) for all isolates concerning voriconazole, itraconazole, caspofungin, and amphotericin B (Sigma-Aldrich) were determined using the CLSI M38-A2 method. For voriconazole antifungal susceptibility, *A. flavus* isolates were categorized as susceptible (MIC 2 mg/mL), intermediate (MIC = 2 mg/mL), and resistant (MIC 2 mg/mL). The primer set Yap-mut was designed using the DNA sequences of isolates XM\_041289406 and XM\_745789. Total RNA was reversely transcribed into cDNA according to the manufacturer's instructions. Relative expression levels were calculated by the comparative cycle threshold ( $\Delta\Delta CT$ ) method, with the  $\beta$ -tubulin gene used as the internal standard.

**RESULTS AND DISCUSSION:** The lowest MIC/MEC values were observed for CFG VRC ITC AMB, respectively. Nine isolates displayed voriconazole MIC values greater than or equal to the epidemiological cutoff value. The expression and mutation of the Yap1 gene were analyzed in voriconazole intermediate/resistant isolates. In *A. flavus*, the A78C replacement in the Yap1 gene led to Q26H amino acid substitutions, which had not been previously reported in voriconazole-resistant *A. flavus*. Although there are still ambiguous points about the mechanisms of azole resistance, our results show that mutations were not seen in most resistant and intermediate isolates, while all of these isolates exhibited overexpression of the studied gene. In conclusion, it appears that the main cause of the emergence of mutations in voriconazole-resistant isolates of *A. flavus* is previous or prolonged exposure to azoles.

**Keywords:** *Aspergillus flavus*, Voriconazole Resistance, Yap1 Gene Mutations

# The clinic-mycological spectrum of *Candida* infection in diabetic foot ulcers in a tertiary care hospital

Mycology

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**BACKGROUND AND OBJECTIVES:** In diabetic foot ulcers, if fungal agents such as *Candida* species penetrate the cutaneous or depth of the ulcer, it can increase the wound severity and make it more difficult to heal. Limited studies on the rate of *Candida* infections in DFUs have been reported in recent years in our region. In many of these studies, detailed molecular studies have not been performed to determine *Candida* species causing the infection. Hence, we undertook the present study to assess the prevalence of *Candida* infection in DFUs and determine the spectrum of *Candida* species as an infectious agent.

**MATERIALS AND METHODS:** A cross-sectional study was performed on 100 diabetic patients with a foot ulcer from December 2019 to November 2020 in northern Iran. Patient data and wound grades were recorded in a questionnaire. *Candida* infection was confirmed by direct microscopic examination and culture. To identify the causative agent, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using *Msp1* enzyme and the partial amplification of hyphal wall proteins (HWP1) gene were performed.

**RESULTS AND DISCUSSION:** Of 100 patients, the mean age  $62.1 \pm 10.8$  years, 95% type 2 diabetes, 83% 10 years duration diabetes, 59% male, 66% poor education level, 99% married, 52% rural, 95% neuropathic symptoms, 88% using antibiotics, 69% HbA1C  $9\%$ , and mean ulcer grade  $2.6 \pm 1.05$  were. *Candida* infection was seen in 13% of the deep tissue and 7% surrounding the wound. The predominant *Candida* isolate was *C. parapsilosis* (71.5%) and *C. albicans* (14.3%). Infections caused by filamentous fungi were not detected. There was a statistically significant relationship between *Candida* infection and gender, rural, HbA1C, and ulcer grade. Mycological evaluations of diabetic foot ulcers often are ignored. Our study revealed that *Candida parapsilosis* is the most common causative agent of deep-seated foot ulcer infection in these patients and may require specific treatment. Therefore, more attention from physicians to *Candida* infections particularly, early diagnosis and prompt treatment, can help faster-wound healing and prevent amputation.

**Keywords:** Diabetic foot ulcer; fungal infection; *Candida*, *Candida parapsilosis*, risk factors

# Investigating the effects of Aloe vera on wounds caused by *Leishmania major* in sensitive laboratory animals

Protozoology

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**BACKGROUND AND OBJECTIVES:** Leishmaniasis is a vector borne parasitic disease that transmitted by sandflies, either *Phlebotomus* (Old world) or *Lutzomyia* (New World). *Leishmania* are among the most diverse of human pathogens, in terms of both geographical distribution and variety of clinical manifestations. Almost 102 countries/areas are endemic for *Leishmania* infections. Globally, the population at risk is 350 million, overall prevalence being 12 million and 2 million new cases occur annually. This disease appears in four major clinical forms, including visceral leishmaniasis (VL, kala-azar), cutaneous leishmaniasis (CL), post-kala-azar dermal leishmaniasis (PKDL), and mucocutaneous leishmaniasis. There are approximately 500 species of the genus *Aloe* (Liliaceae). *Aloe vera* is the most widely used specie. Enzyme carboxypeptidase and bradykinase known to relief pain, an anti-inflammatory compound aloeresin I and dihydrocoumarins with immunomodulatory and antioxidative properties are found in *A. vera*. *Aloe* gels have the ability to protect ulcer formation.

**MATERIALS AND METHODS:** In order to prepare ethanolic extract, 30 grams of dried powder of *Aloe vera* was used. The study was conducted on 50 female mice. To infect the mice, the standard strain of *Leishmania major* (MRHO/IR/I5/ER) was used. Mice were divided into 5 groups of 10, including A1 (positive control, treatment with Glucantime), B1 (treatment with 1 concentration of extract in distilled water), C1 (treatment with 0.1 concentration of extract in distilled water), D1 (treatment with 0.01 concentration of extract in distilled water) and E1 (negative control, treatment with distilled water). 2 ml of Standard strain was inoculated subcutaneously to all mice. To perform the test in vitro condition, 1 ml of the strain prepared under sterile conditions was transferred to tubes containing 5 ml of RPMI 1640 medium and 5 ml of fetal serum, and after successive passages, 10<sup>6</sup> parasites grew in the culture.

**RESULTS AND DISCUSSION:** The best result among the 5 studied groups belonged to B1. The best concentration of *Aloe vera* extract with anti-leishmania effect in the culture medium is 0.1% and 0.01% in the first 15 and 60 minutes of the experiment. This study shows the growth inhibitory and lethal effects of *Aloe vera* extract in laboratory on the promastigote form of *Leishmania major*.

**Keywords:** Leishmaniasis - *Aloe vera*

# A case report of respiratory bacterial, fungal, viral and parasitic infections in a COPD patient

Protozoology

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**BACKGROUND AND OBJECTIVES:** SARS-CoV-2 is the causative agent of COVID-19, a disease that can manifest with mild to severe respiratory symptoms in individuals. Patients, particularly those with severe cases, are susceptible to various infections, including bacterial, fungal and even parasitic infections.

**MATERIALS AND METHODS:** In a specific case, a 70-year-old patient with COPD presented to a hospital in northern Iran complaining of breathing difficulties. The patient had experienced shortness of breath two weeks prior to the visit, which progressively worsened over time. Considering the potential respiratory issues associated with the parasite *Acanthamoeba*, a sample was taken from the nasal and pharyngeal swab and cultured, leading to the detection of the parasite. Additionally, the ability of *Acanthamoeba* to harbor different microorganisms prompted a survey into endosymbiont fungi and bacteria. *Stenotrophomonas maltophilia* (*S. maltophilia*), a bacterium known for its resistance to most antibiotics and its significance as a nosocomial pathogen, was identified. Furthermore, for the first time globally, *Gloeotinia* fungus was discovered as an endosymbiont of *Acanthamoeba*.

**RESULTS AND DISCUSSION:** The patient underwent a successful treatment regimen. Immunocompromised individuals should be particularly concerned about the increasing incidence of nosocomial and community-acquired *S. maltophilia* infections due to the high fatality-to-case ratio associated with this bacterial pathogen. Moreover, *Acanthamoeba* not only possesses pathogenic properties itself but also facilitates the transmission of various microorganisms, leading to diverse infections. Some recent studies have shown that *Acanthamoeba* lacking endosymbionts exhibit mild pathogenicity, whereas those with endosymbionts demonstrate high pathogenicity. Therefore, the presence of *Acanthamoeba* should not be overlooked in respiratory disorders, as it has the potential to carry numerous pathogenic microorganisms as endosymbionts.

**Keywords:** SARS-CoV-2, COPD, *Acanthamoeba*, Endosymbiont, *Stenotrophomonas maltophilia*, *Gloeotinia*

## Amoebiasis or IBD: a case report

### Protozoology

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**BACKGROUND AND OBJECTIVES:** Background and Objectives: Amoebiasis caused by the protozoan *Entamoeba histolytica* is common in tropical and subtropical regions, but uncommon in developed countries. Because amoebic colitis has similar clinical symptoms and endoscopic features to inflammatory bowel disease (IBD), these cases can be misdiagnosed.

**MATERIALS AND METHODS:** Identification and differential diagnosis of this amoeba was done by PCR method. A 36-year-old man with invasive intestinal amoebiasis was referred to the gastroenterology clinic with symptoms of diarrhea, sometimes bloody, along with abdominal pain and discomfort in the right lower quadrant and a weight loss of 18 pounds. Routine blood tests including Complete Blood Count (CBC), Hemoglobin, Electrolytes, Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), Amylase, Lipase, and liver enzymes were normal. Endoscopic biopsy showed mild colitis.

**RESULTS AND DISCUSSION:** : The patient was treated with corticosteroids for Crohn's disease which subsequently rapidly worsened and was hospitalized for acute exacerbation of Crohn's with multiple perforations in the colon and ileum. Re-examination of stool for this patient identified the protozoan *Entamoeba histolytica* and he was treated with anti-parasitic drugs. Invasive intestinal amoebiasis should be considered as a differential diagnosis in the first clinical presentation of adults with IBD-like symptoms. Regardless of negative endoscopic biopsies, due to the low sensitivity of microscopic examination, serological testing for antibodies and molecular testing for *Entamoeba* DNA for accurate diagnosis and identification of *Entamoeba* species, especially in high-risk populations with recent trips to endemic areas and for patients with It is recommended to suppress the immune system.

**Keywords:** Amoebic colitis, Clinical symptoms, *Entamoeba histolytica*, Infected patients.

# Anti Giardial effects of the methanolic extracts of *Rhamnus cathartica*

Protozoology

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**BACKGROUND AND OBJECTIVES:** *Giardia lamblia* (*G. lamblia*) is an intestinal flagellate of important protozoan parasites of medical and public health in Iran and other parts of the world. Given the importance of treatment in patients with Giardiasis, particularly with the use of medicinal plants and parasite resistance to chemical drugs, in the present study the effect of methanolic extract of *Rhamnus cathartica* (*R. cathartica*) on the cystic stage of the *G. lamblia* in vitro was done.

**MATERIALS AND METHODS:** In this experimental study methanolic extracts of *R. cathartica* prepared in 100, 200, 400 and 800 mg/ml concentrations, and cysts of *Giardia* isolated from stools of patient by sucrose solution 0.85M. Then, methanolic extract after diluting affected on *Giardia* cysts, the results were compared with the control groups. Also, for the quality control of this experiment, PBS and met were used instead of plant extract. anti-giardial effects and cytotoxicity of the extracts at concentrations of 100, 200, 400 and 800 g/ml were investigated using eosin1%. Non-toxicity of plant extracts for normal cells were measured by MTT method.

**RESULTS AND DISCUSSION:** Results of this study indicated that the methanolic extracts of *R. cathartica* in all concentrations had acceptable anti-Giardial activity against *G. lamblia* and concentration of 800 mg/ml of methanolic extracted of *R. cathartica* after 13 and 24 hours has the most killing and cytotoxicity activity on *G. lamblia* cysts in vitro. Moreover, the concentration of 800mg/ml of *R. cathartica* after 24 hour has the highest cytotoxicity effect on *G. lamblia* cysts.

**Keywords:** anti-giardiasis, *Giardia lambelia*, methanolic extract, Herbal medicine

# Antimalarial nano-drug delivery system based on graphene quantum dot on *Plasmodium falciparum*: Preparation, characterization, toxicological evaluation

Protozoology

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**BACKGROUND AND OBJECTIVES:** Malaria infects millions of people every year. Emerging drug resistance in many strains of *Plasmodium falciparum* against the currently used antimalarial is one of the major obstacles in the way of elimination of *falciparum* malaria infection.

**MATERIALS AND METHODS:** Sodium hydroxide, citric acid, methanol, ethanol and Giemsa stain were obtained from Merck. Artesunate (Art) (MW = 384.42, cat No. A3731), Mefloquine (Mef) (MW = 414.77, EC No. 257-412-0, MDL No. MFCD00797519), Gentamicin, sorbitol and Dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. Packed red blood cells O+ (PC), Fresh frozen plasma AB+ (FFP) were obtained from Blood Transfusion organization of Iran. Normal Saline Solution was purchased from Iranian parenteral and pharmaceutical co. RPMI 1640 Medium.

**RESULTS AND DISCUSSION:** The FESEM and TEM images of GQD are presented in Fig. 1A and B. The GQDs are well dispersed with a uniform size distribution, whose average sizes are about 6.53 nm. TEM images also show crystalline GQDs with a lattice measurement of 0.203 nm, which coincide with the graphene (002) plane. The images with a hexagonal honeycomb structure demonstrate that the GQDs are nearly defect-free graphene single crystals. Numerous literature reports signify the nanoparticles to be efficient peroral drug delivery systems. However, their efficient uptake is evidently charge and size-dependent with smaller nanoparticles generally. Malaria is one of the most important parasitic infections in the world. More than 40 % of the world's population lives in malaria-endemic areas. Due to the emergence of *P. falciparum* strains resistant to chloroquine, mefloquine, and artesunate in recent decades, developing countries facing difficulties.

**Keywords:** Artesunate Graphene quantum dot Malaria



# Application of HRM (High-Resolution Melting) in the Detection of Blood-Tissue Protozoa

Protozoology

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**BACKGROUND AND OBJECTIVES:** Blood protozoa and tissues are found dispersed throughout the world, thereby presenting substantial challenges in their detection. The technique used for the detection of protozoans is the HRM technique. The objective of this study was to examine the application of HRM in articles about the diagnosis of protozoa.

**MATERIALS AND METHODS:** In this review study, articles related to Application of HRM High-Resolution Melting in the detection of blood-tissue protozoa were reviewed in various databases of Web of science, Pubmed, Scopus, Google Scholar and etc from 2018 to 2024.

**RESULTS AND DISCUSSION:** In a 2020 study in Gonbad and Bam cities, out of 105 skin smears, 89.5% were infected with Leishmania by HRM technique. In a study conducted in Ardabil in 2022 to detect Leishmania among 19 blood donors confirmed by DAT method, 79% were positive by HRM technique. In a study conducted in Chabahar and Tehran, among 81 blood samples, 33 samples of *P. vivax*, 11 samples of *P. falciparum* and 1 sample of mixed infection were detected using HRM technique. In a study conducted in Tehran in 2020 to detect *Toxoplasma gondii* in pregnant women and HIV+ people, out of 242 people, 50 people were positive for the acute phase and 70 people were positive for the chronic phase tested positive with HRM technique. **DISCUSSION:** Early and prompt identification of these protozoa is of great significance for effective patient management. Existing literature suggests that the HRM technique represents one of

**Keywords:** HRM, protozoa, Blood, tissue

# Co-infection with *Entamoeba histolytica* and *Strongyloides stercoralis*

## Protozoology

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**BACKGROUND AND OBJECTIVES:** Amoebiasis is caused by the protozoan *Entamoeba histolytica* and is one of the three common parasites that cause human death. *Strongyloides stercoralis* is a neglected helminthic infection that affects an estimated 30–100 million people worldwide. This parasitic infection can be asymptomatic in immunocompromised individuals but is more vulnerable in patients with underlying disease, and mortality is high due to disseminated infection and hyperinflation.

**MATERIALS AND METHODS:** The patient is a 35-year-old man living in the village who had visited the Hospital in Babol. The patient had a fever, chills, and cough for 2 years. Eleven months ago, he developed a severe cough and disturbed the patient's sleep. Five months before the visit, the patient had been hospitalized four times (two times each, about 20 days) in Amol Hospital and Tehran. The patient stated, "The cough becomes more intense after eating and at night." The patient lost 25 kg of weight and had purulent discharge from the left ear, weakness, lethargy, diarrhea, and severe fever and chills 5 weeks ago. Bronchiectasis was also diagnosed in the patient. A colonoscopy showed gastritis, enteritis, and rectal polyps in the right colon.

**RESULTS AND DISCUSSION:** : The cause of diarrhea was diagnosed as strongyloidiasis infection along with amoebiasis. Diarrhea was cured after two weeks. The patient's fever continued and was accompanied by profuse sweating (so that the patient's clothes got wet) in the evenings. The important findings of the patient included the following; (ESR): 61 mm/hr. (Normal: 1-13 mm/hr.), (CRP): 85 mg/dl (Normal: 1 mg/dl ), (IGC) =600 (Normal: 700-1600), Hemoglobin (Hb)=7.3 g/dl (Normal: 12.4-14.9 g/dl). Conclusion: Mebendazole (100 mg), ivermectin (3 mg), IVIG, blood transfusion, and hydrocortisone were also prescribed to the patient. Treatment of water and electrolyte compensation in the acute phase of diarrhea long-term use of antibiotics such as metronidazole personal hygiene and proper waste disposal are necessary to prevent the spread of the disease. Clinical symptoms are similar in different patients and some cases. Cough, fever, chills, and respiratory and digestive disorders appeared in almost all patients.

**Keywords:** Clinical symptoms, *Entamoeba histolytica*, Immune suppression, Infected patients, *Strongyloides stercoralis*.

# Comparison of the clinical features and outcome of children with hemophagocytic lymphohistiocytosis (HLH) secondary to visceral leishmaniasis and primary HLH

Protozoology

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**BACKGROUND AND OBJECTIVES:** Hemophagocytic lymphohistiocytosis (HLH) is an aggressive and life-threatening syndrome of excessive immune activation. It most frequently affects infants from birth to 18 months of age, but the disease is also observed in children and adults of all ages. HLH can occur as a familial or sporadic disorder. It can be triggered by a variety of events that disrupt immune homeostasis. Infection is a common trigger both in those with a genetic predisposition and in sporadic cases. Prompt treatment is critical. Barriers to a successful outcome are often a delay in diagnosis due to: The rarity of this syndrome, Variable clinical presentation, Lack of specificity of the clinical and laboratory findings. It may be seen either primarily or secondary to infectious or immuno-inflammatory diseases.

**MATERIALS AND METHODS:** The study was conducted in Amir Oncology Hospital, affiliated to Shiraz University of Medical Sciences, Shiraz, Iran. A total of 60 patients who were diagnosed as HLH between September 2014 and December 2018 were registered. Information on demographic characteristics, clinical, laboratory, and radiological findings at presentation, genetic mutation analysis if available, type of treatment and response to treatment, allogenic hematopoietic stem cell transplantation and survival outcomes, underlying disorders including immune deficiency, malignancy, and infectious disorders were collected. New patients were screened for Perforin gene mutation and sCD25. Patients were classified into two groups of either primary or secondary HLH.

**RESULTS AND DISCUSSION:** Thirty-four patients were male and 26 were female, mean-age was 25.7 months. Twenty-one patients (35%) were categorized as secondary HLH which included: 19 patients (90.48 %) with visceral leishmaniasis, 1 patient (4.76%) with Chediak-higashi syndrome, 1 patient (4.76%) with lymphoma-associated HLH. Fever and splenomegaly were the most frequent clinical findings. Serum ferritin and LDH were consistently high in all cases. Soluble CD25 was measured in 20 patients, which was high in 14 (70%), There was no significant correlation between diagnosis of HLH based on HLH-2004 criteria and probability of disease according to H-score (P=0.256). Nearly a third of patients and most of the secondary HLH patients were associated with visceral leishmaniasis, therefore, investigation in the endemic area is recommended.

**Keywords:** Primary HLH, Secondary HLH, Visceral Leishmaniasis, H-Score, Soluble CD25,

# Identification and genotyping of *Acanthamoeba* spp., in bronchoalveolar lavage fluid from immunocompetent patients with chronic respiratory disorders (CRD)

Protozoology

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**BACKGROUND AND OBJECTIVES:** This study aimed to detect *Acanthamoeba* spp., in BALF specimens of immunocompetent patients with respiratory disorders (CRD) using polymerase chain reaction (PCR) and determine their genotypes.


**MATERIALS AND METHODS:** In this study, 211 BALF samples were collected from patients with CRD during the COVID-19 pandemic who were candidates for fiberoptic bronchoscopy (FOB) at Imam Khomeini Hospital, Sari, Mazandaran Province, northern Iran and investigated for *Acanthamoeba* spp., by PCR and sequencing.

**RESULTS AND DISCUSSION:** PCR test showed that *Acanthamoeba* spp. in 5 of the 211 samples (2.36%). Genetic analysis revealed that three of the *Acanthamoeba* strains belonged to the T4 genotype and one to the T2 genotype. This finding highlights the presence of *Acanthamoeba* in the lungs of people with respiratory infections. However, it's important to note that this might be a coincidence and not necessarily a cause of the respiratory problems. More research is needed to determine if *Acanthamoeba* actually plays a role in lung infections.

**Keywords:** Bronchoalveolar lavage, *Acanthamoeba*, PCR, T4 genotype, T2 genotype

# Identify of Intestinal Microsporidia in HIV + /AIDS and Cancer Patients Undergoing Chemotherapy in Mazandaran Province

## Protozoology

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**BACKGROUND AND OBJECTIVES:** The aim of this study was to identify *Enterocytozoon bieneusi* and *Encephalitozoon* spp. in fecal samples of HIV + /AIDS and cancer patients undergoing chemotherapy, and comparing the results to healthy individuals in Mazandaran province, north of Iran.

**MATERIALS AND METHODS:** Stool samples were collected from 50 HIV + /AIDS patients, 50 cancer patients, and 50 healthy samples referred to medical centers in north of Iran. Stool samples were kept in 2.5% potassium dichromate at 4 °C, and stained by modified trichrome for light microscopy examination. The multiplex/nested-PCR targeted the small subunit ribosomal RNA (SSU rRNA) gene. To characterize genotypes, the nested PCR products sequenced by Bioneer Company and was subjected to phylogenetic analyses.

**RESULTS AND DISCUSSION:** 10 of 50 samples (20%) of HIV + /AIDS patients, 5 of 50 samples (10%) of cancer patients, and 1 of healthy individuals (2%) were microscopically positive. From 50 HIV + / AIDS patients, *E. bieneusi* and *Encephalitozoon* spp. were detected in 10 (20%) and 6 (12%) cases, among cancer patients, 7 (14%) and 2 (4%) cases were *E. bieneusi* and *Encephalitozoon* spp. respectively. Out of 50 samples of healthy individuals, 3 (6%) cases of *E. bieneusi* were observed. The genotypes D and M were detected among positive samples of *E. bieneusi*. *E. bieneusi* and then *Encephalitozoon* spp. are common intestinal microsporidia in HIV + /AIDS patients and cancer patients undergoing chemotherapy in Mazandaran province. *E. bieneusi* genotype D seems to be the predominant genotype in Mazandaran province. Due to the considerable prevalence of intestinal microsporidia, physicians are advised to pay more attention to this opportunistic infection in high-risk groups.

**Keywords:** Cancer, *E. bienersi*, *Encephalitozoon* spp., Genotyping, HIV + /AIDS.

# Isolation and pathogenicity assay of *Acanthamoeba* and its endosymbionts in COVID-19 patients and respiratory disorders

Protozoology

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**BACKGROUND AND OBJECTIVES:** *Acanthamoeba* spp., are common free-living amoebae found in nature and occasionally in clinical samples that can serve as reservoirs for certain microorganisms. The SARS-CoV-2 virus is a newly emerged respiratory infection, and the investigation of parasitic infections remains an area of limited research. Given that *Acanthamoeba* can act as a host for various endosymbiotic microbial pathogens and its pathogenicity assay is not fully understood, this study aimed to identify *Acanthamoeba* and its bacterial and fungal endosymbionts in patients with chronic respiratory disorders and hospitalized COVID-19 patients in northern Iran. Additionally, a pathogenicity assay was conducted on *Acanthamoeba* isolates.

**MATERIALS AND METHODS:** Lung samples, urine and nasal swab samples were collected from a total of 150 COVID-19 hospitalized patients and 150 patients with chronic respiratory disorder. Then were cultured on non-nutritive agar medium and were checked for the presence of *Acanthamoeba* for two weeks. Positive samples were passed to a new fresh NNA medium to remove microbial and fungal contamination. Sediment of scraped and washed positive samples were used for extracting DNA using a commercial kit (Roche, Mannheim, Germany). The extracted DNA was amplified using an *Acanthamoeba* specific primer pair. All the positive isolates were sequenced and using the NCBI site based on the highest homology, the *Acanthamoeba* genotype was determined. Endosymbiotic bacteria and fungi were also identified through universal primers. Using the methods of osmotolerance, thermotolerance and cytopathic effect assay, the pathogenicity of the isolates was evaluated.

**RESULTS AND DISCUSSION:** Results: Eight *Acanthamoeba* isolates were identified, and PCR was performed to confirm the presence of amoebae and identify their endosymbionts. Four isolates were found to have bacterial endosymbionts, including *Stenotrophomonas maltophilia* and *Achromobacter* sp., while two isolates harbored fungal endosymbionts, including an uncultured fungus and *Gloeotinia* sp. In the pathogenicity assay, five isolates exhibited a higher degree of pathogenicity compared to the other three. This study provides significant insights into the comorbidity of acanthamoebiasis and COVID-19 on a global scale, and presents the first evidence of *Gloeotinia* sp. as a fungal endosymbiont. Nevertheless, further research is required to fully comprehend the symbiotic patterns and establish effective treatment protocols.

**Keywords:** *Acanthamoeba*, COVID-19, Cytopathic effect assays, Endosymbiont, Osmo-tolerance, Thermo-tolerance

# Prevalence and Risk Factors of Cryptosporidium Infection in Patients Referred Therapeutic Centers of Babol, Northern Iran

## Protozoology

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**BACKGROUND AND OBJECTIVES:** Cryptosporidiosis is a parasitic disease caused by a small cell of the genus. This disease has a global distribution and causes persistent and severe diarrhea in immunocompromised people. In recent decades, Cryptosporidium has been introduced as a new pathogen in humans, which, in addition to healthy people with a normal immune system, can also cause diarrhea in immunocompromised people, so the study of this parasite in different regions in immunocompromised people and Their treatment plays an effective role in maintaining the health of society. At the same time, parameters such as residence, parents' income and educational status, child's nutritional status, drinking water status, contact with animals and the number of family members are also effective in infection with this parasite.

**MATERIALS AND METHODS:** From May to February 1402, a total of 168 stool samples were collected from patients with weak immune system and different degrees of diarrhea referring to different departments of medical centers in Babol city and suburbs. To examine the samples, microscopic diagnosis was immediately performed using the wet spread method, the formalin concentration-ethro staining process with the modified Ziel-Nelson method for coccidia group parasites.

**RESULTS AND DISCUSSION:** Among the parasitic agents observed from the total of different diagnostic methods for the group of coccidia, only Cryptosporidium was observed in 1.2% of patients. Discussion: Regarding the importance of diarrheal diseases, Cryptosporidium is an intestinal protozoan that causes self-limiting diarrheal disease that can cause severe disease in immunocompromised patients. Mazandaran province has the potential of contracting common diseases between humans and animals on a large scale due to free and industrial animal husbandry as well as climatic conditions and high humidity, food culture and abundance of surface water. However, it is necessary to accurately diagnose Cryptosporidium and other parasitic infections in the feces of patients with diarrhea who refer to hospitals or reference laboratories with appropriate parasitological methods.

**Keywords:** Clinical symptoms, Cryptosporidium Infection, Northern Iran, Therapeutic Centers.



# Seroepidemiology of toxoplasmosis and its risk factors in northern Iran

Protozoology

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**BACKGROUND AND OBJECTIVES:** : *Toxoplasma gondii* is an obligate intracellular protozoan and an opportunistic and zoonotic pathogen with global distribution that infects all warm-blooded animals, including humans, from all over the world. There is a great variation in the seroprevalence of *Toxoplasma gondii* infection in different regions of the world, where approximately 1.3 of the world's population is infected with this parasite. This infectious disease is often fatal in people with HIV/AIDS, neoplastic disease, recipients of bone marrow or heart transplants, but it leads to stable protective immunity in healthy people. Although toxoplasmosis is an infectious disease in Iran, little is known about its epidemiology in the general population. This study was conducted with the aim of investigating the epidemiological situation of *Toxoplasma gondii* infection in a population of women and men in Babol city.

**MATERIALS AND METHODS:** This cross-sectional study was conducted with the aim of investigating the seroprevalence of *Toxoplasma gondii* infection in Babol, Iran from December 2017 to January 2018. 722 sera were screened for IgG and IgM anti-*T. gondii* antibodies by enzyme-linked immunosorbent assay (ELISA).

**RESULTS AND DISCUSSION:** Out of 722 participants, 520 (72%) were women and 202 (28%) were men. Among women, 220 (42%) and 82 (41%) men were positive for anti-toxoplasma IgG. Anti-toxoplasma IgM was positive in only 4 of the participants (age 2-16 years). The overall prevalence of anti-*Toxoplasma gondii* IgG and IgM in the studied population was 42% and 0.005%, respectively. Conclusion: These findings provide information about the seroprevalence and epidemiology of *Toxoplasma gondii* infection in Babol, which shows a high chronic or latent infection in this population. Considering the importance of *Toxoplasma gondii* infection and the possibility of its reactivation and transformation from chronic to acute infection, screening and comprehensive evaluation to determine the status of anti-*Toxoplasma* antibodies in infants, patients with immunosuppression and other people with weak immunity, in different regions Iran is needed.

**Keywords:** Cross-sectional study, Epidemiology, Immunoassay, Toxoplasmosis.

# Seroprevalence and Molecular diagnosis of toxoplasmosis in Patients with Thalassemia in Mazandaran, Iran

Protozoology

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**BACKGROUND AND OBJECTIVES:** *Toxoplasma gondii* has a high prevalence in Mazandaran province. This parasite has special importance in pregnant women and immunocompromised individuals that are significantly increasing. Different types of *T. gondii* are able to develop a wide range of toxoplasmosis from an asymptomatic to fatal form. Therefore, the aim of this study was to evaluate the seroprevalence and genotypes of *T. gondii* in patients with thalassemia in Mazandaran province using ELISA and nested PCR method.

**MATERIALS AND METHODS:** A total of 300 blood samples from thalassemia patients in Mazandaran province were collected. A serological test was performed for thalassemia patients. DNA extraction was conducted on the samples and PCR test was performed using RE gene for molecular screening. Finally, genotyping *T. gondii* was performed on positive molecular samples using the nested PCR and GRA6 gene method. The samples were sequenced and the phylogenetic tree was drawn.

**RESULTS AND DISCUSSION:** 59.7 % of thalassemia patients had IgG antibodies anti-*T. gondii* and 0.6 % had IgM. 2.7% of thalassemia patients had DNA of *T. gondii*. The results of phylogenetic analysis showed that 1 of samples were classified in group I (highly pathogenic), 2 in group II and 1 in group III. this study showed that thalassemia patients in northern Iran could be at high risk of acute toxoplasmosis and infection. In addition, this study shows that the majority of *T. gondii* strains in human samples in Mazandaran province are clonal type II. Due to the high prevalence of *T. gondii* in thalassemia patients and the significant prevalence of pathogenic type I clonal and its close varieties, it is necessary to take special measures to prevent toxoplasmic encephalitis and irreversible complications of this disease.

**Keywords:** *Toxoplasma gondii*, thalassemia, Elisa, PCR, genotype, Mazandaran

# The presence of Leishmania RNA virus 2 (LRV2) in Leishmania major infections is associated with more severe skin lesions and reduced effectiveness of meglumine antimoniate treatment.

Protozoology

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**BACKGROUND AND OBJECTIVES:** The present study investigated the possible role of Leishmania RNA virus 2 (LRV2) in the severity of dermal lesions and treatment failure due to Leishmania major.

**MATERIALS AND METHODS:** The drug susceptibility of 14 clinical isolates of L. major, including resistant (n = 7) and sensitive (n = 7) isolates, was checked in the J774A.1 macrophage cell line. The presence of LRV2 among isolates was investigated by the RdRp gene and semi-nested PCR. Moreover, 1 × 10<sup>6</sup> sensitive L. major LRV2<sup>+</sup> and LRV2<sup>-</sup> promastigotes were inoculated subcutaneously into the base tails of the 40 BALB/c mice divided into 4 groups (n = 10 in each group), including clinical LRV2<sup>+</sup>, clinical LRV2<sup>-</sup>, positive control LRV2<sup>+</sup> and negative control LRV2<sup>-</sup>. The groups were infected with a unique isolate. The lesion size and parasite burden were evaluated.

**RESULTS AND DISCUSSION:** Mice infected with L. major strains carrying LRV2 developed significantly larger skin lesions compared to mice infected with LRV2-free strains (p=0.034). Mice inoculated with LRV2-positive L. major strains had a significantly higher parasite burden compared to mice infected with LRV2-negative strains (p=0.002). In other the hand, a higher proportion of meglumine antimoniate (MA)-resistant L. major strains contained LRV2 (6 out of 7 resistant vs. 4 out of 7 susceptible), this difference was not statistically significant (p=0.237). While a higher proportion of drug-resistant Leishmania major strains contained LRV2, this difference was not statistically significant. However, mice infected with L. major strains carrying LRV2 developed significantly larger skin lesions and had higher parasite loads compared to mice infected with LRV2-free strains. This suggests that LRV2 might play a role in worsening cutaneous leishmaniasis (CL) and possibly contributing to treatment resistance, though further research is needed to confirm this connection.

**Keywords:** Leishmania RNA virus 2 , Leishmania major, pathogenesis

# Toxoplasmosis seroprevalence in a drug-abusing population: insights from an Iranian case-control study

Protozoology

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**BACKGROUND AND OBJECTIVES:** *Toxoplasma gondii* (*T. gondii*) is an intracellular protozoan parasite that infects approximately one-third of the global population. Studies have suggested that *T. gondii* infection can induce neurological changes, behavioral alterations, and psychological disorders through various mechanisms. This descriptive-analytical study aimed to measure and compare the levels of *T. gondii* IgG antibodies using the ELISA method in individuals with a history of drug abuse and healthy individuals.

**MATERIALS AND METHODS:** The study included 105 drug addicts as the case group and 105 non-addicted individuals as the control group. All participants were over 15 years of age. Blood samples were collected, and sera were separated to assess the presence of IgG antibodies against *T. gondii* using the ELISA method. The results between the case and control groups were compared using chi-square statistical analysis and a univariate logistic regression model.

**RESULTS AND DISCUSSION:** RESULTS: The results showed that 27 individuals (25.7%) in the case group and 12 individuals (11.4%) in the control group had IgG antibodies against *T. gondii*. The chi-square test revealed a statistically significant correlation between the presence of IgG antibodies and the case/control group status ( $P < 0.05$ ). However, when controlling for the effect of other variables using a univariate logistic regression model, this relationship was not statistically significant ( $P > 0.05$ ). Additionally, the analysis showed that occupation, contact with other animals, and the type of substance used were significantly associated with *T. gondii* infection ( $P < 0.001$ ). DISCUSSION: Although the hypothesis of an association between toxoplasmosis and addiction was not supported in this study, the potential role of *T. gondii* infection in drug abuse cannot be entirely ruled out. Further research is needed in this field to better understand the complex interplay between parasitic infections and substance use disorders

**Keywords:** *Toxoplasma gondii*, drug abuse, seroprevalence, case-control study, substance use disorders

# Strongyloides hyperinfection syndrome: a case report

The important role of the microbiology laboratory in controlling hospital infections

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**BACKGROUND AND OBJECTIVES:** Strongyloides stercoralis is endemic in the northern provinces and in order to identify it, in addition to clinical tests, clinical manifestations of the patient are also needed. The aim of this study was to investigate the clinical manifestations of patients with Strongyloides stercoralis infection.

**MATERIALS AND METHODS:** The patient is a 60-year-old male resident of the village who came to Babol Hospital with a cough, weight loss, nausea, fever, and night sweats. He visited the doctor several times and took medicine to treat and diagnose the cause of his cough. A positive response to the drugs prescribed for the treatment of cough was observed within a few days, but the patient's cough started again after stopping the drug and increased within two weeks, so the patient could not sleep at night due to the severity of the disease. The cough was accompanied by white sputum and became more severe in the early morning. Hemoptysis and respiratory distress were not observed. The patient took a bronchodilator spray, but the severe cough continued. The patient had relatively adequate sleep and rest. The patient was prescribed intravenous immunoglobulin, daily nasal irrigation with normal saline, and a low-salt and low-fat diet.

**RESULTS AND DISCUSSION:** Results: By examining the patient's clinical symptoms and several stages of stool testing, it was determined that he was infected with Strongyloides stercoralis and he was treated with anti-parasitic drugs. Discussion: It seems that the need for awareness and attitude is more in our country as a native region and it is found in the northern cities of Iran due to its humid climate, especially in rural areas more than other areas. Following tests and clinical evidence, rapid identification and treatment are necessary, especially in patients with immune system deficiency.

**Keywords:** Clinical symptoms, Immigrants, Infected patients, Strongyloides stercoralis.

# Development of a multiplex-PCR method for detection and identification of emerging *Lophomonas* spp.

Novel diagnostic technologies

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**BACKGROUND AND OBJECTIVES:** Pulmonary lophomoniasis is an emerging disease caused by the protozoan parasite *Lophomonas* spp. microscopic examination as a routine diagnostic method has limited sensitivity and specificity. Recently, polymerase chain reaction (PCR) methods have been developed and are more accurate. Therefore, this study aimed to compare the diagnostic efficacy of microscopic examination, conventional PCR, and multiplex-PCR for the detection of *Lophomonas* infection.

**MATERIALS AND METHODS:** This study was conducted at the Iranian National Registry Center for Lophomoniasis (INRCL) and included 120 patients clinically suspected of having lophomoniasis, as well as 30 bronchoalveolar lavage (BAL) specimens that were confirmed by microscopic examination. The specimens were examined using three methods: microscopic examination (Giemsa staining), conventional PCR, and multiplex-PCR. Moreover, multiplex-PCR was used for simultaneous identification of two species of *Lophomonas*.

**RESULTS AND DISCUSSION:** Among the three techniques, multiplex-PCR was the most sensitive (100%, 95% CI, 85.9–100), while Giemsa staining had the lowest sensitivity (86.7%, 95% CI, 68.35–95.64%). There was good agreement between multiplex-PCR and conventional PCR in identifying positive samples. The study also confirmed the presence of *L. blattarum* species in all samples using by multiplex-PCR. This study demonstrates that in-house multiplex PCR is a sensitive and accurate diagnostic test for the detection and identification of *Lophomonas* species. Therefore, our findings suggest that this method may be a valuable tool to overcome some diagnostic pitfalls for lophomoniasis.

**Keywords:** *Lophomonas*, Diagnosis, Microscopic examination, Conventional PCR, Multiplex PCR

# A cross-sectional study of the Prevalence of needle stick and its related factors Among the staff of Kowsar Hospital in Semnan during 2014-2018

Epidemiology of infectious diseases & antimicrobial resistance

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**BACKGROUND AND OBJECTIVES:** Introduction: Injuries caused by sharp tools are one of the most important biological risks for hospital employees, leading to dangerous physical and psychological consequences. The present study aimed to determine the prevalence of needle sticks and investigate their related factors among the staff of Kowsar Hospital in Semnan.

**MATERIALS AND METHODS:** Methods: In this cross-sectional study, all hospital employees who suffered from needle sticks during 2014-2018 were examined. The data collection tool was a checklist, including demographic variables, occupation, type of accident, and measures taken after the needle stick, part of which was collected from the personnel file in the infection control office, and another part was collected through interviews. After collecting the relevant data, they were entered into SPSS software (version 26) and subjected to statistical analysis.

**RESULTS AND DISCUSSION:** Findings: A total of 194 cases of needle sticks occurred, with a prevalence of 23.7%. The mean age of the investigated subjects was  $34.57 \pm 9.41$  years, and 41.2% of cases were in the age group of above 35 years. Moreover, 68% of them were women, 60.3% of subjects worked the morning shift, and most cases of needle sticks occurred in employees with less than five years of work experience (47.9%). Most cases of needle stick (60.3%) were related to employees with bachelors' education and nursing jobs (38.7%). In addition, the highest rate of needle sticks was related to the operating room (28.9%), and insertion of the injection needle into the hand (76.8%) was the most common incident of needle stick, and washing with soap and water (95.4%) was the most common procedure performed after needle stick. Discussion & Conclusion: Considering the relatively high prevalence of needle sticks among the medical staff,

**Keywords:** Cross-sectional study, Hospital, Needle stick, Prevalence

# Longitudinal Study of Surgical Site Infections in Orthopedic Procedures: Risk Factors, Microbial Profile and Resistance Patterns from 2012 to 2022, Babol, Iran

Epidemiology of infectious diseases & antimicrobial resistance

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**BACKGROUND AND OBJECTIVES:** Healthcare-associated infections (HAIs), including surgical site infections (SSIs), are a major global health concern affecting many countries. The prevalence of HAIs in Iran reported between 3.1% and 10%. SSIs are the most common and severe HAIs, particularly in patients undergoing surgical procedures. This study aims to provide a comprehensive analysis of the risk factors, microbial profiles, and antibiotic resistance patterns associated with SSIs in orthopedic surgery patients over a ten-year period at the Babol University of Medical Sciences. this research seeks to inform clinical practices and contribute to the development of targeted strategies for reducing SSIs.

**MATERIALS AND METHODS:** This retrospective observational study analyzed medical records of patients who underwent orthopedic surgeries and were diagnosed with SSIs between 2012 and 2022. Patients who underwent elective orthopedic surgeries for conditions such as fractures, deformities, degenerative diseases, or osteopathies. whose complete medical records and follow-up data was accessible, and diagnosed with SSIs based on the Centers for Disease Control and Prevention (CDC) definition were included in this study. Data on patient demographics, comorbidities, surgical details, and laboratory indicators were collected. Microbial analysis involved wound cultures and antibiotic susceptibility testing. Statistical analyses identified significant risk factors for SSIs.

**RESULTS AND DISCUSSION:** Out of 572 patients, 71 developed SSIs. Significant risk factors included smoking, hypertension, diabetes mellitus, recent hospitalization, high BMI, urinary catheterization, general anesthesia, ASA scores below 3, use of open drains, corticosteroid use, and prolonged surgery duration. The frequency of SSI was significantly higher in people with orthopedic implants, so that the highest incidence of SSI (26 cases; 36.6%) was related to screw and plate surgeries. The most prevalent pathogen was *Staphylococcus aureus*, with a notable presence of methicillin-resistant *Staphylococcus aureus* (MRSA). Antibiotic resistance patterns varied, highlighting the need for tailored prophylaxis protocols. In our study, diabetes mellitus (DM) and hypertension (HTN) had a significant relationship with SSI. This study underscores the importance of monitoring risk factors and microbial resistance patterns to develop effective prevention and treatment strategies for SSIs in orthopedic surgery. Targeted interventions addressing identified risk factors can potentially reduce the incidence and severity of these infections.

**Keywords:** surgical site infections, orthopedic procedures, microbial profiles, antibiotics resistance patterns,



# Assessment of Antibacterial potency of Gemifloxacin in combination with Imipenem and Amikacin against Drug-resistance *Escherichia coli* Isolates, In Vitro

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** The increased multiple drug resistance (MDR) in Enterobacteriaceae has limited the options for treating serious infections including sepsis which can quickly lead to organ failure in areas like lungs, kidneys, and liver. One of the ways to deal with this problem is to combine different antibiotics with synergy potency to increase their antimicrobial activity and reduce their toxicity. This study aimed to investigate the antibacterial activity of gemifloxacin in combination with imipenem and amikacin against *Escherichia coli* isolates in laboratory conditions.

**MATERIALS AND METHODS:** In a cross-sectional descriptive study, 376 blood samples from patients with symptoms of sepsis were collected from medical centers during 2020-2023. After performing standard biochemical and microbiology tests, antibiotic sensitivity test was performed by disk diffusion method based on CLSI-2021 guidelines. The Minimum Inhibitory Concentration of gemifloxacin in combination with imipenem and amikacin was determined by the microdilution broth method.

**RESULTS AND DISCUSSION:** Frequency of isolates among 208 positive cultures, belonged to: *Escherichia coli* (62%), *Staphylococcus aureus* (21.5%) and *Streptococcus pneumoniae* (16.5%) respectively. Among *E. coli* isolates, the highest and lowest rates of antibiotic resistance were observed against sulfamethoxazole (72.54%) and gemifloxacin (18.32%), respectively. Among 89(69%) MDR isolates, and 23(17.83%) XDR (extensively drug resistant) isolates the concentration of gemifloxacin in combination with amikacin that inhibited 90% of *E. coli* isolates (MIC<sub>90</sub>) was 0.5µg/mL, 8-fold lower than gemifloxacin + imipenem (MIC<sub>90</sub>= 4µg/mL) (P<0.01). According to the results, a high percentage of the isolates had multi-drug resistance, which is considered a threat to the public health of society. Given the favorable antibacterial effects of combination of gemifloxacin and amikacin on the MDR/XDR *E. coli* isolates, in vitro, the clinical application of this recipe for treatment of blood infections can be investigated in future studies.

**Keywords:** *E. coli*, synergy, gemifloxacin, imipenem, amikacin

# Trends of Antibiotic Resistance Pattern in Nosocomial Infections in Kashan Shahid Beheshti Hospital during 2018 to 2022

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Background: Management of drug-resistant nosocomial infections (Nis) is a major public health concern in both developing and developed countries. The current research aimed to review the trend of antibiotic resistance and the prevalence of resistant isolates involved in NIs in Shahid Beheshti hospital in Kashan over a five-year period to provide valuable information to policymakers.

**MATERIALS AND METHODS:** Methods: This retrospective study was conducted on NIs data collected from Beheshti hospital between January 2018 and December 2022. Antibiotic resistance time trends were evaluated using joinpoint regression to assess mean annual percentage changes (AAPC) in antibiotic resistance, considering p values (0.05) and confidence intervals (95%).

**RESULTS AND DISCUSSION:** Results: A total of 1552 bacterial pathogens were isolated from clinical specimens of patients between 2018 and 2022. Among them, 57.3% were male, and 42.7% were female. Nearly half (49.8%) of the patients were elderly (aged ≥ 65 years). Also, 73.4% were hospitalized in the intensive care unit (ICU) and coronary care unit (CCU). The findings showed that the most common bacterial isolates were *Acinetobacter* spp. (37.7%) and *Klebsiella* spp. (27.2%), followed by *Escherichia coli* (12.1%), *Pseudomonas aeruginosa* (8%), and *Staphylococcus epidermidis* (7.1%). The increasing trend of antibiotic resistance to ciprofloxacin, gentamicin, meropenem, ceftazidime, piperacillin/tazobactam, and ceftriaxone was significant. The prevalence trend of multidrug-resistant (MDR) *E. coli* and *Klebsiella* spp. strains increased from 27.6 to 87.3% and from 79.5 to 97.1%, respectively. The results showed that age, hospital ward, specimen type, hospitalization duration, and mortality were significantly associated with multidrug resistance (p0.001). Conclusions: Despite the stability of overall antimicrobial resistance, an

**Keywords:** Resistance, Antimicrobial agents, Antibiotic, Nosocomial infection, Health care associated

# Analysis of Tetracycline, Vancomycin, and Erythromycin resistance genes in Lactic acid bacteria isolated from Infant feces

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** The most dangerous risk of using antimicrobial agents to treat the disease is antimicrobial resistance (1). The appearance of new methods of resistance, the increase in resistance to multiple drugs, and the ability for genetic material-carrying resistance to be easily transferred horizontally between different bacterial species all contribute to a heightened vulnerability to diseases that were previously believed to be manageable with the advent of antibiotics (2). Lactic acid bacteria are commonly found in the human intestinal tract, making it necessary to check for antibiotic-resistance genes in their genome regularly (3)

**MATERIALS AND METHODS:** In this study, a total of 50 fecal samples were collected for analysis. Subsequently, a series of primary tests including gram-positive, catalase, and negative motility tests were carried out after dilution of the samples. Further diagnostic tests were performed, involving the use of differential culture medium for sugar fermentation, temperature-based differential tests, bile esculin agar test, and NaCl broth 6.5%. Antibiotic resistance patterns of the isolated lactic acid bacterial strains were studied using the Kirby-Bauer disk diffusion method (in accordance with the CLSI guidelines). Additionally, DNA extraction was performed, followed by PCR analysis to detect the tetQ, ERMB, and vanA genes.

**RESULTS AND DISCUSSION:** Lactic acid bacteria were detected in 30 out of 50 fecal samples. From these samples, 30 isolates of lactic acid bacteria were chosen for further testing. Among these isolates, 30% were resistant to vancomycin, 16.7% were resistant to tetracycline, and 20% were resistant to erythromycin. Additionally, PCR analysis revealed that 88.89% of the resistant strains had the tetQ gene, 60% had the vanA gene, and 50% had the ermB gene. This study highlights the alarming prevalence of vancomycin and other resistance genes, indicating a potential risk of resistance development. The transmission of these genes to susceptible bacteria could lead to the rise of resistant strains.

**Keywords:** Lactic acid bacteria, Antibiotic resistance, Feces, Tetracycline, Vancomycin, Erythromycin

# Antibacterial and antibiofilm activity of Zinc oxide nanoparticle against non fermenter bacteria

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** One of the most important nonfermenting gram-negative bacteria is *P. aeruginosa*. This bacterium can cause nosocomial infections. *P. aeruginosa* that are resistant to most drugs. The use of nanoparticles can inhibit the growth of them and kill bacteria. This project objective to produce Zinc oxide nanoparticles and evaluate antimicrobial activity and inhibiting biofilm formation in clinical isolates of non fermenter bacteria. Materials and methods

**MATERIALS AND METHODS:** Zinc oxide nanoparticles were synthesized by chemical method in Kashan University of Medical Sciences. In this study, forty isolates of *P. aeruginosa* was isolated from clinical specimen contains: respiratory tract samples, sterile body fluids, wound, urine, etc. The bacteria isolates were collected of the inpatient and outpatient departments of Shahid Beheshti Hospital in Kashan. Phenotypic and molecular methods (using *oprL* gene by PCR test) were used to identify these bacterial isolates. Then, antibacterial and antibiofilm activity isolates of *P. aeruginosa* were recognized by broth microdilution methods.

**RESULTS AND DISCUSSION:** Zinc oxide nanoparticles made with a size of 35 nm (Figure1, & 2). In this study, 57.5% isolates that have the ability to form a strong biofilm, 26% moderate isolates and 16.5% weak isolates. The minimum inhibitory concentration (MIC<sub>100</sub>) and MIC<sub>50</sub> of Zinc oxide nanoparticles were 250 µg/ml, and 125 µg/ml, respectively. In addition, minimum biofilm inhibitory concentration (MBIC<sub>100</sub>) and MBIC<sub>50</sub> of nanoparticles were 125 µg/ml, and 31.25µg/ml, respectively. Discussion & Conclusion Our outcomes show that multidrug resistance *P. aeruginosa* are yield more biofilm. In this study, it was shown that Zinc oxide nanoparticles could prevent the growth of multidrug resistance bacteria. Therefore, these nanoparticles can be use together with other antimicrobial agents to eliminate biofilm producing *P. aeruginosa* in the patient's body. In the future, we can hope to inhibit the growth and production of biofilm in multidrug resistance non fermenter bacteria by using new compounds and the combinatory

**Keywords:** Zinc oxide nanoparticles, *Pseudomonas aeruginosa*, antimicrobial activity

# Antibiotic resistance pattern of *Escherichia coli* isolated from hospitalized and outpatients with urinary tract infection against fluoroquinolone in Ghaem and Emam Reza hospitals

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Determining the pattern of antibiotic resistance specific to each geographical area can be used to plan the treatment protocol in order to prevent the emergence and spread of multidrug resistant organisms. The present study was conducted to investigate the resistance of *Escherichia coli* to fluoroquinolone antibiotics during the years 1396 to 1399 in urinary tract infections (UTIs) in Ghaem and Imam Reza hospitals of Mashhad.

**MATERIALS AND METHODS:** The present study was conducted retrospectively. First, by referring to the HIS and WHONet system of the hospital, the files related to the UTI in outpatients and inpatients in Ghaem and Imam Reza hospitals of Mashhad during the years 1396 to 1399 were extracted. Then, the patients whose culture was *E. coli* were selected and included in the study, and the information of these patients, including their sex, age, and antibiogram, were extracted and recorded in an Excel file. Obtained information was entered into the SPSS statistical software version 16, and analyzed.

**RESULTS AND DISCUSSION:** A total of 157,849 urine cultures were performed, of which 34,770 cultures (22.02%) were positive, and among the positive culture, *E. coli* was reported in 6382 samples (18.3%). Among the patients whose urine culture was reported to be *E. coli*, 60.5% were female and the rest were male. Also, the average age of these patients was  $55.33 \pm 24.99$  years. The rate of resistance to ciprofloxacin and levofloxacin was 66.7% and 60.4%, respectively. During the years of conducting the study, the resistance to ciprofloxacin in Imam Reza Hospital had an increasing trend. In Ghaem hospital, the general trend was increasing, but a slight decrease in the resistance to this antibiotic was observed in 1399. Most of the samples of *E. coli* in the UTIs of patients of Ghaem and Imam Reza hospitals have become resistant to ciprofloxacin and levofloxacin, and measures should be taken to prevent further increase of antimicrobial.

**Keywords:** *Escherichia coli*, urinary tract infection, antimicrobial resistance

# Antibiotic Resistance Patterns of Bacterial Agents in Pediatric Urinary Tract Infections at Bo Ali Hospital of Sari during 2021-2023

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Urinary tract infections (UTIs) are prevalent bacterial infections in children, necessitating effective antimicrobial therapy. UTIs can manifest in various forms, including cystitis (infection of the bladder) and pyelonephritis (infection of the kidneys), and can lead to severe complications if left untreated. Studies indicate that UTIs are a common pediatric health issue, with a significant number of children experiencing at least one episode during their early years. The emergence of antibiotic resistance among uropathogens poses significant challenges to treatment strategies, making the selection of effective antibiotics increasingly difficult. The objective of this study is to examine the pattern of drug resistance in strains isolated from pediatric patients admitted to Bo Ali hospital of Sari during the years 1400-1402.

**MATERIALS AND METHODS:** A retrospective cross-sectional study was conducted from 1400 to 1402, including children with UTIs admitted to Bo Ali Sina Hospital in Sari. Urine samples were collected from UTI-positive patients and then cultured on blood agar and EMB agar plates. Antibacterial susceptibility was assessed using the disk diffusion method. Demographic and clinical information, as well as microbiological data, were extracted and recorded from patient files. SPSS software was utilized for data analysis.

**RESULTS AND DISCUSSION:** A total of 1502 children with UTIs were included in the study. The majority of participants were girls (1034, 68.8%), with boys accounting for 468 cases (31.2%). Among them, 125 infants (under one month) accounted for 10.1% of cases, while the largest age group comprised 431 individuals (34.7%) over 5 years old. *Escherichia coli* was the most common pathogen, identified in 823 cases (54.80%). Among Gram-negative bacteria, *Pseudomonas aeruginosa* and *Klebsiella* were present in 49 (3.27%) and 47 (3.13%) cases, respectively. *Staphylococcus epidermidis* was the predominant Gram-positive bacterium, identified in 136 cases (9.05%). Other Gram-positive bacteria included *Staphylococcus saprophyticus* (44 cases, 2.93%), *Staphylococcus aureus* (12 cases, 0.80%), and *Enterococcus faecalis* (1 case, 0.07%). Among the evaluated antibiotics, Amikacin showed the highest sensitivity at 98.20%, while Nalidixic Acid and Azithromycin had the lowest sensitivity at 28.6%. Based on the findings of this study, Amikacin is recommended as the most effective antibiotic.

**Keywords:** Urinary tract infection, antibiotic resistance, *Escherichia coli*

# Antibiotic resistance profiles of *Escherichia coli* isolates from commercial chickens in three different times

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** The rapid emergence and high levels of antimicrobial resistance have been detected in chicken populations worldwide, hence posing a global threat to the modern human medicine, veterinary medicine, and food safety. The aim of this study was to determine antimicrobial resistance profiles in *E.coli* isolates originating from commercial chicken flocks of Ardabil province, northwest of Iran in three different times.

**MATERIALS AND METHODS:** In this study drug sensitivity of 390 *E.coli* isolates using Kirby-Bauer disk diffusion method were examined with 30 different antibiotics. The isolates obtained from 85 chickens flocks located in different parts of Ardabil province with clinical signs of colibacillosis submitted in 2018, 2020 and 2022 to private veterinary laboratory.

**RESULTS AND DISCUSSION:** The overall, all isolates were completely resistant to tetracycline, tylosin and nalidixic acid, and more than 80 % were resistant against doxycycline, flumequine, colistin, neomycin, sultrim, enrofloxacin and lincoceptin. Less resistance (less than 30 %) was to ciprofloxacin, imipenem, ceftazidime, danofloxacin, fuzbac, gentamycin, cefalexin, florfenicol. There was antibiotic resistance increase in 2022 isolates compared with 2018 and 2020 in several antibiotics including tylosin, doxycycline, flumequine, fuzbac, gentamycin, colistin, lincoceptin, neomycin, sultrim, ampicillin, cefixime, enrofloxacin, florfenicol, difloxacin, lincomycin and cefalexin. In chlortetracycline, ceftriaxone, ciprofloxacin, danofloxacin, chloramphenicol, kanamycin, furazolidone, ceftazidime, amoxicillin, penicillin, imipenem and co-trimoxazole no change or even resistance decrease were observed. Because of acquired antibiotic resistance and various results of antibiotic therapy, it must be attended to antibiogram test more than before.

**Keywords:** *E. coli*, Antibiotic sensitivity test, Chickens, Ardabil

# Antibiotic resistance, virulence genes and biofilm formation of enterotoxigenic *Escherichia coli* in feces of diarrheic calves in Tabriz region, Iran

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** This study aimed to investigate the antibiotic resistance profile and biofilm formation of Enterotoxigenic *Escherichia coli* (ETEC) isolated from calves with diarrheal disease.

**MATERIALS AND METHODS:** A PCR assay was utilized to screen 97 *Escherichia coli* (*E. coli*) isolates for the presence of virulence genes specific to ETEC. The antimicrobial resistance of *E. coli* isolates against various antimicrobials and biofilm formation was assessed.

**RESULTS AND DISCUSSION:** The prevalence of *E. coli* strains in diarrheal calves was 96.03%, with ETEC isolates accounting for 15.46%. These isolates harbored F4 (5.15%) and K99 (11.34%) genes. All isolates exhibited resistance to multiple antibiotics. Varied levels of adherence were observed in 89.28% of *E. coli* isolates. The study identified a significant correlation between the animals' health status and the type of diarrhea, suggesting that antibiotic administration to animals could lead to the emergence of antibiotic-resistant bacteria that may pose a risk to humans.

**Keywords:** Enterotoxigenic *Escherichia coli*, calves, Iran, Biofilm, Susceptibility test.



# Antimicrobial effect of chitosan-silver-curcumin nanocomposite film on a number of antibiotic-resistant isolates and standard isolates

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Nowadays, with the spread of antibiotic resistance, the synthesis of new antimicrobial compounds is important. Chitosan biopolymer due to its biocompatibility, silver nanoparticles with strong antibacterial properties and curcumin nanoparticles with potential anti-inflammatory and antibacterial properties, can be considered as very good candidates for nanocomposite production.

**MATERIALS AND METHODS:** First, chitosan-silver and chitosan-silver-curcumin nanocomposite films were prepared and then it was cut into circles with a diameter of 7 mm using a punching machine and sterilized using UV. Three Erlenmeyer flasks containing 50 ml of TSB culture medium were prepared. Then, a piece of sterile film weighing 0.1 gram was placed in the first Erlenmeyer flask and 0.5 ml of bacterial suspension with a concentration of 0.5 McFarland was added. Two other Erlenmeyer flasks were used as positive and negative controls. All the Erlens were incubated at 37 °C and 120 rpm. For evaluation antibacterial effect, CFU determination was done at 12, 24, 48 and 72 hours. A number of isolates resistant to clinical antibiotics with the codes K5, PSt14, E18 and S3, which according to biochemical identification were similar to *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* bacteria, as well as a number of standard bacteria

**RESULTS AND DISCUSSION:** The antimicrobial effect of chitosan-silver-curcumin film on antibiotic-resistant isolates is higher compared to chitosan-silver nanocomposite film after 24 hours. Meanwhile, both chitosan-silver and chitosan-silver-curcumin nanocomposite films had 100% bactericidal effect on standard isolates. The results of this study show that the presence of curcumin has increased the microbicidal properties of chitosan-silver nanocomposite, especially in the case of clinical isolates. Considering the increase of antibiotic resistance, chitosan-silver-curcumin nanocomposite is proposed as a new antimicrobial compound.

**Keywords:** Nanocomposite, Chitosan, Silver, Curcumin, Antimicrobial, Antibiotic-resistant isolates

# Assessment of antibiotic resistance and genetic characteristics in *Pseudomonas aeruginosa* strains isolated from Cystic Fibrosis (CF) patients.

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Cystic fibrosis lung disease is specified by chronic lung infections with the opportunistic pathogens such as *Pseudomonas aeruginosa*. *P. aeruginosa* encounters vast genetic adaptation to the Cystic fibrosis (CF) lung environment, and the quorum sensing regulator gene *lasR* will have mutated. *lasR* mutants enforce host inflammatory responses in respiratory epithelial cells, and causes accumulation of proinflammatory cytokines and neutrophil recruitment. *Pseudomonas aeruginosa* isolated from the sputum of Cystic Fibrosis (CF) patients are always antibiotic resistant strains. Based on this fact, in this evaluation, the antibiotic resistance characteristic and genetic properties of *pseudomonas aeruginosa* isolated from patients were assessed applying disk diffusion and molecular methods in the specimens derived from CF patients.

**MATERIALS AND METHODS:** The current study was carried out as a descriptive evaluation on 30 *pseudomonas aeruginosa* strains detected from CF patients from Tehran Children's Medical Center. Isolated strains from sputum have been diagnosed applying standard biochemical tests and then the antibiotic resistance of each one was evaluated. Antibiogram was performed by disk diffusion method, and the sensitivity of bacteria to ceftazidime, cefotaxime, amoxicillin, ciprofloxacin, amikacin, gentamicin, imipenem, ticarcillin and piperacillin antibiotics were investigated. Then, the presence of quorum sensing genes such as *lasR* and *lasI* were evaluated by applying multiplex PCR.

**RESULTS AND DISCUSSION:** Results showed that more than 55% of the strains were resistant to more than 5 antibiotics at the same time. The resistant rate are as followed: 98%, 99%, 95%, 89%, 55%, 50%, 35%, 20% of bacteria were resistant to amoxicillin, cefepime, ticarcillin, cefotaxime, piperacillin, imipenem, gentamicin and ceftazidime, and 20% of bacteria were resistant to ciprofloxacin. Performing multiplex PCR has proven that most of the multi-drug resistant strains have *lasR* and *lasI* genes to improve the lung tissue to cause pulmonary inflammation. In *Pseudomonas aeruginosa* the presence of quorum sensing systems causes that these resistances occur violently. The *lasR* gene is present in 70% of multi-drug resistant strains who are resistant to more than 5 antibiotics, and the *lasI* is present in 56% of multi-drug resistant strains who are resistant to more than 4 antibiotics

**Keywords:** Cystic Fibrosis .*Pseudomonas aeruginosa*. antibiotic resistance

# Assessment of the Frequency and Pattern of Bacterial Antibiotic Resistance in Blood Culture Samples from Patients at Imam Ali Hospital in Amol from June 2023 to February 2024

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** In recent years, antibiotic resistance and the emergence of various resistant bacterial strains have caused concern for the World Health Organization and healthcare workers. Blood culture is one of the most important methods for diagnosing severe infections. Similar to all tests, false positive results are the main limiting factor of this method, which creates significant problems in diagnosis. Therefore, this study aimed to determine the frequency and pattern of bacterial antibiotic resistance and to examine false positive cases in blood culture samples from patients hospitalized at Imam Ali Hospital in Amol.

**MATERIALS AND METHODS:** This descriptive cross-sectional study collected eight months of blood culture data from all patients who visited Imam Ali Hospital in Amol and had blood cultures requested by their physicians.

**RESULTS AND DISCUSSION:** In this study, out of 866 first-round samples examined, 108 patients had positive blood cultures. 57.40% of the participants were female. Most of the patients (57.40%) were hospitalized in NICU. The highest resistance was observed to the antibiotics oxacillin (85.10%) and erythromycin (85.07%), and the highest sensitivity was observed to the antibiotics nitrofurantoin (85.71%) and gentamicin (72.46%). there were 643 infants (under 1year old), with 66 cultures reported as positive. The most common infection-causing bacteria were *Staphylococcus epidermidis* (74.24%), *Listeria monocytogenes* (6.06%) and *Staphylococcus aureus* (4.54%). The highest resistance was observed to the antibiotics oxacillin (89.19%), norfloxacin (85.71%), and erythromycin (85.71%). Additionally, 223 samples were from patients aged 1 year or older (average 45.97 years) which 37 cultures were reported as positive. Among these, *Staphylococcus epidermidis* (29.72%) and *Klebsiella pneumoniae* (10.86%) were the most frequent. The highest resistance was observed to the antibiotics erythromycin (81.25%) and cefazolin (75.00%).

**Keywords:** Antibiotic Resistance, blood culture, Bacterial isolation, Imam Ali hospital

# Biosynthesis of zinc oxide nanoparticles by endophytic bacteria of Persian Gulf algae and investigation of its antimicrobial activity against *Vibrio parahaemolyticus* ATCC 17832

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Among the 80 species in the *Vibrio* family, over 20 can cause gastrointestinal diseases in humans. *Vibrio parahaemolyticus* is a Gram-negative bacterium found in aquatic environments that can infect humans, leading to gastroenteritis and wound infections. According to the CDC, *V. parahaemolyticus* is responsible for approximately 4,500 illnesses and 5 deaths each year. This bacterium has developed new pathogenic strains from marine sources. It has caused severe economic loss for shrimp industry in various countries. With the rise of antibiotic-resistant infections, there is growing interest in alternative antimicrobial agents, such as metal oxide nanoparticles especially biogenic nanoparticles.

**MATERIALS AND METHODS:** In this study, a cell lysed extract (CLS) was prepared from an overnight culture of *Bacillus vallismortis* bacteria, and zinc sulfate salt was added to it. The mixture was incubated in a 100°C water bath for 2 hours, resulting in the formation of zinc oxide nanoparticles (ZnO NPs), indicated by the appearance of a white precipitate. The biosynthesized nanoparticles were then characterized using various techniques, including Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), zeta potential analysis, and UV-visible spectroscopy. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ZnO NPs against a 24-hour culture of *V. parahaemolyticus* were determined using a microtiter plate test.

**RESULTS AND DISCUSSION:** Results showed that cell-free extract from *B. vallismortis* was effective for the biosynthesis of ZnO NPs. The UV-Visible spectroscopy analysis indicated an absorption peak for the ZnO NPs at 219.22 nm. Fourier Transform Infrared Spectroscopy (FTIR) revealed that the stabilization of the biosynthesized zinc oxide nanoparticles is primarily attributed to the hydroxyl, amine, and carboxyl groups present in bacterial proteins. The average particle size of the nanoparticles ranged from 31.89 to 39.35 nm, with a polydispersity index (PDI) of 0.3500. The minimum bactericidal concentration (MBC) analysis demonstrated *V. parahaemolyticus* growth inhibition a concentration of 1 mg/ml. These findings indicate that biogenic ZnO NPs exhibit significant antimicrobial activity against *V. parahaemolyticus*, a bacterium responsible for gastrointestinal diseases.

**Keywords:** Green synthesis; Zinc oxide nanoparticles; *Bacillus vallismortis*; *Vibrio parahaemolyticus*; antibacterial

# Cold Atmospheric Plasma reduces the microbial load of *Pseudomonas* in burn wounds

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* is an opportunistic pathogen causing severe, acute and chronic nosocomial infections in immunocompromised, catheterized or burn patients. Various types of virulent factors have been identified in *P. aeruginosa*, suggesting their contribution to the pathogenesis of the disease. This organism is generally resistant to numerous antimicrobial agents due to natural resistance in particular impermeability or mutations and acquisition of resistant determinants. Since cold atmospheric plasma is a good candidate for *S. aureus* and MRSA biofilm treatment, may therefore be of value in the bacterial resistance crisis.

**MATERIALS AND METHODS:** This study was designed and implemented to investigate the effect of cold plasma in reducing the microbial load of *Pseudomonas*. After local anesthesia and shaving the site, the burn wound was induced using a hot coin (Blab/c mouse). After that, using knowledge intensive helium plasma device (product by plasma technology development company, Tehran, Iran) driven by 5 kV, 20 kHz, the burn was exposed to cold plasmas (1cm<sup>2</sup>/1 min).

**RESULTS AND DISCUSSION:** The results of this study showed that treatment with helium plasma significantly reduces the microbial load of *Pseudomonas* compared to the control group. Since *Pseudomonas* is one of the main causes of infection in burn wounds, and infection is an obstacle to burn healing, cold plasma can be a suitable and effective treatment option to in eliminating the infection caused by *Pseudomonas*, accelerate healing burn wound and shorten the recovery period.

**Keywords:** Cold Atmospheric Plasma, *Pseudomonas aeruginosa*, Burn wound

# Comparison the antibacterial effect of alcoholic extract, essential oil, and nanoemulsion prepared from seed and root of two medicinal plants

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Background and purpose: Due to increasing antimicrobial resistance rates, special attentions have been paid to medicinal plants. This study aimed to evaluate the antibacterial and antifungal effect of the alcoholic extract, essential oil, and nanoemulsion prepared from *Salvadora persica* root and grape seed.

**MATERIALS AND METHODS:** Materials and methods: The alcoholic extract and essential oil of *S. persica* root and grape seeds were prepared by percolation and distillation methods, respectively. Also, the low-energy spontaneous emulsification method was used to prepare the nanoemulsion. Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimum Fungicidal Concentration (MFC) of the antimicrobials were determined to evaluate the antibacterial and antifungal effect of them against some clinically significant bacteria and fungi.

**RESULTS AND DISCUSSION:** Results and Discussion: The alcoholic extract (6.25%) inhibited *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. Meanwhile, the essential oil had a better effect on *Staphylococcus aureus*, *Enterococcus faecalis*, and *Streptococcus mutans*. Also, nanoemulsion (0.09%) inhibited most gram-negative bacteria. The essential oil (12.5%) killed the tested gram-positive bacteria, and the nanoemulsion (0.39%) was able to kill gram-negative bacteria, except *P. aeruginosa*. *Candida albicans* and *Aspergillus fumigatus* were the most sensitive fungi to the *S. persica* root extract, while the essential oil (1.56%) inhibited all fungi, and nanoemulsion (0.09%) inhibited all fungi, except *Aspergillus niger*. The average MIC and MBC of the grape seed extract were 10.71% and 35.71%, respectively, and was more effective on gram-positive bacteria. The *S. persica* nanoemulsion had more antibacterial and antifungal effect than the alcoholic extract and essential oil. However, these agents may be effective as an adjuvant in the prevention or treatment of infections.

**Keywords:** Keywords: *Salvadora persica*, Alcoholic Extract, Essential Oil, Nanoemulsion, Antibacterial, Antifungal.

# Evaluation of the effect of nanoemulsion of *Zataria multiflora* essential oil and carvacrol on standard and biofilm producing bacteria

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** For ages infections due to biofilms have made difficulties for medical team and made patients' treatment complex. The very high resistance of biofilms to drug penetration as well as the resistance of biofilm forming bacterias compared to the planktonic form of them have caused the lack of suitable antibiotic for the treatment of these infections. Many studies show that the activity of Standard antibiotics increase significantly in the presence of nanoparticles. In the present study, the effect of nanoemulsions of carvacrol and *Zataria* essential oil on standard bacteria isolated bacterias from patient were investigated.

**MATERIALS AND METHODS:** Nanoemulsions were prepared by high energy emulsification method using ultrasound and homogenizer. After determining the particle size and dispersion using a nanosizer, long-term stability was evaluated. The antimicrobial effects of nanoemulsions containing essential oils on standard and biofilm- forming bacterias were investigated by 96-well plate method and macrobroth dilution method.

**RESULTS AND DISCUSSION:** The studied compounds, especially *Zataria* essential oil and carvacrol, showed very good antimicrobial and anti-biofilm activity. The use of nanoemulsions, in addition to helping the stability of these compounds, enhanced the antimicrobial effects on standard and isolated strains from the patient. Herbal compounds can obviously have very good antimicrobial and anti-biofilm effects. Making nanoemulsions from carvacrol increases the ability of these two plant compounds to prevent biofilm production, while despite the very good anti-biofilm production effect of eugenol, clove essential oil and their nanoemulsions; making nanoemulsions from them, causes no significant increase in their inhibitory effects on biofilm production.

**Keywords:** Essential oil, *Zataria multiflora*, carvacrol, biofilm, standard bacteria

# Evaluation of the frequency of ace and asaI genes in *Enterococcus faecalis* clinical isolates

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Enterococci are part of the natural flora of the human digestive system. As opportunistic pathogens, they cause nosocomial infections, especially in Intensive Care Unit. By receiving plasmids and different mutations, these bacteria can become resistant to all kinds of antibiotics. The aim of the present study is to investigate the frequency of ace and asaI resistance genes in *Enterococcus faecalis* isolates.

**MATERIALS AND METHODS:** A total of 79 clinical isolates were collected from the hospitals of Borujerd city and were identified and confirmed by 16s rRNA molecular and biochemical methods. The pattern of drug resistance was determined using disk diffusion method and CLSI 2022 criteria. Chromosomal and plasmid genome extraction was performed and PCR was performed with specific primers to identify the frequency of ace and asaI genes.

**RESULTS AND DISCUSSION:** All isolates were sensitive to linezolid and resistant to penicillin. Antibiotic resistance to tetracycline 72%, vancomycin 3% and chloramphenicol 28% were determined. 63.29% of the samples had asaI and ace genes, all of which were resistant to ciprofloxacin. The frequency of ace and asaI genes in *Enterococcus faecalis* isolates is high (P<0.05) and their prevalence is directly related to antibiotic resistance, especially ciprofloxacin. The results can increase information to prevent colonization and infection by bacteria.

**Keywords:** *Enterococcus faecalis*, ace, asaI, IAU science.



# Evaluation Second Line Anti-Tubercular Drug Resistance among Mycobacterium tuberculosis Clinical Isolates of Isfahan Tuberculosis Regional Reference Laboratory

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** BACKGROUND AND OBJECTIVES Tuberculosis remains a severe public health problem worldwide, causing millions of deaths annually. It is estimated that one-third of the world population is infected with latent tuberculosis. Drug-resistant tuberculosis is considered a major and growing global threat. Evidence suggests that drug resistance leads to the development of multidrug-resistant TB. The aim of this study was to evaluate the second-line drug resistance among multidrug resistance Mycobacterium tuberculosis clinical isolates from pulmonary tuberculosis patients.

**MATERIALS AND METHODS:** MATERIALS AND METHODS In this study, out of 438 positive tuberculosis isolates referred to the Regional Reference Laboratory of Tuberculosis in Isfahan province during 2017-2019, 33 resistant isolates were studied. Drug susceptibility testing was performed by the proportion method. Evaluation of second-line drugs consisted of amikacin, kanamycin, capreomycin and ofloxacin.

**RESULTS AND DISCUSSION:** RESULTS AND DISCUSSION Out of 438 isolates 33 isolates were drug resistant to first-line drugs by phenotypic method, the highest and lowest frequency resistance to isoniazid and ethambutol was observed respectively. Out of the 18 isolates (54.5%) resistant to rifampin resistant and MDR, 10 isolates (30.3%) were MDR-TB that 100% of MDR isolates were resistant to capreomycin and kanamycin and 33.3% resistant to amikacin, also the lowest frequency was 5.6% to ofloxacin. **CONCLUSION** Based on the results indicated drug resistance is different in each region and should be surveyed. Therefore, evaluation and identification of drug resistance can improve the available programmatic management for the diagnosis and control of TB. The circulating of Pre-XDR and MDR isolates is alarming the tuberculosis control program in the country.

**Keywords:** Keywords: Mycobacterium tuberculosis, Multidrug resistance, second lineA

# Examining the abundance of gram-positive bacteria resistant to beta-lactam antibiotics in clinical, industrial and environmental samples

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Bacteria have many capabilities to deal with the lethal effects of antibiotics, one of the most important of which is the production of beta-lactamase enzymes. In addition to pathogenic strains, these enzymes can also be produced by antibiotic-producing strains. In this research, the abundance of these enzymes in Gram-positive cocci isolated from pathogenic samples, antibiotic production industrial units, and actinobacteria isolated from environmental samples have been investigated.

**MATERIALS AND METHODS:** 100 clinical isolates from the microbial cultures of patients referred to Nilo Laboratory in Tehran, 20 isolates obtained from the environmental control process of the penicillin production departments of Jaber Ibn Hayyan Pharmaceutical Company and 30 isolates of actinobacteria obtained from environmental samples and prepared by the method Microbiological standards were identified. Antibigram was performed with disc diffusion (amoxicillin, ampicillin, cefazolin and ceftriaxone) on the samples and the results were analyzed.

**RESULTS AND DISCUSSION:** Out of 100 isolated clinical samples, 72 gram-positive cocci samples were detected. No antibiotic resistance was found in blood and ear samples, but the highest resistance in abscess and sputum samples of coagulase negative staphylococci was 100% and 85.7%. 50% of Gram-positive cocci isolated from penicillin production areas showed resistance to ampicillin and amoxicillin. The highest resistance to amoxicillin and ampicillin and the lowest resistance to cefazolin and ceftriaxone. More than 75% of isolated actinobacteria were resistant to all four antibiotics. The information obtained from this study indicates the distribution and abundance of beta-lactamase enzyme as one of the main mechanisms of bacteria's resistance to penicillin and cephalosporin antibiotics in clinical, industrial and environmental environments. This research indicates the need to develop and adhere to a specific antibiotic treatment policy, conduct regular monitoring studies, track emerging drug resistance patterns, and implement effective infection prevention and control

**Keywords:** staphylococcus, antibiogram, antibiotic resistance, actinobacter

# Frequency of Qnr genes in isolates of *Pseudomonas aeruginosa* isolated from hospitalized patients in Sari city, 2023

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* is an opportunistic pathogen and a common cause of nosocomial infections. Excessive antibiotic use has made this bacterium resistant to antibiotics through various mechanisms, including acquiring antibiotic resistance genes. By examining specific genes in the bacterium, it is possible to determine its resistance to certain antibiotics and prevent ineffective prescriptions. Qnr genes encode proteins that protect DNA gyrase and topoisomerase IV against quinolones. This study investigates the frequency of Qnr genes in *Pseudomonas aeruginosa* isolates from patients in Sari city.

**MATERIALS AND METHODS:** In this descriptive-analytical study, *Pseudomonas aeruginosa* isolates were sampled from the laboratories of educational and therapeutic hospitals in Sari and then analyzed in the microbiology research laboratory of Sari Medical School. The strains were identified using laboratory tests including oxidase test, culture in VP, MR, SIM, TSI, lysine decarboxylase, citrate and urea media. Then the identified samples were placed in a medium containing 30-40% glycerol at -70 They were kept to be used for the next stages of the experiment. Disc In agar method based on CLSI was used to check the drug resistance of the strains to the proposed antibiotics. The boiling method was used to extract DNA. The data obtained in this study were analyzed using SPSS version 22 statistical software and Chi-square statistical test.

**RESULTS AND DISCUSSION:** In a study with 100 samples, 71 were men. The highest *Pseudomonas aeruginosa* infection rate was in the 21-30 age group (26%), while the lowest was in those over 61 and 0-10 age groups (9%). *P. aeruginosa* showed high resistance to ticarcillin (96%) and nalidixic acid (87%), but was sensitive to piperacillin-tazobactam, imipenem, and gentamicin at 63%, 49%, and 41% respectively. The prevalence of qnrA, qnrB, and qnrS genes was 31%, 24%, and 35% respectively. Further research on these genes is recommended for better treatment and prevention strategies. The study found ciprofloxacin to be the most effective quinolone for *Pseudomonas aeruginosa* infection. Piperacillin-tazobactam is a good alternative. qnrS and qnrA were prevalent and equally

important for future research. Screening for PMQR-producing *Pseudomonas aeruginosa* is advised for prompt treatment and prevention.

**Keywords:** Antibiotic resistance infection, Qnr gene, PMQR, *Pseudomonas Aeruginosa*

# Gene Expression Analysis of MexCD-OprJ and MexEF-OprN in Hospital Isolates of *Pseudomonas aeruginosa*

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Leila: In recent years, the emergence of multidrug-resistant (MDR) strains of *Pseudomonas aeruginosa* in hospital settings has presented formidable challenges in patient management and infection control. Therefore, understanding the molecular mechanisms of antibiotic resistance is essential due to the significance of mortality resulting from drug resistance in *P. aeruginosa* infections. The aim of this study is to investigate the antibiotic resistance pattern and the expression levels of MexCD-OprJ and MexEF-OprN efflux genes in clinical isolates of *P. aeruginosa* collected in Mashhad between the summer and autumn of 2023.

**MATERIALS AND METHODS:** Leila: A total of 103 *P. aeruginosa* isolates were obtained from clinical samples collected from various hospital units. The isolates were identified using standard phenotypic and biochemical methods. Antibiotic resistance was assessed using the disc diffusion method with antibiotic discs containing Cefepime, Aztreonam, Norfloxacin, Levofloxacin, Ofloxacin, Ceftiofur, and Ceftriaxone. Additionally, the agar dilution method was employed for minimum inhibitory concentration (MIC) technique for levofloxacin. Furthermore, the gene expression of MexC and MexE efflux pumps was evaluated using Real-Time PCR.

**RESULTS AND DISCUSSION:** In a study of 103 *Pseudomonas aeruginosa* isolates, 65% were from male patients and 35% from female patients. All isolates were resistant to ceftriaxone, with high resistance rates to other antibiotics: 93% to ceftiofur, 65% to cefepime, and 54.4% to aztreonam. About 71% of isolates were resistant to fluoroquinolones. Over half (53.4%) of the isolates were identified as multidrug-resistant (MDR). The highest resistance level, observed in 22 samples, was linked to overexpression of efflux pumps. Real-Time PCR results confirmed higher expression of resistance-related genes (*mexE* and *mexC*) in some levofloxacin-resistant MDR strains. In conclusion besides the upregulation of *mexE* and *mexC* pumps, other resistance mechanisms such as mutations and the presence of integrons, contribute to the observed high resistance levels in the studied strains.

**Keywords:** Hospital infections, MexCD-OprJ and MexEF-OprN, *P. aeruginosa*, Multidrug-resistant.

# Genetic relation between virulence factors genes and carbapenemase-producing uropathogenic *Escherichia coli*

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Uropathogenic *Escherichia coli* (UPEC) has developed vast resistance and also carries various virulence factors causing urinary tract infections (UTIs). Our study aimed to evaluate the genetic relationship between virulence factor genes and carbapenemase-producing-UPEC (CP-UPEC) strains isolated from UTIs.

**MATERIALS AND METHODS:** Three hundred UPEC strains were collected from pyelonephritis and cystitis. The susceptibility of isolates was evaluated using the disk diffusion method. Carbapenemase and related genes and virulence factors were detected using the polymerase chain reaction (PCR) technique. The phylogroups and serogroups were also determined using PCR.

**RESULTS AND DISCUSSION:** Results: Seventeen (3.66%) UPEC were CP- coli. A range of 4-128 µg/mL was considered for imipenem minimum inhibitory concentration. The *bla*IMP and *bla* OXA-48 carbapenemase genes existed in 17% and 7% of UPEC (MIC: 64-128µg/ml), respectively. Virulence genes were mostly *iutA* with 97.66% (n=293), *fyuA* with 85.33% (n=256), and *inh* with 83% (n=249). The *csgA*, *fimH*, *papII*, *traT*, *iutA*, and *fyuA* genes were detected in all CP-UPEC isolates. O1 (32%), O16 (15%) and O25 (7%) were predominant serogroups. Nine of 11 CP-UPEC belonged to B2 and two belonged to the B1 phylogroup. Conclusion: CP-UPEC belonging to O25/B2 could carry *bla*IMP and *bla*OXA-48 genes and. There was a high prevalence of virulence genes in CP-UPEC. Hence, surveillance is needed to control the spread of MDR strains.

**Keywords:** Uropathogenic *Escherichia coli*, virulence typing, carbapenemase, serogrouping, phylogroups

# Identification of class I integron in carbapenem-resistant Enterobacteriaceae isolated from patients hospitalized in medical teaching hospitals of Mazandaran University of Medical Sciences

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Carbapenem-resistant Enterobacteriaceae (CRE) and integron genes, especially class I integron is an increasing global challenge of the high morbidity and mortality associated with their infection. This study aimed to determine the prevalence of class I integron in carbapenem-resistant Enterobacteriaceae isolated from patients in the educational hospitals of Mazandaran University of Medical Sciences.

**MATERIALS AND METHODS:** Totally, 100 enterobacteriaceae isolates were collected from March 2022 to March 2023 at the educational hospitals of Mazandaran University of Medical Science using a consecutive sampling method. Isolates were identified by standard microbiological techniques. Disk diffusion Method was used to determine the antibiogram of isolates. 73 carbapenem-resistant isolates were identified by the disk diffusion method and then subjected to genetic conformation using polymerase chain reaction (PCR).

**RESULTS AND DISCUSSION:** Of the 73 carbapenem resistant isolates comprising K.pneumoniae (39.72%) E.coli (30.13%) S.rubidaea (12.32%) P.mirabilis (4.10%) E.aerogenes (4.10%) E.calocae (2.73%) C.freundii (2.73%) E.gergoviae (1.36%) C.diversus (1.36%) and P.vulgaris (1.36%). The isolates showed less resistance to imipenem (51%) in comparison to meropenem (73%). Out of the 73 isolates, 47 (64.38%) expressed the intl gene with K.pneumoniae 26(89.65%) accounting for the majority. The prevalence of class I integron in carbapenem-resistance Enterobacteriaceae pathogens among patients at the educational hospitals of Mazandaran University of Medical Science is relatively high and over 50 percent, and effective infection prevention and identifying this gene should be implemented at the hospitals to prevent the rapid spread of these dangerous organisms

**Keywords:** Enterobacteriaceae, carbapenem-resistance, class I integron.

# Identification of rifampin and isoniazid resistance in *M. tuberculosis* isolates from the southwest of Iran using molecular techniques

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** The emergence and spread of drug-resistant strains of *M. tuberculosis* (MTB) represents a serious threat to the therapy and control of tuberculosis (TB) in the global. Considering in different regions, the amount of gene mutations associated with MTB drug resistance is different, it is necessary to evaluate the prevalence of various types of mutations. The main purpose of this study is to investigate the most frequent mutations associated with resistance to rifampin and isoniazid in MTB strains from the TB reference center in southwest Iran.

**MATERIALS AND METHODS:** A collection of 413 confirmed MTB isolates were collected during November 2015 to February 2018 in the Ahvaz Regional TB Laboratory, Southwest of Iran. Subsequently, the susceptibility of first-line anti-TB drugs was evaluated through the proportional method. Mutations linked to resistance against RIF and INH were identified using PCR analysis and sequencing.

**RESULTS AND DISCUSSION:** In this study, sequence analysis for the detection of mutations linked to antibiotic resistance in the *rpoB*, *katG*, *mab-inhA*, genes in MTB clinical isolates were performed. The rates of resistance to INH + RIF, INH, and RIF were reported as 0.96%, 0.96%, and 1.94% respectively. Among four INH resistant isolates, only 1 isolate showed a mutation in codon 315 of *katG* gene. In relation to rifampin, mutations linked to the *rpoB* gene at codon 533 were found in 3 from 8 rifampin resistant isolates. All four MDR isolates showed mutation in the *rpoB* gene at codons 533, 531, but only one MDR isolate exhibited a mutation in *mabA-inhA* promoter. Numerous isolates showed no mutations. This research emphasizes the importance of evaluating mutations in additional genes associated with resistance to INH and RIF in this region.

**Keywords:** Mycobacterium tuberculosis, Rifampin, Isoniazid, Mutation, Resistance



# Investigating binding factors in *Staphylococcus aureus* strains collected from Cockroaches obtained from hospitals environment in North of Iran

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** The role of disease transmission to humans from insects was shown in the 19th century. Among these insects are cockroaches that can affect human health. For this reason, the importance of these insects in medicine is related to their eating habits and natural habitat (sewage and other environments). *Staphylococcus aureus* is one of the most significant germs that can be spread by cockroaches. The wide distribution of the organism in different environments and the ability to cause a wide range of infections is due to the presence of many factors. the purpose of this study is to investigate the virulence factors in *S.aureus* isolated from cockroaches caught from the teaching hospitals of Babol University of Medical Sciences.

**MATERIALS AND METHODS:** Thirty strains of *Periplaneta americana* and *Blattella germanica* cockroaches were gathered, of which 21 were *P. americana* cockroaches (70%) and 9 were *B. germanica* cockroaches (30%). The internal and external surfaces of the cockroaches were subjected to bacterial isolation. The samples were assayed to detect the presence of virulence factors genes (*eno*, *ebps*, *fib*, *bbp*) by the use of a polymerase chain reaction (PCR) method.

**RESULTS AND DISCUSSION:** A total of 30 cockroaches were collected; out of these, 6 (20%) *S. aureus* strains were isolated. Among the infected cockroaches, 70% were from *P. americana*, while 30% were from *B. germanica*. Furthermore, two strains were isolated from the inner surface of *B. germanica*, whereas three strains were isolated from the inner surface of *P. americana*, and one strain was isolated from the outer surface. the frequencies of the genes *eno*, *ebps*, *fib* and *Bbp* are 50%, 15%, 66% and 0% respectively. The *eno* and *fib* and were the most frequent virulence factors genes. From the results of this study, he found out the prevalence of adhesin factors in *Staphylococcus aureus* living in cockroaches in the hospital. These results helped to make the right decision to reduce environmental contamination from *Staphylococcus aureus* and prevent the mechanical transmission of this pathogen by insects.

**Keywords:** *Staphylococcus aureus*, Cockroaches, Virulence genes, Polymerase chain reaction.

# Investigating the antibiotic resistance pattern of *Escherichia coli* in urine culture samples of Farabi Bastak Hospital

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Introduction: Antibiotic resistance among pathogenic bacteria is a worldwide problem. Considering that *E. coli* is the most important cause of urinary tract infections and hospital acquired infections, this study was designed to determine the antibiotic resistance pattern of *E. coli* in urine samples extracted from Farabi Bastak Hospital patients.

**MATERIALS AND METHODS:** Materials and methods: In this descriptive study, all urine samples sent for culture to the laboratory of Farabi Bastak Hospital were evaluated during a period of 12 months (March 2023 to February 2024). After culture in eosin methylene blue (EMB), blood agar and differential culture media, the diagnosis of *E. coli* strain, antibiogram pattern of this bacterium was performed by Kirby-Bauer disk diffusion method and its non-growth halo was evaluated according to the standards of the National Committee for Clinical Laboratories (NCCLS).

**RESULTS AND DISCUSSION:** Findings: From 433 positive culture samples obtained, 336 strains of *E. coli* (71.6%) were isolated. According to the findings, the most cases of resistance to *E. coli* were related to the antibiotics Co-trimoxazole (51.78%), cefotaxime (48.34%), ceftriaxone (47.97%), and ciprofloxacin (40.69%). On the other hand, the most sensitive antibiotics are Nitrofurantoin (85%), Amikacin (81.7%) and Imipenem (73.9%). Conclusion: In this study, nitrofurantoin has the highest antibiotic sensitivity and is used as the first drug in the treatment of urinary tract infection. In children, cefotaxime and in adults, ceftriaxone are also used in the treatment of urinary infections, and in this study, high antibiotic sensitivity to the two mentioned drugs was observed. Co-trimoxazole has the highest amount of antibiotic resistance, which practically justifies the non-use of this drug in the treatment of urinary infections.

**Keywords:** Key words: Urinary tract infection, *Escherichia coli*, Antibiotic Resistance

# Investigating the sensitivity and drug resistance pattern in the bacterium that causes urinary infections, *Staphylococcus aureus*, in patients referred to the Mousavi clinic treatment center in Gorgan city

Tuberculosis and other mycobacterial infections

حمید رضا<sup>2</sup>, سعیده<sup>1</sup>

دانشجو<sup>1</sup>  
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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus* bacteria, gram-positive cocci and facultative anaerobes, which is considered the most important species in the genus *Staphylococcus* from the medical point of view, and is considered one of the important factors in causing hospital infections. In recent years, the important role of this bacterium in causing hospital infections has led to an increase in research related to this bacterium. *Staphylococcus aureus* causes a wide range of diseases such as endocarditis, food poisoning, septicemia, skin infections and skin peeling syndrome in humans. Antibiotic resistance in this bacterium is controlled by chromosomes and plasmids. Excessive and over-the-counter use of antibiotics over time leads to an increase in resistance and a decrease in the sensitivity of bacteria to different antibiotics. The purpose of this research was to evaluate the antibiotic resistance pattern in *Staphylococcus* bacteria isolated from urinary infection samples in people referred to Mousavi Clinic in Gorgan city.

**MATERIALS AND METHODS:** This descriptive-cross-sectional study was conducted in a four-year period (April 1999 - May 1403) on 9034 samples of urinary tract infection (UTI) in the microbiology department of Mousavi Clinic. Determining the antibiotic sensitivity and resistance pattern of *Staphylococcus aureus* isolates was done by disk diffusion method.

**RESULTS AND DISCUSSION:** Out of 7034 cultured urinary tract infection (UTI) samples, 267 samples were isolated from *Staphylococcus aureus* strain, and in its antibiogram culture, the highest antibiotic resistance was related to erythromycin (75.3%), penicillin (64.5%) and amoxicillin (64 %) and the highest antibiotic sensitivity included nitrofurantoin (90.4%), vancomycin (76.3%) and cefoxitin (65.8%). Since the distribution of antibiotic resistance of isolates carrying UTI in patients is variable. Therefore, it is important to determine the antibiotic resistance of UTI caused by *Staphylococcus aureus* in diagnostic-treatment centers in different geographical areas. Also, due to the limited number of investigated samples and the difference of antibiotic resistance in different geographical locations, the result is approximate and more investigation is needed in different regions with more clinical samples.

**Keywords:** *Staphylococcus aureus*, Diffusion disk, urinary tract infection, Antibiogram culture

# Investigation of the prevalence of *Staphylococcus aureus* at various levels in ICU and NICU wards of Taleghani hospital in Tehran, Iran

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Nosocomial infections, also known as healthcare associated infections, are hospital infections that arise 42–72 hours after a patient receives medical care in the hospital. Infections in hospitals lead to high mortality rates. Risk factors for these infections include patient health status, infection control methods, age, immune system function, multiple chronic conditions, frequent exposure to health facilities, use of invasive procedures, chemotherapy and immunotherapy, and local pathogen prevalence. Contamination of environmental surfaces, particularly those in contact with patients, is a major cause of nosocomial infections. *Staphylococcus aureus* is a major bacterium that causes surface contamination. This study investigated the prevalence of *Staphylococcus aureus* contamination on various surfaces in ICUs and NICU in Iran.

**MATERIALS AND METHODS:** 320 samples were taken from various levels of Taleghani Hospital's ICU and NICU. Catalase, coagulase, and DNase tests were utilized for bacterial identification. The samples underwent an antibiotic susceptibility test (antibiogram). The abundance of the genes TSST-1, PVL, and *mecA*, and Chromosome *mec* (SCC*mec*) typing was investigated after extracting bacterial DNA.

**RESULTS AND DISCUSSION:** 205 samples (64%) were identified as belonging to the Staphylococcaceae family. according to the findings of the antibiotic sensitivity test, All of the *S. aureus* isolates were susceptible to vancomycin, while the majority (90%) were resistant to ceftaroline. Out of a total of 21 *S. aureus* isolates, five strains (23.8%) had the *mecA* gene, and four strains (19%) had the PVL gene. There was no TSST-1 gene found in any of these samples. SCC*mec* type I and Healthcare-Acquired Methicillin-resistant *S. aureus* (HA-MRSA) was found in 87.5% of isolates, while only 22.5% contained SCC*mec* IV and Community-Associated Methicillin-Resistant *S. aureus* (CA-MRSA). There was a high level of non-*Staphylococcus aureus* contamination on surfaces in the ICUs. High incidence of SCC*mec* type I proved that bacterial surface contamination in hospitals originates from MRSA-infected or MRSA-carrier patients.

**Keywords:** Nosocomial bacteria, Multidrug resistance, MRSA

# Investigation of the prevalence of Streptococcus Group A and their drug resistance pattern among children with pharyngitis during 2021-2022

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Drug-resistant group A Streptococcus (GAS) is microbial agents capable of causing pharyngitis and invasive infections, particularly among children worldwide. In this research, the frequency of antibiotic resistance of GAS was investigated in children with pharyngitis during 2021-2022.

**MATERIALS AND METHODS:** Clinical examination of symptomatic children was performed according to Centor criteria in Mofid Children's Hospital and Children's Subspecialty Clinic in Tehran, Iran. Pharyngeal swab samples were prepared in Amies transfer medium and transferred to the laboratory along with demographic and clinical information. Cultivation in Blood agar medium and incubation in the presence of 5% carbon dioxide in a candle jar were done for 24 h. Beta hemolytic colonies were further investigated in terms of morphology after Gram staining, catalase, sensitivity to bacitracin, and PYR test. Sensitivity to penicillin and meropenem was tested using Etest, and sensitivity to ampicillin, erythromycin, clindamycin, tetracycline, moxifloxacin, cefotaxime, levofloxacin, and linezolid antibiotics was done by disc diffusion method according to CLSI standard 2022.

**RESULTS AND DISCUSSION:** Out of a total of 241 symptomatic patients, GAS infection was confirmed in 14.1% (34 patients). All the isolates were sensitive to penicillin, ampicillin, meropenem, cefotaxime and linezolid (100%). The highest resistance was estimated to erythromycin (61.3%), moxifloxacin and levofloxacin (30% and 30%), followed by clindamycin (12.9%) and tetracycline (19.3%). Also, intermediate resistance was detected in 70% of the isolates against levofloxacin. Single, double, and triple drug resistance was determined in 12.9%, 41.9%, and 9.6% of the isolates, respectively, while sensitivity to all antibiotics was detected in 35.5% of them. In conclusion our results showed  $\beta$ -lactam antibiotics can be effective against GAS-pharyngitis in children in the absence of colonization or co-infection with  $\beta$ -lactamase producing bacteria. The increase in resistance to macrolides, lincosamides, fluoroquinolones and tetracyclines in these isolates indicates serious therapeutic problems in the treatment of invasive GAS infections and the treatment of patients with beta-lactam allergy.

**Keywords:** Group A Streptococci; Antibiotic resistance; Children; Pharyngitis

# Isolation, Identification and antibiotic resistance of *Proteus mirabilis* from commercial poultry carcasses in Ardabil province, Iran

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** *Proteus mirabilis* is an important pathogen in human that contaminated poultry products can be a source for human infections and multi-resistant strains. The aim of this study was to survey the contamination level of commercial poultry carcasses by *P. mirabilis* and to determine the antimicrobial sensitivity of the isolated bacteria

**MATERIALS AND METHODS:** Samples were taken from heart blood and liver's visceral surface of 125 carcasses obtained from 30 commercial flocks (including 27 broiler flocks, 1 laying flocks and 2 breeder flocks) located in different parts of Ardabil province, northwest of Iran. *P. mirabilis* isolates were identified based on microbiological standard methods. The antibiotic susceptibility test was performed using the Kirby-Bauer disk diffusion method.

**RESULTS AND DISCUSSION:** Out of 125 collected carcasses, bacteria isolates were confirmed 98. Among them, 7 (7.14 %) of isolates were diagnosed to be *P. mirabilis*. The strains exhibited high percentages of resistance to penicillin G (85.7 %) and ampicillin (71.4 %) and low percentages of resistance to vancomycin (14.3%) and cefoxitin (0.0 %). Sulfamethoxazole-trimethoprim, ciprofloxacin and gentamicin were resistant with 57.1 %, 42.8 % and 28.5%. The present study showed that poultry products, especially infectious lesions of liver and heart of poultry carcasses can be an important source of contamination and dissemination of drug-resistance *P. mirabilis*. Therefore, performing antimicrobial sensitivity test is necessary prior to prescribing any antibiotic in a farm.

**Keywords:** Drug resistance, *Proteus mirabilis*, Poultry carcasses, Ardabil

# Molecular study and expression of virulence and antibiotic resistance genes in *Staphylococcus aureus* isolated from cows suffering from acute mastitis treated with *Zataria multiflora* extract.

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus* is one of the most important causes of mastitis in dairy cows. The occurrence of antibiotic resistance in this bacterium is a major concern in veterinary medicine and public health. Considering the identification of genes encoding resistance to erythromycin in this bacterium, the aim of the current research is to study the molecular and expression of virulence and antibiotic resistance genes in *Staphylococcus aureus* isolates isolated from cows suffering from acute mastitis treated with *Zataria multiflora* extract.

**MATERIALS AND METHODS:** In this study, the identity of 125 samples of *Staphylococcus aureus* bacteria was confirmed using gram staining and biochemical tests. Antibiotic sensitivity of all isolates was evaluated with antibiotic disks according to the table proposed by CLSI 2021. Evaluation of slime production and biofilm formation was done using Congo Red Agar and 96-well Micro titer Plate methods, respectively. Investigating the antimicrobial effect of *Zataria multiflora* essential oil on *Staphylococcus aureus* bacteria was also done by disk diffusion agar method. The abundance of *ermA*, *ermC*, *IcaD*, *IcaC* and *IcaA* genes was investigated by multiplex PCR. Finally, the expression of *ermA* and *IcaD* genes was evaluated in *Staphylococcus aureus* cells treated with *Zataria multiflora*.

**RESULTS AND DISCUSSION:** The highest level of resistance to erythromycin (67 isolates) and the lowest resistance to gentamicin (5 isolates) were observed. In the first 24 hours, out of 67 samples of *Staphylococcus aureus* resistant to erythromycin, 60 samples produced strong slime, 4 samples produced medium slime and 3 samples produced weak slime, and no sample without slime was obtained. All samples of *Staphylococcus aureus* resistant to erythromycin had the ability to form biofilm, 66.6% of them had the ability to produce a strong biofilm and the other 33.3% had the ability to form an average biofilm. The results of the effect of essential oil on *Staphylococcus aureus* by disc diffusion method show that with increasing concentration of essential oil, the diameter of the growth halo increases. The results showed that 55 isolates had *ermC* gene, 44 isolates had *ermA* gene, 60 isolates had *IcaD* gene, 28 isolates

**Keywords:** Key words: mastitis, *Staphylococcus aureus*, biofilm, antibiotic resistance, *Zataria multiflora*,

# Molecular typing of clinical isolated methicillin-resistant *Staphylococcus aureus* in Mazandaran

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most clinically important bacteria with the antibiotic resistance challenges. The present study aimed to determine the antibiotic resistance pattern and the prevalence of SCCmec and ccr types in MRSA isolates in North of Iran.

**MATERIALS AND METHODS:** In this study, 100 clinical isolates of *S. aureus* were collected from Hospitalized patient during a period of 10 months, from March to December 2022. The isolates were identified using standard microbiological and biochemical tests, and were confirmed by determination of the nuc gene. The antibiotic susceptibility pattern of the isolates was evaluated by the disk agar diffusion and micro broth dilution methods. Then, all MRSA isolates were checked for the presence of mecA, and were typed as the presence of SCCmec types I, II, III, IVa, IVb, IVc, and Ivd, and ccr types 1, 2, 3, 4, and 5 genes using the PCR method.

**RESULTS AND DISCUSSION:** The isolates of *S. aureus* were obtained from patients hospitalized in the teaching hospitals. All isolates were sensitive to quinupristin- dalfopristin and linezolid, while 99 isolates were susceptible to rifampin, gentamicin and vancomycin. In addition, 80 isolates were resistant to cefoxitin. There was no significant difference in antibiotic resistance between MRSA and Non-MRSA strains. Overall, 73 (91.25%) MRSA isolates had SCCmec III genotype, while 2 (2.5%) had SCCmec IV genotype and 5 (6.25%) MRSA isolates were untypeable using these primers. None of the 80 MRSA isolates showed positive results for ccr1 and ccr4. 24 (30%), 7 (8.75%), and 23 (28.75%) MRSA isolates had genotypes carrying ccr2, ccr3, and ccr5 genes, respectively. 26 (32.5%) MRSA isolates were non-typeable using the primers used for ccr genes in this study. Conclusion: The strong presence of SCCmec III among the isolates of this region indicates the common source of these isolates among the hospitals of this region.

**Keywords:** Methicillin-resistant *S. aureus*; SCCmec, ccr; Typing



# Nosocomial infection in burn intensive care unit: A Retrospective Study in a referral burn center in, Ahvaz, Iran

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Burn-related nosocomial infections are challenging conditions to manage and can cause several complications entailing a complicated treatment. So, in the present study, nosocomial infections in burn patients hospitalized in the burn intensive care unit (BICU) of the referral burn hospital of Ahvaz City were investigated.

**MATERIALS AND METHODS:** This retrospective study was conducted between January 2021 and December 2023 in the BICU of Taleghani Burn Hospital in Ahvaz, Iran. The study did not include patients with an ICU stay of less than 48 hours and survival of less than 72 hours. The demographic and clinical data, referral status, the type of infections and the isolated microorganisms, antibiotics resistance, and the mortality rate are evaluated retrospectively from patient files and the hospital registries.

**RESULTS AND DISCUSSION:** A total of 1239 patients were hospitalized in BICU 469 cases (37%) acquired infections and 525 isolates were obtained. The most commonly isolated microorganisms were *Pseudomonas aeruginosa* (64.04%) in burn wounds, bloodstream, and urinary tract infections, *Enterobacter* spp. (8.80%), *Escherichia coli* (7.68%), *Acinetobacter baumannii* (4.87%), Fungus (3.93%), *Staphylococcus epidermitis* (3.93%), *Staphylococcus aureus* (2.43%), *Citrobacter* spp. (1.5%), *Enterococcus* spp. (0.56%), *Klebsiella pneumoniae* (0.37%), group D streptococcus (0.19%). The median time from the patients being hospitalized to infection was 10.89±9.42 days. Also, extensive resistance rates (more than 80%) were found against penicillin, piperacillin, meropenem, gentamicin, amikacin, Trimethoprim/sulfamethoxazole, and imipenem antibiotics. Burn wound infections were the most prevalent infection (82.38%), followed by bloodstream (20.57%) and urinary tract infections (6.85%). *S. aeruginosa* was the main pathogen of infections in burn patients. In each burn center treatment and infection-control strategies should be performed based on the characteristics of burn patients and drug susceptibility

**Keywords:** Burn intensive care unit, Nosocomial infection, antibiotic resistance

# Phenotypic and genotypic investigation of *Streptococcus agalactiae* collected from pregnant women in North Iran

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Background: *Streptococcus agalactiae* colonizing the genital tract of pregnant women transfers to infant and causes different diseases along with the septic abortion. We aimed to determine the prevalence of antibiotic resistance and capsular genes of different clonal types of this bacterium in pregnant women.

**MATERIALS AND METHODS:** Methods: Four hundred twenty non-repeated vaginal and rectal specimens from pregnant women were cultured on a selective medium, and the grown bacteria were identified by standard tests. Antimicrobial resistance pattern of the isolates was determined using the disk agar diffusion method. The genomic DNAs were extracted using a kit, and the antibiotic resistance and capsular genes and RAPD types were detected using the PCR method.

**RESULTS AND DISCUSSION:** There was a significant relationship between the weeks of pregnancy and the positive bacterial cultures. Moreover, 31 and 18 participants had a history of abortion and membrane rupture, respectively. Among 106 *S. agalactiae* isolated, 94.33% were resistant against tetracycline, while all were susceptible to linezolid. Moreover, 15, 15, 42, and 7 isolates showed an iMLS B, M-, cMLS B, and L-phenotype. The *ermB* was the most prevalent resistance gene (94.33%), while 38 (35.84%), 8 (7.54%), 79 (74.52%), 37 (34.9%), and 20 (18.86%) isolates were contained the *ermTR*, *mefA/E*, *tetM*, *tetO*, and *aphA3* gene, respectively. The high-level antibiotic resistance and prevalence of resistance genes may be due to the arbitrarily use, livestock industry consumption, and the preventive use of antibiotics in pregnant women. In addition, 20 (18.86%), 32 (30.18%), 4 (3.77%), and 6 (5.66%) isolates carried genes encoding capsular types Ia, Ib, III, and V, respectively. Nine clones

**Keywords:** *Streptococcus agalactiae*, Pregnant women, Antibiotic resistance, capsular genes, Molecular typing

# Prevalence and antibiotic resistance of bacteria in blood cultures of patients hospitalized in Zare Hospital of Sari in 2014-2017

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Bacteremia can have severe consequences, including death. The diversity of bacteria leading to bacteremia and septicemia presents significant challenges for patients. Additionally, the growing antibiotic resistance among these bacteria underscores the need for epidemiological studies and continuous assessment of antibiotic efficacy in every treatment center. Hospitalized patients, especially patients with burn injury are particularly at risk of blood infections from opportunistic bacteria. This study aims to investigate the prevalence of bacteria isolated from blood cultures in hospitalized patients at Zare Hospital in Sari from 2015 to 2018 and to determine their antibiotic susceptibility and resistance.

**MATERIALS AND METHODS:** This retrospective study involved reviewing 1734 patient files and sampling at Zare Hospital in Sari from 2015 to 2018. Observations were conducted on all requested 10 cc blood culture samples and antibiogram test was performed to determine their antibiotic sensitivity

**RESULTS AND DISCUSSION:** Out of 1,734 requested blood cultures, 299 cases (17.24%) were positive. The most common gram-positive microorganisms were *Staphylococcus aureus* (25.1%) and coagulase-negative *Staphylococcus* (2.7%). The most common gram-negative microorganisms were *Acinetobacter* (37.8%) and *E. coli* (7.7%). The most effective antibiotics against gram-positive microorganisms were Vancomycin(96.3%) and Cephalexin(72%), while Amikacin(100%) and Meropenem(76.9%) were most effective against gram-negative microorganisms. The highest resistance in gram-positive microorganisms was observed with Ampicillin (82.7%), Oxacillin(74.6%), and Ciprofloxacin(56%); for gram-negative microorganisms, the most resistance was seen with Ampicillin(90.9%) and Cefotaxime(90.9%).

**Keywords:** Bacteremia, bloodculture, Antibiotic Resistance, burn

# Prevalence and Antibiotic Resistance Patterns of *Staphylococcus aureus* Strains Associated with Surgical Site and Bloodstream Infections in Hospitalized Patients from Tehran, Iran

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus* (*S. aureus*) is a leading cause of surgical site infections (SSIs) and bloodstream infections (BSIs), posing a significant challenge in healthcare settings due to its increasing antibiotic resistance. This study aimed to investigate the prevalence and antibiotic resistance patterns of *S. aureus* strains associated with SSIs and BSIs in hospitalized patients from Tehran, Iran.

**MATERIALS AND METHODS:** A total of 362 *S. aureus* isolates were obtained from patients with SSIs and BSIs across five major hospitals in Tehran between March 2023 and November 2023. Antimicrobial susceptibility testing was conducted using the broth microdilution, disk diffusion (Kirby-Bauer), and E-test methods, following the Clinical and Laboratory Standards Institute (CLSI) protocols. The study determined the prevalence of methicillin-resistant *S. aureus* (MRSA), multidrug-resistant (MDR), and extensively drug-resistant (XDR) strains, as well as the resistance and sensitivity patterns to a range of antibiotics.

**RESULTS AND DISCUSSION:** Out of 362 isolates, 59.94% (217/362) were associated with SSIs, and 40.06% (145/362) with BSIs. The prevalence of MRSA was 63.25% (229/362). High resistance rates were observed against  $\beta$ -lactams, with 95.58% resistance to penicillin, 83.98% resistance to oxacillin, and 80.66% to ceftriaxone. Glycopeptide resistance was minimal, at 1.93% for vancomycin. Among the isolates, 59.94% were MDR and 16.02% were XDR. Remarkably, 82.53% (189/229) of MRSA isolates were MDR, and 25.33% (58/229) were XDR. The resistance patterns also revealed that 66.85% of the isolates were resistant to erythromycin, 64.91% to tetracycline, 62.7% to clindamycin, 59.94% to ciprofloxacin, 53.59% to trimethoprim/sulfamethoxazole, and 29.0% to linezolid. The study highlights the alarming prevalence of MRSA and MDR/XDR *S. aureus* strains in SSIs and BSIs in Tehran, Iran. The extensive resistance to multiple antibiotic classes underscores the critical need for enhanced infection control strategies, antibiotic stewardship programs, and the development of new antimicrobial agents.

**Keywords:** *Staphylococcus aureus*, Antibiotic resistance, Surgical site infections, Bloodstream infections, Multidrug-resistant

# Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from Commercial poultry carcasses in Ardabil province, Iran

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Poultry especially broiler is one of the most common forms of protein in Iran. Farmers nonetheless use antibiotics for prevention, treatment, and growth enhancement. The objective of this investigation was to determine antibiotic resistance patterns of *S. aureus* isolated from commercial poultry carcasses in Ardabil province, northwest of Iran

**MATERIALS AND METHODS:** Samples were taken from heart blood and liver's visceral surface of 125 carcasses obtained from 30 flocks (including 27 broiler flocks, 1 laying flocks and 2 breeder flocks) located in different parts of Ardabil province. The isolates of *S. aureus* were determined based on microbiological standard methods. The antibiotic susceptibility test was performed using the Kirby-Bauer disk diffusion method

**RESULTS AND DISCUSSION:** Out of 98 isolates, 8 (8.16%) *Staphylococcus aureus* were detected while in 34 cases, no bacteria were found. The *S. aureus* isolates showed resistance to Doxycycline (100 %), Ampicillin (87.5%), followed by Methicillin (75.0%), Sulfamethoxazole-trimethoprim (62.5%), Ciprofloxacin (62.5%), Erythromycin (50.0%), Chloramphenicol (37.5%), Gentamicin (37.5%), Vancomycin (25.0%) and Cefoxitin (12.5%). Our data indicated that antimicrobial resistance of *S. aureus* was in poultry flocks of Ardabil province, northwest of Iran, and that antibiotics, especially tetracycline and ampicillin, to treat in poultry should be used with caution

**Keywords:** Antimicrobial resistance †*Staphylococcus aureus* †Poultry carcasses †Ardabil province

# Prevalence of class 1 integron in carbapenem-resistant Enterobacteriaceae in patients admitted to Sari Teaching Hospitals in 2022

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** The high prevalence of class 1 integron in carbapenem-resistant Enterobacteriaceae (CRE) is a significant concern. Class 1 integrons are genetic elements that can capture and express resistance genes. The integrons play a crucial role in the spread of antibiotic resistance among bacteria. In CRE, the presence of class 1 integrons can contribute to the limited treatment options and increased difficulty in controlling infections. Understanding the prevalence of class 1 integron in these bacteria is essential for effective surveillance and management strategies. The aim of this study is to determine the prevalence of class I integron in CRE isolated from patients in the educational hospitals of Mazandaran University of medical sciences

**MATERIALS AND METHODS:** In this cross-sectional study, 100 isolates of Enterobacteriaceae strains were collected from March 2022 to March 2023 from hospitalized patients. The isolates were identified by standard microbiological methods and disc-diffusion was used to determine the susceptibility profiles of the isolates and, then the presence of class I integron gene was assessed by polymerase chain reaction (PCR) technique.

**RESULTS AND DISCUSSION:** Totally, 73 carbapenem-resistant isolates were identified by disk-diffusion method. In the studied carbapenem class, the isolates showed less resistance to imipenem (%51) in comparison than meropenem (%73). Out of 73 isolates, 47 (%64.38) expressed the intI gene with *K. pneumoniae* 26 (% 89.65) accounting for the majority. The prevalence of class I integron in carbapenem resistance Enterobacteriaceae among hospitalized patients at the educational hospitals of Mazandaran University of Medical Sciences is over % 50. The effective infection prevention and identifying this gene should be implemented at the hospitals to prevent the rapid spread of these dangerous organisms.

**Keywords:** Enterobacteriaceae, carbapenem-resistant, class I integron

# Prevalence of CRISPR-Cas systems and their association with antibiotic resistance in *Enterococcus faecalis* and *Enterococcus faecium* isolated from hospitalized patients

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** CRISPR-Cas system, as an adaptive immune defense system plays an important role in controlling horizontal gene transfer and limiting the spread of antibiotic resistance. In this study we analyzed the presence of CRISPR genes and their association with antibiotic resistance patterns of *Enterococcus faecalis* and *Enterococcus faecium* isolated from hospitalized patients.

**MATERIALS AND METHODS:** A total of 75 *Enterococcus* isolates were collected from urinary tract infections (UTI), Blood and Wound infections. Isolated bacteria were identified by their biochemical reaction profile and polymerase chain reaction-based methods. The resistance and susceptibility of the isolates were assessed using disc diffusion method. CRISPR loci were screened using PCR, and the relationship between CRISPR-Cas systems and antibiotic resistance was statistically analyzed. PCR products were sequenced. Following that, Phylogenetic, structural and conservational analysis were used to draw phylogenetic trees, identify conserved regions and mutations within the CRISPR-associated endonuclease Cas9 protein, and generate sequence logos, respectively.

**RESULTS AND DISCUSSION:** Among 75 *Enterococcus* strains, the CRISPR-Cas loci was detected in 65 (86.6%) isolates. The incidence of CRISPR-Cas was more common in *E. faecalis*. CRISPR1-cas, CRISPR2, and CRISPR3-cas were identified in 76%, 82.6%, and 64% of *Enterococci* isolates, respectively. Interestingly, the prevalence of CRISPR-Cas genes was lower in extensively drug-resistant (XDR) isolates (32%) compared to multidrug-resistant (MDR) isolates (68%) (p-value = 0.0001). DNA sequencing revealed high similarity between our samples and reference strains, with variations at specific positions in CRISPR3-Cas *csn1* samples. Additionally, a specific mutation (V42I and A424S) was identified in one of the CRISPR3-Cas *csn1* sample. It is concluded that antibiotic resistance levels were inversely correlated with the existence of CRISPR/Cas systems. With the increase in the degree of antibiotic resistance (MDR, XDR to PDR), the occurrence ratio of the (CRISPR)/CRISPR-associated sequence decreased.

**Keywords:** *Enterococcus faecalis*, *Enterococcus faecium*, CRISPR-Cas, Antibiotic resistance, Phylogenetic analysis

# Prevalence of multidrug resistant *Pseudomonas aeruginosa* among covid\_19 patients at ICU of Tehran hospitals with UTI (Urinary tract infection)

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Opportunistic bacteria have caused Urinary Tract infection. *Pseudomonas aeruginosa* is the main cause of opportunistic infections associated with the intensive care unit. Several virulence factors are involved in the colonization and invasion of *Pseudomonas aeruginosa*, which make the infection more severe. The purpose of this study is Prevalence of multidrug resistant *Pseudomonas aeruginosa* among covid\_19 patients with UTI at ICU of Tehran hospitals.

**MATERIALS AND METHODS:** This cross-sectional study was conducted on 60 *Pseudomonas aeruginosa* isolates among covid\_19 patients at ICU of Tehran hospitals with UTI. All isolates were identified by standard microbiological tests. Antimicrobial sensitivity test was done by disk diffusion methods.

**RESULTS AND DISCUSSION:** In this study, (78.3%) of the patients were male and (21.6%) were female. The highest rate of drug resistance was for gentamicin (90%) and tobramycin (88.3%). Also, 3.3% of the samples were resistant to colistin. The highest antibiotic sensitivity was for Ceftazidime, Avibactam (91.7%) and Colistin (96.7%). The rate of resistance to imipenem (80%) and meropenem (86.6%) was reported. In this study, 43 samples (71.6%) had metalloβ-lactamase enzyme. The incidence of secondary infections as well as antibiotic resistance in this study is a cause of concern, thus, to control infection and prevent the spread of drug-resistant bacteria, there is a need for cautious management in medication delivery and detection of resistant isolates

**Keywords:** *Pseudomonas aeruginosa*, Urinary Tract Infection, antimicrobial resistance



# Production of silver nanoparticle using isolated actinomycets from soil

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Actinomycets are Major order of filamentous gram-positive bacteria, which considered as normal flora and opportunistic pathogens. Nowadays, these bacteria are noted as special due to their ability for production of enzymes, vitamins, growth hormones, anti-cancer agents, antibiotics, bioactive Nano-particles and other bioactive compounds. The aim of this study is isolation and characterization of actinomycet with capability to produce gold, silver and zinc nanoparticles from wheat fields in Marvdasht province with antibacterial effect on pathogenic bacteria.

**MATERIALS AND METHODS:** To perform this study, 30 soil samples were collected from the wheat fields of Marvdasht. After the isolation and identification of actinomycetes using phenotypic and genotypic methods, the ability to produce nanoparticles of silver by adding silver nitrate to the solutions was determined. Reduction of silver ions in the solution was determined through spectrophotometry studies and the size and shape of nanoparticles were studied by transmission electron microscopy. In addition, presence of silver nanoparticles by X-ray diffraction (XRD) was confirmed and the isolates were molecularly identified usin PCR technique

**RESULTS AND DISCUSSION:** The results showed that three strains of actinomycetes were isolated from 30 soil samples that all three isolated strains were capable to produce silver nanoparticles. Based on applied molecular test the isolates were identified as *Streptomyces minutiscleroticus* and *Streptomyces rochei* with more than 99% similarity. Generally, most of soil microorganisms such as actinomycetes are capable to produce silver nanoparticles and their production and antibacterial properties can be evaluate by optimizing the physical and chemical factors. Therefore, further study try to evaluate antibacterial effect of the nanoparticles.

**Keywords:** Actinomycet, Silver nanoparticles, molecular identification

# Study of beta-lactam resistance genes in clinical isolates of *Pseudomonas aeruginosa* in Mazandaran province

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* is considered as one of the most important causes of nosocomial infections due to the presence of various antibiotic resistance genes. Beta-lactams are the important ones for treatment of infections and the beta-lactamases are the main mechanisms to resistance. Among them, PER, GES, VEB (Class A), OXA2, OXA10 (Class D) play the important role in resistance to beta-lactams. This study aimed to investigate the frequency of these genes in clinical isolates of *P. aeruginosa*.

**MATERIALS AND METHODS:** In this study, 100 non-repetitive isolates of *P. aeruginosa* prepared from wounds, catheters, blood, stool, eye secretions, urine, and sputum. The antibiotic resistance pattern of the isolates was determined by disk agar diffusion method using piperacillin, piperacillin-tazobactam, aztreonam, ceftazidime, imipenem, meropenem, and doripenem. The DNAs were extracted using alkaline lysis method (SDS + NaOH). The prevalence of beta-lactam resistance genes was identified by PCR method.

**RESULTS AND DISCUSSION:** Aztreonam was the least effective antibiotic with 39% resistance rate, while 12% of isolates were resistant against piperacillin-tazobactam. Also, the highest resistance rate was found in the wound isolates. There was a significant relationship between the presence of these genes and the development of resistance to beta-lactams. In addition, the prevalence of blaVEB, blaGES, and blaPER genes in this study was 93.02%, 83.72%, and 81.39%, respectively. Among the three blaVEB-negative isolates, only one was susceptible to piperacillin-tazobactam, and others were only sensitive to ceftazidime. The VEB enzyme is an effective factor for resistance to aztreonam, and can be inhibited by clavulanate. However, there was a relationship observed between the presence of blaVEB and blaGES genes and the hospital department, from which, the highest resistance was related to the burn ward. The high prevalence these genes in beta-lactam-resistant isolates in this area indicated the inappropriate antibiotic usage and prescription in Mazandaran.

**Keywords:** *Pseudomonas aeruginosa*, Beta-lactam resistance, blaVEB, blaGES, blaPER

# Study on the development of antibiotic resistance in pet owners (sample of forty dogs and their owners) following long-term antibiotic use in their pets

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Antibiotic resistance is one of the greatest global health threats in the present era. Recently, research has proposed that the indiscriminate use of antibiotics in domestic animals, particularly dogs, can lead to an increase in antibiotic-resistant bacteria in these animals. Additionally, the transfer of antibiotic resistance genes from bacteria in pets to human bacteria is possible through horizontal gene transfer mechanisms, such as transposons. Moreover, resistant bacteria can be excreted into the environment through pet feces, affecting environmental microbiomes and leading to the transfer of resistance genes to humans through contact with soil, water, and other environmental sources. In this study, we evaluated samples from forty dogs undergoing long-term antibiotic treatment and their owners to examine antibiotic resistance in pets and its correlation with their owners. The results showed a significant correlation between antibiotic resistance in the dogs and their owners.

**MATERIALS AND METHODS:** In this study, blood and fecal samples were collected from forty dogs that had been under long-term treatment with antibiotics including enrofloxacin, doxycycline, metronidazole, amoxicillin, and cephalixin. Additionally, blood samples were collected from their owners. The samples were then cultured on blood agar plates to allow bacterial growth. Following bacterial growth, the disk diffusion method was used to determine the sensitivity of the bacteria to the aforementioned antibiotics.

**RESULTS AND DISCUSSION:** According to the results and data analysis, The bacteria *Escherichia coli* and *Staphylococcus aureus* were the most abundant in the samples and approximately seventy percent of the pet owners exhibited antibiotic resistance, particularly to amoxicillin and cephalixin. This percentage was higher among those who had more frequent contact with their pets. Given the increasing trend of pet ownership, there is a serious issue concerning the management of antibiotic use in pets and its relationship with humans. This situation necessitates more preventive measures for pet owners and closer monitoring of antibiotic use in veterinary hospitals and clinics.

**Keywords:** Antibiotic/bacterium/microbiome/microbial resistance/*Escherichia coli*/ *Staphylococcus aureus*

# The antibiotic resistance patterns against *Staphylococcus aureus* isolates of patients hospitalized in the intensive care unit

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus* is a Gram-positive pathogen and a common cause of various infections in community and hospital settings. Common clinical symptoms include fever, pneumonia, endocarditis, septic arthritis, and osteomyelitis [1]. Methicillin-resistant *S. aureus* (MRSA) is a multidrug-resistant (MDR) bacterium that can cause various infectious diseases and is related to increased mortality [2]. Treatment of MRSA infections remains challenging due to its ability to develop resistance to different classes of antibiotics [3]. In this study, the antimicrobial resistance pattern was performed among *S. aureus* isolates collected from patients administered in the ICU.

**MATERIALS AND METHODS:** In a cross-sectional study, 290 respiratory specimens were collected from ICU-admitted patients of two teaching hospitals of Tehran University of Medical Sciences. The *S. aureus* isolates were identified using biochemical tests and confirmed by PCR amplifying *nuc-A* and *mec -A* genes. The Kirby–Bauer disk diffusion and minimal inhibition concentration (MIC) methods were used to perform the antimicrobial susceptibility test against *S. aureus* isolates [4]. The proportion of methicillin-resistant *S. aureus* isolates was also documented

**RESULTS AND DISCUSSION:** *S. aureus* isolates were confirmed in 43 (14%) of 290 respiratory specimens. A high resistance rate of 74.4%, 62.8%, and 55.8% to azithromycin, ofloxacin, and ciprofloxacin was observed, respectively. The proportion of MRSA isolates was 48.8% (21 out of 43). Approximately 76.2% of MRSA isolates and 31.8% of MSSA isolates were resistant to at least five different antibiotics. Also, 53.3% of expired patients were infected with MRSA (non-significant). The emergence of MDR MRSA strains, which may be accompanied by increased mortality, is a growing concern. As a result, implementing antimicrobial therapy following antibiotic resistance protocols and exploring new therapeutic approaches is crucial.

**Keywords:** antibiotic resistance, *S. aureus*, MRSA infections, ICU-admitted patients

# The effect of Chitosan/*Urtica dioica* and Chitosan Nanoparticles Against the Growth and the Expression of Two Virulence Genes (*kpsMT* and *ompT*) of Multiple Drug Resistant (MDR) *Escherichia coli* strains Isolated from Diabetic Ulcers patients in Montazeri Hos

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Background: Diabetes wound infection is a common disease in society. Multidrug-resistant *E. coli* bacteria often cause infections and diabetic ulcers in people. Objectives: This study aimed to evaluate the antibacterial effect of newly synthesized Chitosan /*U. dioica* NPs on multidrug-resistant *E. coli* bacteria isolated diabetic wounds.

**MATERIALS AND METHODS:** This study was conducted on samples containing *E. coli* bacteria, which were collected from diabetic wounds of people for eight months. After antibiogram test, colloidal Chitosan and Chitosan-*U. dioica* nanoparticles were prepared in 100 nm sizes in spherical, and spindle-shapes at 0.032 to 0.256 g/ml for these NPs. Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of nanoparticles were studied using the microdilution method. The expression rate of *kpsMT* and *ompT* genes were assessed by Real-Time PCR. data were analyzed by SPSS Software Version 20.

**RESULTS AND DISCUSSION:** : twenty-one of 100 (21%) diabetic ulcer samples were infected with *E. coli*. The highest resistance of *E. coli* to antibiotics belonged to Polymyxin B, Amoxicillin, Erythromycin, Penicillin, Nalidixic acid, Tetracycline, Imipenem, and Vancomycin. The results showed that Chitosan -*U. dioica* and Chitosan NPs (100 nm) as well as the spherical and spindle-shape after 24 h at 37°C can be used to treat diabetic ulcer infections caused by *E. coli*. Further research is recommended to consider the effects of different infections of diabetic ulcers in vitro conditions. The best antibacterial property was achieved by Chitosan / *U. dioica* nanoparticles at 0.256 g/ml. The results show a decrease in *ompT* and *kpsMT* genes expression among MDR *Escherichia coli* isolates with a significant difference compared to the control sample in the presence of nanoparticles. Conclusions: According to the results, this study confirmed the high prevalence of MDR *E. coli* strains in diabetic

**Keywords:** Chitosan/ *Urtica dioica*, *E. coli* spp, Nanoparticles, Diabetic ulcers, Green-synthesized

# The effect of green synthesized nanoparticles on the biofilm formation and gene expression in clinical isolates of *Acinetobacter baumannii*

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Background and purpose: *Acinetobacter baumannii* is a biofilm-producing and antibiotic-resistant pathogen causing human infections. This study investigated the expression of the *bap* gene in clinical isolates of *A. baumannii* and the effect of green silver nanoparticles obtained from *Astrodaucus persicus* and *Nepeta pogonosperma* plants on biofilm formation and *bap* gene expression.

**MATERIALS AND METHODS:** Materials and methods: One hundred clinical isolates were collected from hospitalized patients and identified using phenotypic and genotypic tests. The Antibiotic resistance pattern of the isolates and biofilm production ability (with and without nanoparticles) were evaluated by disk agar diffusion and micro titer plate assay. The minimum inhibitory concentration of nanoparticles was determined by micro broth dilution method. Finally, the *bap* gene expression was evaluated before and after treatment with nanoparticles using the Real-time PCR.

**RESULTS AND DISCUSSION:** Results and Discussion: Most isolates (99%) were MDR, and ciprofloxacin was the least-effective. In addition, 49%, 49% and 2% of the isolates were strong, moderate, and weak biofilm-producer, respectively. Also, 99 isolates showed an increased expression of *bap* gene, while 46 (46.46%) had an increased expression by 2-5-times. All isolates with a 11-20-times overexpressed *bap* gene belonged to the strong biofilm-producer group. Also, silver nanoparticles of *A. persicus* (1.171 µg/ml) and *N. pogonosperma* (1 µg/ml) decreased the expression of the *bap* gene, while we found a statistically significant difference between the effect of nanoparticles in 24 and 48 h treatment. The results of the present study indicate a relationship between the *bap* gene expression level and the increase in drug resistance and biofilm formation of *A. baumannii* isolates. Also, the results showed that silver nanoparticles effectively inhibit biofilm formation and significantly reduce the expression of the *bap* gene in strong biofilm-producer isolates.

**Keywords:** Keywords: *Acinetobacter baumannii*, *Astrodaucus persicus*, *Nepeta pogonosperma*, biofilm, *bap*, nanoparticles.

# The effect of mesenchymal stem cell conditioned medium incorporated within chitosan nanostructure in clearance of common gastroenteritis bacteria in-vitro and in-vivo

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Gastroenteritis infection is a major public health concern worldwide, especially in developing countries due to the high annual mortality rate. The antimicrobial and antibiofilm activity of human mesenchymal stem cell-derived conditioned medium (hMSCsCM) encapsulated in chitosan nanoparticles (ChNPs) was studied in vitro and in vivo against common gastroenteritis bacteria.

**MATERIALS AND METHODS:** The synthesized ChNPs were characterized using Zeta potential, scanning electron microscopy (SEM), and dynamic light scattering (DLS) techniques. HMSC-derived conditioned medium incorporated into chitosan NPs (hMSCsCM-ChNPs) composite was fabricated by chitosan nanoparticles loaded with BM-MSCs (positive for CD73 and CD44 markers). The antimicrobial and antibiofilm activity of composite was investigated against four common gastroenteritis bacteria (*Campylobacter jejuni* ATCC29428, *Salmonella enteritidis* ATCC13076, *Shigella dysenteriae* PTCC1188, and *E. coli* ATCC25922) in-vitro and in-vivo.

**RESULTS AND DISCUSSION:** Majority of ChNPs (96%) had an average particle size of 329 nm with zeta potential 7.08 mV. The SEM images confirmed the synthesis of spherical shape for ChNPs and a near-spherical shape for hMSCsCM-ChNPs. Entrapment efficiency of hMSCsCM-ChNPs was 75%. Kinetic profiling revealed that the release rate of mesenchymal stem cells was reduced following the pH reduction. The antibacterial activity of hMSCsCM-ChNPs was significantly greater than that of hMSCsCM and ChNPs at dilutions of 1:2 to 1:8 (P 0.05) against four bacteria. The number of bacteria present decreased more significantly in the group of mice treated with the hMSCsCM-ChNPs composite than in the groups treated with hMSCsCM and ChNPs. The antibacterial activity of hMSCsCM against common gastroenteritis bacteria in an in vivo assay decreased from 106 CFU/ml to approximately (102 to 10) after 72 h. These novel treatment approach to combat gastroenteritis bacteria in the context of more challenging infections.

**Keywords:** Antibacterial, Antibiofilm, Mesenchymal stem cells, Chitosan, Gastroenteritis

# The Impact of Aminoglycoside-Modifying Enzymes and 16S rRNA Methylase (ArmA) on Aminoglycoside Resistance in *Acinetobacter baumannii* Clinical stains

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** As a pathogen, *Acinetobacter baumannii* is known to have various antibiotic resistance genes, and its resistance against common antimicrobial substances such as Aminoglycosides, making it difficult to eradicate. Aminoglycosides are mainly used to treat health care related diseases. *Acinetobacter baumannii* shows various resistance mechanisms against aminoglycoside through the production of aminoglycoside modifying enzymes. Due to the widespread of this disease in hospitals and among patients, it is necessary to discover patterns and mechanisms that cause resistance in these bacteria. In our study, *Acinetobacter baumannii* strains were investigated and aminoglycoside modifying genes were identified molecularly

**MATERIALS AND METHODS:** This cross-sectional descriptive study conducted on 100 clinical isolates of *Acinetobacter baumannii* in hospitals in Sari include Zare, Imam Khomeini (RA), Bo Ali Sina and Razi Ghaemshahr hospital in a period of six months from March to December 2018. These clinical strains were isolated from patients who were admitted to the burn and infectious departments in the mentioned hospitals. Antibiotic sensitivity was determined using the disk agar diffusion method, while the microbroth dilution method was used to evaluate resistance to aminoglycosides.

**RESULTS AND DISCUSSION:** The frequency of the studied genes among the isolates were as follows: aph (3) - VIa (aphA6) (77%), ANT (2) - Ia (aadB) (73%), ANT (3) - Ia (aadA1) (33%), AAC (6) - Ib (aacA4) (33%), armA (22) and AAC (3) -IIa (aacC 2) (19%). All isolates were resistant to ciprofloxacin, while amikacin and neomycin showed the most effectiveness in disk agar diffusion testing. Moreover, isolates were seen to be the least resistance (68%) to neomycin, while 94% of them were resistant to gentamicin, tobramycin, kanamycin and streptomycin in MIC test. The MDR in this research was 94%. Aminoglycoside resistance genes are common in *Acinetobacter baumannii* isolates in the studied area. This is due to overuse of antibiotics and poor infection control. Monitoring and preventing resistance is crucial. Choosing the right antibiotic based on sensitivity testing is vital for effective treatment and minimizing further resistance development.



**Keywords:** *Acinetobacter baumannii*, Aminoglycosides, Aminoglycoside Modifying Enzymes, armA

# The Spectrum of Nasal Enterobacteriaceae: Frequency, Phenotypic Traits, and Genotypic Profiles in Diabetes versus Non-Diabetes Population

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Members of the family Enterobacteriaceae are causing morbidity and mortality worldwide. Due to the production of extended-spectrum beta-lactamase (ESBL), antibiotic resistance among members of the family Enterobacteriaceae is increasing rapidly. Some evidence suggests that nasal colonization with resistant bacteria can increase the risk of opportunistic and sometimes life-threatening infections, especially in people with underlying medical conditions including diabetes. Little is known about the occurrence and characterization of Enterobacteriaceae as colonizers among the population. Therefore, the present study aimed to detect and characterize ESBL-producing Enterobacteriaceae in nasal samples of diabetes and non-diabetes populations in Isfahan, Iran.

**MATERIALS AND METHODS:** A total of 240 participants (120 diabetes and 120 non-diabetes) were included in the study. Each subject completed a brief checklist, and specimens were obtained from the anterior nares for Enterobacteriaceae screening. Antibiotic susceptibility patterns, phenotypic ESBL disk diffusion test, and biofilm formation assay were carried out on the isolates. The genotypic analysis included bla TEM, bla SHV, and bla CTX.

**RESULTS AND DISCUSSION:** Out of 240 participants, 4 (3.3%) and 9 (7.5%) were colonized with Enterobacteriaceae isolates in the diabetes and non-diabetes groups respectively. The highest proportion of antibiotic resistance was to cefixime (50%) and trimethoprim-sulfamethoxazole (50%) among diabetes participants. Although none of the isolates were detected ESBL-producers by phenotyping disk diffusion method, 50.0% and 44.4% of the isolates were positive for all three beta-lactamase genes including bla CTX, bla TEM, and bla SHV in diabetes and non-diabetes participants respectively. Regarding to biofilm formation assay, 30.8%, 46.2%, 7.7%, and 15.4% of the isolates were non-adherent, weekly, moderate, and strong biofilm producers respectively. In the present study, nasal colonization of Enterobacteriaceae isolates was not at a high level in diabetes and non-diabetes participants. No ESBL-producing isolates were detected by the phenotypic disk diffusion method but some isolates were positive for all three beta-lactamase genes.

**Keywords:** Antibiotic resistance, Enterobacteriaceae, Extended-spectrum beta-lactamase, Diabetes Mellitus, Nasal colonization.

# Three Iranian monofloral honey samples as therapeutic candidate against highly resistant *Pseudomonas aeruginosa*

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Due to the increase in antibiotic resistance, today researchers are looking for a new treatment method to deal with resistant bacteria. Among these bacteria is *Pseudomonas aeruginosa*, which can be the cause of wound infection in burn, hospitalized or diabetic patients. Natural and herbal materials such as honey have been effective since the past until today due to their antibacterial and antioxidant properties. In this research, we investigate the effect of different Iranian honeys in inhibiting or reducing the growth of this bacterium.

**MATERIALS AND METHODS:** Seven types of Iranian honey were tested against a clinical multi-drug resistance (MDR) isolate of *Pseudomonas aeruginosa*. The initial screening was done using the well diffusion method to assess the antibacterial activity at various concentrations by measuring the inhibition zones. This was followed by the Minimum Inhibitory Concentration (MIC) test using 96-well microplates to determine the lowest concentration at which honey inhibits bacterial growth. Absorbance was measured at 540 nm to quantify bacterial growth. Finally, the Minimum Bactericidal Concentration (MBC) test was performed to identify the lowest concentration of honey that kills the bacteria.

**RESULTS AND DISCUSSION:** The types of honey tested showed different degrees of antibacterial activity. Some honey showed effective bacterial inhibition in all concentrations (100% to 75%), while Konar and Bahar Naranj honeys were effective in lower concentrations (80% and 75%). These results show that different honeys have different efficiency based on their type and concentration. The findings suggest that certain Iranian honeys have potent antibacterial properties against *Pseudomonas aeruginosa*, with potential applications in wound care. Continued research may provide further insights into optimizing the use of honey in medical treatments to combat bacterial infections.

**Keywords:** *Pseudomonas aeruginosa*, Honey, MDR

# Toxin-antitoxin Genes Expression in Multidrug-resistant *Mycobacterium tuberculosis* Isolates under Drug Exposure

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Toxin-antitoxin systems (TAs) are highly conserved in *Mycobacterium tuberculosis* (Mtb). The TAs role in maintaining and disseminating drug resistance in bacterial populations has been indicated. So, we aimed to analyze the expression level of mazEF-related genes in drug-susceptible and multidrug-resistant (MDR) Mtb isolates under isoniazid (INH) and rifampin (RIF) stress.

**MATERIALS AND METHODS:** We obtained 23 Mtb isolates, including 18 MDR and 5 susceptible isolates, from the Ahvaz Regional TB Laboratory collection. The expression levels of mazF3, mazF6, and mazF9 toxin genes, and mazE3, mazE6, and mazE9 antitoxin genes in MDR and susceptible isolates were evaluated by quantitative real-time PCR (qRT-PCR) after exposure to sub-inhibitory concentrations of RIF and INH.

**RESULTS AND DISCUSSION:** The mazF3, F6, and F9 toxin genes were overexpressed in at least two MDR isolates in the presence of RIF and INH, in contrast to mazE antitoxin genes. More MDR isolates were induced to overexpress mazF genes by RIF than INH (72.2% vs. 50%). Compared to the H37Rv strain and susceptible isolates, the expression levels of mazF3,6 by RIF and mazF3,6,9 by INH were significantly upregulated in MDR isolates (p0.05), but no remarkable difference was detected in the expression level of mazF9 genes by INH between these groups. In susceptible isolates, the expression levels of mazE3,6 by RIF and mazE3,6,9 by INH were induced and enhanced significantly compared to MDR isolates, but there was no difference between MDR and H37Rv strain. We propose that mazF expression under RIF/INH stress may be associated with drug resistance in Mtb in addition to mutations.

**Keywords:** INH; *Mycobacterium tuberculosis*; RIF; Toxin-antitoxin systems; drug resistance; gene expression.

# Two Iranian Honey as natural antibiofilm agent against *Klebsiella pneumoniae*

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Increasing antibiotic resistance among bacterial pathogens requires the identification of alternative therapeutic agents, especially the natural ones. Bacteria living in a biofilm can exhibit a 10 to 1,000-fold increase in antibiotic resistance compared to similar bacteria living in a planktonic state. *Klebsiella pneumoniae* an important biofilm-forming pathogen is the most common cause for pneumonia, bacteremia, and septicemia. The aim of study is to evaluate the effect of some native Iranian honeys on biofilm formation in multidrug-resistant clinical strains of *Klebsiella pneumoniae*.

**MATERIALS AND METHODS:** Clinically *K.pneumoniae* isolated from hospitalized patients were included in this study. The antibacterial activity of some native Iranian honeys was evaluated against bacterial isolates. Six concentrations (100, 95, 90, 85, 80 and 75%) of honeys which showed antimicrobial activity in primary screening were prepared and further assessed for antibiofilm activity using standard crystal violate microplate method.

**RESULTS AND DISCUSSION:** Somr honey and Konar honey as natural antimicrobial agents showed the ability to inhibit *K.pneumoniae* biofilm formation. Konar honey has a greater biofilm inhibitory effect on this bacterium than Somr honey. In Konar honey, higher concentrations (100, 95 and 90%) have more biofilm inhibitory effect but in Somr honey, different concentrations had different antibiofilm activity which needs further study. The present study shows that Iranian Somr and Konar honeys have a positive effect in inhibiting *K. pneumoniae* biofilm and these honeys can be a suitable alternative to combat diseases related to antibiotic resistance in this bacterium.

**Keywords:** Honey, *Klebsiella pneumoniae*, biofilm inhibition

# Utilizing PLGA Carriers for Downregulating Expression of *Klebsiella pneumoniae* Adhesion Genes

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** *Klebsiella pneumoniae* poses a serious threat to public health due to its increasing antibiotic resistance and virulence. Adhesion to host tissues is a critical step in *K. pneumoniae* pathogenesis, facilitated by various adhesion genes encoding for surface structures such as fimbriae and capsule polysaccharides. Targeting these adhesion mechanisms represents a promising approach to combat *K. pneumoniae* infections. Poly (lactic-co-glycolic acid) (PLGA) carriers offer advantages such as biocompatibility, biodegradability, and tunable release kinetics, making them suitable for delivering therapeutic agents to modulate bacterial adhesion.

**MATERIALS AND METHODS:** Isolation of *Klebsiella pneumoniae* Strains: 1. Clinical samples were collected from hospitalized patients, resulting in the isolation of 100 *K. pneumoniae* strains using standard microbiological methods. 2. These strains were then subjected to further analysis. Antibiotic Resistance Profiling: 1. The susceptibility of *K. pneumoniae* strains to antibiotics was assessed. 2. The highest prevalence of resistance was observed against ciprofloxacin (75%), trimethoprim–sulfamethoxazole (73%), and nitrofurantoin (68%). Virulence-Associated Genes Analysis: 1. The presence of virulence-associated genes in *K. pneumoniae* strains was investigated. 2. Genes such as *entB*, *traT*, *ybts*, *magA*, *iucC*, *htrA*, and *rmpA* were detected in varying proportions among the isolates.

**RESULTS AND DISCUSSION:** *Klebsiella pneumoniae* adhesion genes. Targeting bacterial adhesion mechanisms using PLGA-based delivery systems offers a novel approach to attenuate virulence and enhance antimicrobial strategies against *K. pneumoniae* infections. Further research is warranted to optimize PLGA carrier formulations, elucidate the mechanisms of action, and evaluate the clinical applicability of this approach in combating *K. pneumoniae* infections.

**Keywords:** PLGA; *Klebsiella pneumoniae*; Adhesion Genes

# Why do prisoners contract tuberculosis at more than 100 times the rate of the general population?

Tuberculosis and other mycobacterial infections

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**BACKGROUND AND OBJECTIVES:** Tuberculosis is a contagious disease and the second leading infectious killer worldwide. In 2022, 10.6 million people were infected globally, with about a quarter of the population carrying the bacteria. Studies show tuberculosis incidence in prisons is 100 times higher than in the general population. Overcrowding, malnutrition, and weak immune systems in prisons facilitate the disease's spread, making them major reservoirs. This high rate also impacts societal disease spread. Given the higher prevalence in crowded settings, especially prisons, this study aimed to determine tuberculosis prevalence among prisoners in Qaimshahr city.

**MATERIALS AND METHODS:** In this descriptive-analytical study, prisoners in Qaimshahr city were examined for tuberculosis. Using an insulin syringe and five units of PPD, the test was administered by experienced personnel, and the results were analyzed after 48-72 hours. Individuals with an induration greater than 5 mm were considered PPD positive. Additionally, to investigate pulmonary involvement, chest X-ray imaging was performed on affected patients. The data were recorded in SPSS software version 22 and analyzed using descriptive statistical methods, including mean, standard deviation, and frequency tables.

**RESULTS AND DISCUSSION:** In this study, out of 652 prisoners in Qaimshahr city, all of them were men, 550 prisoners were evaluated using the PPD tuberculin skin test. After 48-72 hours, 63 individuals (11.45%) had an induration of 5-10 mm, 26 individuals (4.72%) had an induration of 10-15 mm, and 33 individuals (4.18%) had an induration of more than 15 mm. Considering that the test result is considered positive for a diameter greater than 5 mm, 122 prisoners (22.18%) were diagnosed with tuberculosis. Among these individuals, chest X-rays were taken for 76 people, and the analysis showed that 32 people (5.81%) had pulmonary involvement of tuberculosis, which is more than 100 times higher than the rates reported among the general population. Understanding tuberculosis prevalence in prisons is crucial for planning actions to reduce rates, including improving conditions, speeding up diagnosis and strain identification, implementing treatments, and ensuring patient support and follow-up.

**Keywords:** Epidemiological surveillance; Prisons; Tuberculosis; prevention; prison; pulmonary tuberculosis.

# Investigating the Efficacy of Dapsone in Treating Sepsis Induced by Cecal Ligation and Puncture Surgery in Male Mice

Severe sepsis, bacteraemia & endocarditis

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**BACKGROUND AND OBJECTIVES:** Background and objective: Sepsis is a life-threatening condition caused by the body's response to an infection. Dapsone is a sulfone with antibiotic, and immunomodulatory properties. Experimental evidence suggests that dapsone has important anti-oxidative stress and anti-inflammatory effects. The objective of this study is to investigate the efficacy of dapsone in mice after CLP surgery.

**MATERIALS AND METHODS:** Materials and Methods: The study involved dividing animals into five groups: CLP, sham, and three treatment groups receiving different doses of Dapsone (0.5, 1, 2 mg/kg). Sepsis was induced through CLP surgery, followed by Dapsone administration. Half mice in groups were monitored for survival rates after 96 hours, while others underwent examinations after 24 hours.

**RESULTS AND DISCUSSION:** Results and discussion: The results of this study showed that single-dose administration of dapsone at (0.5, 1, 2 mg/kg) after CLP surgery in mice with sepsis improved survival compared to the CLP group. Also, it was associated with a significant reduction in proinflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , IL-6, NO, and MPO, lactate, and creatinine serum levels ( $p < 0.05$ ), while dapsone didn't have any significant effect on urea serum level. Our data suggest that dapsone treatment leads to increased survival in septic mice after CLP, and due to dapsone reduced TNF- $\alpha$ , IL-1 $\beta$ , IL-6, MPO, Lactate, and nitrite levels, has anti-inflammatory effects in sepsis. The sepsis treatment with dapsone in mice has protective effects against inflammation and oxidative stress.

**Keywords:** Sepsis, Inflammation, Dapsone, Clp, Mice



# Assessment of the Frequency and Pattern of Bacterial Antibiotic Resistance in Sputum Culture Samples from Patients at Imam Ali Hospital in Amol from June 2023 to May 2024

Respiratory infections

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**BACKGROUND AND OBJECTIVES:** Sputum culture is one of the important methods for diagnosing respiratory infections and evaluating the microbiological status of patients. This method helps physicians identify the bacteria present in the sputum, determine the type of infection, and choose the appropriate treatment. In recent years, the increase in antibiotic resistance among bacteria has become a serious challenge in the treatment of bacterial infections. Bacterial isolates identified in sputum cultures may be resistant to antibiotics, which can lead to inadequate treatment and serious complications for patients. Therefore, this study aimed to determine the frequency and pattern of bacterial antibiotic resistance in sputum cultures of patients hospitalized at Imam Ali Hospital in Amol.

**MATERIALS AND METHODS:** This descriptive cross-sectional study collected twelve months of sputum culture data from all patients who visited Imam Ali Hospital in Amol and had sputum cultures requested by their physicians.

**RESULTS AND DISCUSSION:** In this study, out of 83 first-round samples examined, 71 patients were observed to have positive sputum cultures. 59.20% of the positive samples were from male patients, and most of the patients were admitted to the ICU. the frequency percentages of the observed microbial agents were as follows: Staphylococcus haemolyticus: 14.08% Pseudomonas aeruginosa: 12.67% Enterobacter: 11.26% Klebsiella pneumoniae: 11.26% Additionally, The highest antibiotic resistance in the samples from this center, in terms of percentage and frequency, was as follows: Cefazolin, Oxacillin, Azithromycin, and Nalidixic Acid (with 100% resistance) Cefixime (with 95.23%) Erythromycin (with 92.59%) Cefotaxime (with 91.66%).

**Keywords:** Sputum culture, Bacterial isolates, antibiotic resistance, Imam Ali Hospital

# Introducing inhibitory chemical structures with high affinity against *Klebsiella pneumoniae* OXA-48 protein based on the chemical structure of imipenem

Respiratory infections

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**BACKGROUND AND OBJECTIVES:** With the rapid emergence of bacteria resistant to a wide range of antibiotics, the effectiveness of existing treatments has been greatly reduced. These resistances are mainly caused by excessive and incorrect use of antibiotics, as well as the reduction of economic incentives for the development of new drugs. *Klebsiella pneumoniae* bacteria is one of the most important pathogens causing hospital infections, which has become resistant to carbapenems, one of the strongest antibiotic groups. Beta-lactamase OXA-48, an enzyme produced by this bacterium, is one of the main factors of this resistance.

**MATERIALS AND METHODS:** This study was conducted with the aim of designing a specific inhibitor against the OXA-48 protein of *Klebsiella pneumoniae*. Molecular docking and virtual screening methods were used to identify compounds with high affinity to OXA-48 protein. At first, the chemical structure of the antibiotic imipenem and the crystal file 7KH9.pdb were used as the basis for designing the compounds. Then the identified compounds were evaluated using advanced molecular docking software and pharmacological and toxicity analyses.

**RESULTS AND DISCUSSION:** The results showed that the identified compounds have a high affinity for OXA-48 protein. Using molecular docking tests, these compounds were able to effectively interact with the target protein and inhibit beta-lactamase activity. In addition, pharmacological analyzes showed that the studied compounds have suitable properties in terms of absorption, distribution, metabolism and excretion (ADME). Toxicity evaluations also showed that these compounds are safe in terms of being toxic to human cells. This study showed that the identified compounds can act as effective inhibitors of OXA-48 protein in *Klebsiella pneumoniae* bacteria. These results can be a basis for the development of new drugs against infections caused by carbapenem-resistant bacteria. More efforts are needed to optimize these compounds and conduct clinical trials to confirm their effectiveness and safety.

**Keywords:** OXA-48 protein, *Klebsiella pneumoniae*, Virtual screening, Molecular docking, pharmaceutical properties,

# Investigating the chemical content and cytotoxicity of Shirazi thyme (*Zataria multiflora*) essential oils in MDR *Klebsiella pneumoniae* bacteria with KPC and OXA-48 genes

Respiratory infections

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**BACKGROUND AND OBJECTIVES:** Infectious diseases are one of the biggest threats to human health and despite extensive efforts to control them, they are still one of the most important causes of death in the world. Bacterial resistance to antibiotics, especially in multidrug-resistant (MDR) bacteria such as *Klebsiella pneumoniae*, which have KPC and OXA-48 genes, has created a great challenge in the treatment of infectious diseases. This study investigates the chemical content and cytotoxicity of Shirazi thyme (*Zataria multiflora*) essential oils and propose the molecular mechanism of the antibacterial properties of these essential oils in MDR *Klebsiella pneumoniae* bacteria using bioinformatic methods.

**MATERIALS AND METHODS:** First, essential oils of Shirazi thyme were prepared and their chemical content was determined using gas chromatography and mass spectroscopy techniques. Cytotoxicity of these essential oils was investigated by different bioinformatics tools. To determine the molecular mechanism of the antibacterial property, various bioinformatic tools such as molecular docking methods were used, in which the interactions between the active compounds of the essential oils and the target proteins of the bacteria were investigated.

**RESULTS AND DISCUSSION:** The results showed that essential oils of Shirazi thyme have various chemical compounds that include phenolic and oxidant compounds. These compounds create strong antimicrobial properties in this essential oils. The cytotoxicity of these essential oils was investigated in different concentrations and the results showed that this essential oil is less toxic in low concentrations. Molecular docking showed that the active compounds of essential oil can effectively bind to the target proteins of *Klebsiella pneumoniae* and inhibit their activity. Shirazi thyme essential oils can be used as natural and effective antimicrobial agents against resistant bacteria such as MDR *Klebsiella pneumoniae* due to their phenolic and oxidant compounds. With low cytotoxicity and strong antimicrobial effects, this essential oil can be a suitable alternative to chemical antibiotics in the treatment of infectious diseases. This study shows that the use of plant essential oils can be a suitable and sustainable solution to combat drug

**Keywords:** Cytotoxicity, Shirazi thyme essential oil, Molecular mechanism, MDR *Klebsiella pneumoniae*

# Investigating the level of resistance to carbapenems and the production of carbapenemase enzyme in clinical isolates of *Pseudomonas aeruginosa* in Gilan.

Respiratory infections

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* is a gram-negative bacterium and one of the most important opportunistic pathogens causing hospital infections, which usually acquires resistance to several drugs simultaneously. This study was conducted with the aim of determining the level of resistance to carbapenems and the level of production of metallo-beta-lactamases in clinical isolates of *Pseudomonas aeruginosa*, and the frequency of metallo-beta-lactamase genes blaIMP and blaVIM in resistant strains was investigated.

**MATERIALS AND METHODS:** A total of 107 isolates of *Pseudomonas aeruginosa* were collected from different laboratories in Rasht city and identified using biochemical methods. Disc diffusion method was used to determine antibiotic resistance of strains. In order to identify broad-spectrum  $\beta$ -lactamase and metallo- $\beta$ -lactamase-producing strains, the combined disk method was used. The presence of blaIMP and blaVIM genes in resistant strains was investigated in PCR reaction.

**RESULTS AND DISCUSSION:** Out of 107 *Pseudomonas aeruginosa* isolates, 50 isolates (46.7%) were resistant to imipenem. Also, in the phenotypic test, 29 isolates (27.1%) were carbapenemase-producing and 33 isolates (30.8%) were resistant to ceftazidime and broad-spectrum beta-lactamase-producing. Out of 50 isolates resistant to imipenem, in 21 isolates (42%), a fragment with an approximate length of 382 bp was produced in the PCR reaction and was identified as having the VIM gene. Also, 5 isolates (10%) were identified as positive for the presence of IMP gene by producing a fragment with an approximate length of 587 bp. Out of this number, 3 isolates (6%) have both genes at the same time. All the isolates carrying IMP and/or VIM genes were carbapenemase producers in the phenotypic test.

**Keywords:** Metallobthalactamase, *Pseudomonas aeruginosa*, antibiotic resistance, blaIMP, blaVIM.

# The Prevalence and Antibiotic Sensitivity of Bacteria Isolated from Endotracheal Tube Cultures in Sari Hospitals, 2023

Respiratory infections

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**BACKGROUND AND OBJECTIVES:** ventilator-associated pneumonia (VAP) represents one of the most common ICU-acquired infections, carrying a significant morbidity and risk of mortality. Microorganisms responsible for VAP differ according to geographic areas, ICU patients' specific characteristics, durations of hospital and ICU stays before onset of the disease, and risk factors for MDR pathogens. Increasing antibiotic resistance among the common bacterial pathogens associated with VAP, has made the choice of empiric treatment of these infections increasingly challenging. Recent findings emphasize the importance of prompt and appropriate antimicrobial therapy. The purpose of this study is to investigate the prevalence of ventilator-dependent bacteria and their drug resistance.



**MATERIALS AND METHODS:** This cross-sectional study was conducted in 2023 on the patients of Fatemeh Zahra and Razi hospitals. After sampling and cultivation, identification of grown bacteria was done based on microbiological and biochemical standards. To evaluate the drug resistance pattern of the isolates, the disc diffusion method in agar was used according to the instructions of the CLSI standard institute. In this study, the drug sensitivity of bacteria to 13 antibiotics (oxacillin, cephalothin, ampicillin, vancomycin, clindamycin, colistin, amikacin, cotrimoxazole, ciprofloxacin, meropenem, imipenem, ceftriaxone, and cefixime) was investigated.

**RESULTS AND DISCUSSION:** A total of 129 samples were taken from 107 patients. Of these samples, 12.9% were Gram-positive and 87.1% were Gram-negative. Among the isolated microorganisms, Enterobacter was the most common, accounting for 23.4%. Ampicillin (96%) and Cefixime (84%) showed the highest resistance among Gram-negative bacteria, and Colistin (90%) showed the highest sensitivity. Among Gram-positive bacteria, Azithromycin (75%) and Clindamycin (75%) had the highest resistance, and Vancomycin had the highest sensitivity at 80%. Considering the various bacterial factors in ICU and lack of sufficient information regarding the pattern of antibiotic resistance related to ventilator, antibiogram test is considered to start the treatment. Epidemic data collection is needed in order to provide the treatment staff with specific prescriptions to use these antibiotics. Another result of this action is preventing the creation of resistant strains and reducing the side effects of biotic resistance.

**Keywords:** ventilator-associated pneumonia, Drug resistance, Hospital infection

# Biofilm, hemolysin and extended spectrum beta lactamase production in uropathogenic *Escherichia coli*

Abdominal/gastrointestinal, urinary tract & genital infections

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**BACKGROUND AND OBJECTIVES:** Urinary tract infection is one of the most common bacterial infections among all ages and in both sexes. Multidrug-resistant (MDR) uropathogenic *Escherichia coli* (UPEC) harboring several virulence factors including biofilm and hemolysin, play an important role in causing urinary tract infections. The aim of this study was to investigate the antibiotic resistance pattern, biofilm formation and hemolysin production of isolates causing urinary tract infections in Salman Farsi Bushehr Hospital.

**MATERIALS AND METHODS:** This cross-sectional study was conducted on 105 UPEC isolates isolated from patients with urinary tract infection. All isolates were identified by standard microbiological diagnostic tests. Antibiotic sensitivity pattern was determined by disc diffusion method. Double disk synergy test was used to determine ESBL (extended spectrum beta lactamase) isolates. Biofilm production was investigated by microtiter plate method.

**RESULTS AND DISCUSSION:** Result: Out of 105 samples, the frequency of ESBL isolates was 52.4%. The most resistant antibiotics were ampicillin (83.8%) and nalidixic acid (75.2%). Most isolates (46.7%) produced weak biofilm, then 21.9% and 12.4% of isolates produced moderate and strong biofilm, respectively. Approximately 24% of isolates also produced hemolysin. Discussion: The results of this study showed a high prevalence of ESBL strains with a remarkable rate of biofilm-producing isolates from symptomatic patients, making them a serious health concern in the region. This survey highlights the ciprofloxacin cannot be used for empirical treatment of UTI and alternative drugs and evaluation of the susceptibility profile for UPECs are recommended.

**Keywords:** Urinary Tract Infection, uropathogenic *Escherichia coli*, Biofilm, Hemolysin, Extended Spectrum

# Biofilm, hemolysin and extended spectrum beta lactamase production in uropathogenic *Escherichia coli*

Abdominal/gastrointestinal, urinary tract & genital infections

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**Keywords:** Urinary Tract Infection, uropathogenic *Escherichia coli*, Biofilm, Hemolysin, Extended Spectrum

# Determining the frequency of bacterial causes of urinary tract infection in patients referred to Imam Khomeini Hospital in Shirvan city in 2023

Abdominal/gastrointestinal, urinary tract & genital infections

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**BACKGROUND AND OBJECTIVES:** Urinary tract infection (UTI) is one of the most common causes of bacterial infection in the community and hospitals. Urinary tract infection is more common in women than in men. The economic and public health costs caused by urinary tract infections and the increase in antibiotic resistance are significant and have a great impact on the quality of life of affected patients.

**MATERIALS AND METHODS:** In this descriptive-cross-sectional study, during a study in 2023, urine samples of outpatients referred to Shirvan Imam Khomeini Hospital were collected using the Clean Catch Midstem method. First, the samples were cultured in McConkey agar medium and incubated at 37-35°C for 24-48 hours. A colonization rate equal to or greater than 10<sup>5</sup> colonies per ml was considered as a positive sample. Then the morphology of the colonies was examined and standard biochemical tests including: urea, SIM, MR/VP, TSI and Simon Citrate, phenylalanine deaminase and gram staining were performed.

**RESULTS AND DISCUSSION:** In the investigation of the total number of 3020 people who referred to the laboratory of Imam Khomeini Hospital in Shirvan, 147 urine cultures (4.86%) were positive. The most common bacterial isolates are *Escherichia coli* with 101 (68.71%), *Klebsiella* species with 19 species (12.93%), *Enterococcus* 5 (3.40%), *Staphylococcus aureus* 4 (2.72%), *Streptococcus* 3 (2.04%), *Staphylococcus epidermidis* 3 (2.04%), *Streptococcus* Group B 2 (1.36%), *Enterobacter* 2 (1.36%), *Citrobacter* 2 (1.36%), *Proteus mirabilis* 2 (1.36%), *Ceraria* 1 (0.68%), *Staphylococcus saprophyticus* 1 (0.68%), *Streptococcus* group D (0.68%) 1 and *Pseudomonas aeruginosa* 1 (0.68%) was isolated. According to the obtained results, *Escherichia coli* bacteria is the most common cause of urinary tract infection, which is more than other isolates and is the most common bacterium in causing urinary tract infections. And the number of Gram-negative bacteria, especially the Enterobacteriaceae family, is more than Gram-positive bacteria in causing urinary infections.

**Keywords:** urinary infection, urinary pathogens, Shirvan



# Investigating the antibiotic resistance of *Escherichia coli* isolated from patients hospitalized in Sina hospital, Hamadan and evaluating the frequency of the quinolone resistance gene (*qnrA*).

Abdominal/gastrointestinal, urinary tract & genital infections

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**BACKGROUND AND OBJECTIVES:** Background and purpose: *Escherichia coli* bacteria is responsible for 80% of urinary infections in outpatients and 50% of urinary infections in hospitalized patients in Iran. Improper antibiotic treatment has caused an increase in antibiotic resistance in *E. coli* strains isolated from urinary tract infections over the past few decades. The purpose of this study was to investigate the antibiotic resistance profile and evaluate the frequency of antibiotic resistance genes of *E. coli* strains isolated from human infections in relation to quinolones.

**MATERIALS AND METHODS:** A number of 100 *E. coli* isolates isolated from urinary tract infections of patients admitted to Sina Hospital in Hamadan were collected. After phenotypic and genetic tests, the confirmed strains were subjected to antibiotic sensitivity testing. PCR was performed using *qnrA* specific primers.

**RESULTS AND DISCUSSION:** Of the 100 final confirmed isolates, 70% of the strains were resistant to nalidixic acid, 53% to levofloxacin, and 59% to ciprofloxacin. In total, the frequency of *qnrA* resistance gene was 28%. It seems necessary to use genotypic methods along the phenotypic methods to detect resistance to promote effective and fast treatment and prevent the spread of resistant strains.

**Keywords:** Urinary tract infection, *E. coli*, antibiotic resistance, quinolone, *qnrA*

# Molecular detection of SEA, SEC and SEQ enterotoxins in *Staphylococcus aureus* clinical isolates

Abdominal/gastrointestinal, urinary tract & genital infections

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**BACKGROUND AND OBJECTIVES:** Background: *Staphylococcus aureus* (*S. aureus*) is one of the most significant pathogens, causing severe infectious disease. Staphylococcal food poisoning remains the third most prevalent reason behind food-borne illnesses worldwide. Objectives: In this study we aimed to examine the antimicrobial susceptibility profile and prevalence of SE genes including sea, sec and seq in *S. aureus* clinical isolates.

**MATERIALS AND METHODS:** Methods: During the period of April to September 2017, a number of 96 *S. aureus* isolates were collected from Shahid Madani university teaching hospital, Tabriz, Iran. Antibiotic susceptibility test was carried out by modified Kirby-Bauer disk diffusion method. The presence of Staphylococcal enterotoxin genes, sea, sec, and seq was evaluated by polymerase chain reaction (PCR) assay.

**RESULTS AND DISCUSSION:** Results: High levels of resistance was observed to erythromycin (54%), cotrimoxazole (16%), clindamycin (59%), gentamicin (44%), linezolid (54%), and ceftiofuran (51.04%). All isolates were sensitive to vancomycin. The most commonly detected enterotoxin gene was sea (19.79%). None of the isolates harbored sec or seq genes. Conclusion: The present study indicates a high prevalence of antibiotic resistance among *S. aureus* clinical isolates and highlights the potential risk of *S. aureus* as a threat for public health. There is an urgent need for implementation of appropriate antibiotic use guidelines to prevent further antibiotic resistance. Also, the sea gene was found as the predominant enterotoxin gene among clinical *S. aureus* isolates. Further studies are needed to monitor the presence of Staphylococcal enterotoxin genes to reduce the food poisoning.

**Keywords:** : *Staphylococcus aureus*, Staphylococcal enterotoxin, SEA, SEC, SEQ.

# Evaluation of silver sulfadiazine 1%-cerium nitrate 2.2% cream efficacy and safety in moderate to severe burn patients: a single-blind randomized clinical trial

skin, soft tissue, bone & joint infections

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**BACKGROUND AND OBJECTIVES:** Burn injury is a major global health crisis. Topical antimicrobials such as silver sulfadiazine (SSD) are commonly used for superficial burn wounds. SSD has a broad-spectrum antimicrobial activity and also anti-inflammatory property, but also suffers from some limitations. Therefore, some studies suggest to add cerium nitrate (CN) to SSD, as an immunomodulatory and tanning agent with antitoxic properties, but its effect on patients' mortality, length of hospital stay, and bacterial colonization is controversial. Objectives: In this research, we evaluated the efficacy and safety of SSD 1%+CN 2.2% cream in patients with moderate to severe burn

**MATERIALS AND METHODS:** Twenty-two patients who fulfilled the inclusion criteria randomly were assigned to the intervention (n=7) or control (n=15) group and received SSD 1%+CN 2.2% or SSD cream 1% respectively, once daily until the complete re-epithelization or preparation of the burned skin for grafting. Intensity of pain, re-epithelialization time, required interventions, laboratory and clinical findings and final outcome were recorded

**RESULTS AND DISCUSSION:** There was no significant difference in re-epithelialization time between the treatment and control groups (P>0.05). The same findings were reported about the required interventions and laboratory and clinical parameters. However, the final outcome and the pain score on third day were significantly better in the treatment group (P=0.017). On the other hand, all patients in the treatment group needed graft surgery. Use of SSD 1%+CN 2.2% cream did not significantly improve re-epithelialization time or infection occurrence and patients' pain, but also increased graft surgery rate in comparison with SSD 1% cream in moderate to severe burns.

**Keywords:** Silver sulfadiazine, cerium nitrate, flammacerium, moderate-severe burn, infections

# Investigating the effect of antiseptic shower before surgery, in the prevention of postoperative surgical infection, in patients hospitalized for elective inguinal hernia surgery.

skin, soft tissue, bone & joint infections

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**BACKGROUND AND OBJECTIVES:** Surgical site infections (SSI) are one of the most common and important complications that always cause many problems and costs. Many methods and measures to reduce it are being implemented and reviewed. One of the ways to reduce it is to decrease the microbial load by using antiseptic materials for the skin surface, and in this study, the effect of chlorhexidine shower was investigated.

**MATERIALS AND METHODS:** This study was conducted as an interventional and randomized controlled trial. 300 patients who referred for elective inguinal hernia surgery were divided into 3 groups of 100. The intervention group received a 4% chlorhexidine shower the night before the operation. Control group 1 received a bath with usual detergents and control group 2 did not take any bath. Patients were examined for the incidence of SSI up to 30 days after surgery.

**RESULTS AND DISCUSSION:** Results: Out of 300 patients who underwent surgery in this study, 12 (4%) suffered superficial SSI. Among these numbers, 1 person was in the intervention group, 3 people were in the control group 1, and 8 people were in the control group 2. Comparison between the incidence of infection in three groups showed that this difference is statistically significant ( $P=0.034$ ). Conclusion: Based on the findings of this study, chlorhexidine shower can be effective in reducing the incidence of SSI.

**Keywords:** chlorhexidine, antiseptic, surgical site infection, inguinal hernia surgery

# High Prevalence of Multidrug-Resistant ESKAPEE Pathogens in Nosocomial Infections among Tehran Hospitals

Resistance surveillance & epidemiology

Mansour Segighi © ⓘ

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**BACKGROUND AND OBJECTIVES:** Hospital-acquired infections (HAIs) are a major public health concern, with the ESKAPEE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and *Escherichia coli*) posing a significant threat due to their ability to develop multidrug resistance (MDR). This study investigates the prevalence and resistance profiles of these ESKAPEE pathogens in HAIs among patients admitted to major hospitals in Tehran, Iran.

**MATERIALS AND METHODS:** A number of 326 isolates from clinical samples including wounds, blood, urine, cerebrospinal fluid, sputum, bronchoalveolar lavage and burns were collected from patients diagnosed with HAIs in major Tehran hospitals during April 2023 to November 2023. Bacterial species were identified by microbiological and biochemical laboratory tests. Disc diffusion (Kirby-Bauer) method and E-test strip was used to determine antimicrobial resistance profile, Extensive drug resistance (XDR), and MDR strains following CLSI guidelines.

**RESULTS AND DISCUSSION:** The study found a high prevalence of ESKAPEE pathogens associated with HAIs, with a concerning level of MDR observed across all species. Our results indicated *Staphylococcus aureus* (24.84%; 81/326) and *Escherichia coli* (21.47%; 70/326) as the most prevalent among the ESKAPEE clinical strains. Particularly alarming were the MDR rates: *Pseudomonas aeruginosa* (83.76%), *Acinetobacter baumannii* (77.62%), and *Klebsiella pneumoniae* (71.94%). *Staphylococcus aureus* (68.21%), *Escherichia coli* (62.93%), *Enterococcus faecium* (52.43%) and *Enterobacter* spp. (34.50%) also exhibited substantial MDR prevalence. XDR was observed in 18.1% of total isolates (59/326), most of which were *Acinetobacter baumannii*. This study reveals a significant burden of HAIs caused by MDR-ESKAPEE pathogens in Tehran hospitals. The high resistance rates, particularly for *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, pose a critical challenge for effective treatment. These findings necessitate robust antibiotic stewardship programs, intensified infection control measures, and continuous surveillance to combat the growing threat of untreatable hospital-acquired infections.

**Keywords:** Hospital-acquired infections; Multidrug resistance; Extensive drug resistance; ESKAPEE pathogens; Tehran

# prevalence and antibiotic resistance of *Acinetobacter* isolated from patients hospitalized in the intensive care unit (ICU) Sari hospitals in 2013-2014

Resistance surveillance & epidemiology

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**BACKGROUND AND OBJECTIVES:** *Acinetobacter* is a gram-negative bacterium. The most common species of *Acinetobacter* is *Acinetobacter Baumannii*, which is an opportunistic pathogen in humans and affects people who have a weak immune system, especially patients admitted to the intensive care unit (ICU) [2]. Importance of this bacterium as a hospital infection is its increasing antibiotic resistance due to the continuous use of antibiotics [3] (Dijkshoorn, Nemec and Seifert 2007). The purpose of this study is to assess the rate of antibiotic resistance of *Acinetobacter* species isolated from patients in ICU of university affiliated Hospitals of Mazandaran University of Medical Sciences.

**MATERIALS AND METHODS:** In this cross-sectional descriptive study, 50 *Acinetobacter* samples from ICUs of four academic hospitals covered by Mazandaran University of Medical Sciences during 2013 and 2014 were collected. After cultivation in standard medium, the existence of *Acinetobacter* was confirmed. The experiment of determining antibacterial sensitivity was done by disc diffusion method based on CLSI standard on Mueller Hinton agar medium. Also, E-test method was used to measure the minimal inhibitor concentration (MIC). To determine the outcome of the disease, the patients were followed up for 3 to 6 months.

**RESULTS AND DISCUSSION:** A study of 50 patients, predominantly aged 60-80, found that 74% of the isolates were taken from endotracheal tubes. The main risk factors for *Acinetobacter* infection were diabetes (42%), heart and pulmonary diseases (30%), and recent antibiotic use. No significant difference was observed in the influence of risk factors on ICU stay duration. The disc diffusion method revealed 100% resistance to Amikacin and Cefepime, 96% to Meropenem and Ciprofloxacin, 76% to Imipenem, and 16% to Colistin. The E-test showed 100% resistance to Amikacin, Imipenem, and Meropenem, 96% to Ciprofloxacin, and 8% to Colistin. Over 96% of isolates were resistant to conventional ICU antibiotics, with Colistin being the only effective treatment, though resistance to Colistin was noted. The study highlighted the critical issue of antibiotic resistance, with 62% of patients dying within 3-6 months.

**Keywords:** *Acinetobacter Baumannii*, Gram-negative, E-test, antibiotic resistance

# Several ways to fight against antibiotic resistance.

Resistance surveillance & epidemiology

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**BACKGROUND AND OBJECTIVES:** A great war is coming. war with a strong but very small enemy. an enemy called bacteria. is it easier to prevent fires or to put out flames? Daily antibiotic-resistance led to the death of hospitalized patients, so we have to think of a solution. a large share of antibiotic-resistance is caused by self-inflicted use of antibiotics by the patient, improperly performing the antibiogram-test by the laboratory, and inappropriate antibiotic prescription by the doctor.

**MATERIALS AND METHODS:** In this study first, questionnaires with the following themes were prepared: Do you know the dangers of arbitrary use of antibiotics or improper use of antibiotics? Do you know the Antibiotic agent and the uses of each one? Do you know what antibiotic resistance is? Then patients, nurses and treatment staff, infection-control-committee, general and specialist doctors were asked these questions. Furthermore, in this study, the data related to the culture of all types of patient samples in a year (2023-2024) were analyzed and the level of resistance and sensitivity of each antibiotic and sample type was investigated. Unfortunately, many of our statistical population did not have much information in this regard. By analyzing the data, I have found that most of the cases of antibiotic resistance were in secretion culture samples such as wounds and tracheal tubes.

**RESULTS AND DISCUSSION:** In such clinical cases, what is our plan to prevent the Occurrence of antibiotic\_resistance? My suggestion is: 1: In every hospital or every treatment center, there should be an infectious disease specialist doctor to provide the necessary guidelines for prescribing\_antibiotics to other doctors. 2: Due to the fact that in many hospitals there may be several patients in one room at the same time, it is necessary to clean and sampling the ventilation\_and\_cooling\_system regularly to prevent the colonization of bacteria there. 3: There should be a computer-system in the entire pharmacy-system of the country, through which any antibiotics can be prescribed and sold only. 4: Finally, at the same time as trying to prevent the Occurrence of bacterial resistance, it is necessary to use new drugs (including use of metals and nanomaterials) in regarding with new ways to overcome bacteria.

**Keywords:** Antibiotic\_Resistance, prescribing\_antibiotics.

# A comparative study on synergistic effects of Asteraceae, Fabaceae, and Zingiberaceae on *Pseudomonas aeruginosa*

Susceptibility testing methods

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**BACKGROUND AND OBJECTIVES:** Herbal extracts contain secondary metabolites which have antibacterial effects, among them flavonoids, tannins, and saponins are mentionable and widely used for medicinal purposes. Although numerous studies have been performed to access their antibacterial effects, synergistic effects remained overlooked. As such the synergistic effect of *Silybum marianum* and *Echinacea angustifolia* from Asteraceae, *Glycyrrhiza glabra* and *Alhagi maurorum* from Fabaceae, *Zingiber officinale* and *Curcuma longa* from Zingiberaceae were tested against *Pseudomonas aeruginosa*.

**MATERIALS AND METHODS:** Extracts were prepared using hydroalcoholic method. And disk diffusion method was used to determine the antibacterial effects of each herbal extracts alone and in combination to verify any synergistic effects on bacterial culture. Inhibition zone was measured after 24h incubation at 35°C. All testes were repeated 3 times.

**RESULTS AND DISCUSSION:** Zingiberaceae with 8.67 mm inhibition zone was the most effective family against *P. aeruginosa*. Fabaceae was next with 7.67 mm diameter, and Asteraceae with 5.33mm showed lowered effect. The synergistic effect was higher compare to every herbal extract tested alone, i.e: *Curcuma longa* (8.33 mm), *Zingiber officinale* (7.67 mm), *Alhagi maurorum* (5.33 mm), *Glycyrrhiza glabra* (5 mm). In case of *Silybum Marianum* (7 mm), *Echinacea angustifolia* (7.33 mm) from Asteraceae, the synergistic effect was not seen (5.33 mm). Conclusion: Zingiberaceae seems to have better effective secondary metabolites compare to other families tested here which could be due to the presence of metabolites such as gingerol and shogaol which showed to be effective against *P. aeruginosa* as an example of gram-negative bacteria which is commonly found in hospital infections. Knowing synergistic effects, active metabolites, and their effects on bacterial causative agents would help with better management of such infections

**Keywords:** Herbal extracts, Fabaceae, Zingiberaceae, *Pseudomonas aeruginosa*



# Investigation of the antimicrobial resistance rates in clinical burn center isolates of *Pseudomonas aeruginosa* causing invasive infections in the south of Iran

Susceptibility testing methods

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**BACKGROUND AND OBJECTIVES:** This research aimed to examine the frequency of resistance to antimicrobial agents and their variations in *Pseudomonas aeruginosa* (*P. aeruginosa*) clinical isolates, which are accountable for invasive infections in the southern part of Iran, from 2018 to 2022.

**MATERIALS AND METHODS:** A retrospective study was conducted, involving the gathering of microbiological data from Taleghani Burn Center Hospital from 2018 to 2022. The primary variables under scrutiny were the antimicrobial susceptibility testing, which utilized disc diffusion and strip methods. The interpretation criteria employed for the study were the CLSI, and the percentage of resistant isolates was also considered.

**RESULTS AND DISCUSSION:** The disc diffusion and strips method are the most commonly used approach for antimicrobial susceptibility testing. According to CLSI, resistance rates ranged from 3.64% (colistin) to 77.38% (amikacin). The rates of antimicrobial resistance remained relatively constant over time, with 2018-2022. 67.62% of isolates were MDR, and 9.46% were XDR. Wound and urine isolates demonstrated higher resistance, except to amikacin and piperacillin than those from blood culture and biopsy. Antimicrobial resistance is widely prevalent in *P. aeruginosa*, a common bacterium in southern Iran. The study reveals the highest resistance rates for commonly used antibiotics such as amikacin, piperacillin, ceftazidime, and meropenem. However, colistin and nitrofurantoin are more effective against this bacterium. The wound and urine isolates have shown the highest resistance rates, indicating the need for prompt and appropriate treatment. Interestingly, the resistance rates for most antibiotics remained relatively stable during the study period, emphasizing the need to develop alternative treatments for

**Keywords:** Burn, *Pseudomonas aeruginosa*, Invasive infection, Microbiology

# Evaluation the frequency of ExoA, ExoS, ExoU, ExoY and type 3 secretion system genes in drug-resistant *Pseudomonas aeruginosa* isolated from burn patients during 2022-2023

## Resistance mechanisms

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**BACKGROUND AND OBJECTIVES:** This study aims to determine the prevalence of ExoA, ExoS, ExoU, ExoY and type 3 secretion system genes in drug-resistant *P. aeruginosa* isolates from burn patients in southern Iran (2022-2023), contributing to filling a research gap despite limited past studies on this topic in the specified setting.

**MATERIALS AND METHODS:** This descriptive cross-sectional study was conducted at Namazi and Abu Ali Sina Hospitals between March 2022 and March 2023. Samples were collected and cultured to identify *P. aeruginosa*. Confirmed isolates were tested for antibiotic susceptibility using disc diffusion method and CLSI guidelines. MIC assay was performed on colistin and imipenem resistant strains. Bacterial DNA was extracted using a commercial kit and stored for molecular experiments. PCR was conducted using specific primers for exotoxin genes and results were analyzed using gel doc system and SPSS software.

**RESULTS AND DISCUSSION:** The *exoA* gene (81.5%) and *exoT* gene (77.8%) had the highest frequencies, while the *exoS* gene (36.1%) and *exoU* gene (46.3%) had the lowest frequencies, respectively. The T3SS gene had a prevalence of 66.7%. Statistical analysis showed that presence of T3SS gene was significantly associated with increased antibiotic resistance for all antibiotics (P<0.001) except for clindamycin (P=0.109). Conclusion: Most *P. aeruginosa* isolates harbor *exoA* and *exoT* genes. A significant association exists between T3SS gene possession and antibiotic resistance, emphasizing its relevance in infection management. Additional investigations are necessary to comprehend the complete impact and inform appropriate treatments.

**Keywords:** T3SS, *Pseudomonas*, burn, ExoA, ExoS, ExoU, ExoY,

# Frequency of *cfr* and *repS* genes in methicillin-resistant *Staphylococcus aureus* clinical isolates in Kermanshah city

## Resistance mechanisms

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus* is an opportunistic pathogen. Methicillin-resistant strains play a major role in causing hospital-acquired infections. Meanwhile, several genes are important in disease exacerbation and bacterial resistance to antibiotics. The aim of this study is to evaluate the frequency of *cfr* and *repS* genes in methicillin-resistant isolates in Kermanshah.

**MATERIALS AND METHODS:** 100 clinical isolates of *Staphylococcus aureus* from urine, blood culture, pleural membrane, wound, nose, brain tumor and catheter of dialysis department were collected from Kermanshah hospitals and identified and confirmed by 16s rRNA, *mecA* and biochemical methods. The pattern of drug resistance was determined using disk diffusion method and CLSI 2022 criteria. Chromosomal and plasmid genome extraction was performed and PCR was performed with specific primers to identify the frequency of *cfr* and *repS* genes.

**RESULTS AND DISCUSSION:** Out of 100 isolates, 75 methicillin-resistant isolates were identified. The highest resistance to oxacillin was determined by 95%, followed by linezolid and clindamycin by 86% and the highest sensitivity to chloramphenicol by 98%. Among 75 isolates, 7 isolates equivalent to 9.3% had *cfr* gene and 4 isolates equivalent to 5.3% had *repS* gene. Only one isolate (1.3%) had both studied genes. The frequency of *cfr* and *repS* genes in *Staphylococcus aureus* isolates is not high ( $P < 0.05$ ), but the frequency of these genes is significant with the isolates in blood culture and dialysis department and their resistance to the studied antibiotics. The results can increase information to prevent colonization and infection by bacteria.

**Keywords:** *Staphylococcus aureus*, *cfr*, *repS*, IAU science.

# Investigating efflux pump activity and prevalence of MexAB and oprD genes in multidrug-resistant *Pseudomonas* isolated from burn patients during 2022-2023

## Resistance mechanisms

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**BACKGROUND AND OBJECTIVES:** Due to their nutritional versatility, *Pseudomonas* is found almost everywhere. Although carbapenems are the most effective antibiotics for the treatment of multidrug-resistant aeruginosa infections, an increase in the emergence of high carbapenem-resistant isolates has been observed worldwide. Increased expression of the MexAB-OprM and MexXY(-OprA) systems, suppression or inactivation of the OprD porin, and overproduction of the chromosomal cephalosporinase AmpC generally contribute to antimicrobial resistance. The purpose of this study is to investigate the efflux pump activity and the prevalence of MexAB and oprD genes in multidrug-resistant *Pseudomonas* isolated from burn patients during 2022-2023.

**MATERIALS AND METHODS:** The statistical population of this cross-sectional study included all burn patients of Amirul Mominin Hospital in Shiraz in 2023 and 2024. First, wound samples of burn patients were taken. Then, *Pseudomonas* cases were diagnosed in suitable culture media with phenotypic tests. After the diagnosis of *Pseudomonas*, antibiogram was performed and cases with multidrug resistance (MDR) were identified. Then the presence of efflux pump in MDR strains was checked and by using the genome extraction method, the gene in question was measured with a specific primer using the PCR method. SPSS version 25 software was used for statistical analysis.

**RESULTS AND DISCUSSION:** *Pseudomonas* shows the highest antibiotic resistance to Piperacillin(79.6%), Meropenem and Amikacin(77.8%), Cotrimoxazole and Imipenem(75.9%), Gentamicin(74.1%), Ceftazidime(72.2%) and Carbapenem(69.4%), respectively. They had the lowest resistance to Colistin(1.9%). Prevalence of mexA and oprD genes were 72.2%, 41.7%, and efflux pump activity was reported as 74.1%. So, Colestin is the most effective antibiotic for MDR *Pseudomonas* in burn patients. Also, antibiotic resistance has a direct relationship with increased efflux pump activity and increased prevalence of MexAB and decreased oprD.

**Keywords:** MexAB, oprD, *Pseudomonas*, Antibiotic Resistance

# Investigation of the presence of fibronectin genes (fnbA and fnbB) and fibrinogen (clfA and clfB) in staphylococcus aureus samples isolated from patients' wound specimens

Resistance mechanisms

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**BACKGROUND AND OBJECTIVES:** In this study, the presence of fibronectin genes (fnbA and fnbB) and fibrinogen (clfA and clfB) in staphylococcus aureus samples isolated from patients wound specimens was investigated.

**MATERIALS AND METHODS:** In this descriptive cross-sectional study, 100 isolates of Staphylococcus aureus were collected from patients admitted during the period from April to September of the same year. Isolates were confirmed using common microbiological methods. Genomic DNA of isolates was extracted by boiling method and PCR method was used to identify pathogenic genes. The results were analyzed using Chi-square test.

**RESULTS AND DISCUSSION:** The results showed that the highest sensitivity to penicillin (47%) and the highest half sensitivity was observed for gentamicin (35%) antibiotics. Also, 61% of strains were resistant to cotrimoxazole. Also, the lowest antibiotic susceptibility (12%), cotrimoxazole (12%), the least susceptibility to tetracycline (26%), and the lowest antibiotic resistance were obtained with septrloxacin. The results also showed that the highest and lowest incidence was related to clfB and fnbA genes with 90% and 46% dispersion, respectively, and the highest prevalence was related to clfA and fnbB genes with 53% and 87% dispersion. Also, the results of statistical analysis showed that there is no significant difference between the frequency of genes in Staphylococcus aureus based on sex and age and type of clinical specimen (P 0.05). Also, the association between the frequency of antibiotics in Staphylococcus aureus was determined based on sex, age and type of clinical specimen.

**Keywords:** Staphylococcus aureus, fibrinogen, fibronectin, PCR

# Phenotypic and genotypic investigation of biofilm production capability in clinical isolates of *Staphylococcus aureus* collected from patients hospitalized in Mazandaran

## Resistance mechanisms

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**BACKGROUND AND OBJECTIVES:** The pathogenicity of *Staphylococcus aureus* is significantly attributed to its capacity to create biofilms, which augment bacterial resistance against antibiotics and host immune responses. This study aimed to explore the involvement of *icaA*, *icaB*, *icaC*, *icaD*, *fnbA*, and *fnbB* genes in the biofilm formation ability of clinical *S. aureus* isolates

**MATERIALS AND METHODS:** We collected 100 clinical *S. aureus* isolates from patients hospitalized in educational hospitals of Mazandaran in 2023. The isolates were identified using standard biochemical tests and confirmed by the presence of the *nuc* gene. Antibiotic susceptibility patterns were determined through the disk agar diffusion and micro broth dilution method. The potential for biofilm formation in the isolates was assessed using a microplate colorimetric test. Subsequently, a PCR screening was conducted to detect the presence of the *icaA*, *icaB*, *icaC*, *icaD*, *fnbA*, and *fnbB* genes.

**RESULTS AND DISCUSSION:** Among the 100 clinical *S. aureus* isolates, penicillin exhibited the highest resistance rate at 94%, while vancomycin displayed the lowest resistance rate at 6%. Approximately 32% of the isolates demonstrated a multidrug resistance (MDR) phenotype, while 29% were identified as methicillin-resistant *S. aureus* (MRSA). Notably, 89% of the isolates displayed the capability to produce biofilms. Among these, 54 isolates (60.67%) exhibited strong biofilm production, 28 isolates (31.46%) displayed moderate production, and 7 isolates (7.86%) showed weak production. In terms of genetic analysis, PCR results revealed a prevalence of 90%, 92%, 92%, 94%, 91%, and 17% for the *icaA*, *icaB*, *icaC*, *icaD*, *fnbA*, and *fnbB* genes, respectively. Intriguingly, the MDR isolates exhibited a 100% prevalence of these genes. Similarly, the MRSA isolates displayed prevalences of 96.55%, 89.65%, 89.65%, 96.55%, 89.65%, and 20.68% for these genes, respectively. Furthermore, the ability to produce biofilms was observed in 100% of MDR isolates and 93.1% of MRSA isolates.

**Keywords:** *Staphylococcus aureus*, Biofilm, *icaADBC*, *fnbA*, *fnbB*

# Phenotypic and molecular investigation of antibiotic resistance pattern and molecular typing of non-fermenting bacteria isolated from blood cultures of patients admitted to Mofid Hospital

Resistance mechanisms

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**BACKGROUND AND OBJECTIVES:** The study aims to investigate the phenotypic and molecular characteristics of antibiotic resistance patterns and to perform molecular typing of non-fermenting bacteria isolated from blood cultures of patients at Mofid Hospital. The presence of bacteremia, which indicates bacterial infection in the bloodstream, poses significant public health challenges due to its potential to cause severe diseases and economic burdens on the healthcare system. By understanding the genetic and resistance profiles of these bacteria, the research seeks to provide insights that could aid in better managing infections and improving patient outcomes.

**MATERIALS AND METHODS:** Over five months, blood culture samples from the Pediatric Infectious Research Center, Shahid Beheshti University, were studied for *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Positive samples in Bactec vials were cultured on Blood Agar and McConkey media. After incubation at 37°C, colonies were evaluated morphologically and Gram-stained. Gram-negative bacilli were further identified using biochemical tests like TSI, SIM, and oxidase. For the antibiogram, bacteria at half McFarland's concentration were cultured on Mueller Hinton Agar, and antibiotics were administered per CLSI 2023 guidelines. DNA extraction involved boiling bacteria in distilled water, centrifuging, and collecting the DNA-containing supernatant. PCR used specific primers to identify target genes at 50-60°C. For molecular typing, RAPD-PCR was performed on carbapenem-resistant strains, with electrophoresis used to analyze gel patterns.

**RESULTS AND DISCUSSION:** Investigating the phenotypic and molecular aspects of antibiotic resistance in non-fermenting bacteria isolated from blood cultures of hospitalized patients provides essential insights for managing these infections and improving patient outcomes.

**Keywords:** Phenotypic and molecular investigation, antibiotic resistance, molecular typing, non-fermenting bacteria

# Prevalence and drug resistance of isolated bacteria from middle ear infections with serous (Otitis media with effusion) in children referred to ENT clinic of Bu-Ali Sina Hospital in Sari during 2016-2017

## Resistance mechanisms

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**BACKGROUND AND OBJECTIVES:** Otitis media with effusion (OME) is a middle ear infection with fluid accumulation, which was characterized by serous secretions and inflammation and it is very common in children. Bacterial infections can contribute to the pathogenesis of the OME. This study was aimed to investigate the prevalence and drug resistance of isolated bacteria from middle ear infections with serous (Otitis media with effusion) in children.

**MATERIALS AND METHODS:** This cross-sectional study was performed on patients with OME (2-16 years) which was referred to the ENT clinic of Bu-Ali Hospital in Sari during 2016-2017. Patients after Three months of non-response to treatment were subjected to myringotomy and 0.5 cc of aspirated fluid from their ears separated and subsequently cultured on blood agar, chocolate agar and EMB, and incubated for 24 hours at 37 °C. After determining the strain of bacteria by differential tests, antibiotic resistance was determined by disc diffusion method. The data analyzed using GraphPad Prism 6 software and SPSS20.

**RESULTS AND DISCUSSION:** Our results showed that patients who had positive culture results, had significant high-level of ear pus secretions ( $P=0.006$ ). The prevalence *Staphylococcus epidermidis* was highest (23.3%) in OME patients following that, *Staphylococcus aureus* and *Staphylococcus saprophyticus* were the highest (13.3%) in comparison to other gram-positive bacteria, while in gram negative bacteria *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were the highest (6.7%). Antibiogram results showed that gram positive bacteria isolated from the OME patients are the high antibiotic resistance to ceftazidime (63.20%) and azithromycin (63.20%), while these bacteria were the most susceptible to ceftriaxone (78.90%) and gentamicin (78.90%) and gram-negative bacteria showed that the high resistance to cefixime (100%), ceftazidime (100%) and ceftriaxone (50%), and high susceptibility to amikacin (100%) and gentamicin (100%). Conclusion: It seems that treatment of these patients, especially for *Staphylococcus* species and the amount of antibiotic resistance, may be useful in choosing appropriate therapies for these patients.

**Keywords:** Otitis media, Effusion, Antibiotic Resistance, Common Bacteria, OME.



# The Inhibition effect of *Lactobacillus plantarum* and *Lentilactobacillus* sp. cell free supernatant against growth and biofilm formation of *Pseudomonas aeruginosa*

Resistance mechanisms

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**BACKGROUND AND OBJECTIVES:** Biofilm is a structure formed by a group of microorganisms, Bacteria in the Biofilm lead to much more serious problems. *Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen causing various infections, it has acquired various mechanisms of resistance to numerous groups of antibiotic agents, and the Biofilm-formation is one of them. Lactic acid bacteria produce various beneficial metabolites, including antibacterial and anti-Biofilm agents. The present study aims to verify the Antibacterial and Anti-biofilm effects of *Lactobacillus* probiotic strains on *Pseudomonas aeruginosa* (ATCC 27853) in laboratory conditions.

**MATERIALS AND METHODS:** For Preparation the Cell-free Culture Supernatants of *Lactobacillus plantarum* and *Lentilactobacillus* sp, the overnight culture of both *Lactobacillus* strains was used in MRS broth medium and then the supernatants were separated by centrifugation. Antibiogram was done using standard disk diffusion technique of Kirby–Bauer as approved by CLSI 2021 guidelines. the minimum inhibitory concentration (MIC) values of each CFSs on *Ps. aeruginosa* (ATCC 27853), were determined by the microdilution method in volume concentrations from 1 to 1/64. The ability to form Biofilm by the *Ps. aeruginosa* was investigated by tube method. The potential of Antibiofilm activity of both CFSs against multidrug resistance *P. aeruginosa*, was measured by tube method.

**RESULTS AND DISCUSSION:** Based on the Antibiogram results, the *Ps. aeruginosa* (ATCC 27853) was resistant to Ampicillin, Imipenem, and Tetracycline whereas it was sensitive to Ceftazidime, Amikacin, Gentamicin, Meropenem and Ciprofloxacin. MIC results for both CFSs from *L. plantarum* and *Lentilactobacillus* sp were ¼ concentrations. Found on the result of tube method, the *Ps. aeruginosa* was able to form Biofilm with moderate intensity. Furthermore, CFS from *L. Plantarum* had more effects in inhibiting Biofilm forming than CFS from *Lentilactobacillus* sp at the same SUB-MIC concentrations. Based on this study, CFSs from *L. plantarum* and *Lentilactobacillus* sp are effective in inhibiting the growth and Biofilm-forming of *Ps. aeruginosa* (ATCC 27853). Therefore, seems these substances can hopefully prevent various *pseudomonas* infections.

**Keywords:** cell-free supernatant, Biofilm, *Lactobacillus plantarum*, *Lentilactobacillus* sp., *Pseudomonas aeruginosa*, Antibiofilm.

# The Role of Horizontal Gene Transfer in the Emergence and Dissemination of Multidrug Resistance in Methicillin-Resistant *Staphylococcus aureus* (MRSA)

## Resistance mechanisms

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**BACKGROUND AND OBJECTIVES:** Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a formidable challenge to public health due to its capacity to resist multiple antibiotic treatments. The mechanisms underlying this resistance are predominantly driven by horizontal gene transfer (HGT). This study aims to elucidate the contribution of HGT to the development and propagation of multidrug resistance in MRSA strains.

**MATERIALS AND METHODS:** A comprehensive cross-sectional analysis was conducted, encompassing 200 MRSA isolates procured from diverse hospital settings. The study population included 110 males and 90 females, with a mean age of 45.6 years. Advanced molecular methodologies, including polymerase chain reaction (PCR) and whole-genome sequencing (WGS), were employed to identify resistance genes and elucidate the HGT mechanisms facilitating their spread.

**RESULTS AND DISCUSSION:** The findings revealed that 85% of the 200 MRSA isolates possessed multiple resistance genes. The predominant HGT mechanisms facilitating this gene acquisition were conjugation (70%), transformation (20%), and transduction (10%). The resistance genes most frequently disseminated through these mechanisms were *mecA* (95%), *ermC* (60%), and *aacA-aphD* (50%). This study highlights the pivotal role of horizontal gene transfer in the emergence and dissemination of multidrug resistance in MRSA. Insights into these mechanisms are crucial for the development of effective strategies to curb the spread of resistant MRSA strains and inform clinical treatment protocols. Ongoing surveillance and the application of advanced molecular techniques are recommended to monitor and manage the evolution of antibiotic resistance in MRSA.

**Keywords:** MRSA, Methicillin-resistant *Staphylococcus aureus*, horizontal gene transfer, multidrug resistance, antibiotic

# Comparison of ERIC-PCR pattern and antibiogram of Escherichia coli strains isolated from infection and feces of the same patients referring to Sanandaj health centers, 2022-2023

Resistance detection/prediction approaches

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**BACKGROUND AND OBJECTIVES:** Most Escherichia coli strains are harmless, but some strains cause urinary tract infections. The high resistance of this bacterium to various antibiotics and its rapid spread are among the most important challenges in treating infections caused by this organism. Examining the stool and urine samples of patients can be of great help in identifying the source of infection and the cause of the disease, and it can provide the doctor with various strategies in the treatment of the disease according to the diagnosis of the source of the disease. The purpose of this study is examined urine and stool samples in a patient to detect intrinsic or extrinsic infection using the pattern of ERIC-PCR.

**MATERIALS AND METHODS:** 50 urine samples and 50 stool samples are taken in pairs from the patients who refer to the laboratories of Sanandaj city. After culturing and diagnosis of E.coli, the sensitivity of bacteria measured by disk diffusion method and the sensitivity of bacteria to colistin is measured by colistin disk elution method. Gene extraction and PCR was done for attachment genes (afa.sfa.pap). Also, ERIC-PCR to identify repetitive sequences in E.coli strains that were isolated from urine and feces.

**RESULTS AND DISCUSSION:** In examination of the antibiogram of the urine samples, it was found that there is the highest sensitivity to FM OFX NOR antibiotics and the highest resistance to AMX NA SXT in the urine samples. In examining the antibiogram of stool samples, the most sensitive to FM OFX NOR and the most resistant to AMX CFM SXT. In the examination of paired samples of urine and feces, there was the highest sensitivity in FM OFX NOR and the highest resistance in AMX NA CFM. In examining binding genes by PCR, pap gene played the most important role in binding bacteria present in urine and stool samples to host tissue surfaces. Comparison of ERIC PCR results of Escherichia coli samples obtained from urine samples of patients with urinary tract infections and feces of the same patients shows that 88% (44 samples out of 50 paired samples) have 90% genetic similarity.

**Keywords:** Antibiotic resistance, Urinary tract infection, E.coli, PCR and ERIC PCR

# Evaluation of Antibiotic Resistance of Bacteria Isolated from Patients with Nosocomial Infections Hospitalized in Imam Khomeini Hospital, Sari in 2020-2021

Resistance detection/prediction approaches

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**BACKGROUND AND OBJECTIVES:** Nosocomial infections refer to infections that spread in the hospital environment, medical personnel, and medical equipment, which can cause an increase in mortality, the length of hospitalization of patients, treatment costs, and a decrease in the quality of healthcare. One of the challenges of hospital infections is antibiotic resistance. This resistance can occur due to incorrect and unnecessary use of antibiotics, non-compliance with health standards, as well as a combination of other factors. Antibiotic resistance in hospital infections causes serious problems in the treatment of patients, because less antibiotic drugs are available for treatment. For this reason, knowing hospital infections and their antibiotic resistance is important because of economic losses and damage to hospitalized patients. The purpose of this study is to investigate the prevalence of nosocomial infections and their antibiotic resistance pattern in Imam Khomeini Hospital in 2020.

**MATERIALS AND METHODS:** In this descriptive-cross-sectional, the files of all patients admitted to Imam Khomeini Hospital in Sari were examined in a period of two years (2020-2021). Demographic variables were examined. Samples were taken from patients' pleura, ascites, eyes, urine and sputum. All samples were cultured to measure antibiotic sensitivity and antibiogram test was performed on them. The obtained information was analyzed in SPSS statistical software.

**RESULTS AND DISCUSSION:** An examination was conducted on 500 suspected cases of hospital infections in 2020 and 2021. The average age of the patients was  $53.01 \pm 19.95$  years, and their typical hospitalization duration amounted to  $20.94 \pm 13.10$  days. The highest frequency belonged to *Stenotrophomonas maltophilia* with 72 samples (16.7%), *Staphylococcus epidermidis* with 59 positive samples (13.7%) and *Escherichia coli* with 50 positive samples (11.6%). The sensitivity of *Pseudomonas* was to polymyxin (100%), amikacin (51.2%) and meropenem (46.3%), respectively. Notably, Tetracycline demonstrated the highest sensitivity (63.5%) and after that, amikacin (47.8%) and gentamycin (43.9%) were the most sensitive against gram-negative bacteria, while the highest resistance of gram-negative bacteria was observed against ampicillin (6.2%), cefotaxime (10.7%) and cefixime (11.4%) antibiotics. All strains related to gram-positive bacteria were sensitive to amikacin and doxycillin (100%), while all strains of gram-positive bacteria were resistant to cefepime, cefprozone, and cefoxitin and ciprofloxacin.

**Keywords:** bacteria, nosocomial infection, pattern of antibiotic resistance,

# Evaluation of ERG11, CDR1 and CDR2 gene expression in *Candida albicans* isolated from patients with recurrent cervicovaginal candidiasis and human papillomavirus (HPV) co-infection

Resistance detection/prediction approaches

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**BACKGROUND AND OBJECTIVES:** Cervicovaginal infections caused by *Candida albicans*, along with human papillomavirus (HPV), have been linked to the development of cervical cancer. Therefore, it is crucial to conduct research on *C. albicans* strains that display resistance to azole drugs as the first line of treatment. Additionally, it is of utmost importance to assess the expression of resistance genes associated with azole drugs in these resistant strains. Hence, the objective of this study is to evaluate antifungal susceptibility patterns and the expression of efflux pump genes, specifically ERG11, CDR1, and CDR2, in fluconazole-resistant *C. albicans* isolates obtained from women experiencing recurrent candidiasis and HPV co-infection referred to Babol Medical Center.

**MATERIALS AND METHODS:** We conducted a study involving 18 patients diagnosed with recurrent cervicovaginal candidiasis in combination with HPV. Molecular biology analysis of the fungal isolates was conducted using the PCR-RFLP method, and an antifungal susceptibility test was performed using the broth microdilution method according to the CLSI M27-S4 guideline. Additionally, the mRNA expression levels of resistance genes were determined using real-time PCR.

**RESULTS AND DISCUSSION:** The study involved 18 participants, with an average age of 42 years. Among them, half had previously undergone laser therapy for the removal of genital hair. The colposcopy results showed that one patient had high-grade squamous intraepithelial lesions (HSIL). The culture results, using Sabouraud Dextrose Agar and chromogenic medium, indicated that all isolates were *C. albicans*. The antifungal sensitivity results revealed that five isolates were resistant to fluconazole, clotrimazole, ketoconazole, and miconazole, as determined by the minimum inhibitory concentrations (MIC). The mRNA expression levels of the ERG11, CDR1, and CDR2 genes in the resistant isolates were found to be  $(2/74 \pm 1/77)$ ,  $(32/31 \pm 3/72)$  and  $(2/74 \pm 1/62)$ , respectively. This study demonstrates an increase in the average expression of the CDR1, CDR2, and ERG11 genes in drug-resistant *C. albicans* strains found in individuals with HPV, when compared to susceptible isolates.

**Keywords:** Cervicovaginal candidiasis; HPV; CDR1; CDR2; ERG11; *Candida albicans*

# Study of Clinical and laboratory on antibiotic resistance and infection control in hospitals northern Iran

Resistance detection/prediction approaches

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**BACKGROUND AND OBJECTIVES:** Currently, with the daily spread of bacterial resistance and the significant risk of bacterial infections, they are threatening factors for people's health all over the world. On the other hand, antibiotic resistance has increased and the discovery of new antibiotics has been continuously decreasing. Because of this, researchers are always looking for new solutions so that as soon as the colonization of the pathogen becomes resistant to the first-generation antibiotics, the treatment should be changed to second or third generation antibiotics. The aim of present study was to examine the development of antibiotic resistance prevention and infection control programs.



**MATERIALS AND METHODS:** In This Clinical laboratory study, after collecting clinical samples, various biochemical and microbial laboratory tests were performed and then Antibiotic susceptibility testing was evaluated by disc diffusion method according to the CLSI guidelines with following antibiotics from different groups of the various wards of the hospital. According to, Multiplex PCR test showed the presence of a phenotype of resistance to one or more antimicrobial agents, were investigated for identify virulence and resistance genes. The data were analyzed utilizing expressive statistics in SPSS software version 23.

**RESULTS AND DISCUSSION:** In This research showed that the classes of antibiotics have become resistant, Antibiotics have not yet entered the microbial resistance phase and Antibiotics have the potential to develop resistance very high. The results showed that by using the Multiplex PCR method, there was possible to identify several genes simultaneously and prevent the creation of resistant strains and the transmission of these genes in human populations. According to research findings, the level of resistance of tested microorganisms in patients with nosocomial infections in northern Iran was observed a significant association decrease to be provided in combination with the optimal use of selected drugs, infection control strategies and were used with combination antibiotics. Also, in order to prevent the increase of resistance, the indiscriminate and arbitrary use of antibiotics should be prevented so that the appropriate antibiotic is selected for the treatment of patients and before that, culture and antibiogram are done.

**Keywords:** nosocomial infections, antibiotic resistance, Multiplex PCR, infection control,

# The prevalence of Integron classes in *Acinetobacter baumannii* and the relationship with its drug resistance in Sari Hospitals

Resistance detection/prediction approaches

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**BACKGROUND AND OBJECTIVES:** Among the microorganisms that cause infection in hospital environments, the drug resistance of *Acinetobacter baumannii* is one of the biggest concerns of doctors in clinical treatment, especially in the ICU. Some studies relate the ability of this microorganism to acquire the mechanism of resistance against antibiotics to the presence of the Integron gene. The purpose of this study is to investigate the prevalence of integron classes in *Acinetobacter baumannii* and verify its relationship with antibiotic resistance.

**MATERIALS AND METHODS:** This cross-sectional descriptive study, 260 clinical samples were examined, and through the extraction of OXA51 gene, the presence of *Acinetobacter baumannii* was detected in 104 samples. Then, identification of class 1, 2 and 3 Integron genes on bacteria was done by PCR method.

**RESULTS AND DISCUSSION:** PCR results show that 66 samples have Integron 1, 56 samples have Integron 2 and Integron type 3 did not have positive results in any sample. In total, out of 104 *Acinetobacter baumannii* isolates, 38 samples had both types of Integron 1 and 2, and 20 samples had none of these two types. The present study shows the resistance of 90.4% of *Acinetobacter baumannii* to Imipenem, which is the most effective treatment, which emphasizes the importance of developing new treatment methods.

**Keywords:** *Acinetobacter baumannii*, antibiotic resistance, Integron

# Innovative Nanoparticle Formulations to Combat MRSA: Quorum Sensing Inhibition and Synergistic Antibiotic Effects

Policy aspects of AMR

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**BACKGROUND AND OBJECTIVES:** Disrupting bacterial quorum sensing (QS), specifically the accessory gene regulator (agr) system of *Staphylococcus aureus*, offers a promising approach to mitigate virulence and enhance antibiotic susceptibility. Natural compounds such as quercetin, ferulic acid, and chitosan have shown potential as QS inhibitors, but their combined effects on MRSA infections remain completely unknown.

**MATERIALS AND METHODS:** Nanoparticles were prepared via ionic gelation, creating formulations of ferulic acid with chitosan (FC), quercetin with chitosan (QC), and a combination of all three compounds (CFQ). The nanoparticles were characterized using dynamic light scattering (DLS), zeta potential measurements, scanning electron microscopy (SEM), X-ray diffraction (XRD), and thermogravimetric analysis (TGA). The effects of these formulations on agr QS inhibition were evaluated using real-time PCR and luciferase assays. Biofilm formation assays were conducted to assess the impact on MRSA biofilms, and antibiotic susceptibility testing was performed to determine the synergistic effects with clindamycin.

**RESULTS AND DISCUSSION:** Characterization confirmed successful encapsulation and appropriate physicochemical properties of the nanoparticles. CFQ formulation demonstrated optimal particle size (219.4±13.2 nm) and stability (zeta potential of -26.1 mV). Real-time PCR revealed significant downregulation of agr-regulated genes and reduced QS activity. Biofilm assays indicated that CFQ exhibited superior inhibition of biofilm formation compared to FC and QC alone. Antibiotic susceptibility testing showed enhanced efficacy of clindamycin in the presence of the encapsulated compounds. Targeting the agr QS system with a combination of quercetin, ferulic acid, and chitosan encapsulation presents a potent strategy to combat MRSA infections. This approach not only mitigates virulence and antibiotic resistance but also holds potential to reduce selective pressure for resistance development. It is in this regard that the study highlights QS inhibitors as holding great potential for the development of next-generation antimicrobial therapies in responding to the growing global crisis need for effective treatments against antibiotic resistance.

**Keywords:** Quorum Sensing Inhibition; MRSA; Nanoparticles; Biofilm Formation; Antibiotic Synergy



# Using natural inhibitors, a new and practical strategy to inhibit antibiotic resistance by increasing the expression of efflux pumps in *Pseudomonas aeruginosa*

Policy aspects of AMR

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**BACKGROUND AND OBJECTIVES:** Increasing antibiotic resistance in *Pseudomonas aeruginosa* has highlighted the need to evaluate the mechanisms involved in antibiotic resistance to improve treatment. Increased expression of efflux pumps is one of the most important mechanisms of antibiotic resistance in *P. aeruginosa*. The aim of this study was to investigate the relationship between increased expression of MexCD-OprJ and MexEF-OprN efflux pumps and the development of Multi-drug Resistant (MDR) clinical isolates of *P. aeruginosa*, and determine the inhibitory effects of three natural inhibitors against these efflux pumps.

**MATERIALS AND METHODS:** In this descriptive-analytical study, 100 clinical isolates of *P. aeruginosa* were collected from 5 hospitals. The isolates were identified by standard diagnostic tests, and the MDR strains were identified using the disk agar diffusion method. The expression levels of the MexCD-OprJ and MexEF-OprN efflux pumps were evaluated by Real-time PCR. Then, the inhibitory effects of natural inhibitors (*Fraxinus excelsior*, *Glycyrrhiza glabra* and curcumin) against these efflux pumps were detected by Real-time PCR after treatment of the bacteria with these inhibitors for different times.

**RESULTS AND DISCUSSION:** Out of 100 clinical isolates, 41 showed multidrug-resistance phenotype (MDR). Piperacillin- tazobactam and levofloxacin were the most and least effective antibiotics, respectively. Also, all MDR isolates showed more than 10-fold increase in expression of mexD and mexF genes. In this study, a significant relationship was observed between resistance to tested antibiotics and increased expression of MexEF-OprN and MexCD-OprJ efflux pumps. In the inhibition test of the efflux pumps' expression, as the number of days of bacterial treatment with inhibitors increased, the reduction of gene expression also increased. This reduction was observed in combination of these inhibitors with ciprofloxacin and tobramycin, too. An effective strategy to treat infections caused by MDR isolates can be the use of an antibiotic in combination with an Efflux Pump Inhibitor (EPI), which inhibits the release of the antibiotic and restores the effect of the antibiotic to increase bacterial cell death.

**Keywords:** *Pseudomonas aeruginosa*, MexCD-OprJ, MexEF-OprN, Multi-drug Resistant (MDR)

# Carbapenemase genes distribution in clonal lineages of *Acinetobacter baumannii*: a comprehensive study on plasmids and chromosomes

Healthcare-associated infections

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**BACKGROUND AND OBJECTIVES:** The global spread of plasmids carrying carbapenemase genes within carbapenem resistant *Acinetobacter baumannii* (CRAB) strains poses a worldwide public health issue. In this study, we conducted a comprehensive genetic analysis of plasmids and chromosomes harboring the major carbapenemase genes (bla NDM, bla KPC, bla VIM, bla IMP, bla GES, bla OXA-58-like, bla OXA-24/40-like, bla OXA-143-like, and bla OXA-23-like) in CRAB strains using bioinformatic tools.

**MATERIALS AND METHODS:** We retrieved plasmids and chromosomes carrying the major carbapenemase genes from GenBank. The size, replicon type, and conjugal apparatus of the plasmids were also determined. Furthermore, allele types, co-existence of other antimicrobial resistance genes alongside carbapenemases in plasmids or chromosomes, co-occurrence of carbapenemase genes, gene repetition, and sequence types (ST) of whole genomes were characterized.

**RESULTS AND DISCUSSION:** Results The database contained 113 plasmids and 38 chromosomes harboring carbapenemase genes. This investigation revealed that bla NDM and bla OXA-58-like were the predominant allele types in both the plasmids and chromosomes. Nine (7.96%) plasmids with bla NDM-1 were potentially conjugative. The most common replicon types of the plasmids were R3-T1, R3-T8, R3-T2, R3-T23, and RP-T1. The analysis revealed that bla NDM-1 and bla OXA-58-like genes possessed the highest variety of co-existence with other antibiotic resistance genes. The co-occurrence of dual carbapenemases was identified in 12 plasmids and 19 chromosomes. Carbapenemase gene repetitions were identified in 10 plasmids and one chromosome. Circular alignment revealed that the plasmids carrying the co-occurrence of bla NDM-1 and bla OXA-58 were more homogeneous. However, there was heterogeneity in certain regions of these plasmids. According to the minimum spanning tree (MST) results, the majority of the plasmids belonged to the genomes of ST2Pas, ST1Pas, ST422Pas,

**Keywords:** *Acinetobacter baumannii*, carbapenem resistant, carbapenemase, gene repetition, sequence type

## “Visceral leishmaniasis in a Sarabi breed dog presented to the veterinary clinic of Shahrekord University.”

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Leishmaniasis, a chronic parasitic disease affecting humans and animals, is caused by *Leishmania infantum* and transmitted by sandflies. Dogs, particularly susceptible to the visceral form, can be primary human infection sources. Early detection and treatment in dogs can reduce human infection, especially in rural areas. This article reports a case of a Sarabi breed dog with visceral leishmaniasis. The dog, showing disease symptoms, was examined at a veterinary clinic, and diagnostic tests were conducted for definitive diagnosis.

**MATERIALS AND METHODS:** A Sarabi dog, aged 5, was brought to a clinic showing symptoms of anorexia, diarrhea, blood in stool, swollen lymph nodes, respiratory distress, and mucosal pallor. Post clinical examination and laboratory tests, it was found that the dog was suffering from anemia, decreased red blood cell count, hematocrit, hemoglobin, and changes in biochemical indices such as decreased total serum protein, albumin, and increased globulin level, phosphate, creatinine, and urea, indicating acute glomerulonephritis and kidney failure. A key finding was the observation of the amastigote stage of *Leishmania* in the macrophages in the lymph nodes, a pathognomonic lesion in leishmaniasis, causing swelling and suppuration of the lymph nodes. This case underscores the importance of early detection and treatment of leishmaniasis in dogs.

**RESULTS AND DISCUSSION:** Leishmaniasis, transmitted by sandflies, is a systemic disease affecting various organs in the body, with the liver, kidneys, and bone marrow being severely involved. If diagnosed in the early stages, the possibility of treating and improving the condition of the animal is very high. However, if diagnosed in the final stages, especially when kidney failure has occurred due to acute glomerulonephritis, the chances of treatment and recovery in the animal are very slim. Other researchers and veterinarians can conduct further research in this area to find solutions for definitive and early diagnosis of the disease that limit the need for other laboratory tests. Also, treating this disease in affected animals is one of the existing challenges in veterinary medicine that requires further research and investigation.

**Keywords:** Visceral leishmaniasis, sandfly, clinical symptoms, acute glomerulonephritis.

# AI and ND Seromonitoring in Green chicks and small parrots 2022-2023

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Currently pet birds are going to be most popular due changing the life cycle and more incoming of the humans. Parrots are one of the birds with most popularity in new civil social and locations, in current study some different strains of parrots which were referred to the Isfahan birds clinic will be studied by serology for ND and AI, There was no any suspected clinical sign and no any vaccination done, Due to a close relationship between parrots and the owners also regarding to owner request, public health and epidemiological reports the study have been done.

**MATERIALS AND METHODS:** In current study during 2022- May to 2023 -April about 50 green chicks parrots including different strains parrots (Yellow, Red, Blue, Orange, White) and small parrots (Budgies, Parrotlets, Love birds, Small Conures, Cockatiels) were studied at the birds garden, the sampling method were Blood which prepared using wing vein, the sera were tested for AI and ND by HI

**RESULTS AND DISCUSSION:** Regarding to the results the ND titer were ranged from 1 to 8, with the average of 5 and CV of 169%, The titer of the sera for H5N1 were 0 but for H9N2 were ranged from 1 to 6 and the mean titer were 4.5 with C.V. of 140%. so, biosecurity, vaccination and nutritional management would be done regularly under veterinary avian specialist care.

**Keywords:** Green chicks, Parrot, AI, ND, Seromonitoring

# An outbreak of tetanus in a sheep flock and the risk of transmission to veterinary technicians during vaccination and ear tagging

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Tetanus caused by *Clostridium tetani* is a fatal zoonotic disease which usually occurs sporadically. In contaminated animals there is usually a wound background. Human infection may happen through direct contact with animal's infected wounds. Subsequently, people who are engaged with animals are at a higher risk of the infection. Ear tagging of small ruminants is a veterinary technicians responsibility following vaccination against brucellosis. Lack of hygiene and injuries on the animal's ear during the procedure may lead to tetanus outbreak among small ruminants and the possibility of the technician's infection. The aim of the present study is to report an outbreak of tetanus in a sheep flock following ear tagging and emphasizes on the risk of human infection through skin injuries of animals.

**MATERIALS AND METHODS:** In a flock of 600 ear-tagged sheep, 50 animals showed tetanus symptoms from which wound exudate was collected. Ear tagging was done without gloves. The samples were stained by gram staining and cultured on blood agar anaerobically. Further biochemical tests were conducted according to the colonies appearance and the staining result.

**RESULTS AND DISCUSSION:** A total of 50 sheep showed clinical signs including limb stiffness, muscle spasm, lateral and sternal recumbency. The presence of gram-positive drumstick-like appearance of the bacteria and the colony morphology, biochemical tests including starch hydrolysis, gelatin hydrolysis and nitrate reduction were accomplished. The results of all the bacteriological tests revealed the infection with *Clostridium tetani*. Vaccination is not usually carried out except in the region with a high prevalence of tetanus, so it is important to prevent wound infection regarding hygiene during ear tagging. Using contaminated instruments makes both animals and contacted humans at a higher risk of tetanus. In addition, technicians should use gloves, especially when they have wounds, burns and injuries on their skin. Using antibiotic spray after each injury during ear tagging is another practical preventive method. Tetanus is a life-threatening disease in both sheep and human and the latter is more susceptible to the infection.

**Keywords:** *Clostridium tetani*, Ear tagging, Sheep, Vaccination, Veterinary technician

# Anaplasma ovis prevalence in the sheep and goats by PCR, Sistan, Iran

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Anaplasma ovis is a gram-negative rickettsia bacterium that causes anaplasmosis in small ruminants. The transmission of the pathogen is primarily through ticks, which are prevalent in sheep and goats. This disease usually presents itself in a subacute form, characterized by symptoms such as fever, anorexia, jaundice, fatigue, abortion, and decreased milk production. Although the mortality rate is generally low, it can be high in acute cases. The diagnosis of anaplasmosis is typically based on Giemsa-stained blood smears and microscopic detection of the pathogen. However, this method is not suitable for detecting the disease in subclinical cases. Molecular tests are considered the most appropriate diagnostic tool for asymptomatic cases. In this study, Anaplasma ovis was detected in asymptomatic sheep and goats in Sistan, located in southeastern Iran, using molecular tests.

**MATERIALS AND METHODS:** Blood samples were collected from 100 sheep and goats from the Sistan region of Sistan-Va-Baluchistan province, Iran. following DNA extraction, the 852 bp fragment of the msp4 gene of Anaplasma ovis, was amplified using polymerase chain reaction (PCR) with the specific primers.

**RESULTS AND DISCUSSION:** 64 out of 100 samples were found to be positive for Anaplasma ovis. This study revealed a high rate of asymptomatic infection among small ruminants in the area. The results of this study are in line with previous reports on the prevalence of anaplasmosis in cattle in the region. The findings suggest that the disease is endemic and prevalent in the area. Given the economic impact of sub-clinical infections, it is recommended that future studies investigate specific risk factors belonging to the disease in the region.

**Keywords:** Anaplasmosis, Anaplasma Ovis, Sistan, PCR, small ruminants

# Antimicrobials Resistance of Healthy Horse Eyes Microbiota in Tehran Province, Iran

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Various bacteria colonize the mucous surface of the eye conjunctiva. In cases of environmental conditions such as post-surgery, trauma or other underlying factors, these bacteria can cause eye infections such as conjunctivitis, corneal infections or endophthalmitis. The aim of this study was to determine the bacterial population and antibiotic resistance of the conjunctiva in healthy horses.

**MATERIALS AND METHODS:** Swab samples were collected from the eyes of 20 healthy, in different breeds, ages, and gender groups in Tehran province, Iran in the winter of 2019. The swabs were cultured on blood agar, MacConkey agar and broth agar and incubated. The grown bacterial colonies were separately cultured again in the above-mentioned mediums. The isolated bacteria were characterized using cellular morphology, gram-staining, and biochemical tests. Sensitivity and resistance to 10 common and effective antibiotics for horses in similar studies, including colistin, ceftiofur, florfenicol, amoxicillin, ampicillin, enrofloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole, neomycin, and gentamycin were evaluated by antibiogram test. The frequency of bacteria isolated from ocular swabs and the association between the frequency of the isolated bacteria and either age or sex of the studied horses along with the correlation between antibiotic sensitivity and resistance of bacteria and sex of horses as well as age groups were evaluated by chi-square and Fisher's exact test.

**RESULTS AND DISCUSSION:** Results showed that various species of bacteria include *Bacillus cereus*, *Micrococcus luteus*, *Bacillus licheniformis* and *Pasteurella multocida* being the top 4 most frequent isolated bacteria, respectively. There were no statistically significant differences in the frequency of isolated bacteria between sexes and age groups. There were no statistically significant differences in the sensitivity of bacterial flora of the eyes to colistin, ceftiofur, florfenicol, amoxicillin, and ampicillin between neither different age groups nor different sexes of the horses, respectively ( $p > 0.05$ ). However, sensitivity to enrofloxacin and ciprofloxacin was significantly associated with the age groups of the horses ( $p < 0.05$ ). Sensitivity to trimethoprim sulfamethoxazole between male and female horses was statistically significant ( $p < 0.05$ ). The results of this study suggests that the frequency of bacterial flora in healthy horses eyes is influenced by housing and management conditions rather than age and sex.

**Keywords:** Microbial flora, Conjunctiva, Eyes, Horse, Antimicrobials Resistance

# CRISPR Based Strategies applications in Zoonotic Diseases

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** The prevention and control of zoonosis is not only of great significance for the development of animal husbandry, but also an important safeguard for global public health. For the purpose of preventing and controlling zoonosis, we need to develop genome-editing tools such as CRISPR/Cas, nucleases induce DNA double-strand-breaks at specific locations within an organisms' genome. So far, two classes of CRISPR/Cas systems have been developed, encompassing six types and multiple subtypes. The class 1 employ an interference machinery comprising multiple Cas proteins. On the other hand, the Class 2 systems utilize a single Cas protein for interference. CRISPR/Cas-based gene editing has been extensively employed in various pathogens to investigate the roles of gene and protein in molecular pathogenesis.

**MATERIALS AND METHODS:** Virus: CRISPR/Cas system allows scientists to perform genetic manipulation on viral genes, such as targeted knockout, substitution, and codon modification, to obtain information on viral pathogenic genes, proteins, and their interactions with host proteins. Bacteria: CRISPR/Cas system can also be effectively employed for genetic manipulation on bacteria, including gene mutation, deletion, and replacement. CRISPRi system involves simultaneous expression of the catalytically inactive form of RNA-guided DNA endonuclease from the type II CRISPR system, known as dead Cas9, along with a small guide RNA specific to a target sequence. Parasite: A rapid method for generating custom guide RNA libraries utilizing arrayed single-stranded oligonucleotides is developed. This approach enables reproducible pooled cloning of CRISPR/Cas libraries and is applied to create mutant pools of various sizes in the protozoan parasite. We conduct an in vivo genetic screen in the murine host, leading to the identification of several known and novel virulence factors.

**RESULTS AND DISCUSSION:** Like other gene editing methods, CRISPR/Cas system also has its limitations. once the sgRNA recognizes a nonspecific site rather than the target sequence in the genome and mediates the cleavage, an "off-target" effect will occur. The enhanced comprehension of CRISPR/Cas biology has led to the broadened applications of this technique in the field of zoonosis. The CRISPR/Cas offers instruments that hold the potential to elucidate virulence-related gene, facilitate the development of the treatment and prevention of zoonosis.

**Keywords:** Zoonotic Diseases, CRISPR, Gene editing



# Cryptosporidium spp. infections (zoonotic protozoa) in calves and lambs as a source of environmental contamination (A case study: Mazandaran province)

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Cryptosporidium spp. are obligate, intracellular, protozoan parasites belonging to the phylum Apicomplexa (1). Cryptosporidiosis becomes a major public health and veterinary concern by affecting in human and various host range species of animals (2). All domestic animal, livestock, wildlife, and human can be potential reservoirs that contribute Cryptosporidium spp. to food and surface waters and transmitted to other hosts through fecal-oral route (3). The oocyst stage of Cryptosporidium spp. can remain infective and resistant to various environmental exposure and also resistant to many general disinfecting agents including chlorination which normally used in water treatment (4). Domestic animals play an important role in environmental pollution due to the spread of protozoa through feces. This study deals with cryptosporidium infection in domestic ruminants (calves 0-6 months and lambs 0-3 months) in Mazandaran province.

**MATERIALS AND METHODS:** This research was done to identify Cryptosporidium spp. protozoa in calves and lambs of Mazandaran province. For this purpose, animal samples (stool) were collected from 708 heads of lambs (0-3 months) and 713 heads of calves (0-6 months) for one year. It should be noted that the examined calves and lambs did not have any disease symptoms. The samples were examined after staining using modified zihil - nelson technique. Results showed, 29 samples of lambs (4.09%) and 28 samples of calves (3.92%) were positive.

**RESULTS AND DISCUSSION:** In this study, the presence of Cryptosporidium spp. oocysts in calves and lambs of Mazandaran province was recorded. Calves and lambs can excrete oocysts in their feces for up to 14 days. Although the presence of Cryptosporidium spp. protozoan in livestock is not always associated with disease, research results show that in diarrheal animals, this parasite worsens the course of the disease (6). The association of Cryptosporidium protozoa with other pathogenic agents can increase the severity of pathogenicity in its host. In particular, calves and lambs shed millions of oocysts, resulting in enormous environmental contamination and a risk of infection to other animals and humans (7). Considering that in this research, infestation rate in animals without clinical signs is high, so this subject is an important problem for public health.

**Keywords:** Cryptosporidium spp, calves, lambs, Mazandaran province

## Detection of Rickettsia species in collected ticks from Southeast Iran

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Rickettsioses are emerging zoonotic diseases caused by bacteria of the genus Rickettsia, transmitted to humans through the bite of infected ticks. In Iran, limited information is available regarding the prevalence of Rickettsia spp. in ticks, particularly in rural regions. The present study aimed to investigate the possible circulation of Rickettsia species and identify the variables associated with ticks infesting ticks collected from rural areas of southeastern Iran.

**MATERIALS AND METHODS:** A total of 2100 ticks were collected from domestic animals and dogs in rural regions of Kerman Province. The ticks were identified to species level and tested for the presence of Rickettsia spp. using molecular techniques. DNA was extracted from the ticks, and PCR amplification targeting the gltA gene was performed to detect Rickettsia DNA. Positive samples were further sequenced for species identification.

**RESULTS AND DISCUSSION:** Out of the 2100 ticks examined, 24.9% tested positive for Rickettsia spp. Subsequent sequencing analysis revealed the presence of four distinct species: *R. aeschlimannii*, *R. conorii israelensis*, *R. sibirica*, and *R. helvetica*. In addition, there was a significant association between tick species and host animals (dogs and domestic animals) ( $p < 0.001$ ), Rickettsia spp infection in ticks ( $p < 0.001$ ), and Rickettsia spp. ( $p < 0.001$ ). The prevalence of Rickettsia spp. exhibited variability among different tick species and collection sites, with certain regions demonstrating a notably higher prevalence.

**Keywords:** Rickettsia spp., ticks, dogs, rural areas, Kerman province

# Determination of the antimicrobial resistance rate of *Staphylococcus aureus* isolated from milk samples collected from milk suppliers in Sistan, southeast of Iran

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus aureus*, a gram-positive bacterium, is one of the most important pathogenic bacteria caused staphylococcal poisoning. The presence of a nanogram of staphylococcal enterotoxin per gram of food may lead to staphylococcal poisoning, indicating the importance of the bacterium to investigate. Moreover, the major concern of food safety is multidrug resistant *Staphylococcus aureus* in clinical and environmental samples. Data reporting antibiogram of the bacterium from milk source in southeast of Iran is scarce. Therefore, this study investigated antibiogram of isolates from milk sources from Sistan, Iran.

**MATERIALS AND METHODS:** For a six months period, a total of 100 confirmed isolates of *S. aureus* were recovered from milk samples collected from milk suppliers in Sistan, southeast of Iran. using standard biochemical characterization, such as growth on mannitol salt agar, and coagulase, DNase and catalase tests, the isolates were confirmed. Disk diffusion method was run to assess the bacterial antibiogram (wi. e., six customary antibacterial agents including amoxicillin, Ceftriaxon, Sulfamethoxazole, Gentamicin, Enrofloxacin, and Florfenicol) according to the current guidelines recommended by the Clinical and Laboratory Standards Institute.

**RESULTS AND DISCUSSION:** *S. aureus* showed the highest resistance against amoxicillin (100%), followed by Ceftriaxone(74%), Sulfamethoxazole (34%), Gentamicin(31%), and Enrofloxacin (26%), and the least resistance was observed against Florfenicol (6%). There is a statistically significant association between the antibiotic and resistance pattern of *S. aureus* ( $\chi(10) = 325.8$ ,  $P = .000$ ). 77% (77/100) of *S. aureus* were MDR. Among MDR isolates, three different MDR patterns (P1 (54.5%; 42/77); P2 (32.5%; 25/77); P3 (13%; 10/77)) were detected. The majority of MDR *S. aureus* with statistically significant differences showed P1 (resistance to three antibiotics;  $\chi(2) = 20$ ,  $P = 0.000$ ) MDR pattern. Eighteen antibiotypes belonged to *S. aureus*. The prevalent antibiotype of the isolates was CRO-AMX-SXT (3.1%;  $\chi(6) = 40.2$ ,  $p = 0.000$ ). Our findings shows that multidrug resistance strains of *S. aureus* can be isolated from milk suppliers in Sistan, Iran, hence, a public health concern that calls for urgent

**Keywords:** Antibiogram, Milk, Sistan, *Staphylococcus aureus*

## Determining the molecular identity of biofilm-producing genes in *Neisseria* isolated from dog dental plaques

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Periodontal disease is a very common disease that affects many dogs and its prevalence reaches 85% in dogs over 4 years old. It affects the supporting and protective structures of the teeth, and its etiological factor is the bacterial plaque that forms on the surface of the teeth and is an immune response to infection. Brushing is the most appropriate method to prevent periodontal disease through the mechanical removal of dental plaque.

**MATERIALS AND METHODS:** This study aims to determine the molecular identity of biofilm-producing genes in *Neisseria* isolated from dog dental plaques. This study is of descriptive type. The sample size includes 60 dental plaques from dogs referred to the veterinary clinic, which have been confirmed to have dental plaque by performing examinations and tests. First, the DNA of each sample was isolated by the column kit of SinaClone Company and after extraction, template DNA amplification was done by PCR method.

**RESULTS AND DISCUSSION:** Among 60 samples under investigation, 6 samples (10%) were positive in terms of presence. Positive cases of PCR in agarose gel were observed for *Neisseria* 139 bp and for biofilm generating gene 344 bp. Oral bacteria, especially *Neisseria*, are known to be effective factors in causing tooth decay and periodontal diseases. According to the obtained reports, tooth decay exists in 95% of the population. The most important factor in tooth decay is attachment of bacteria to different surfaces of the mouth and teeth.

**Keywords:** Biofilm, *Neisseria*, Dental Plaque, Molecular Identity

# Ephrin Protein: A Promising Vaccine Candidate Against *Haemonchus contortus*

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Ephrin domain-containing protein (EPH) was identified as one of the HcESPs that can be isolated from different stages of this helminth. In this study, we investigated the physicochemical and antigenic properties of the Ephrin protein (GenBank: CDJ87334.1) using bioinformatics tools.

**MATERIALS AND METHODS:** The antigenicity was evaluated using the ANTIGENpro server. Additionally, the ProtParam server was employed to assess the physicochemical characteristics of this protein. Moreover, the ABCpred webserver and IEDB database were used to predict linear B-cell epitopes and cytotoxic T lymphocyte (CTL) epitopes from the studied protein, respectively.

**RESULTS AND DISCUSSION:** Our findings showed that this protein is antigenic (0.566420). The molecular weight was 29769.50 Daltons. The theoretical isoelectric point (pI) of the vaccine was calculated to be 5.16. Furthermore, the GRAVY index of the vaccine was -0.287, indicating its polar nature and strong interaction with water, suggesting high solubility. Additionally, the count of residues with negative charge (Asp + Glu) and positive charge (Arg + Lys) was found to be 34 and 22, respectively. The protein formula was identified as C1329H2028N352O405S11. Also, the solubility of Ephrin protein was determined 0.412. Furthermore, our study showed the Ephrin protein has many linear B-cell epitopes and cytotoxic T lymphocyte (CTL) epitopes with high antigenicity that are suitable for vaccine designing for *H. contortus*. Our results show that the Ephrin protein is an antigenic protein and can be utilized for vaccine design or serological detection of *H. contortus*.

**Keywords:** *Haemonchus contortus*, Ephrin protein, Epitopes.

# Evaluation of antibacterial effect of *Teucrium Polium* against *Staphylococcus aureus*

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Antibacterial drugs are among the first line of medicaments in many countries. Many antibacterial agents have been derived from medicinal plants. *Teucrium polium* (Lamiaceae), has been used in traditional medicine as antifungal, antipyretic, antispasmodic, antibacterial, antidiabetic, antioxidant, and anticancer. The effects of this plant have been investigated in kidney, liver, and brain. This species is known for its richness in secondary metabolites such as essential oils and polyphenols. *S. aureus* is an important human pathogen. This gram-positive bacterium is a main cause of bacteremia, endocarditis, and respiratory tract infections. The prevalence of bacteremia due to *S. aureus* has been reported 5015 people per 1000-individual population in hemodialysis patients in the USA. A 34% increase in infection with *S. aureus* in Europe caused much concern. The aim of this study is Evaluation of antibacterial effect of *Teucrium Polium* against *Staphylococcus aureus*.

**MATERIALS AND METHODS:** The antimicrobial effects of extracts were evaluated on *Staphylococcus aureus* by disk agar diffusion method. 100 gram *T. polium* L. powder was Added to 500 ml ethanol 96 degree. The mixture was preserved at laboratory temperature for 24 hours. The aqueous mixture was boiled for 20 minutes with low flame. The collecting supernatant was centrifuged by 6000 rpm for 5 min. Samples were stored into the dark container at refrigerator temperature after filtering by 0.45  $\mu$  Whatman filter paper. 0.2 gram of aqueous and ethanol extract were added to 5 ml of sterile distilled water. 1 ml of this solution was added to sterile plates. The final concentration of the extract was 2000  $\mu$ g/ml. Mueller Hinton agar medium were sterile and used for bacteria were added to the plates and placed at room temperature. After incubation, the diameter of free zone was measured exactly by using a ruler.

**RESULTS AND DISCUSSION:** Aqueous and ethanolic extracts were quite effective in 2000  $\mu$ g/ml concentration on *Staphylococcus aureus* and were prevented from growth on medium. The anti-microbial efficacy of the ethanolic extract of the *Teucrium polium* plant against *Staphylococcus aureus*, as the results showed the presence of anti-microbial activity towards 75% of the isolates of *Staphylococcus aureus* bacteria.

**Keywords:** *Teucrium Polium* - *Staphylococcus aureus*

# Evaluation of genetic link between *Mycobacterium avium* ssp. paratuberculosis colonization and Crohn's Disease: an insilico study

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** *Mycobacterium avium* subspecies paratuberculosis (MAP) has been frequently associated with Crohn's disease (CD), a type of inflammatory bowel disease (IBD). The pathogenesis of IBD presents significant challenges in medical research, particularly regarding the initial disease trigger, diagnostic processes, underlying mechanisms, and the development of effective therapies. Furthermore, MAP is an obligate intracellular organism that causes Johne's disease (JD), in the gut of ruminants and other mammals. These animal species can transmit the MAP-resistant pathogen to humans through dairy products. The purpose of this study was investigation of the potential causal and genetic relationship between MAP and CD, to better understand the mechanisms of IBD pathogenesis.

**MATERIALS AND METHODS:** This study analyzed differential expression genes (DEGs) via bioinformatic tools. One NCBI-GEO microarray experiment with the accession ID GSE16879 was identified, extracted, and analyzed with GEO2R online tools and R software. Genes with the highest differential expression were identified using parameters P 0.05 and -2 LogFC 2. Then, the expression of the related genes between humans, cattle, and sheep was separated. For the genes that had an increase in expression, the protein network was predicted through the STRING database and visualized with the Gephi software. Afterward, each study's design, novel gene expression, and links to genes with significant expression increases were analyzed.

**RESULTS AND DISCUSSION:** DEGs were obtained for 2049 genes (654 upregulate, 1395 downregulate). Through genomic analyses, it has been determined that the genes PTEN, HSPA5, INS, VDAC1, and PPARG in the colon, as well as IL1B, CTNNB1, PTEN, HIF1A, PTGS2, and SIRT1 in the ileum, are expected to humans, sheep, and cattle. These genes are implicated in IBD and JD pathogenesis. PTEN gene is consistently expressed in both the colon and ileum across these species, highlighting its potentially pivotal role in these diseases. Furthermore, analysis results revealed that PTEN is a tumor suppressor gene that regulates cellular activities crucial for pathogen resistance. The PTEN gene and JAK/STAT signaling pathway are critical components of the host immune defense against MAP infection. Studying these genes' roles and effects may offer insights into developing preventive strategies against

**Keywords:** *Mycobacterium avium* subspecies paratuberculosis, Crohn's disease, Bioinformatics analysis, in silico.

# Evaluation of Immune response impact in controlling zoonotic fungal infections: A case study on *Aspergillus fumigatus* in animal models

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** *Aspergillus fumigatus* is an opportunistic fungus that can cause serious infections especially in individuals undergoing immunosuppressive therapy, people with HIV and diabetic patients. The pathogenic mechanism of this fungus is through inhalation of spores, with the primary target being the host's lung tissue. However, in invasive cases, the fungus can spread from the lungs to the bloodstream and other organs, leading to both pulmonary and systemic symptoms. This study investigates the role of the immune system in managing these infections and analyzes the impact of cellular and humoral immune responses on disease severity and progression in animal models. The results obtained may contribute to improving therapeutic strategies and preventive measures for similar fungal infections.

**MATERIALS AND METHODS:** In this study two groups of ten BALB/c mice each were used due to their immunological similarity to humans. *Aspergillus fumigatus* spores were injected intravenously into one group of healthy mice to induce infection. The second group of mice was designated as the control group. Cellular and humoral immune responses of the mice were assessed using ELISA assays to measure antibody levels and flow cytometry analysis to evaluate T cells and macrophages.

**RESULTS AND DISCUSSION:** Inflammatory cytokines such as interferon-gamma and interleukin-17 were significantly increased in response to this infection. Additionally, T cells and macrophages in the fungal infected models were more active. Neutrophilia, as well as elevated levels of immunoglobulins M and G, were observed in mice with fungal infections. Increased levels of TNF-alpha and gamma were also noted.

**Keywords:** Microbiology/mycology/immunology/*Aspergillus fumigatus*/zoonotic fungal infection/cellular immunity/humoral immunity/zoonosis



# Evaluation of the prevalence of *Bacillus cereus* in Raw milk available in dairy product shops of Zabol and Zahedan in 2020-2021

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** *Bacillus cereus* is particularly important for food safety and public health due to its ability to spoil and being food born disease through the production of various toxins. The aim of this study was to determine the prevalence of *Bacillus cereus* in raw milk from dairy retailers in Zabol and Zahedan. The resistance of this bacterium to some antibiotics was also evaluated.

**MATERIALS AND METHODS:** 100 samples of raw milk were collected from Zabol (n=50) and Zahedan (n=50), southeast of Iran. Using disk diffusion method, the antimicrobial susceptibility against tetracycline, erythromycin, colistin, norfloxacin, gentamicin, and chloramphenicol was tested in *B. cereus* isolates.

**RESULTS AND DISCUSSION:** Eighteen samples were contaminated with *B. cereus*, of which 10 samples belonged to Zabol and 8 samples belonged to Zahedan. The isolates recovered from Zabol (BSZB) and Zahedan (BSZH) were 100% resistant to beta-lactam and sulfamethoxazole. 90%, 50%, 80%, 10%, 0%, 10% of Zabol isolates were resistant to tetracycline, erythromycin, colistin, norfloxacin, gentamicin, and chloramphenicol, respectively, while 87.5%, 75%, 100%, 0%, 0%, 12.5% of BSZH were resistant to tetracycline, erythromycin, colistin, norfloxacin, gentamicin, and chloramphenicol, respectively. There was no statistically significant difference in the prevalence or antibiotic resistance of *Bacillus cereus* between Zabol and Zahedan. Interestingly, all isolates (100%) have multi-antibiotic resistance (MDR). It is recommended that more hygiene be monitored, and that proper ventilation in dairy retail outlets can reduce milk contamination. Educate veterinarians not to over-prescribe antibiotics, and conduct further studies on resistance.

**Keywords:** Antibiogram, *Bacillus cereus*, Dairy retail, Milk, Prevalence, Zabol, Zahedan

# Identification and characterisation of alpha toxin in isolated clostridium novyi from sheep in Iran

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Clostridium novyi is a gram-positive, anaerobic bacterium that based on the type of toxin produced, C. novyi is divided into pathogenic types A and B, and the non-pathogenic type C. Alpha toxin from Clostridium novyi type B is the major virulence factor that causes necrotic hepatic (black disease) in sheep and cattle. The identification of C. novyi and isolation of its pathogens by conventional methods is a time-consuming process, necessitating a simple and rapid method for isolating and detecting pathogenic C. novyi. Therefore, this study aimed to molecularly and in vivo and in vitro assay identify  $\alpha$ -toxin in local C. novyi isolates from sheep livers.

**MATERIALS AND METHODS:** 75 livers suspected of Black disease were collected and cultured in chopped liver broth medium under anaerobic conditions for 24-72 h in 37 °C in a gas jar. For molecular confirmation, the DNA of isolates was extracted from colonies grown on 5% sheep blood agar, using specific  $\alpha$ -toxin primers. The PCR on  $\alpha$ -toxin produced a band in the range of 609 bp, indicating that the samples belonged to C. novyi type B. Biochemical tests such as catalase, sugar fermentation and ... were performed for sample screening. Lecithinase activity of C. novyi isolates in the egg yolk agar plate was evaluated by anaerobically inoculating isolates by a sterile loop on the plate at 37°C for 72 h. The cell-free supernatant of isolated were tested on Vero 96 cells that were observed for 5 days. The supernatant of isolated was inoculated (IV) in mice.

**RESULTS AND DISCUSSION:** C. novyi was isolated from the 18 liver of a sheep and was confirmed as C. novyi type B by differential PCR. The C. novyi isolate fermented glucose and maltose and demonstrated lecithinase activity, and the cell-free culture supernatant of the C. novyi isolate exhibited cytotoxicity toward Vero cells, demonstrating that the isolate produces toxins. The mice die that prove the toxin is produce. This C. novyi isolated from a sheep in Iran and confirmed by biochemical and molecular characterization. Furthermore, by cell culture and animal assay confirm.

**Keywords:** clostridium noviy- sheep-PCR-cell cultur- toxin

# Infection status to thermophilic *Campylobacter* species in backyard chickens in Amol, northern Iran

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Thermophilic *Campylobacter* spp., primarily *C. jejuni* and *C. coli*, are the leading causes of food-borne human gastroenteritis worldwide, and have been designated as one of the high-priority antimicrobial-resistant pathogens by the World Health Organization. Domestic poultry are considered the important reservoirs for *Campylobacter* spp. Despite extensive colonization in the intestinal tract, *Campylobacter* infections in poultry produce little or no clinical. Hence, transmission of *Campylobacter* to humans occurs mainly through the consumption of contaminated poultry meat, as well as direct contact with live poultry or their feces. *Campylobacter* possesses many virulence factors. The *Campylobacter* adhesion to fibronectin (CadF), encoded by *cadF* gene, is prevalent among *Campylobacter*s. The cytolethal distending toxin (CDT), encoded by the *cdtA*, *cdtB*, and *cdtC* genes, has been well characterized in *Campylobacter*s. The present study aimed to investigate *Campylobacter* infection and their antibiotic resistance and virulence genes in backyard chickens in northern Iran.

**MATERIALS AND METHODS:** In total, 160 cloacal swab samples were obtained from apparently healthy backyard chickens in different rural areas of Amol, northern Iran. After isolation with bacteriological method, *Campylobacter*s were confirmed and speciated by multiplex PCR. All isolates were also evaluated for resistance to seven antibiotics by means of the Kirby-Bauer disk diffusion method, and for presence of virulence genes *cadF*, *cdtA*, *cdtB*, and *cdtC* using PCR assay.

**RESULTS AND DISCUSSION:** Overall, 58 (36.3%) out of 160 cloacal samples were positive for *Campylobacter* spp., including 28 (48.3%) *C. coli*, 9 (15.5%) *C. jejuni*, 6 (10.3%) mixed infection with *C. coli* and *C. jejuni*, and 15 (25.9%) other spp. A high rate of resistance was observed to ciprofloxacin (100%), tetracycline (92.3%), nalidixic acid (76.9%), ampicillin (65.4%), and erythromycin (63.5%), while resistance to amoxicillin/clavulanic acid (co-amoxiclav) and gentamicin was 34.6% and 23.1%, respectively. Moreover, 80.8% of isolates were multidrug-resistant. The *cadF* and three *cdt* genes were detected in 80.8% and 30.8% of *Campylobacter* isolates, respectively. Our findings suggest that backyard chickens could be a source of *Campylobacter* infections and drug resistance transmission in village people of northern Iran.

**Keywords:** Chicken, Backyard, *Campylobacter*, Antibiotic, Iran, Zoonosis

# Influenza monitoring in serums of some waterfowl birds

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Influenza A viruses are classified by subtypes based on the properties of their hemagglutinin (H or HA) and neuraminidase (N or NA) surface proteins so 18 different HA subtypes and 11 different NA subtypes distinguished. The subtypes such H9N2, H5N1 and H5N8 or H7N5 as reported in birds of Iran. Influenza type A viruses are most significant to public health due to their potential cause an influenza pandemic. Waterfowl birds are the primary natural reservoir for most subtypes of influenza A viruses. There are a few waterfowl birds included Cranes, Swans, Coots, Sea gulls, Ducks, Flamingos and Goose in the Isfahan bird garden and tested for AIV by serological and molecular tests.

**MATERIALS AND METHODS:** Using sterile syringe about 2 ml in wing vein blood collected and transferred for AIV titration, meanwhile the choanal cleft and cloacal swabs were prepared for molecular test. Regularly monitoring of 5% of sensitive birds were examined randomly every month; The technical method for serology were HI, ELISA and RT-PCR for molecular and confirmation of suspected titers.

**RESULTS AND DISCUSSION:** All of the birds showed positive titer for H9N2 but the highest titer were 8 for Coots, Goose and Green head duck, the lowest titer were 1 and related to pelicans, Flamingos and Cranes. The sera were negative for H5N1 and H5N8 and H7 but the swabs were checked by RT-PCR technique which confirmed the negative titers, this monitoring monthly well done and sanitization of gates and all areas with wide antiseptic materials carried out daily.

**Keywords:** AI, waterfowl birds, Monitoring

# Investigating the potential interference resulting from the combination of the metabolite derived from bacteria, exhibiting probiotic properties isolated from dog's milk, with routine antibiotics in the inhibitory effect against the *Staphylococcus* spp

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Today, mastitis is one of the most critical diseases causing economic losses in livestock herds. According to extensive studies, probiotics are considered safe compounds with few side effects. This research study aims to explore the combined effects of lactobacillus metabolite compounds (*Parvibaculum* spp., and *Lactiplantibacillus plantarum*) with probiotic potential alongside common antibiotics in combating mastitis-causing bacteria (*Staphylococcus aureus* and *Staphylococcus saprophyticus*) in dogs.



**MATERIALS AND METHODS:** After cleaning the animal's teat with alcohol, the secretions were collected in sterile tubes and transferred to the laboratory near ice packs. The next steps included cultivating, isolating, and biochemically confirming the bacteria, followed by conducting an antimicrobial susceptibility test. We prepared a 0.5 McFarland standard of bacteria and used the spread plate method on the Mueller–Hinton Agar (MHA) medium. After cultivating the desired lactobacilli in MRS broth medium and incubating, we performed centrifugation and collected the resulting extract as a metabolite. The sensitivity test to probiotic metabolite in different concentrations was carried out using a blank disk on the MHA medium. In the next step, *Lactobacillus* metabolite was combined with antibiotic discs, and after the test was dried, the sensitivity of the bacteria to this combination was determined by measuring the diameter of the zone of inhibition. The sensitivity test was repeated three times.

**RESULTS AND DISCUSSION:** The study results indicated that the metabolite by itself did not have any effect on the bacteria responsible for causing mastitis (*Staphylococcus aureus* and *Staphylococcus saprophyticus*) as no inhibition zone was observed. However, when *Lactiplantibacillus plantarum* was combined with various antibiotics, including Penicillin (PEN), Erythromycin (ERY), Sultrim (SLT), Fosfomycin (FO), Ciprofloxacin (CP), and Cephalexin (CEX) (from Padtan Teb Co., Iran), it exhibited an antagonistic effect. Moreover, this combination also interfered with *Staphylococcus aureus* when used with Tetracycline. *Parvibaculum* spp., in combination with all antibiotics, demonstrated an antagonistic effect on *Staphylococcus aureus*, while all lactobacilli displayed an antagonistic effect on *Staphylococcus saprophyticus* with all antibiotics. In conclusion, the effects of microbial metabolite combinations vary depending on the specific type of antibiotic utilized.

**Keywords:** probiotic, lactobacillus, metabolite, canine, mastitis, *Staphylococcus* spp

# Investigation of Hospital Cockroaches as Carriers of Pathogenic Bacteria in the Hospitals of Sari, 2014

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Cockroaches play a significant role as carriers of nosocomial bacteria in hospitals, thriving in environments with abundant food, warmth, and moisture. They harbor and spread pathogens through mechanical transmission, contaminating surfaces and medical equipment as they move. Additionally, their saliva and feces contribute to bacterial dissemination within hospital settings. The bacteria they carry can lead to serious health complications such as urinary tract infections, wound infections, and sepsis, which are often difficult to treat due to their resistance to antibiotics. This study examines the bacterial contamination on the external surfaces and gastrointestinal tracts of cockroaches present in hospitals affiliated with Mazandaran University of Medical Sciences.

**MATERIALS AND METHODS:** In 2014, cockroach samples collected from Imam Khomeini Hospital in Sari were methodically identified and documented according to their specific collection locations within the hospital. They were then transported to the medical faculty laboratory for further analysis. Bacterial isolation was meticulously carried out on both the external surfaces and the gastrointestinal tracts of the cockroaches, following stringent standardized protocols to ensure the accuracy and reliability of the findings.

**RESULTS AND DISCUSSION:** Bacteriological analysis revealed that 100% of the examined cockroaches carried bacteria. The predominant bacteria isolated in this study included *Proteus mirabilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *acinetobacter* species, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Antibiotic sensitivity testing of these bacteria also indicated the presence of multi-drug resistant pathogens.

**Keywords:** Cockroaches, Pathogenic Bacteria, Nosocomial Infections, Sari

# Investigation of the prevalence of Imerian and cryptosporidial species in native chickens of Lordegan city

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Coccidiosis, a parasitic disease affecting poultry, is primarily caused by Eimeria and cryptosporidial species, posing significant challenges to the poultry industry globally. This study aims to inspect the prevalence of Eimeria and cryptosporidial species in native chickens of Lordegan city, focusing on the impact of these parasites on chicken health and productivity. The objectives include collecting fecal samples from native chickens across nine villages in Lordegan city, identifying the presence and abundance of Eimeria and cryptosporidial species, and assessing the overall prevalence of coccidiosis in the area.

**MATERIALS AND METHODS:** Fecal samples were randomly collected from 100 native chickens distributed across nine villages in Lordegan city. The samples underwent analysis using the modified McMaster flotation method to quantify the number of oocysts per gram of feces. Oocysts were then sporulated in a 2.5% potassium dichromate solution to facilitate the identification of the genus and species of the parasites.

**RESULTS AND DISCUSSION:** The analysis revealed an overall prevalence of coccidiosis in the sampled chickens, with various Eimeria species identified, including Eimeria serulina, Eimeria maxima, Eimeria acervulina, Eimeria tenella, and Eimeria mitis. Co-infections involving multiple Eimeria species were also detected. Cryptosporidial species were not identified in this study. The prevalence of individual Eimeria species varied, with Eimeria maxima and Eimeria mitis being the most prevalent. Studies conducted in various regions have reported the prevalence of coccidiosis in poultry, with Eimeria species identified as the primary causative agents. The findings of this study align with these reports, emphasizing the widespread nature of coccidiosis in poultry populations. The identification of multiple Eimeria species in co-infections highlights the complexity of managing this disease and the need for comprehensive control strategies. Additionally, the absence of cryptosporidial species in this study suggests regional variations in parasite ecology and highlights the importance of localized research in disease management.

**Keywords:** Coccidiosis, Eimeria Species, Cryptosporidial Species, Native Chickens, Lordegan City

# Isolation and identification of mycoplasmas causing contagious agalaxia disease from sheep and goats in Chaharmahal Bakhtiari province

Veterinary microbiology/ Zoonotic diseases

Mohammad Hosein Pakrooh © ®, Mohammad Kazemi, Mohammad Javad koohian afzal, Rahmat allah Fatahian dehkordi

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**BACKGROUND AND OBJECTIVES:** Contagious agalaxia, an infectious disease affecting livestock, particularly sheep and goats, is a significant concern in regions with dense livestock breeding, including the Chaharmahal Bakhtiari province of Iran. This disease, characterized by fever, anorexia, lameness, and reproductive issues in affected animals, leads to considerable economic losses. Despite its prevalence, limited research exists on the isolation and molecular identification of the causative agent, *Mycoplasma agalactiae*, in these regions. This study aims to fill this gap by isolating and identifying *Mycoplasma agalactiae* from sheep and goats exhibiting contagious agalaxia symptoms in the Chaharmahal Bakhtiari province, using culture and Polymerase Chain Reaction (PCR) methods.

**MATERIALS AND METHODS:** Samples were collected from sheep and goats presenting contagious agalaxia symptoms in three cities of the Chaharmahal Bakhtiari province: Lordegan, Felard, and Khanmirza. Following collection, samples underwent culture and PCR analysis to detect the presence of *Mycoplasma agalactiae*. The PCR method was specifically designed to amplify a segment of the 16S rRNA gene of *Mycoplasma agalactiae*, allowing for the identification of the causative agent based on the expected size of the amplified product.

**RESULTS AND DISCUSSION:** From the 33 samples analyzed, 14 were confirmed to contain *Mycoplasma* species, as evidenced by the appearance of a specific band of 163 base pairs (bp) on agarose gel electrophoresis. Culture results further confirmed the presence of *Mycoplasma agalactiae* in one of these samples, marking the successful isolation of the causative agent of contagious agalaxia in sheep and goats within the Chaharmahal Bakhtiari province. Previous studies have highlighted the widespread occurrence of contagious agalaxia in various regions, including Mediterranean countries, Africa, Europe, America, and West Asia. Similar to the findings of this study, these investigations have emphasized the role of *Mycoplasma agalactiae* as the primary etiological agent of the disease. The successful isolation and identification of *Mycoplasma agalactiae* in the Chaharmahal Bakhtiari province underscore the importance of targeted surveillance and control measures in regions with high livestock density.

**Keywords:** Contagious Agalaxia, *Mycoplasma agalactiae*, Sheep, Goats, Chaharmahal Bakhtiari Province, PCR



# Isolation, Identification, and Molecular Investigation of *Saprolegnia parasitica* in Aquatic Environments

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** *Saprolegnia parasitica*, an oomycete, is a ubiquitous genus of water molds responsible for considerable economic losses in aquaculture, and its impact on natural fish populations is also of concern. This study aimed to isolate, identify, and molecularly investigate the *Saprolegnia parasitica* from aquatic environments in Sari and Babolsar, Iran, to comprehend their distribution, pathogenicity, and ecological role, thereby mitigating potential economic losses in aquaculture and maintaining the health of natural fish populations and ecosystems.

**MATERIALS AND METHODS:** fifteen water samples were collected from fish breeding ponds and rivers in Sari and Babolsar cities and cultured directly on YGC medium and in sterile plates with hemp seed at 25°C. Suspected mold samples were transferred to a PDA medium and stained by LCB for microscopic observation. The DNA was extracted from selected samples using the phenol-chloroform-isoamyl alcohol method, followed by PCR amplification with universal primers ITS-1 and ITS-4. Agarose gel electrophoresis was used to evaluate PCR products. Samples confirmed as *Saprolegnia* sp. were further analyzed through DNA sequencing to gain insights into their genetic diversity and phylogenetic relationships. Sequencing results were compared to existing *Saprolegnia* sequences in public databases for species identification and phylogenetic analysis.

**RESULTS AND DISCUSSION:** Three of the 15 water samples showed suspicious morphology and were confirmed as *Saprolegnia parasitica* by molecular assay. Further analysis through DNA sequencing provided additional insights into the genetic diversity and phylogenetic relationships among the identified *Saprolegnia* species. The findings of this study contribute to the understanding of *Saprolegnia parasitica* distribution and highlight the need to continuously monitor and develop effective control strategies to protect fish populations and the aquaculture industry.

**Keywords:** *Saprolegnia parasitica*, aquatic environments, molecular characterization, species distribution, pathogenicity.

# Listeria monocytogenes in Meat and Its Impact on Human Health

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** *Listeria monocytogenes* is a gram-positive, non-sporulating, motile bacillus that can cause serious foodborne illnesses, particularly in pregnant women, the elderly, and immunocompromised individuals. This study aims to investigate the prevalence of *L. monocytogenes* in meat products and its potential impact on human health. The United States has experienced about 30 *Listeria* outbreaks in the last decade, with 524 listeriosis cases and 80 deaths. Contaminated meat products, such as hot dogs and deli meats, have been associated with several outbreaks.

**MATERIALS AND METHODS:** A total of 100 meat samples, including beef and chicken, were collected from local markets and supermarkets. The samples were tested for the presence of *L. monocytogenes* using standard microbiological methods, including enrichment, selective plating, and biochemical identification. Positive isolates were further characterized using molecular techniques, such as multiplex PCR, to confirm the presence of *L. monocytogenes*.

**RESULTS AND DISCUSSION:** Out of the 100 meat samples tested, 15 were found to be positive for *L. monocytogenes*, indicating a prevalence rate of 15%. The highest contamination rate was observed in chicken (15%) and followed by beef (10%). The presence of *L. monocytogenes* in meat products poses a significant risk to public health, as it can cause serious infections, such as sepsis, meningoenzephalitis, and endocarditis. The mortality rate for listeriosis ranges from 20% to 50%. Essential oils have shown potential as natural alternatives to synthetic antimicrobials in preventing and treating *L. monocytogenes* infections.

**Keywords:** *Listeria monocytogenes*, Zoonosis, public health, meat, beef, chicken

# Mass investigation on the prevalence rate of zoonotic internal parasites in cats from Arak County, Iran

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Zoonosis diseases are of special importance from both economic and public health aspects. In many parts of the world, many losses and damages are caused by these diseases. The purpose of this study is to investigate the prevalence of zoonotic internal parasites in cats from Arak City, Iran.

**MATERIALS AND METHODS:** In this study, 220 cats (87 homeless cat collars and 133 domestic cat collars) were sampled. Sampling of feces was done directly from the rectum of the animals. 220 stool samples collected were classified according to animal gender and age.

**RESULTS AND DISCUSSION:** The results show that 17.7% of the studied cats were infected with common parasites. In this study, the highest rate of infection with internal parasites related to Isospora at 6.8%, *Toxocara cati* at 7.3%, and *Cryptosporidium* at 2.3% was observed. Statistical analysis shows that there is a significant relationship between the level of infection in homeless cats compared to domestic cats (P0.05). Also, the contamination rate in the studied population has a statistically significant relationship with the age of the cats (P0.05), but no significant relationship was observed with the sex of the cats (P0.05).

**Keywords:** cat, internal parasite, Zoonosis diseases

# Molecular Assessment of *Coxiella burnetii* in Horses in Northwestern Iran

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Q fever is a prevalent illness caused by the bacterium *Coxiella burnetii* (*C. burnetii*) that can affect humans and different animals. It specifically attacks macrophage cells in body tissues and circulating monocytes. A research study was carried out in the West Azerbaijan and Ardabil provinces of northwestern Iran to examine *C. burnetii* infection.

**MATERIALS AND METHODS:** Swab samples were collected from 140 mares (70 from each province) and 20 stallions (10 from each province) that appeared to be healthy. Their DNA was analyzed using real-time PCR targeting the IS1111 element of the bacterium.

**RESULTS AND DISCUSSION:** The analysis revealed that only 0.625% of the samples tested positive for *C. burnetii*. One mare from the Ardabil province was found to be carrying the bacterium, indicating that horses, even when asymptomatic, can harbor *C. burnetii* and contribute to its transmission. This underscores the importance of considering the role of horses in the spread of Q fever.

**Keywords:** *Coxiella burnetii*, horse, real-time PCR, Iran

# Molecular identification of *Salmonella enteritidis* isolated from poultry flocks in Tabriz city (northwest of Iran) in 2022

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Salmonellosis is one of the important zoonotic diseases, which has a wide geographic distribution. Most of the *Salmonella* spp isolated from infected people are *Salmonella enterica* serovar enteritidis. *Salmonella enteritidis* is one of the three serovars which is important and dominant in most countries of the world. *Salmonella enteritidis* is frequently isolated from poultry and humans. Chicken meat and egg are two important sources of *Salmonella enteritidis* infection for humans. The aim of this study was molecular identification of *Salmonella enteritidis* isolated from poultry flocks in Tabriz city (northwest of Iran) in 2022.

**MATERIALS AND METHODS:** 100 poultry feces samples from different flocks were considered for this study. The samples were cultured and the specific biochemical tests were done for identification of *Salmonella enteritidis*. Then, the positive samples were confirmed molecularly by PCR method.

**RESULTS AND DISCUSSION:** The results showed that 45 samples (45%) were positive as *Salmonella enteritidis* by conventional staining and culturing in differential media. All of tested samples contained the *hliA* gene and confirmed as *Salmonella enteritidis*. According to high frequency of *Salmonella enteritidis* in poultry flocks in Tabriz city, these findings are very important for poultry industry and the control strategy for *Salmonella enteritidis* infection.

**Keywords:** *Salmonella enteritidis*, Poultry, PCR, Tabriz

# Molecular prevalence of *Anaplasma phagocytophilum* in small ruminants in Sistan, southeastern, Iran

Veterinary microbiology/ Zoonotic diseases

Amirhosein Azadmehr <sup>1</sup> © ©, Mehdi Rasekh <sup>2</sup>, Ali Sarani <sup>3</sup>

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**BACKGROUND AND OBJECTIVES:** *Anaplasma phagocytophilum* is a tick-borne bacterium that infects white blood cells in both animals and humans, causing a disease known as anaplasmosis that is not widely recognized, especially in developing countries. In sheep and goats, anaplasmosis can lead to symptoms such as fever, anemia, lethargy, and decreased milk production, ultimately resulting in economic losses for livestock farmers. Based on clinical signs and paraclinical documents tick-borne infections particularly anaplasma species are endemic in sistan area due to climate, poorness and other known or unknown conditions. This study was conducted to detect *A. phagocytophilum* infection among sheep and goats in the Sistan region and attempt to identify the prevalence of this microorganism using a controversial PCR method.

**MATERIALS AND METHODS:** Blood samples from 100 sheep and goats were randomly collected from Sistan. After DNA extraction, samples were evaluated for *A. phagocytophilum* by using specific primers, PCR and amplifying 641 bp fragment of MSP4 gene.

**RESULTS AND DISCUSSION:** The results revealed that *A. phagocytophilum* was detected in approximately 11 out of 100 tested samples. The prevalence rate of 11% indicates a moderate level of infection in the studied population. Further investigations are needed to understand the factors contributing to the spread of *A. phagocytophilum* in the region, such as tick vectors and possible reservoir hosts. Effective control measures, including tick control and appropriate treatment protocols, should be implemented to reduce the impact of anaplasmosis on sheep and goat health and productivity.

**Keywords:** Anaplasmosis, *Anaplasma phagocytophilum*, sistan, PCR, sheep, goats, blood parasite

# Phylogenetic group determination and genetic diversity of *Escherichia coli* isolated from domestic animals' stool specimens and human clinical samples

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** *Escherichia coli* consists of a wide range of strains with huge diversity in their genome, distributed in nature and the alimentary tracts of animals and humans. This study analyzed the phylogenetic group determination and genetic diversity of *E. coli* strains isolated from domestic animals and human clinical samples.

**MATERIALS AND METHODS:** Twenty *E. coli* isolates from domestic animals were analyzed for phylogenetic grouping. Also, 100 clinical samples and 20 animal samples were evaluated by the enterobacterial repetitive intergenic consensus–polymerase chain reaction (ERIC-PCR) technique. The results and the similarity between the strains were determined based on the Dice similarity coefficient in the SAHN program of the NTSYS-pc software.

**RESULTS AND DISCUSSION:** The frequency of phylogroups among animal samples were A = 5%, B1 = 65%, B2 = 20%, and D = 10%. Based on the ERIC-PCR results, the clinical strains were allocated into 19 clusters. Most strains were in the E7 cluster. Fifty percent of the *E. coli* isolated from animal specimens belonged to the E4 group, and the lowest number of strains was in the E3 and E5 (1 strain) groups. The results confirmed the efficiency and usefulness of the ERIC-PCR tool for the identification and classification of bacteria. Also, we demonstrated the most phylogroup among animal samples.

**Keywords:** *Escherichia coli*, domestic animals, human, ERIC-PCR

# Prevalence of Extended-Spectrum Beta-Lactamase (ESBL) genes in Escherichia Coli isolates recovered from poultry colibacillosis and human urinary tract infections

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Escherichia coli harboring Extended-spectrum  $\beta$ -lactamase (ESBL) genes is considered a leading pathogen contributing to the global burden of antimicrobial resistance. The aim of our study was to determine the frequency of the four most common ESBL genes in E. coli isolates recovered from broilers colibacillosis and Urinary Tract Infections (UTIs) cases in one community, Semnan, Iran. E. coli isolates from consecutively collected urine samples of patients with symptoms of urinary tract infections (UTIs) at a university-affiliated hospital and broilers suspected to have colibacillosis were enrolled in our study.

**MATERIALS AND METHODS:** Materials and methods: In this study, of 100 (human: 50 and poultry: 50) E. coli isolated from the viscera of broilers suspected of colibacillosis and urine of patients with urinary tract infection identified by differential biochemical tests, the presence of blaTEM, blaCTX-M, blaCTX-M1 and blaCTX-M2 genes. genes were determined by PCR amplification.

**RESULTS AND DISCUSSION:** The results of this study showed that 54% of human samples (27/50) and 24% of poultry samples (12/50) of strains harbored blaTEM and 64% of human samples (32/50) and 6% of poultry samples (3/50) produced blaCTX-M genes. And also 40% (20/50) and 18% (9/50) of strains harbored blaCTX-M1 in humans and poultry respectively. Also, no positive sample was observed for gene blaCTX-M2. According to the results, the prevalence of ESBL-producing E. coli is relatively high in both studied groups. This finding shows that broilers in Semnan poultry farms harbour ESBL producing E. coli isolates that could play an important role in contamination of the food chain. Therefore, it is crucial to continuously monitor livestock rearing and food industries to detect microorganisms carrying resistance markers.

**Keywords:** Keywords: Antimicrobial resistance, Escherichia coli, Extended-spectrum  $\beta$ - lactamase (ESBLs)



# Prevalence of Shiga Toxin-Producing *Escherichia coli* isolates in Fresh Beef Meat Samples from an Industrial Slaughterhouse

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** The aims of the current study are the identification of O157 and non-O157 STEC serogroups isolated from fresh beef samples at an industrial slaughterhouse, determination of antimicrobial resistance patterns, and genetic linkage of STEC isolates.

**MATERIALS AND METHODS:** A total of 110 beef samples were collected from the depth of the rump of cattle slaughtered at Hamadan industrial slaughterhouse. After detection of *E. coli* isolates, STEC strains were identified according to PCR for *stx1*, *stx2*, *eaeA*, and *hlyA* virulence genes, and STEC serogroups (O157 and non-O157) were identified by PCR. The genetic linkage of STEC isolates was analyzed by ERIC (Enterobacterial Repetitive Intergenic Consensus)-PCR method. The antimicrobial susceptibility of STEC isolates was detected by the disk diffusion method according to CLSI guidelines.

**RESULTS AND DISCUSSION:** Among 110 collected beef samples, 77 (70%) were positive for *E. coli*. The prevalence of STEC in *E. coli* isolates was 8 (10.4%). Only one *E. coli* isolate was identified as Enteropathogenic *E. coli*. The overall prevalence of O157 and non-O157 STEC isolates was 12.5% (one isolate) and 87.5% (7 isolates), respectively. The hemolysin gene was detected in 25% (2 isolates) of STEC strains. Evaluation of antibiotic resistance indicated that 100% of STEC isolates were resistant to ampicillin, ampicillin-sulbactam, amoxicillin-clavulanic acid, and cefazolin. Resistance to tetracycline and ciprofloxacin was detected in 62.5% and 12.5% of isolates, respectively. The analysis of the ERIC-PCR results showed five different ERIC types among the STEC isolates. The isolation of different clones STECs from beef and the presence of antibiotic-resistant isolates indicates that more attention should be paid to the hygiene of slaughterhouses.

**Keywords:** *Escherichia coli*, STEC, Beef, Shiga toxin, O157, Non O157

# Prevalence of Theileriosis in small ruminants in Sistan, Iran

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Theileriosis is one of the most common tick-borne diseases that can affect a wide range of domestic and wild animals worldwide. theileria is a blood protozoon that classified in the family of Theileriidae and in the order of Piroplasmida. Various species of Theileria can cause different virulence in animals. infection with theileriosis can cause a significant economic loss so, monitoring the infection prevalence and its control is a major issue. According to clinical and para-clinical observations, this disease is endemic in Sistan's livestock industry and every year it causes extensive economic damage in the region. In this study, we determined the infection of theileriosis in the asymptomatic small ruminants by PCR based on the 18S rRNA gene in Sistan region, Iran

**MATERIALS AND METHODS:** Blood samples were collected (August to December 2023) randomly from asymptomatic sheep and goats from 5 districts of the Sistan including Zabel, Hamoon, Zahak, Hirmad and Nimrooz. after the DNA extraction with the commercial extraction kit, the quality of the DNA samples were checked with the nanodrop. the prevalence of the infection was determined using PCR and the 18S rRNA gene detection.

**RESULTS AND DISCUSSION:** A total of 17 (28%) from 60 blood samples, were positive for Theileria spp. by using PCR. Regarding the findings of previous studies and this study, it is clear that the disease is endemic, and economic effects should be considered in the region. Identifying specific risk factors belonging to the area is essential to mitigate economic losses.

**Keywords:** Sheep, Goats, Theileriosis, Sistan

# Rabies and Immunocompromised Populations: Exploring the Intersection of Zoonotic and Chronic Diseases

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Rabies, a fatal zoonotic disease caused by the rabies virus, poses a heightened risk to immunocompromised individuals, such as those with HIV/AIDS, cancer, or undergoing immunosuppressive therapy. This study aims to investigate how immunocompromised status affects rabies infection progression, clinical outcomes, and the efficacy of post-exposure prophylaxis (PEP).

**MATERIALS AND METHODS:** The data collected included specific metrics on disease progression, such as time to symptom onset and severity scoring, as well as detailed responses to post-exposure prophylaxis (PEP), including dosage, timing of administration, and patient adherence. Clinical outcomes were assessed based on patient survival rates, duration of hospitalization, and any reported complications. Additionally, a controlled laboratory study was performed using immunocompromised animal models, specifically BALB/c mice with induced immunodeficiency. These models were exposed to a standardized dose of the rabies virus, and subsequent observations focused on quantifying viral load in the central nervous system and peripheral tissues at various time points post-infection. The immune response was measured through cytokine profiling and T-cell activity assays. Survival rates were meticulously recorded, with the experimental groups receiving varying regimens of PEP to assess the efficacy of different treatment protocols.

**RESULTS AND DISCUSSION:** Immunocompromised patients exhibited accelerated disease progression and poorer clinical outcomes compared to immunocompetent individuals. The efficacy of PEP was significantly reduced, with lower survival rates in both human and animal models. These findings highlight the critical need for enhanced rabies management strategies in immunocompromised populations, emphasizing the importance of early intervention and tailored therapeutic approaches to improve prognosis and survival rates.

**Keywords:** Rabies, Public health, Immunocompromised, Zoonotic Diseases

# Seroepidemiological Analysis of Leptospiral infection using MAT in Stray Dogs in Mazandaran, Iran

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Leptospirosis is caused by the spirochete *Leptospira*, a zoonotic bacterial pathogen that infects a wide variety of mammals and poikilothermic animals worldwide (1). Animals serve as a source of transmission through the shedding of *Leptospira* in their urine (2). As a mammal, the dog is infected with a variety of pathogenic *Leptospira* serovars. Therefore, it is important for public health. Clinical signs of dogs with leptospirosis can vary from subclinical or minimal clinical disease or mild fever to severe kidney, liver, and pulmonary disease (3). The advent of animal vaccines against specific serotypes has reduced the risk of transmission to humans (4). The aim of this research was to identify serovars for making effective dog vaccines.

**MATERIALS AND METHODS:** The studied group consisted of 60 dog collars, in the age range of 1 to 5 years, which were selected from stray dogs in different areas of Mazandaran province from April to March 1402. After the blood samples were taken, they were transferred to the National *Leptospira* Reference Laboratory, Razi Karaj Vaccine and Serum Research Institute, Iran. In the laboratory, the samples were centrifuged at 3000×g. Serum samples were tested for leptospira antibodies in 20 leptospira serovars (5). After that, serum samples were added to live *Leptospira* cell suspensions in 96-well round-bottom microtiter plates at room temperature in the dark for 2 hours. After that, a small amount of wells was added on a slide and observed at 20x magnification using a dark field microscope (6).

**RESULTS AND DISCUSSION:** In total, 60 blood samples were collected from stray dogs to detect the antibodies against leptospira interrogans serovars by the MAT. The prevalence rate of positive MAT tests in stray dogs was estimated at 40%. The following protocol confirmed that the most common titers were 1:400 (50%) and 1:800 (46%). In addition, common serovars in order of abundance include: *Leptospira canicola*, *Leptospira icterohaemorrhagiae*, *Leptospira pomona*, *Leptospira autumnalis*, *Leptospira sergio hargo*, *Leptospira grippotyphosa*. The results also showed a high prevalence of leptospirosis in stray dogs of Mazandaran province. Since Leptospirosis is a zoonosis disease, it should be studied continuously in humans and animals, especially dogs.

**Keywords:** Seroepidemiology, *Leptospira*, MAT, Stray Dogs, Mazandaran

# Seromonitoring (ND –AI) of Ostrich chicks during 2021-2022

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Industry of ostrich breeding in Iran is developing and well increased in Isfahan as the first ranking in the country, It was about 20 years ago which a few African Ostrich chicks imported to Iran and at the moment we arrive to more than 5000 breeder just in Isfahan Province with about 7200 chicks for laying or meat production in future. One of the most complicated diseases of the ostrich chicks is viral Newcastle and Influenza. The clinical signs coupled with management and nutritional difficulties but the contagious signs of disease order for the biosecurity and vaccination...

**MATERIALS AND METHODS:** Sampling of bloods done via jugular and wing vein in the infected chicks aged 2 to 6 months from 6 farms in Isfahan and Chahar mahal provinces from April 2021 to march 2022. The samples transported to serology laboratory and the sera were prepared for HI test for ND and AI and also ELISA test kit of biochek used for chicken Aadenovirus 1 and Borna Virus investigation

**RESULTS AND DISCUSSION:** : Regarding to the results of HI test for ND and AI were positive and valid, in which the CV for ND were 167% with the Maximum of 10 ,Minimum were 2 and average of 8 ,the CV for AI ( H9N2 ) were 158% with the Maximum titer of 9 ,Minimum titer of 3 and average of 6 , Fortunately no any infection of H5s serotypes were positive According to the clinical and paraclinical results the syndrome were related to per acute ND (enteric form) co working with AI. Ostriches ND vaccination in all ages, disinfecting and biosecurity would be preventive for downer syndrome.

**Keywords:** Ostrich Chicks, , seromonitoring, AI ,ND

# Survey the prevalence and pathophysiology of Dengue fever virus in stray dogs

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Dengue fever is an infectious viral disease that transmitted by mosquitoes of the Culicidae family, genus Aedes, which leads to different serotypes of Bone-breaking fever, Dengue shock syndrome, and Haemorrhagic syndrome. Dengue virus is an RNA virus of the Flavivirus and based on antigenic properties, it has four serotypes (DENV 1-4) that are close to each other. today, Dengue fever is considered as a re-emerging or new emerging zoonotic. we decided to investigate its prevalence and pathophysiological effects on stray dogs. This study is by investigating the habitat of the Aedes mosquito in garbage dumps and swamp areas.

**MATERIALS AND METHODS:** from 75 dog collars from shelters in different urban areas of Mazandaran that were in garbage dumps and had suspicious symptoms, Blood samples in the amount of 5 cc (2 cc for PCR and 3 cc for hematology and biochemistry) were taken from the Cephalic vein. after transferring the samples to the laboratory with a cold chain, the extraction process was carried out with the molecular detection method of Arbohemorrhagic Geneva with the following sequence done DEN1 TCAATATGCTGAAACGCGGAGAAACCG DEN2 TTGCACCAACAGTCAATGTCTTCAGGTTC at 511 bp. the positive samples of the molecular test were examined to investigate the pathophysiology and clinical complications.

**RESULTS AND DISCUSSION:** Out of 75 dog collars, the result of 35 samples was positive, which was divided into: 19 collars from Sari, 9 collars from Kiakola and 2 collars from Babol city. ages between 1 and 5 years had the highest number of patients (48%) and puppies had the lowest number of patients (1.3%).72% of dogs with Dengue Haemorrhagic fever and 28% did not show acute clinical signs and the most common clinical sign was fever (in 91% of dogs). Vomiting was observed in 32%, loss of appetite in 21%, Diarrhea in 10% and Mucosal bleeding in 15%. Hepatomegaly was found in 80% of dogs. The most common bleeding observed was Haemorrhagic and mucous. disease complications were liver dysfunction in 19.8%, coagulation disorder in 2.7%, kidney dysfunction in 5.7%, skin disorder in 2.4%, and disseminated intravascular coagulation (DIC) in 3% was seen. mortality rate was not observed in this study.

**Keywords:** Dengue fever Aedes Zoonotic Stray dogs

# The antibacterial and anti-biofilm potential of *Zataria multiflora* essential oil against *Staphylococcus aureus* isolated from bovine mastitis

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Bovine mastitis, a major economic burden, is often caused by infectious bacteria including *Staphylococcus aureus*. This study investigated the antibacterial and anti-biofilm properties of *Zataria multiflora* essential oil against *Staphylococcus aureus* isolates from bovine mastitis.

**MATERIALS AND METHODS:** Fifteen *Staphylococcus aureus* isolates were obtained from clinical mastitis cases. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oil were determined using the broth microdilution method. Anti-biofilm activity was assessed via the microtiter plate test. Data were analyzed using the t-test.

**RESULTS AND DISCUSSION:** *Zataria multiflora* essential oil exhibited antibacterial activity against *Staphylococcus aureus* (MIC= 25 µg/ml and MBC= 100 µg/ml). In addition, *Zataria multiflora* essential oil significantly inhibited biofilm formation at sub-MIC concentrations (MIC/2 and MIC/4). These findings suggest potential therapeutic applications for bovine mastitis, warranting further investigation into this promising natural compound.

**Keywords:** *Zataria multiflora* essential oil, *Staphylococcus aureus*, bovine mastitis

# The assessment of antibiotic resistance and virulence profiles of *Proteus mirabilis* isolated from broiler chickens

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** *Proteus mirabilis* is an opportunistic zoonotic pathogen commonly associated with a broad spectrum of human infections, both in community settings and healthcare facilities. Antimicrobial resistance in *P. mirabilis* has been exacerbated by the overuse of antibiotics, an issue currently being addressed worldwide. This study aimed to investigate the association between biofilm formation, virulence gene expression, and antibiotic resistance in *P. mirabilis* isolates collected from broiler chickens (n = 50) in Qazvin, Iran, from August to December 2023.

**MATERIALS AND METHODS:** A total of 50 isolates were confirmed as *P. mirabilis* from fecal and tissue samples of 100 broiler chickens by polymerase chain reaction (PCR). The disk diffusion method was used to evaluate antibacterial susceptibility, ESBL production was determined using a double-disk synergy test following CLSI guidelines. Phenotypically confirmed ESBL- and AmpC-producers were analyzed for the presence of genes encoding blaCTX-M, blaSHV, and blaTEM, blaACC, blaCIT, blaEBC, blaFOX, blaMOX, and blaDHA genes and nine virulence genes were screened by PCR. We also assessed the biofilm formation capability of the isolates using the crystal violet staining technique.

**RESULTS AND DISCUSSION:** The results of the drug susceptibility tests revealed that 56% of the isolates were multidrug-resistant (MDR). *P. mirabilis* was found to have the highest antibiotic resistance rate against Chloramphenicol and Trimethoprim/ Sulfamethoxazole at 60%(30/50) and the lowest for Meropenem and Cefepime at 0% (0/50), and Gentamicin at 12% (6/50). The blaTEM (12%) and blaDHA genes (12%) were the most detected genes in phenotypically confirmed ESBL- and AmpC-producing isolates, respectively. All 50 *P. mirabilis* isolates harboured the ireA, ptA, and zapA, pmfA, atfA, and hpmA virulence genes. ucaA was found in 18 isolates, whereas mrpA was not detected in any isolate. Approximately, 88% of the isolates were biofilm producers, whereas 12% were non-producers. Among the virulence genes, ucaA was found to be significantly higher in biofilm producers than in non-biofilm producers *P. mirabilis*. Our research indicates that poultry farms in Iran could be a major reservoir of antibiotic resistance.

**Keywords:** *Proteus mirabilis*; Antimicrobial resistance; Virulence gene; Biofilm formation; Broiler chickens



# The effect of *Kluyveromyces marxianus* on *Aspergillus parasiticus*

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Aflatoxins, produced by *Aspergillus* species, are potent mycotoxins that pose serious health risks. *Kluyveromyces marxianus* yeast is a heat-resistant and fast-growing yeast and is often isolated from dairy products. It has antibacterial and antifungal activity and is predicted as a safe biological control against mycotoxins.

**MATERIALS AND METHODS:** In this study, the effect of *Kluyveromyces marxianus* metabolite (PTCC 5188) on *Aspergillus parasiticus* (PTCC 5286) was investigated by disc diffusion method, MIC determination by macro dilution method with 11 tubes and MFC. Also, the inhibition rate of the growth of this yeast on this mold was obtained by the two-layer culture method in the Sabouraud dextrose agar culture medium.

**RESULTS AND DISCUSSION:** The average MIC of the metabolite of *Kluyveromyces marxianus* on *Aspergillus parasiticus* was determined to be 125 µL/ml (Landa) and its average MFC was 250 µL/ml. The amounts of 115, 125 and 135 Landa metabolites of *Kluyveromyces marxianus* on *Aspergillus parasiticus* by disk method caused 10, 12 and 18 mm Inhibition Zone Diameter (IZD), respectively, and the diameter of the IZD of itraconazole drug was 22 mm. The inhibition rate of the growth of this yeast on this mold was obtained in a two-layer culture with the average diameter of the IZD of 23 mm. The ability of *K. marxianus* to inhibit *A. parasiticus* offers a promising strategy for reducing aflatoxin contamination in food and feed products. This biological control approach could be integrated with other management practices to enhance food safety and protect public health. Further research is needed to identify these compounds and understand their modes of action

**Keywords:** *Kluyveromyces marxianus*, *Aspergillus parasiticus*, Aflatoxins, Biological control, Antifungal activity

# The growth inhibitory effect of *Trichoderma* fungus on *Fusarium*

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** *Fusarium* constitutes a large number of soil microbial populations. Some of its species produce mycotoxins in grains, which can affect human and animal health. *Trichoderma* species are distributed globally and they are saprophytic have minimal nutritional requirements and are effective against a variety of microbes.

**MATERIALS AND METHODS:** In this research, the MIC effect of *Trichoderma reesei* metabolite (PTCC 5307) on *Fusarium graminearum* (PTCC 573) was investigated by macrodilution method in sabouraud dextrose broth medium with 11 tubes, MFC, and disk diffusion method in Sabouraud dextrose agar culture medium. Also, the Percentage Inhibition of Radial Growth (PIRG) of these two molds at two opposite points was kept and analyzed in a Sabouraud dextrose agar medium for one week at 37 degrees.

**RESULTS AND DISCUSSION:** The average MIC of the metabolite of *Trichoderma reesei* on *Fusarium graminearum* was 62.5 µL/ml and the average MFC was 125 µL/ml. The values of 53, 63 and 73 Landa metabolites of *Trichoderma reesei* on *Fusarium graminearum* by disc method created 11, 12 and 14 mm Inhibition Zone Diameter, respectively, and the diameter of itraconazole drug Inhibition Zone Diameter was 19 mm. The percentage of radial growth inhibition of these two molds was 18.18%. The study confirms the efficacy of *Trichoderma reesei* metabolites in inhibiting the growth of *Fusarium graminearum*, suggesting further research on the effects of various *Trichoderma* species on pathogenic and mycotoxin-producing fungi. These findings underscore the potential of *Trichoderma* as a biocontrol agent, offering a sustainable alternative to chemical fungicides.

**Keywords:** *Trichoderma*, *Fusarium*, growth inhibition, mycotoxins, biocontrol

# The Role of Genetics in Susceptibility to Bovine Spongiform Encephalopathy (BSE)

Veterinary microbiology/ Zoonotic diseases

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**BACKGROUND AND OBJECTIVES:** Bovine Spongiform Encephalopathy (BSE), commonly known as mad cow disease, is a neurodegenerative disease affecting cattle, with significant implications for animal health and the agricultural economy. Understanding the genetic factors contributing to susceptibility can aid in managing and potentially reducing the incidence of this disease. This study aims to investigate the role of genetics in the susceptibility to BSE in cattle.

**MATERIALS AND METHODS:** A cohort of 200 cattle was selected for this study, comprising 100 males and 100 females, with a mean age of 4.5 years. Blood samples were collected, and genetic analysis was conducted to identify specific genetic markers associated with increased susceptibility to BSE. The study employed Polymerase Chain Reaction (PCR) and sequencing techniques to analyze the genetic profiles of the subjects.

**RESULTS AND DISCUSSION:** The genetic analysis revealed that 58 cattle (29%) exhibited markers linked to increased susceptibility to BSE. Of these, 34 were females and 24 were males. Additionally, specific genetic mutations were identified in 42 cattle, with 28 females and 14 males showing these mutations. The presence of these genetic markers was significantly correlated with the observed susceptibility to BSE. Conclusion The study concludes that there is a significant genetic component to the susceptibility of cattle to BSE, with certain genetic markers being more prevalent in affected individuals. This understanding can inform breeding programs and disease management strategies to reduce the incidence of BSE in cattle populations.

**Keywords:** Bovine Spongiform Encephalopathy, Genetics, Susceptibility, Cattle, Neurodegenerative Disease, Genetic Markers

# Acinetobacter phage AbTJ ORF43 as putative endolysin for fighting *A. baumannii* MDR-TJ: In silico study

Phage therapy

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**BACKGROUND AND OBJECTIVES:** *Acinetobacter baumannii* is recognized as an opportunistic pathogen that poses a significant threat in the context of nosocomial infections. The clinical significance of this bacterium primarily stems from its exceptional capacity to develop resistance against a wide range of antibiotics, thereby establishing itself as one of the most formidable multidrug-resistant microorganisms, ultimately challenging the efficacy of existing antibiotic therapies (1). The 2024 World Health Organization list has made this bacterium a critical priority to find a new treatment. In this context, AbTJ, a new phage infecting *A. baumannii* MDR-TJ, was first isolated in China in 2019 (2, 3). In the recent study, we aim to use bioinformatics tools to find the protein in AbTJ phage, because knowing its function can be used as an antibiotic to treat *A. baumannii* MDR.

**MATERIALS AND METHODS:** First, phage sequences related to *A. baumannii* MDR-TJ were obtained from the Phage Scope database. Among the 62 proteins coded with this phage, the ORF (Open reading frame) of 43, which produced a secretion protein with an unknown function, was selected for further analysis. To determine the functions of this protein, along with NCBI protein BLAST, the I-TASSER and Swiss model servers, which are mainly used for protein structure prediction, were used.

**RESULTS AND DISCUSSION:** Our result showed that ORF 43 of the AbTJ phage had two binding and catalytic domains. Its binding domain could interact with bacterial peptidoglycan and is the catalytic domain belonging to TtsA-like glycoside hydrolase family. Its sequence also had about 50 percent identity with *A. baumannii* glucosyl hydrolase 108 family and *Acinetobacter* phage RL2015 endolysin. Moreover, its sequence showed 50.29 and 42% similarity with typhoid toxin secretion-associated muramidase of *Salmonella enterica* and *Salmonella Typhi*. Moreover, its predicted structure was similar to that of *Salmonella* muramidase with RMSD 0.42 and 1.74. *Salmonella* muramidase facilitates typhoid toxin transport through the peptidoglycan layer. Therefore, according to our functional analysis, ORF 43 of MDR-TJ phage could hydrolysis *Acinetobacter*, *Salmonella enterica*, and *Salmonella Typhi* peptidoglycan. Therefore, it can be said that this protein can be phage endolysin for the treatment of drug-resistant bacteria.

**Keywords:** *Acinetobacter* phage AbTJ, secretion activator protein, functional annotation, potential targets

# Antimicrobial Activity of Silver Nanoparticles Synthesized from Red Onion Peels Extract

Phage therapy

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**BACKGROUND AND OBJECTIVES:** Due to bacterial resistance to antibiotics, alternative methods to inhibit bacterial activity are urgently needed. In this study, we propose the use of silver nanoparticles (AgNPs) synthesized using red onion skin as a green synthesis method. These nanoparticles are environmentally friendly and cost-effective, and their production can complement antibiotic treatments for microbial infections. This research aims to present the properties and characteristics of silver nanoparticles synthesized from red onion skin through green synthesis.

**MATERIALS AND METHODS:** For this study, 1 g cleaned and sliced red onion peels in 100ml distilled water was heated at 90°C for 30 minutes. The extract was then cooled at room temperature, filtered, and the aqueous extract was collected in a bottle. 1 mM AgNO<sub>3</sub> was gradually added drop by drop to the onion peel extract in a 3:7 ratio. The reaction mixture was heated at 90°C for 30 minutes. A color change in the solution indicated the synthesis of AgNPs. The precipitate was filtered and then re-dispersed in 25 mL of deionized water and centrifugation to purify the AgNPs. Following this, the solution was freeze-dried for 48 hours to produce powdered AgNPs. The properties and presence of nanoparticles were verified using various analyses, including SEM, FTIR, XRD, and UV-Vis spectroscopy. Additionally, the Kirby-Bauer method was employed to determine the antibiotic activity of AgNPs (200 mg/ml) against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus*

**RESULTS AND DISCUSSION:** Results: SEM images revealed that the synthesized nanoparticles were spherical and nanosized. UV-Vis analysis, with the highest peak in the 440 to 460 nm range, and also XRD confirmed the synthesis of silver nanoparticles. This study investigated the activity of silver nanoparticles synthesized with red onion skin extract against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram-positive bacteria (*B. cereus* and *S. aureus*). Conclusion: This study successfully synthesized spherical, nanosized silver nanoparticles using red onion skin extract. The nanoparticles exhibited significant antimicrobial activity against both Gram-negative (*E. coli*, *P. aeruginosa*) and Gram-positive bacteria (*B. cereus*, *S. aureus*). These findings indicate that silver nanoparticles from red onion skin extract are promising as effective, eco-friendly antimicrobial agents

**Keywords:** Keywords: red onion skin, silver nitrate, antibacterial, green synthesis

# Antimicrobial Efficacy of Aloe Vera Extracts: Investigating Novel Applications in Infection Control

Phage therapy

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**BACKGROUND AND OBJECTIVES:** Aloe Vera, a succulent plant known for its therapeutic properties, has gained significant attention in recent years for its potential antimicrobial activity. The antimicrobial properties of Aloe Vera have been attributed to its rich phytochemical composition, including polysaccharides, phenolic compounds, and saponins. These bioactive compounds have shown promising results in inhibiting the growth of various pathogenic microorganisms, including bacteria, fungi, and viruses. This paper aims to evaluate the antimicrobial activity of Aloe Vera and highlight its potential applications in infection control.

**MATERIALS AND METHODS:** Fresh Aloe Vera leaves were collected and washed thoroughly. The outer skin was removed, and the gel was extracted using a sterile gel was homogenized and filtered to obtain a clear extract. Streptococcus strain (specify strain details) was cultured in nutrient broth overnight at 37°C. The culture was adjusted to a specific optical density (OD) at 600 nm to standardize the inoculum. Sterile filter paper discs were impregnated with different concentrations of Aloe Vera extract (e.g., 25%, 50%, 75%). The discs were placed on Mueller-Hinton agar plates inoculated with the standardized Streptococcus culture. Plates were incubated at 37°C for 24 hours, and the diameter of the inhibition zones was measured. To evaluate Minimum Inhibitory Concentration (MIC), A series of dilutions of Aloe Vera extract were prepared in nutrient broth. Each dilution was inoculated with the standardized Streptococcus culture. The tubes were incubated at 37°C for 24 hours, and the lowest concentration inhibiting

**RESULTS AND DISCUSSION:** Aloe Vera extract exhibited a concentration-dependent antimicrobial effect against Streptococcus, with clear inhibition zones observed around the discs. The largest inhibition zone was observed at the highest concentration of Aloe Vera extract (75%). The MIC of Aloe Vera extract against Streptococcus was determined to be 30%, indicating the lowest concentration at which visible growth inhibition occurred. A significant decrease in bacterial counts was observed within the first 2 hours of exposure, reaching a complete eradication by 24 hours. These important results highlight the potent antimicrobial effect of Aloe Vera extract on Streptococcus, suggesting its potential as a natural alternative for combating bacterial infections.

**Keywords:** Antimicrobial Activity, Aloe vera, Gel, Streptococcus

# Bioinformatics approaches for designing a novel multi-epitopes vaccine against Crimean-Congo hemorrhagic fever virus

Phage therapy

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**BACKGROUND AND OBJECTIVES:** In this study, we implemented a set series of bioinformatics approaches to design an efficient multi-epitopes vaccine against Crimean-Congo hemorrhagic fever virus (CCHFV).

**MATERIALS AND METHODS:** The various immunodominant epitopes from Envelopment polyprotein, and RNA-directed RNA polymerase L proteins of the Nigeria strain of CCHFV were selected. These peptides promote cellular and humoral immune responses. Conservation, antigenicity, and allergenicity of them was assayed in all CCHFV strains. To improve the immunogenicity of the vaccine, we used two powerful adjuvants. The characteristics of the vaccine were analyzed. The two and tertiary structure of the vaccine were predicted, refined, and validated. Interaction of it with toll-like receptors (TLRs) 2, 3, and 8 was evaluated. Stability of the secondary structure of mRNA and in silico cloning were analyzed. ability of vaccine for stimulation of immune system after injection in human body was evaluated by in silico immune simulation.

**RESULTS AND DISCUSSION:** The designed vaccine has good physicochemical properties, antigenicity, allergenicity, and high-quality structure. Docking and molecular dynamic simulation with TLRs 2, 3, and 8 proved a stable interaction between the vaccine and these pattern recognition receptors. Gene expression assay showed the vaccine is prone for expression in the Escherichia coli. Furthermore, in silico immune simulation showed the vaccine may increase effective adaptive immunity. Over all, the multi-epitope vaccines could generate humoral and cell-mediated immune responses against CCHFV. It has a valid structure and appropriate properties including stability and high expression capacity in the prokaryotic system.

**Keywords:** Crimean-Congo hemorrhagic fever virus, multi-epitope vaccine, humoral immunity, cellular immunity,

# Co-delivery of streptomycin and hydroxychloroquine by labeled solid lipid nanoparticles to treat brucellosis: an animal study

Phage therapy

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**BACKGROUND AND OBJECTIVES:** Can brucellosis-related biochemical and immunological parameters be used as diagnostic and treatment indicators? The goal of this project was to look at biochemical parameters, trace elements, and inflammatory factors in the acute and chronic stages of brucellosis after treatment with streptomycin and hydroxychloroquine-loaded solid lipid nanoparticles (STR-HCQ-SLN).

**MATERIALS AND METHODS:** The double emulsion method was used for the synthesis of nanoparticles. Serum levels of trace elements, vitamin D, CRP, and biochemical parameters were measured in rats involved in brucellosis. The therapeutic effect of STR-HCQ-SLN was compared with that of free drugs.

**RESULTS AND DISCUSSION:** In both healthy and infected rats, serum concentrations of copper, zinc, iron, magnesium, potassium, and biochemical parameters of the liver were significantly different. By altering the serum levels of the aforementioned factors, treatment with STR-HCQ-SLN had a positive therapeutic effect on chronic brucellosis. Vitamin D levels declined (46.4%) and CRP levels rose (from 7.5 mg to less than 1 mg) throughout the acute and chronic stages of brucellosis. This study showed that by comparing the biochemical parameters and the levels of trace elements in the serum of healthy and diseased mice in the acute and chronic stages of brucellosis, it is possible to get help from other routine methods for diagnosis.

**Keywords:** streptomycin, hydroxychloroquine, solid lipid nanoparticles, brucellosis



# Designing a Phage-loaded Hydrogel for the Healing of Wounds Infected with *Enterococcus faecalis*

Phage therapy

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**BACKGROUND AND OBJECTIVES:** Background: Chronic wounds caused by *Enterococcus faecalis* represent a major health concern, and their management is particularly challenging due to the emergence of antibiotic-resistant bacteria. Bacteriophages have thus gained immense prominence as one of the upcoming alternatives for treating antibiotic-resistant bacteria. Bacteriophage-Delivering Hydrogels have been reported as a strategy to locally deliver phages for treating wound infections. Objective: The main purpose of this study was to create a phage-containing wound dressing for the management of *E. faecalis* infected wounds.

**MATERIALS AND METHODS:** The hydrogel was prepared from sodium alginate (SA), carboxymethyl cellulose (CMC), and hyaluronic acid (HA) (2). The isolated phage (SAM-E.f 12) was incorporated into the SA-CMC-HA hydrogel. The swelling index and water absorption rate of the hydrogel was measured after 24 hours and the degradation was investigated over seven days. The surface morphology and composition of hydrogel were analyzed using Scanning Electron Microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR). The antibacterial activity of SAM-E.f 12-based phage-loaded hydrogel was investigated using disk diffusion method. Wound healing and antibacterial properties of hydrogel were evaluated using a mouse model (3,4).

**RESULTS AND DISCUSSION:** The hydrogel showed a swelling index of 0.43, water absorption rate of 30 in 7 days and a degradation of 23 % over seven days. The hydrogel remained stable for four weeks. The phage-loaded hydrogel significantly inhibited bacterial growth (optical density 0.3 compared to 1.1 for the controls). Histological analysis showed that the structure of the skin layers in the group treated with phage loaded Hydrogel was restored. Compared with the blank dressing and control, the repaired tissue in the phage dressing group had new capillary vessels and no sign of inflammation in its dermis, and its epidermis had a higher degree of re-epithelialization (p .05). The slow-released phage has demonstrated positive effects in repairing infected wounds. These findings highlight the potential of a SAM-E.f 12-based phage-loaded hydrogel as a straightforward and effective formulation for addressing *E. faecalis*

**Keywords:** Keywords: *Enterococcus faecalis*, Phage therapy; Infected wounds; Hydrogel; Phage SAM-E. f

# Effect of *Pseudomonas aeruginosa* specific bacteriophage PB10 on *lasI* gene expression

Phage therapy

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**BACKGROUND AND OBJECTIVES:** A common opportunistic pathogen that plays a major role in nosocomial, acute, and chronic infections is the Gram-negative bacillus *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* can bind to various surfaces and form biofilms leading to chronic infections by increasing resistance to antibiotics, disinfectants, various irradiation treatments, environmental conditions, and the immune system. The communication between cells known as quorum sensing is in charge of the expression of several virulence genes, including those for pyocyanin, proteases, poisons, and biofilm. Quorum quenchers, which are various substances that obstruct bacterial cell-to-cell communication, reduce the expression of virulence genes that are in charge of proteases, toxins, siderophores, swarming, and biofilm formation. This study aims to investigate the effect of *Pseudomonas aeruginosa* bacteriophage on *lasI* gene expression.

**MATERIALS AND METHODS:** RT-PCR method was used to investigate the effect of bacteriophage PB10 on *LasI* gene expression in *P. aeruginosa* bacteria. First, RNA of the desired bacteria was extracted. For this purpose, 6 well plates were used. Each well was incubated with 5 ml of TSB medium cultured with bacteria with a concentration of 10<sup>6</sup> CFU/ml and 5×10<sup>5</sup> PFU/ml of bacteriophage at 37°C. At intervals of 2, 4, 8, 24 and 48 hours, the contents of the wells were transferred into the microtube and microfuged. Then the supernatant of the microtubules was drained and the sediment was used for RNA extraction. cDNA synthesis was performed using Pars Tos kit and protocol. The microtubes were then subjected to real-time PCR.

**RESULTS AND DISCUSSION:** The results of the present study were consistent with the results obtained from study of Oliveira et al., with the difference being that as the incubation time increased, the *lasI* gene expression decreased. In the group treated with bacteriophage PB10, *lasI* gene expression was significantly reduced in 24 hours and 48 hours compared to the control group (P 0.01).

**Keywords:** *Pseudomonas aeruginosa*, Bacteriophage PB10, *LasI*, biofilm, Quorum sensing

# Engineering a Staphylococcus aureus-Specific Endolysin Fusion Protein for Targeting Methicillin-Resistant *S. aureus* (MRSA)

Phage therapy

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**BACKGROUND AND OBJECTIVES:** The misuse of antibiotics has resulted in a concerning rise in antibiotic resistance. As a result, there is an urgent need to discover an effective alternative to traditional antibiotics. Endolysins, enzymes produced during the phage replication cycle, can destroy the peptidoglycan of bacterial cell walls, leading to the lysis of their host bacterial cells. Lysozyme subfamily 2 (LYZ2) is a modular region of the gene 61 (gp61) of phage  $\phi$ MR11 that possesses lytic activity against *S. aureus*. However, it lacks a cell wall recognition domain, typically found in lysins that target gram-positive bacteria.

**MATERIALS AND METHODS:** In this study, we designed and produced a recombinant Anti-Staphylococcus Endolysin (ASEnd) by fusing the appropriate Cell wall-binding Domain to the C-terminus of LYZ2 through a flexible linker (G4S)<sub>3</sub>. The recombinant chimeric enzyme was over-expressed in *E. coli* BL21(DE3) cells and purified under native conditions using Immobilized Metal affinity chromatography method. The antibacterial properties of the produced enzymes were assessed by disk diffusion, turbidity reduction, and MTT assays.

**RESULTS AND DISCUSSION:** ASEnd in concentrations higher than 0.5 mg/ml inhibited the growth of MRSA. Moreover, the inhibitory effect of ASEnd increased by 16 folds compared to LYZ2 alone, indicating an increase in its lytic activity. Additionally, the ASEnd eliminates MRSA but not *E. coli* cells. However, the enzyme showed mild anti-growth activity against *S. pyogenes*, indicating the fairly low specificity of the enzymes for gram-positive bacteria. ASEnd was non-cytotoxic to HEK293 cells and retained its stability for one week at 4°C, and in a pH range of 6.0 to 8.0. Our findings showed that the ASEnd can effectively inhibit the growth of MRSA and *S. aureus* cells. Moreover, the stability of the enzyme to low pH conditions may be enhanced by protein engineering to empower the enzyme to kill the intracellular *S. aureus* bacteria. Ultimately, the findings give hope that the ASEnd can be used to limit nosocomial infections caused by MRSA.

**Keywords:** antibiotic resistance, methicillin-resistant staphylococcus aureus, endolysin, protein engineering, Lysozyme subfamily

# Evaluating Recombinant Endolysins for Antibacterial Therapies Against *Pseudomonas aeruginosa*

## Phage therapy

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa*, a prevalent microorganism notorious for its role in healthcare-associated infections, has become increasingly problematic due to the rise of multidrug-resistant strains, a consequence of excessive antibiotic use. To combat this growing threat, researchers are delving into the potential of endolysins – enzymes originating from bacteriophages – as a means to target and dismantle the peptidoglycan layer encasing bacterial cell walls. This avenue of research holds promise for the development of innovative antibacterial treatments that could help address the challenges posed by antibiotic resistance.

**MATERIALS AND METHODS:** In the pursuit of identifying homologous sequences to LysPa, a *P. aeruginosa* phage endolysin, a BlastP search was conducted. The sequence exhibiting the highest identity to the query, LysPa, within the Protein Data Bank (PDB) was selected as the foundation for modeling through the Swiss-Model platform. The models generated for the original query and its various recombinant forms underwent validation using tools like SAVESv6.0, MolProbity, and ProSA servers to ensure their accuracy and reliability.

**RESULTS AND DISCUSSION:** Through the BlastP analysis against the extensive Protein Data Bank (PDB) database, 9 sequences sharing more than 30% identity with the query were identified. The endolysin boasting the highest sequence identity (58.99%) was chosen as the template for homology modeling. The subsequent evaluation of the predicted LysPa model demonstrated that 94.2% of its residues resided in preferred regions, as confirmed by assessments from PROCHECK, ERRAT, VERIFY3D, MolProbity, and ProSA Z-score, all indicative of a high-quality model. In conclusion, the study's primary focus revolved around exploring the efficacy of combining LysPa endolysin with an outer membrane-penetrating peptide for combating the challenges posed by multidrug-resistant infections attributed to *P. aeruginosa*. The homology modeling results not only point towards the considerable potential of harnessing these enzymes as potent antibacterial agents but also emphasize the need for further research and development in this direction to bolster their therapeutic applicability in real-world scenarios.

**Keywords:** Phage Endolysin, Homology modeling, *P. aeruginosa*

# Evaluation of anti-parasitic activities of new quinolones containing nitrofuranyl moiety against *Toxoplasma gondii*

## Phage therapy

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**BACKGROUND AND OBJECTIVES:** Toxoplasmosis, a globally prevalent disease caused by *Toxoplasma gondii*, is commonly treated with pyrimethamine and sulfadiazine. However, these drugs are associated with side effects, necessitating the development of new, less toxic alternatives. Quinolones, known for their DNA replication inhibitory properties against various pathogens, show promise in this regard. This in vitro study aims to evaluate the antiparasitic activities of novel quinolone derivatives (NFQ-2, NFQ-5, and NFQ-6) containing nitrofuranyl moiety against *T. gondii*, potentially offering new leads for anti-*Toxoplasma* drug development

**MATERIALS AND METHODS:** Vero cells were incubated with various concentrations of new quinolones and pyrimethamine (positive control) to determine their viability. Subsequently, they were infected with *T. gondii* (RH strain) and then subjected to drug treatment.

**RESULTS AND DISCUSSION:** The obtained IC<sub>50</sub> values were 3.60, 4.84, 5.59, 3.44 and 2.75 µg/mL for NFQ-2, NFQ-5, NFQ-6, ciprofloxacin and pyrimethamine, respectively. The CC<sub>50</sub> values for the NFQ-2, NFQ-5, and NFQ-6 were 25.20, 29.89, and 28.43 µg/ mL, indicating the selectivity indexes more than 5 for these compounds. The anti-*Toxoplasma* efficiency was determined by evaluating infection index, number and size of plaques, and *T. gondii* intracellular proliferation. As the results indicated, the administration of new quinolone derivatives resulted in the reduction of intracellular proliferation, infection index, and the number and size of plaques in comparison to uninfected treated cells (P 0.05). The results were indicative of a considerable synergetic effect when each of the derivatives was used in combination with pyrimethamine, compared to when used alone. Based on our results, the nitrofuranyl-derived quinolones can be considered as new leads for the design of new anti-*Toxoplasma* agents.

**Keywords:** Toxoplasmosis Treatment Quinolones 5-Nitrofuranyl Anti-parasitic activity

# Evaluation of in vitro effects of Anethol against *Candida albicans*/*Staphylococcus aureus* Dual-Species Biofilms.

Phage therapy

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**BACKGROUND AND OBJECTIVES:** Polymicrobial (fungal-bacterial) infections are the most complex and challenging clinical problems to treat, researchers are now looking for effective treatments for such infections. Studies have shown that polymicrobial infections often occur as biofilms. Therefore, finding antibiotics with antimicrobial properties that can enhance the synergistic effect of these compounds with common standard antibiotics can be effective in reducing the dosage and subsequent side effects caused by chemical drugs and counteracting or reducing the resistance created. The aim of this study was to investigate the effect of anethol on homogeneous and heterogeneous biofilms of *Candida albicans* and *Staphylococcus aureus*.

**MATERIALS AND METHODS:** Antifungal activity of anethol against the planktonic phase of the *Candida* and *Staphylococcus* isolates was evaluated based on standard M27-A3 and M7-A8 standard CLSI methods, respectively, and the MIC, MBC and MFC values were determined. MTT Reduction assay and Crystal Violet Staining methods were used to investigate the biofilm development ability of these isolates as well as inhibitory effect of anethol on them after the formation of biofilms in 96 well cell culture plates. Inhibitory effect of anethol against heterogeneous biofilms was also evaluated using MTT Reduction Assay and scanning electron microscopy.

**RESULTS AND DISCUSSION:** The average of anethol MIC for all *Candida albicans* and *Staphylococcus aureus* isolates evaluated as 1035.9 and 612.3  $\mu\text{g} / \text{ml}$ , respectively. Meanwhile, the average of anethol MFC against non-biofilm / Planktonic cells of *Candida albicans* and *Staphylococcus aureus* cells was calculated as 1597.1 and 994.8.  $\mu\text{g}/\text{ml}$ , respectively. All of the *Candida albicans* and *Staphylococcus aureus* isolates were able to develop In vitro biofilms. Meanwhile, 70% of the *Staphylococcus aureus* isolates and 20% of the *Candida albicans* isolates formed a dense and strong biofilm mass. The sessile Minimum Inhibitory Concentration (SMICs) of anethol was 2478.5  $\mu\text{g}/\text{ml}$  for *Candida albicans* isolates and this value was calculated for *Staphylococcus aureus* isolates equivalent to 168.9 $\mu\text{g}/\text{ml}$ . It is worth noting that the highest SMICs calculated for *Candida albicans* and *Staphylococcus aureus* was related to fluconazole and methicillin-resistant isolates. Scanning electron microscopy data of treated *Candida albicans* / *Staphylococcus aureus* biofilms with anethol indicate that

**Keywords:** Heterogenic Biofilm, *Staphylococcus aureus*, *Candida albicans*, Anethol, Scanning Electron Microscope

# Investigating the effect of the hydroalcoholic extract of the stem bark of *Rhamnus cathartica* on the tachyzoite stage of *Toxoplasma gondii*

Phage therapy

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**BACKGROUND AND OBJECTIVES:** Toxoplasmosis is a parasitic infectious disease caused by a single-celled eukaryotic parasite of Sprozoa called *Toxoplasma gondii*. The infection of this parasite causes a wide broad of manifestations from mild flu-like symptoms, muscle pains, swelling in the lymph nodes in acquired form and chorioretinitis, microcephaly and hydrocephaly in congenital forms. Primethamine and sulfadiazine are the first step treatment for toxoplasmosis that these drugs are accompanied by different side effects. So, search to find a suitable anti-toxoplasmic drug with low toxicity and even possible natural products is a priority.

**MATERIALS AND METHODS:** The RH strain of *T. gondii* is intraperitonealy injected to of the Balb/c mice and after 3 to 5 days, the parasites were extracted from mice and washed three times and  $5 \times 10^5$  parasites added to the 96 well plate. The effect of *Rhamnus cathartica* plant extract in concentrations of 100, 200, 400, 800 and 1000  $\mu\text{g/ml}$  at 3, 6, 12, 24 and 48 hours on *T. gondii* parasite tachyzoite was investigated.

**RESULTS AND DISCUSSION:** The results indicated that the effectiveness of the *R. cathartica* increases with increasing drug concentration and time. The best effectiveness of the *R. cathartica* was at a concentration of 1000  $\mu\text{g/ml}$  after 24 hours. Considering the lethality of *R. cathartica* plant extract on *T. gondii* parasite and its better effect than positive controls, it can be concluded that *R. cathartica* drug can be an acceptable candidate for treatment of toxoplasmosis after supplementary studies.

**Keywords:** *Toxoplasma gondii*, anti-toxoplasmosis, *Rhamnus cathartica*, tachyzoite

# Investigating the persistence of bacteriophage in blood in the rat model

## Phage therapy

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**BACKGROUND AND OBJECTIVES:** An important nosocomial infectious bacterium that is increasingly multidrug resistant is *Pseudomonas aeruginosa*. After the discovery of *P. aeruginosa*, it created serious obstacles to the therapeutic use of antibiotics and increased morbidity, mortality and financial burden. Bacteriophages are a good alternative to antibiotics because they have the ability to selectively lyse bacteria. Phages eradicate bacteria and sometimes exhibit beneficial immunomodulatory effects. Bacteriophages can show different levels of persistence in blood. This study was conducted with the aim of investigating the persistence of *Pseudomonas aeruginosa* bacteriophage in blood without causing infection in a rat model.

**MATERIALS AND METHODS:** Four healthy female rats weighing 250±50 grams were obtained from Urmia Animal Center. The animals were acclimated to the new laboratory environment for seven days before the experiment. Rats were housed in sterile cages with sterile water and maintained under conditions specifically designed to prevent the spread of pathogens. To start the work, anesthesia was performed using ketamine 60 mg/kg, xylazine (pre-anesthesia) 5 mg/kg. Using angiocath, 0.2 ml of bacteriophage PB10 with a concentration of 10<sup>8</sup> PFU/ml was injected into the tail area of the rat. After 10 minutes, 1, 3, 24 and 48 hours, blood samples were taken from the eyes of rat and to confirm the presence of phage, double-layer agar culture was performed according to the standard method.

**RESULTS AND DISCUSSION:** The results showed that until 24 hours later, the population of bacteriophages in the cultured blood sample did not change significantly. During this period, the blood bacteriophage population did not show much change compared to the blood taken containing bacteriophages in the early hours, but after 24 hours of bacteriophage injection, the results of double-layer culture showed that nearly half of the initial bacteriophage population decreased. The results showed that the population of bacteriophages in blood changes over time.

**Keywords:** *Pseudomonas aeruginosa* bacteriophages, Rat, Blood



# Isolation and identification of bacteriophage effective against *Klebsiella pneumoniae* isolated from patients

## Phage therapy

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**BACKGROUND AND OBJECTIVES:** *Klebsiella pneumoniae* has high virulence, the ability to form biofilms, and strong resistance to many antibiotics, especially hospital-acquired strains. This causes the use of effective antibiotics to treat patients with *K. pneumoniae* infection to be limited. Therefore, alternative treatments are urgently needed. The aim of this study is to evaluate the effect of phage therapy in order to provide a therapeutic method for the treatment of infections caused by multi-drug resistant *K. pneumoniae*.

**MATERIALS AND METHODS:** In this cross-sectional study, 50 clinical strains of *K. pneumoniae* were collected from Zanjan hospitals and phenotypic and genotypic confirmation. MDR strains were identified by disk diffusion method. Primary bacteriophage isolation and plaque collection were done from sewage treatment plant. Then bacteriophage titer and phage specificity were done by host determination method. The sensitivity of phage on *K. pneumoniae* isolates was similar to the method of determining the host and was performed by the Spot test method. Phage identification was done by TEM electron microscope.

**RESULTS AND DISCUSSION:** The isolated phage was from the myoviridae family and 32 of 50 strains of *K. pneumoniae* were sensitive to it. The phage titer was maintained after 1 hour of incubation at 30, 35 and 40°C and was stable at different pH. The isolated lytic bacteriophage formed small transparent plaques with a latent period of 50 min and a burst time of 55 min, corresponding to 30 phage particles per infected cell. The results of this study indicate that phage therapy is a useful treatment method for treating infections caused by *K. pneumoniae*.

**Keywords:** *Klebsiella pneumoniae*, bacteriophage, myoviridae, MDR, TEM

# Long-Term Refrigeration Effects on Myoviridae Phage Therapeutic Potential

Phage therapy

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**BACKGROUND AND OBJECTIVES:** The increasing prevalence of antibiotic-resistant bacteria, particularly gram-negative strains, has highlighted the need for alternative treatments such as phage therapy. To effectively utilize phages as antibacterial agents, it's crucial to develop reliable methods for preserving their potency. Phage infectivity, a key characteristic for clinical applications, is significantly influenced by storage conditions, primarily temperature. While studies have shown that sub-zero temperatures are optimal for phage preservation, this research aims to evaluate the stability of three *Pseudomonas aeruginosa* phages belonging to the Myoviridae family when stored at refrigerator temperature (4°C) and regularly refreshed over more than a year.

**MATERIALS AND METHODS:** To monitor the concentration and efficacy of the stored Myoviridae phages (PA45, PA32, PA6) against *Pseudomonas aeruginosa*, phage stocks were prepared and enriched approximately every 2 months. A double-layer agar method was used to determine phage titer by counting plaque-forming units (PFU). Following phage enumeration, the phage suspension was purified by centrifugation and filtration before being stored at 4°C. This process was repeated for more than 12 months, and the final phage concentrations were compared to their initial levels.

**RESULTS AND DISCUSSION:** The results don't demonstrate a significant decline in phage titer (pfu/ml) for all three Myoviridae phages over more than 12-month storage period at 4°C. While phage PA32 (as a jumbo phage) and PA45 exhibited more stability compared to phage PA6, the overall trend indicated a partial loss of infectivity. These findings align with some previous reports suggesting that refrigeration is optimal for long-term phage preservation, but two-month enrichment of phages should not be forgotten.

**Keywords:** Myoviridae, Phage Storage, Temperature, *Pseudomonas aeruginosa*

# Relationship between Adherence Genes and Sensitivity to the Lytic Bacteriophage of *Staphylococcus saprophyticus*

Phage therapy

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**BACKGROUND AND OBJECTIVES:** *Staphylococcus saprophyticus* has been identified as the second leading cause of uncomplicated urinary tract infections (UTIs) in young females. In 1961, Torres Pereira isolated a coagulase-negative *Staphylococcus* carrying 51 antigens from the urine of women with acute UTI. The organism was later classified as part of subgroup 3 of *Micrococcus*, which was subsequently identified as *S. saprophyticus*. The antibacterial activities of phages against staphylococcal species and their receptors, such as *Staphylococcus aureus*, have been extensively demonstrated. The present study aimed to investigate adherence genes and sensitivity to the lytic bacteriophage of *S. saprophyticus* in Golestan Province.

**MATERIALS AND METHODS:** *S. saprophyticus* isolates from UTI patients were collected from laboratories. The phage was isolated from urban wastewater using the spot test and Double-Layer Agar method. The association between the presence of adhesion genes in bacterial isolates and phage sensitivity was investigated using the polymerase chain reaction (PCR) method.

**RESULTS AND DISCUSSION:** The phage formed round and clear plaques on bacterial culture, and had a large head (approximately 106 nm) and a long tail (approximately 150 nm), indicating that it belongs to the Siphoviridae family. It was able to lyse 12 of the 35 clinical isolates (34%). The most common virulence gene detected in these isolates was Aas, which is a multifunctional protein with adhesive properties that binds to fibronectin. The next most frequent virulence gene was UafA, which mediates adherence to human bladder epithelial cells. Finally, the relationship between phage sensitivity and adherence genes was assessed, revealing no significant correlation between phage sensitivity and the frequency of adherence genes.

**Keywords:** Keywords: *Staphylococcus saprophyticus*, Bacteriophage, Siphoviridae, Wastewater

# Terminalia chebula as a novel, effective and safe medicinal plant against Toxoplasma gondii

## Phage therapy

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**BACKGROUND AND OBJECTIVES:** Toxoplasmosis is a disease caused by *Toxoplasma gondii* parasite. Considering the increase in the prevalence of this disease and the increasing reports of resistance to the common anti-toxoplasmic drugs and the possible teratogenicity of these drugs, it is necessary to study and investigate the possibility of finding an alternative medicine, even if it is herbal, for the treatment of toxoplasmosis. The aim of the present study is to investigate the anti-toxoplasmic effects of the hydroalcoholic extract of the *Terminalia chebula* plant on the *Toxoplasma gondii* parasite in vitro.

**MATERIALS AND METHODS:** Rh strain *Toxoplasma gondii* parasite is obtained from the toxoplasmosis research center, Faculty of Public Health, Tehran University of Medical Sciences and inoculated into the peritoneum of balb/c mice until the number of parasites reaches 500000 parasites/ml. The hydroalcoholic extract of the *Terminalia chebula* was prepared in serial concentrations of 100, 200, 400 and 800 µg/ml. The effect of different concentrations at 60, 120 and 180 min on the parasite in 24-well plates containing RPMI-1640 was investigated.

**RESULTS AND DISCUSSION:** The hydroalcoholic extract of *Terminalia chebula* in all concentrations had acceptable anti-parasitic effects against *Toxoplasma gondii* parasite in comparison to the negative and positive groups. At a concentration of 800 micrograms/ml of the hydroalcoholic extract of *Terminalia chebula* after 60 minutes, better effects than the positive control have been reported. According to the killing power of the hydroalcoholic extract of the *Terminalia chebula* on the *Toxoplasma gondii* parasite and its better effect than the positive controls, it can be concluded that the above compounds can be suitable candidates for treatment after further studies.

**Keywords:** *Toxoplasma gondii*; anti-toxoplasmosis; hydroalcoholic extract; *Terminalia chebula*

# The effectiveness of mixed natural products-ointment on the Cutaneous leishmaniasis caused by *Leishmania major*, In vivo

## Phage therapy

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**BACKGROUND AND OBJECTIVES:** Cutaneous leishmaniasis (CL) is a widespread protozoan parasitic disease in which glucantime and other pentostams are the main treatments. Today, there are many reports about the side effects of these drugs and resistance to them. Natural products are now a first step in treatment for many infections, like artemisinin for malaria. This study evaluates a novel topical ointment containing extracts from some medicinal plants and natural products against *Leishmania major* in an animal model, aiming to develop a more effective and accessible treatment for CL.

**MATERIALS AND METHODS:** *Leishmania major* promastigotes (MRHO/IR/75/ER strain) were cultured and injected subcutaneously into the tail bases of female BALB/c mice. After lesions developed, mice were divided into three groups (n = 6 mice per group): a test group treated with a topical herbal ointment formulation containing *Rhamnus cathartica*, *Eryngium campestre* methanolic extracts emodin, crocin, and quercetin, glucantime, and amphotericin B (as a positive control), and PBS (as the negative control group). Treatments were applied topically twice a day for 28 consecutive days. Lesion sizes were measured weekly using digital callipers. Parasite burden in lesions was assessed by microscopic examination of Giemsa-stained smears at the end of treatment.

**RESULTS AND DISCUSSION:** The results of the current study revealed that the composition had acceptable efficacy against the lesions caused by *L. major*. Our compositions significantly dose- and time-dependently decrease the size of lesions and reduce the parasite burden of *L. major* in infected mice; the variations were significantly better than positive controls (p0.001). Within 28 days, the ointment led to the complete healing of wounds caused by CL. The number of amastigotes in lesions was significantly reduced in mice treated with the herbal formulation (p0.001). The new formulation showed very appropriate therapeutic effects with no observed adverse reactions

**Keywords:** Cutaneous leishmaniasis, *Leishmania major*, Natural products, In vivo

# The Legacy of George Eliava in Phage Therapy and Its Modern Applications Against Antibiotic-Resistant Infections

Phage therapy

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**BACKGROUND AND OBJECTIVES:** Any class of virus that infects bacteria is known as a bacteriophage. Studies on bacteriophage started in 1915 with British bacteriologists. Frederick William Twort mentioned “glassy transformation” but couldn’t describe his observation exactly. By 1917, independently in other parts of the world, Felix d’Herelle observed “clear circles” in cultures of a coccobacillus. His note was entitled “On an invisible microbe antagonistic to dysentery bacilli,” and this was the beginning of the anti-bacterial treatment by bacteriophage. Professor George Eliava a Georgian physician and bacteriologist who played a major role in promoting therapeutic uses of bacteriophage by supporting the felix, In the Union of Soviet Socialist Republics and other countries. Phage therapy was used by George on *Vibrio cholera*. After he heard about phage therapy, he did the examination. He left a culture on his desk for a while, and after that, the bacteria just vanished on their own. This was

**MATERIALS AND METHODS:** This Essay discusses fundamental phage biology, provides an explanation of the long history of phage therapy, shows the advantages of applying phages as antibacterial agents, and provides a summary of recent phage therapy clinical results. The following databases were used to perform a systematic review of the literature in order to accomplish this from PubMed, Google Scholar, and Springer Link, while I used Specific search terms included “phage therapy,” “bacteriophage biology,” “history of phage therapy,” “phage therapy clinical trials,” “George Eliava” and “advantages of phage therapy”.

**RESULTS AND DISCUSSION:** Like with other treatment methods, phage therapy also has some pros and cons. Bacteriophage therapeutic applications in the modern approach are at the center of attention these days. Despite conventional phage therapy, there are several more therapeutic options for treating infections, like modified phage, dual therapy, and phage-derived proteins. As long as one of the main issues facing the medical field and a global health concern is antimicrobial resistance, due to a lack of knowledge among people about this group of drugs, phage therapy do appear to have a bright future as pharmacologic interventions for the treatment of antibiotic-resistant due to bacterial infections.

**Keywords:** bacteriophage, Phage Therapy, Antibiotic-Resistant, George Eliava, history of phage

# Comparative Synergistic Interactions between Thymol/Ceftazidime and Thymol/Cefotaxime against *Staphylococcus epidermidis*

Antimicrobial peptides

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**BACKGROUND AND OBJECTIVES:** This study aimed to compare the antimicrobial effects of thymol/cefotaxime and thymol/ceftazidime on *S. epidermidis* bacteria.

**MATERIALS AND METHODS:** Antimicrobial effects of thymol/cefotaxime and thymol/ceftazidime were performed first individually and then combined on *S. epidermidis* ATCC1457 by the MIC-MBC method. Therefore, the antimicrobial effects of the compounds that had a synergistic impact were performed on eighteen clinical strains using the MIC-MBC method. Identification of chemical bonds, functional groups, and molecular interactions of the mentioned compounds were investigated with an FTIR device. Checkered method, time killing curve, and biofilm inhibition on *S. epidermidis* ATCC1457, investigation of cytotoxicity on red blood cells (RBCs) by hemolysis method and human skin fibroblast cells (Ffk) by MTT method were performed. Thymol/cefotaxime and thymol/ceftazidime had synergistic effects. Finally, the results of the tests were compared between the two compounds.

**RESULTS AND DISCUSSION:** The results of this study showed that the antimicrobial effects of the thymol/cefotaxime (1/1 µg/ml) were better than the thymol/ceftazidime (16/4 µg/ml) in both clinical and ATCC strains. In the examination with the FTIR device, both compounds had bonds of OH carbohydrates proteins, polyphenols, C=O Amide I band, C-O-Cpolysaccharide, C-Namide III band, but one band named C=C conjugated, C≡C in both compounds showed the connection between thymol with cefotaxime and ceftazidime. The biofilm inhibition effects of thymol/cefotaxime (61.81%) were better than thymol/ceftazidime (49.09%) on *S. epidermidis* ATCC1457. The bacterial killing time curve of the thymol/cefotaxime at a lower concentration and time was better than the thymol/ceftazidime. Cytotoxicity of synergistic compounds on RBCs and human Ffk cells was not different and was lower than that of Triton X-100.

**Keywords:** *Staphylococcus epidermidis*, Thymol, Cefotaxime, Ceftazidime, Synergistic

# Effects of specific antibodies against *Mycobacterium paratuberculosis* infection

Antimicrobial peptides

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**BACKGROUND AND OBJECTIVES:** IgY antibodies derived from chicken egg yolks offer a non-invasive method for producing a huge amount of antibodies. Due to their ability to bind antigen with high affinity, specificity, and limited cross-reactivity, IgY antibodies could be used in preventing infections such as those caused by *Mycobacterium avium* subspecies paratuberculosis (MAP), the pathogen responsible for Johne's disease in animals and possibly linked to Crohn's disease in humans. This study aimed to evaluate the efficacy of IgY antibodies in preventing mice from MAP infection.

**MATERIALS AND METHODS:** Specific IgY antibodies were produced by immunizing hens with formalin-killed MAP strains III & V. The IgY was extracted from yolks using PEG 6000, identified by SDS-PAGE, and its activity measured by ELISA. The protein concentration of purified IgY was determined using the Kjeldahl method. The MIC of IgY measured using broth susceptibility test. In vivo studies were conducted on female Balb/c mice (23-28 days old) over twelve weeks. The mice were divided into three groups consist of positive control (challenged with MAP cfu/ml 109), Negative control (not challenged with MAP and did not receive the treatments) & treatment group (received IgY antibody orally four weeks prior to challenge). Pathology studies were conducted at weeks 1, 3, 6, and 12 after challenge to measure tissue damages.

**RESULTS AND DISCUSSION:** ELISA results showed specific activity of purified IgY against MAP, and growth inhibition assays indicated MIC of 50 mg/ml. PCR with specific primers performed on feces after mice oral challenge with MAP, confirmed MAP localization in the mice intestines. PCR results were positive for both the treatment and positive control groups on the first and second day, but while positivity persisted in the positive control group up to the second week, results turned negative in the IgY-receiving group after the second day. Pathological findings of the targeted organs (intestines, mesenteric nodes, liver, and spleen) including necrosis, vacuolar degeneration, surface and crypt degeneration of intestine epithelial, submucosal edema, and lymphoid gland depletion revealed significantly fewer lesions in IgY-treated mice compared to positive control group (p0.05).

**Keywords:** IgY antibodies, *Mycobacterium avium* subsp. Paratuberculosis, in vivo studies, Balb/c



# Enhancing the Effectiveness of Cefotaxime against *Acinetobacter baumannii* by Using the Natural Compound Thymol

Antimicrobial peptides

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**BACKGROUND AND OBJECTIVES:** This study aimed at the antimicrobial effects of thymol/cefotaxime on *Acinetobacter baumannii* (*A. baumannii*) bacteria.

**MATERIALS AND METHODS:** Antimicrobial effects of thymol/cefotaxime were performed first individually and then combined on *A. baumannii* ATCC19606 by the MIC-MBC method. Therefore, the antimicrobial effects of the compounds that had a synergistic impact were performed on eighteen clinical strains using the MIC-MBC method. The identification of chemical bonds, functional groups, and molecular interactions of the mentioned compounds was investigated using an FTIR device. Checkerboard method, time killing curve, and biofilm inhibition on *A. baumannii* ATCC19606, investigation of cytotoxicity on red blood cells (RBCs) by hemolysis method and human skin fibroblast cells (Ffk) by MTT method were performed. Thymol/cefotaxime had synergistic effects.

**RESULTS AND DISCUSSION:** The study's findings demonstrated that when applied to *A. baumannii* ATCC19606, the antimicrobial activities of thymol, cefotaxime, and thymol/cefotaxime (A1 compound) were, respectively, 256, 128, and 512/128 (FICI: 1 µg/ml). The A1 compound exhibited antibacterial activity of 1024-512/256-128 µg/ml on clinical strains of *A. baumannii*, respectively. Compared to the individual modes, the combined mode had a longer time curve for eliminating *A. baumannii*. Examination with FTIR showed that these two compounds have C=C conjugated, C≡C compound. Thymol, cefotaxime, and other chemicals have biofilm inhibition rates of 29.69%, 16.28%, and 39.28%, respectively against *A. baumannii* bacteria. The toxicity of thymol, cefotaxime, and A1 compound against human RBCs were 36.12, 8.33, and 8.38, and against human Ffk cells were 19.66, 7.08, and 9.03 respectively.

**Keywords:** *Acinetobacter baumannii*, Thymol, Cefotaxime, Antimicrobial

# In silico Design of a Hybrid anti-cancer Peptide Derived from Melittin and TAT antimicrobial peptides

Antimicrobial peptides

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**BACKGROUND AND OBJECTIVES:** Melittin (GIGAVLKVLTTGLPALISWIKRKRQQ) as a small peptide constituent of bee venom is considered as one of the promising candidates for anticancer therapy. It can target interaction between CD147 and CypA molecule to inhibit the caspase pathway. However, its clinical applicability is limited because of low penetration into cancer cells. TAT is an arginine-rich, cell-penetrating peptide that is widely used to deliver different therapeutic molecules into cells.

**MATERIALS AND METHODS:** For this, PDB, CAMPR3, and APD databases were used to evaluate physicochemical properties and also the selection of amino acid regions with high anti-cancer activity in melittin and Tat peptides. AntiCP server was used to predict novel analogs of melittin with higher anti-cancer property based on SVM score. Cluspro was used to study interaction between peptide and CD147 /CypA complex. Molecular dynamic and Coarse grained simulations were employed to examine the stability of designed peptide as well as the interaction between peptide with cancer membrane model.

**RESULTS AND DISCUSSION:** A hybrid peptide was designed with more stability and penetration into cancer cells through fusion between analogs of melittin and TAT. An amino acid region with high anti-cancer activity in Melittin was selected based on the physicochemical properties. Based on the results, a truncated Melittin peptide with 15 amino acids by the GGGS linker was fused to a TAT peptide (nine amino acids) to increase the penetration rate into the cell. A new hybrid peptide analog was designed via replacing the glycine with serine through random point mutation. Based on Clus Pro Docking results, the designed peptide acts as an inhibitory peptide with high binding energy when interacting with CD147 and the CypA proteins. RMSD and RMSF results confirmed the high stability of designed peptide in interaction with CD147. Also, the coarse-grained simulation showed the penetration potential of TM peptide into the DOPS-DOPC model membrane. In conclusion Our findings indicated.

**Keywords:** Antimicrobial peptide, anticancer peptide, melittin, TAT, hybrid peptide.

# Investigating the antimicrobial effects of mouse bone marrow-derived mesenchymal stem cells encapsulated in collagen and fibrin hydrogel scaffolds on wound infection caused by *Bacteroides fragilis* in vivo

Antimicrobial peptides

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**BACKGROUND AND OBJECTIVES:** Wound infection is a common type of nosocomial infection. One of the most significant causes of this infection is *Bacteroides fragilis*. This study aimed to compare and evaluate the antimicrobial effect of mesenchymal cells derived from the bone marrow of mice using collagen and fibrin hydrogel scaffolds on the rat wound infection model.

**MATERIALS AND METHODS:** The stem cells were extracted from mouse bone marrow tissues and then were confirmed by surface markers using flow cytometry analysis. The possibility of differentiation of stem cells into bone marrow was also checked. The extracted stem cells were encapsulated in the collagen and fibrin scaffold. 24 hours after making wound infection in rats, collagen and fibrin-encapsulated mesenchymal stem cells were applied to dress the wound. Finally, the bacterial load was monitored in the infected rat by a standard colony count test after one week. Statistical analysis was performed using SPSS Version 26.

**RESULTS AND DISCUSSION:** The results of this study showed that bone marrow mesenchymal stem cells encapsulated with both collagen and fibrin scaffolds had beneficial effects. The results also showed that bone marrow mesenchymal stem cells with a collagen scaffold have a greater effect than bone marrow mesenchymal stem cells with a fibrin scaffold. According to the results of the present study and to avoid the emergence of bacterial antibiotic resistance, the use of the collagen-hydrogel scaffold of the mouse bone marrow mesenchymal stem cells is recommended as a suitable and new option for the treatment of wound infections caused by *Bacteroides fragilis*.

**Keywords:** *Bacteroides fragilis*, Bone marrow, Mesenchymal stem cells, Wound infection

# Investigating the Synergistic Antimicrobial Effects of Thymol/Cefotaxime on Escherichia coli Bacteria

## Antimicrobial peptides

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**BACKGROUND AND OBJECTIVES:** This study looked into the antibacterial synergism of the thymol/cefotaxime against Escherichia coli (E. coli) bacteria.

**MATERIALS AND METHODS:** The MIC-MBC method was used to assess the antimicrobial effects of thymol, and cefotaxime, both separately and in combination at varying doses, against E. coli ATCC25922. Using the MIC-MBC method, the antibacterial activity of a compound that exhibited a synergistic effect on twenty clinical strains of E. coli bacteria was assessed. Additionally, the FTIR device was utilized to analyze the substance's molecular interactions, chemical bonds, and functional groups. The checkerboard technique, the time killing curve and biofilm inhibition on the E. coli ATCC25922 bacteria, the hemolysis method, the MTT method, and the hemolysis method were used to investigate the cytotoxicity on human skin fibroblast cells (Ffk), and red blood cells (RBCs). There was a synergistic impact from the A3 compound.

**RESULTS AND DISCUSSION:** The study's findings demonstrated that when applied to E. coli ATCC25922, the antimicrobial activities of thymol, cefotaxime, and thymol/cefotaxime (A3 compound) were, respectively, 256, 32, and 128/16 (FICI: 1 µg/ml). The A3 compound exhibited antibacterial activity of 1024-16/256-4 µg/ml on clinical strains of E. coli, respectively. Compared to the individual modes, the combined mode had a longer time curve for eliminating E. coli. These compounds had chemical bonds OH carbohydrates proteins, polyphenols, C=O Amide I band, C-O-Copolysaccharide, and C-Namide III band, but C=C conjugated, C≡C compound, shows the connection between thymol/cefotaxime. Thymol, cefotaxime, and other chemicals have biofilm inhibition rates of 29.69%, 16.28%, and 39.28%, respectively against E. coli bacteria. The toxicity of thymol, cefotaxime, and A3 compound against human RBCs were 36.12, 8.33, and 8.38, and against human Ffk cells were 19.66, 7.08, and 9.03 respectively.

**Keywords:** Escherichia coli, Thymol, Cefotaxime, Synergistic

# Investigation antibiofilm effects of Jelleine-1 peptide and the expression of biofilm genes in methicillin-resistant *Staphylococcus aureus* (MRSA) clinical strains

Antimicrobial peptides

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**BACKGROUND AND OBJECTIVES:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen which is known for its ability to form biofilm (1). Currently, researches are underway to introduce new antibiotic options with the effect of disrupting biofilm formation (2). jelleine-1, an antimicrobial peptide, may have potential antimicrobial activity against multi-drug resistant bacteria (3). The aims of this study were to investigate the effects of Jelleine-1 peptide on the expression of *icaA*, *icaD*, and *fib* genes. Also, we evaluated the inhibition and destroying the peptide on the biofilm of MRSA strains.

**MATERIALS AND METHODS:** Micro-broth dilution and time-kill assay were used to determine antibacterial activities. The effects of Jelleine-1 on bacterial biofilm formation and mature biofilm disruption were determined by crystal violet staining. Finally, the effects of Jelleine-1 on the expression of biofilm-associated genes (*icaA*, *icaD*, and *fib*) in strong biofilm-forming MRSA strains was measured after 12 h using Real-Time PCR.

**RESULTS AND DISCUSSION:** The MIC and MBC of Jelleine-1 were both 128  $\mu$ M, and the MIC level of Jelleine-1 could kill MRSA completely after 240 min treatment. Jelleine-1 could effectively inhibit biofilm formation and the removal of mature biofilms. This peptide could significantly downregulate the expression of the *fib* gene, and the expression of *icaA*, and *icaD* was reduced slightly. Discussion & Conclusions: Jelleine-1 showed favorable antibacterial and anti-biofilm activity against biofilm-forming MRSA clinical strains. These findings confirm that Jelleine-1 could be a promising therapeutic agent against MRSA biofilm-associated infections

**Keywords:** Methicillin-Resistant *Staphylococcus aureus*, Jelleine-1, biofilm, expression of genes, Real-Time PCR

# Synergistic Activity of Thymol with Ceftazidime Antibiotic against *Escherichia coli* Pathogenic Bacteria

Antimicrobial peptides

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**BACKGROUND AND OBJECTIVES:** This study looked into the antibacterial synergism of thymol/ceftazidime on *Escherichia coli* (*E. coli*) bacteria.

**MATERIALS AND METHODS:** The MIC-MBC method was used to assess the antimicrobial effect of thymol and ceftazidime, both separately and in combination, at varying doses on *E. coli* ATCC25922. The MIC-MBC approach was used to assess the antimicrobial properties of a drug that showed synergistic effects on twenty clinical strains of *E. coli* bacteria. Using an FTIR device, the compound's molecular interactions, functional groups, and chemical bond identification were examined. In this study, the checkerboard method, time killing curve, biofilm inhibition on *E. coli* ATCC25922 bacteria, and hemolysis method and MTT method were used to investigate the cytotoxicity on human skin fibroblast cells (Ffk) and red blood cells (RBCs) in human subjects. The effects of the A1 compound were synergistic.

**RESULTS AND DISCUSSION:** The study's findings demonstrated that when applied to *E. coli* ATCC25922, the antimicrobial activities of thymol, ceftazidime, and thymol/ceftazidime (A1 compound) were, respectively, 256, 16, and 16/8 $\mu$ g/ml (FICI: 1). The A1 compound exhibited antibacterial properties on clinical strains of *E. coli* that were 8-1024/4-128  $\mu$ g/ml, respectively. The combined mode demonstrated a longer time curve for killing the aforesaid bacteria compared to the individual modes. These compounds included OH bonds, proteins, polyphenols, C=O Amide I band, C-O-C polysaccharide, and C-Namide III band; however, one compound, C=C conjugated, C $\equiv$ C, demonstrates the relationship between thymol/ceftazidime. Thymol, ceftazidime, and A1 compound all showed biofilm inhibition rates of 29.51%, 22.14%, and 75.51% against *E. coli*, respectively. The corresponding toxicity values for thymol, ceftazidime, and A1 compound were 36.12, 9.54, and 6.83 for human RBCs and 19.66, 8.36, and 6.68 for human Ffk cells.

**Keywords:** *Escherichia coli*, Thymol, Ceftazidime, Synergistic

# The effect of M1 analog of Melittin in Nitric Oxide levels related to wound healing

Antimicrobial peptides

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**BACKGROUND AND OBJECTIVES:** An imbalance in the regulation of the wound healing mechanism may result in the emergence of different types of persistent ulcers or abnormal excessive scarring. Hence, it is crucial to discover innovative pharmaceutical approaches for enhancing wound healing and reinstating the structural stability of damaged tissue. Nitric oxide assumes a pivotal function in overseeing three primary components of the wound healing process: vascular homeostasis, inflammation, and antimicrobial activity. The goal of the present study was to evaluate a novel M1 analogue with modified properties as compared to Melittin in the production of nitric oxide (NO) related to wound healing.

**MATERIALS AND METHODS:** 84 Six-week Balb/c male mice (18–23 g) were obtained from the animal facility, Tabriz university of medical science. Mice were randomly divided into six categories (n=14 per category) including the control group, the witness, the phenytoin group, the bee venom group, the Melittin group, and the M1 peptide group. The assessment of wound healing progress was conducted on the 7th and 14th days. NO production levels were assessed by biochemical analyses.

**RESULTS AND DISCUSSION:** Based on results, a significant percentile of wound healing was found in Melittin analog group. Based on NO analysis result, the treatment of mice with melittin and M1 analog ointments significantly increased the NO level compared to control and bee venom groups. Our findings highlight the therapeutic application of Melittin and Melittin analog in wound healing.

**Keywords:** Antimicrobial peptide, melittin, Nitric oxide, wound healing.

# Thymol Increases Sensitivity of Clinical *Klebsiella pneumoniae* Bacteria to Cefotaxime

Antimicrobial peptides

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**BACKGROUND AND OBJECTIVES:** This study aimed at the antimicrobial effects of thymol/cefotaxime on *Klebsiella pneumoniae* (*K. pneumoniae*) bacteria.

**MATERIALS AND METHODS:** Antimicrobial effects of thymol/cefotaxime were performed first individually and then combined on *K. pneumoniae* ATCC100031 by the MIC-MBC method. Therefore, the antimicrobial effects of the compounds that had a synergistic impact were performed on ten clinical strains using the MIC-MBC method. The identification of chemical bonds, functional groups, and molecular interactions of the mentioned compounds was investigated using an FTIR device. Checkerboard method, time killing curve, and biofilm inhibition on *K. pneumoniae* ATCC100031, investigation of cytotoxicity on red blood cells (RBCs) by hemolysis method and human skin fibroblast cells (Ffk) by MTT method were performed. thymol/cefotaxime had Synergistic effects.

**RESULTS AND DISCUSSION:** The study's findings demonstrated that when applied to *K. pneumoniae* ATCC100031, the antimicrobial activities of thymol, cefotaxime, and thymol/cefotaxime (A3 compound) were, respectively, 256, 8, and 4/2 (FICI: 1 µg/ml). The A3 compound exhibited antibacterial activity of 4-1024/1-128 µg/ml on clinical strains of *K. pneumoniae*, respectively. Compared to the individual modes, the combined mode had a longer time curve for eliminating *K. pneumoniae*. Examination with FTIR showed that these two compounds have C=C conjugated, C≡C compound. Thymol, cefotaxime, and other chemicals have biofilm inhibition rates of 29.69%, 25.68%, and 46.36%, respectively against *K. pneumoniae* bacteria. The toxicity of thymol, cefotaxime, and A3 compound against human RBCs were 36.12, 8.33, and 8.38, and against human Ffk cells were 19.66, 7.08, and 9.03 respectively.

**Keywords:** *Klebsiella pneumoniae*, Thymol, Cefotaxime, Antimicrobial



# Anti-bacterial effect of white tea extract on *Streptococcus mutans* and *Streptococcus salivarius*

Oral microbiology

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**BACKGROUND AND OBJECTIVES:** Due to the increasing frequency of drug-resistant strains among different types of microorganisms, the finding of antimicrobial and antifungal compounds of natural substances, which certainly has fewer side effects, has long been of interest to researchers. Therefore, the aim of this investigation was to study the effect of white tea extract on reducing *Streptococcus mutans* and *Streptococcus salivarius*.

**MATERIALS AND METHODS:** White tea was prepared in powder form and then its hydroalcoholic extract of it was prepared. The antimicrobial effects of extracts on *S. mutans* and *S. salivarius* were performed using well diffusion and broth microdilution methods. All experiments were performed in triplet replications.

**RESULTS AND DISCUSSION:** Antibacterial susceptibility testing revealed that white tea extract at concentration of 500 µg/ml had a remarkable antibacterial activity against *S. mutans*, and *S. salivarius* with a zone of inhibition, 25±1 mm and 30±1, respectively. The minimum inhibitory concentration (MIC) values of the both tested strains were estimated 31.25 µg/ml. Moreover, the minimum bactericidal concentration (MBC) values of white tea extract were estimated 62.5 µg/ml against both streptococci. Considering the inhibitory power of the white tea extract on the gram-positive bacteria of *S. salivarius* and *mutans*, it can be concluded that this plant can be used in various industries, including the pharmaceutical and sanitary industry and it can also improve oral health as *S. mutans* and *salivarius* are the most important bacteria causing decays.

**Keywords:** *Camellia Sinensis*, *Streptococcus mutans*, Tea, Microbial Sensitivity Tests

# Antimicrobial Activity of *Thymus vulgaris* Essential Oil on Oral Pathogens and In-Vitro Study

Oral microbiology

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**BACKGROUND AND OBJECTIVES:** Thymus essential oil, which is derived from the Thymus plant, has strong antimicrobial properties against various pathogens. The primary components responsible for this activity are thymol and carvacrol, which have potent antibacterial, antifungal, and antiviral properties. Thymol and carvacrol have also been shown to interfere with the enzymes and proteins essential for the survival of pathogens. Thymus essential oil has the potential to be a natural alternative for combating microbial infections, but further research is needed to fully understand its mechanisms of action and potential applications in clinical settings. The study aims to evaluate the potential of Thymus essential oil antimicrobial agent for oral health applications, targeting pathogens commonly associated with oral infections and diseases.

**MATERIALS AND METHODS:** A stock solution of Thymus vulgaris oil was prepared in 10% aqueous dimethyl sulfoxide containing 0.5% Tween 80 for easy diffusion. The antimicrobial activities of this oil were determined using a 1 mg/mL solution. Thymus vulgaris oil showed strong inhibitory activity on *Treptococcus pyogenes* and *Streptococcus mutans* at concentrations of 16 to 256 µg/mL, as measured by the agar disk diffusion method.

**RESULTS AND DISCUSSION:** The Thymus vulgaris essential oil was extracted three times by hydrodistillation, and the average yield was  $2.3 \pm 0.14$  g oil/100 g dried leaves. Positive results were inhibition zones above 4 mm in diameter. All microbial isolates were sensitive at concentrations of 56 to 190 µg/mL, with inhibition zones ranging from  $6.7 \pm 0$  to  $35 \pm 0.6$  mm in diameter. *S. pyogenes* was the most sensitive isolate, with all clinical isolates producing the widest inhibition zones against all Thymus vulgaris oil concentrations (5-190 µg/mL). The most sensitive microorganisms tested were *S. pyogenes* with the minimum inhibitory concentration  $2.3 \pm 0.13$  µg/mL followed by *S. mutans* with minimum inhibitory concentration  $4.8 \pm 0.6$  µg/mL. Thymus vulgaris oil has potent antimicrobial activity against clinical isolates of *S. pyogenes* and *S. mutans* in vitro. As a result, it could be utilized in mouthwash, toothpaste, or aromatherapy to prevent and treat oral infections.

**Keywords:** Antimicrobial, Thymus vulgaris, Essential Oil, Oral pathogens, Streptococcus

# Appraising of biofilm productions in streptococcus mutants contain GtfB and GtfC in Clinical Sample

Oral microbiology

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**BACKGROUND AND OBJECTIVES:** One of the most important problems facing general health is tooth decay. Despite the effect of various factors on the development of tooth decay, the activity of acid producing, and make biofilm bacteria especially streptococcus mutants, is the main cause of this complication. The creation of the biofilms, coded by gtf gene. The aim of this study was to evaluate the genes of GtfB and GtfC of streptococcus mutants in the formation of dental plaque biofilm.



**MATERIALS AND METHODS:** Swabs of dental plaque samples were collected. Blood agar was used to isolate streptococcus mutants. Then the biofilm was formed on a polystyrene surface using a microtiter plate and stained with a crystal violet through an ELISA reader at a wavelength of 550 nm. In order to confirm streptococcus mutants' colonies, biochemical and fermentative tests were performed. The kit of gram-positive bacteria (Cinna Pure DNA KIT-PR881614) was used to extract DNA. Then optimum pH and the effect of different concentrations of sucrose on bacterial growth were measured by turbidity method at 630 nm using spectrophotometer

**RESULTS AND DISCUSSION:** The results showed that out of 460 samples, 190 isolates (41.30%) had gtfB gene and 80 (17.4%) had gtfC gene. The absorbance of the biofilm produced by the samples is different and the highest absorption is 1.43. The highest growth rate of streptococcus mutants isolated from tooth plaque was obtained at a concentration of 0.5 g / L sucrose pH = 4.5.

**Keywords:** streptococcus mutants, Biofilm, PCR, Tooth Decay

# Comparative Antibacterial Activity of AH Plus, MTA-Fillapex, and AH26 Endodontic Sealers against *Enterococcus faecalis*

Oral microbiology

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**BACKGROUND AND OBJECTIVES:** Root canal treatments rely on thorough removal of microorganisms and their byproducts, crucial for treatment success. Effective cleaning, irrigation with antibacterial agents like calcium hydroxide, and proper canal sealing with materials such as AH Plus, MTA Fillapex, and AH26 are essential steps. However, challenges persist due to the ability of microorganisms like *Enterococcus faecalis* to endure harsh conditions and penetrate gaps between materials and canal walls. This study focuses on comparing the antibacterial properties of AH Plus, MTA Fillapex, and AH26 sealers to assess their efficacy in preventing microbial proliferation within root canals.

**MATERIALS AND METHODS:** In this laboratory study conducted at the School of Dentistry, Sari University of Medical Sciences in spring 2017, 25 blood agar plates were prepared, each containing 5 wells. Three wells were filled with the endodontic sealers AH26, MTA Fillapex, and AH Plus. An ampicillin disc served as the positive control in the fourth well, while distilled water was used as the negative control in the fifth well.

**RESULTS AND DISCUSSION:** Following 48 hours of observation and plate analysis, the study found that AH26 exhibited the largest zone of inhibition (average diameter: 16.44 mm), indicating its superior efficacy against *Enterococcus faecalis*. MTA Fillapex followed closely with an average diameter of 15.44 mm, while AH Plus showed the least effectiveness with an average diameter of 10.2 mm in removing these microorganisms.

**Keywords:** Sealer, *Enterococcus faecalis*, AH Plus, AH26, MTA-Fillapex

# Comparing the Effect of Probiotic and Non-probiotic Yogurt Drinks on Two Common Oral Microorganisms: An In Vitro Study

Oral microbiology

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**BACKGROUND AND OBJECTIVES:** Background and purpose: Decrease in the number of oral microorganisms leads to reduction in caries score. Bacterial therapy, such as using probiotic bacteria, is an alternative procedure in treatment of infections caused by microorganisms. The purpose of this study was to compare the effect of probiotic and non-probiotic yogurt-drink on two common oral microorganisms.

**MATERIALS AND METHODS:** Materials and methods: In this experimental study, the minimum inhibitory concentration (MIC) of yogurt drinks and diameter of the growth inhibition zone of *Streptococcus mutans* and *Enterococcus faecalis* in probiotic and non-probiotic yogurt drinks were measured using micro broth dilution and disk agar diffusion methods, respectively. Data analysis was done in SPSS V22 applying Mann-Whitney test.

**RESULTS AND DISCUSSION:** Results: The MIC of non-probiotic yogurt drink against *Enterococcus faecalis* and *Streptococcus mutans* was significantly higher than the probiotic yogurt drink ( $P=0.002$ ). The two yogurt drinks did not show any significant differences in the diameter of growth inhibition zone for *S. mutans* ( $P=0.061$ ) and *E. faecalis* ( $P=0.99$ ). Conclusion: The study showed that probiotic yogurt drink can inhibit *E. faecalis* and *S. mutans* more than the non-probiotic yogurt drink, and it may be considered as a preventive agent for oral and dental diseases.

**Keywords:** Keywords: yogurt, probiotic, *Enterococcus faecalis*, *Streptococcus mutans*

# Evaluation of Bacterial status in Clinical Environment of Sari Dental School

Oral microbiology

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**BACKGROUND AND OBJECTIVES:** Bacterial contamination of clinical surfaces of dental units that have been touched or been exposed to patients' blood or saliva can be a reservoir for infections, leading to cross-contamination. This study aimed to evaluate bacterial contamination in the clinical environment of Sari Dental School.

**MATERIALS AND METHODS:** Materials and Methods: In this cross-sectional (descriptive-analytical) study, samples were randomly collected from 15 active dental units of five departments of Sari Dental School, including surgical, pediatrics, prosthodontics, endodontics, and restorative dentistry departments. Samples were collected from headrests, light handles, and dental seats using moist sterile swabs, and air samples were collected using agar plates. Sampling was carried out before and after dental practice. The samples were transferred to the microbiology laboratory to determine the number of various microorganism colonies. Data were analyzed using Chi-square, McNemar, and Kruskal-Wallis tests. P-values lower than 0.05 were considered significant.

**RESULTS AND DISCUSSION:** A significant difference was found between the frequency of contamination before and after clinical practice based on McNemar test results. Staphylococci were more prevalent on the surfaces. Kruskal-Wallis test revealed no significant difference in the total number of microorganisms between different departments after dental practice. Bacterial contamination of air was greater than other parts, followed by dental seats. Conclusion: Microbial contamination of dental units considerably increases after treatment of each patient. Therefore, disinfection of dental unit surfaces and seats between each patient is essential. Also, methods of infection control must be supervised to prevent cross-infection.

**Keywords:** Equipment Contamination, Dental Infection Control, Disinfection, Microorganism

# Investigating the antimicrobial effect of nanoliposomes containing *Melissa officinalis* extract on *Streptococcus mutans*

Oral microbiology

Zahra Noroziyan <sup>1</sup> , Solmaz Shahla <sup>1</sup> 

<sup>1</sup> Investigating the antimicrobial effect of nanoliposomes containing *Melissa officinalis* extract on *Streptococcus mutans*

**BACKGROUND AND OBJECTIVES:** The use of medicinal plants undergoes a significant increase related to their affordability and widespread availability. In this study, a drug was performed using liposomes containing *Melissa officinalis* extract and investigating its properties and the antimicrobial efficacy of the resulting nanoparticle against *Streptococcus mutans*.

**MATERIALS AND METHODS:** Aqueous extraction of the plant was carried out, and nanoliposomes were synthesized using the thin-layer technique by combining cholesterol and phosphatidylcholine according to the thin-layer hydration method. *Melissa officinalis* extract was subsequently included in the nanoliposomes. Particle size was assessed using the dynamic light scattering device (DLS) and zeta potential was determined through Zeta measurement. The morphology of nanoparticles was examined using a SEM microscope, as well as the antimicrobial properties of the extract and the nanoparticles containing the extract against *Streptococcus mutans* bacteria using the minimum growth inhibitory concentration (MIC) method and the minimum lethal concentration (MBC) method.

**RESULTS AND DISCUSSION:** The findings of the present study showed that nanoliposomes derived from *Melissa officinalis* extract have a size of 124 nanometers and a Zeta potential of 64/36 nanometers. In addition, analysis of the electron microscope (SEM) showed that nanoliposomes have a normal spherical morphology. The minimum inhibitory concentration (MIC) and minimum lethal concentration (MBC) against *Streptococcus mutans* bacteria were determined to be 500 and 1000 mg/mL respectively. In addition, MIC and MBC extract-containing nanoliposome results show that liposomal formulations show greater cytotoxic effects on *Streptococcus mutans* compared to free extract. The findings suggest that nanocarriers containing *Melissa officinalis* extract, despite showing appropriate physicochemical properties, reduce the survival of *Streptococcus mutans* compared to liposome-free plant extract. Hence, it can be used as a suitable carrier to affect the bacterium *Streptococcus mutans*.

**Keywords:** Extraction, *Melissa officinalis* plant, antimicrobial effect, nanoliposome, bacterium *Streptococcus mutans*

# Investigating the antimicrobial effect of nanoliposomes containing *Thymus vulgaris* extract on *Porphyromonas gingivalis*

Oral microbiology

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**BACKGROUND AND OBJECTIVES:** The use of medicinal plants is experiencing a significant increase due to their affordability and widespread availability. Within the scope of this research, a formulation was prepared using liposomes that encapsulate *Thymus vulgaris* extract. The study analyzed the physicochemical properties of the resulting nanoparticle, along with investigating its antimicrobial effect on *Porphyromonas gingivalis*.

**MATERIALS AND METHODS:** The aqueous method was used to make the plant extract. It was used to produce a thin film nanoliposome using cholesterol and phosphatidylcholine based on the thin film hydration approach. The nanoliposomes were then loaded with *Thymus vulgaris* extract. Particle size was determined using a dynamic light scattering device (DLS), the zeta potential was determined using Zetasizer, and the morphology of nanoparticles was examined with a SEM microscope. The antimicrobial properties of the extract and nanoparticles containing the extract against the bacterium *Porphyromonas gingivalis* were evaluated by the method of minimum growth inhibition concentration (MIC) and minimum fatal concentration (MBC).

**RESULTS AND DISCUSSION:** The findings of the study suggest that following extraction from *Thymus vulgaris* and capsulation in nanoliposomes, nanoliposomes containing *Thymus vulgaris* extract with a size of 132 nm and a zeta potential of -51/13 mV show. In addition, analysis of the electron microscope (SEM) suggests that nanoliposomes have a spherical morphology. The minimum inhibitory concentration (MIC) and the minimum bacterial lethal concentration (MBC) against *Porphyromonas gingivalis* are determined to be 500 and 1000 µg/ml, respectively. In addition, the results of the MIC and MBC of this extract-containing nanoliposome indicate that the liposomal extract shows equivalent toxicity to *Porphyromonas gingivalis* compared to the free extract. The findings of the study suggest that nanoliposomes containing *Thymus vulgaris* extract, despite showing favorable physicochemical characteristics, did not have a significant effect on the survival of *Porphyromonas gingivalis* compared to the sole extract.

**Keywords:** extraction, *Thymus vulgaris*, antimicrobial effect, nanoliposome, bacterium *Porphyromonas gingivalis*



# Investigating the antimicrobial effect of nanoliposomes containing *Thymus vulgaris* extract on *Streptococcus mutans*

Oral microbiology

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**BACKGROUND AND OBJECTIVES:** The use of medicinal plants is on the rise, primarily attributed to their affordability and widespread availability. In the context of this research, a compound was prepared using liposomes to contain *Thymus vulgaris* extract. The study examined the physicochemical properties of the resulting nanoparticle, as well as its antimicrobial effect on *Streptococcus mutans*.

**MATERIALS AND METHODS:** A process involving extracting plant extract was carried out in an aqueous method. The thin film method was used to produce nanoliposomes. These nanoliposomes were prepared using cholesterol, and phosphatidylcholine based on the thin film hydration technique, and then *Thymus vulgaris* extract was included in them. Particle size was measured using a dynamic light scattering device (DLS), the zeta potential was evaluated with Zetasizer, and the morphology of nanoparticles was examined using a SEM microscope. To investigate the antimicrobial properties of the extract and nanoparticles containing the extract against *Streptococcus mutans* bacteria, the technique of minimum growth inhibitory concentration (MIC) and minimum fatal concentration (MBC) was used.

**RESULTS AND DISCUSSION:** The present study's findings showed that after separating from the *Thymus vulgaris* and capsulation within nanoliposomes, nanoliposomes containing *Thymus vulgaris* extract with a diameter of 132 nm have a zeta potential of -51.13 mV. In addition, microscopic surveys using (SEM) showed that nanoliposomes have regular, spherical morphology. The concentration set for inhibiting growth to a minimum and causing lethality in *Streptococcus mutans* was identified as 250 and 500 µg/ml, respectively. The results showed that the minimum inhibitory concentration (MIC) and the minimum lethal concentration of bacteria (MBC) for the extract encapsulated in the nanoliposome cause greater toxicity on *Streptococcus mutans* than the free extract. Research indicates that nanoliposomes with *Thymus vulgaris* extract can reduce *Streptococcus mutans* survival compared to unprocessed liposomal extract due to suitable physicochemical properties. This indicates nanoliposomes as an effective delivery method for *Thymus vulgaris* extract against *Streptococcus mutans*.

**Keywords:** extraction, *Thymus vulgaris*, antimicrobial effect, nanoliposome, bacterium *Streptococcus mutans*

# Investigation the prevalence of *Helicobacter pylori* in dental plaques of children undergoing endoscopy in 2023

Oral microbiology

Zahra Jamshidi , Ali Asghar Soleimani, Naghi Dara, Mehrzad Sadredin Amin, Mojdeh Hakemi Vala 

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**BACKGROUND AND OBJECTIVES:** *Helicobacter pylori* infection is a widespread bacterial disease in humans. The digestive system, which houses this bacterium, may contribute to tooth decay due to the synergistic effect of this bacterium with oral microbiota. Our study focused on assessing the prevalence of *H. pylori* in dental plaques among children undergoing endoscopy, both with and without dental caries in 2023

**MATERIALS AND METHODS:** From June to November 2023, a total of 100 children aged 4-18 years, who were candidates for endoscopy, participated in the study. Parents completed a questionnaire and consent form. Before endoscopy, all children underwent an oral examination to determine their DMFT index, which is a standard dental statistical index measuring decayed, missing, and filled teeth. A DMFT index of 3 or higher indicates a high risk of tooth decay, while an index of less than 1 indicates a low risk. Supra-gingival plaques were removed from the lower first permanent molar teeth and/or upper central teeth using a sterile scaler and placed into a sterile Falcone tube, then sent to the lab for DNA extraction using the Sambio kit. The presence of *Helicobacter pylori* was detected using PCR and electrophoresis methods. Data was analyzed using SPSS software version 27.

**RESULTS AND DISCUSSION:** The study examined the relationship between *Helicobacter pylori* in dental plaques and dental caries in 45 boys and 55 girls. The results showed that 50.9% of girls tested positive for *Helicobacter pylori*, with 78% of these girls having a high level of tooth decay (DMF/dmf 3) and 81% having severe decay (Decay 3). In contrast, only 29.6% of girls who tested negative for *Helicobacter pylori* had DMF/dmf 3, with 50% having severe decay. Among boys, 53% tested positive for *Helicobacter pylori*, with 95% having DMF/dmf 3 and 100% having Decay 3). In contrast, only 42% of boys who tested negative for *Helicobacter pylori* had DMF/dmf 3, with 66% having severe decay. However, further research is needed to establish a conclusive link between *Helicobacter pylori* and caries development, as the presence of the bacteria does not necessarily imply aggressive behavior.

**Keywords:** *Helicobacter pylori*, Dental plaque, Endoscopy, DMF/dmf index

# Laboratory Evaluation of the Antibacterial Properties of 0.63% Stannous Fluoride as an Intracanal Medication Against Enterococcus Faecalis Compared with 2% Chlorhexidine Gel and Calcium Hydroxide

Oral microbiology

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**BACKGROUND AND OBJECTIVES:** The primary goal of using intracanal medications is to eliminate infections and bacteria from the canal and dentinal walls. Despite thorough cleaning techniques, about 50% of microbial agents may persist due to anatomical variations and inaccessible areas such as lateral canals. Methods like mechanical instrumentation and chemical irrigation are used, but residual bacteria can remain, especially in complex structures. To enhance treatment efficacy, intracanal medicaments with strong antibacterial properties are utilized. Stannous fluoride, chlorhexidine gel, and calcium hydroxide are effective alternatives. This study aims to evaluate the antibacterial properties of 0.63% stannous fluoride against *Enterococcus faecalis* and compare it with 2% chlorhexidine gel and calcium hydroxide.


**MATERIALS AND METHODS:** A total of 25 blood agar plates were prepared in 2017 at the Microbiology Laboratory of the Mazandaran University of Medical Sciences School of Medicine and then inoculated with *Enterococcus faecalis*. Five wells, each 6 mm in diameter, were created in each plate, and cream mixtures of calcium hydroxide, 2% chlorhexidine gel, and 0.63% stannous fluoride were placed in the wells (25 wells for each material). Additionally, 25 wells with ampicillin discs were used as positive controls, and 25 wells with distilled water served as negative controls. The plates were incubated for 48 hours, after which the inhibition zones were measured. Differences between the groups were analyzed using the Kruskal-Wallis and Mann-Whitney tests, with a p-value of less than 0.05 considered significant.

**RESULTS AND DISCUSSION:** In this study, the intracanal medications (2% chlorhexidine gel, calcium hydroxide, 0.63% stannous fluoride) were evaluated for their inhibition zones against *Enterococcus faecalis*. The inhibition zones were measured from the edge of the wells using a caliper in millimeters. The results showed no inhibition zone around the negative control group (distilled water), while the largest inhibition zone was observed around the positive control (ampicillin), measuring 22 mm. The mean inhibition zone diameters for chlorhexidine and stannous fluoride were 18.88 mm and 12.88 mm, respectively, with the smallest inhibition zone diameter for calcium hydroxide at 9.32 mm.

**Keywords:** Stannous fluoride, chlorhexidine, calcium hydroxide, intracanal medication, antimicrobial effect

# Microbial Contamination Assessment of the Clinical Environment in the Faculty of Dentistry, Sari, in the Year 2018

Oral microbiology

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**BACKGROUND AND OBJECTIVES:** Research on bacterial contamination in dental school environments highlights significant concerns regarding patient safety and infection control. Studies underscore the pervasive presence of bacteria in clinical settings, emphasizing the need for stringent infection control protocols to prevent cross-infections. Additionally, microbial aerosol contamination poses risks to both patients and healthcare workers, underscoring the critical importance of strict hygiene measures. These findings stress the necessity for continuous research and effective implementation of safety protocols to enhance infection control in dental practices. This study aimed to assess bacterial contamination in the clinical environment of Sari Dental School during 2018.

**MATERIALS AND METHODS:** Samples were randomly collected in 2018 from 15 active dental units within the Surgery, Pediatric Dentistry, Prosthodontics, Endodontics, and Restorative Dentistry departments of the dental school in Sari. Using sterile swabs, samples were obtained from the headrest, light handle, and seat, while air samples were collected using agar plates. Sampling was conducted at two intervals: prior to the commencement of dental procedures and following their completion. The collected samples were transported to the microbiology laboratory for quantification of various microbial colonies. Data analysis was performed utilizing Chi-square, McNemar, and Kruskal-Wallis tests, with a P-value of less than 0.05 considered statistically significant.

**RESULTS AND DISCUSSION:** The McNemar test results indicated a significant increase in contamination frequency following clinical procedures compared to before. Staphylococcus was predominantly found on surfaces. According to the Kruskal-Wallis test, there was no significant difference in the total number of microorganisms across different departments at the conclusion of dental practices. Airborne bacterial contamination was the highest, followed by contamination on the dental chairs.

**Keywords:** Bacterial contamination, Dental units, Clinical surfaces, Microorganisms

# Microbial Contamination in the Waterlines of the Dental Units in Sari School of Dentistry

Oral microbiology

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**BACKGROUND AND OBJECTIVES:** Microbial contamination of water sources in dental units is one of the problems in dentistry as such contamination can lead to the occurrence of dangerous infections. Given the importance of infection control and creating a healthy environment for the treatment of patients, this study conducted a microbiological analysis of water in the dental units of Sari School of Dentistry.

**MATERIALS AND METHODS:** In this descriptive-analytical and cross-sectional study, three units from each of the endodontic, restorative, surgical, pediatric, and prosthetic units of Sari School of Dentistry were randomly selected. Samples of 3-way syringe water, turbine water before and after flushing, and glass water were prepared and transferred to the microbiology department in sterile tubes. The samples were then cultured in Müller Hinton agar and blood agar and placed in an incubator at 37 °C for 24 hours. After incubation, gram staining was performed on the samples in which the bacterial colony had grown. The bacteria were examined for morphology and gram reaction.

**RESULTS AND DISCUSSION:** Results: Out of 61 samples, 23 samples (37.7%) were infected with bacterial colonies. The highest frequency of bacterial infection was found in the mixed bacterial group (gram- positive cocci and gram-positive bacilli). The results of the chi-square test and Fisher's exact test showed no significant relation between the sampling site and contamination ( $P = 0.309$ ). Conclusion: Water contamination in the pediatric and endodontic units in Sari School of Dentistry is high. Thus, effective measures should be taken to reduce water pollution in these units and reduce the risk of infection in staff and patients.

**Keywords:** Keywords: Dental unit, Dental unit waterline, Biofilm, Dental infection control

# The Anti-Biofilm potency of Zinc Oxide Nanoparticles against Dental Plaque-Forming *Streptococcus mutans* Isolated from Schoolchildren: in Vitro

Oral microbiology

Leila Fozouni <sup>1</sup> © ®, Zahra Shaddel <sup>1</sup>, Ghazal Rouhifar <sup>1</sup>, Masoumeh Kolagar <sup>1</sup>, Mahla Tajik <sup>1</sup>, Mohammad Kordkatouli <sup>1</sup>

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**BACKGROUND AND OBJECTIVES:** Dental caries is one of the most common infections in children which is mainly caused by the bacteria living in the mouth. *Streptococcus mutans* is an important bacterium that colonizes on tooth surfaces. The present study aimed to evaluate the role of streptococcal biofilms in the formation of dental plaque and investigate the in vitro anti-biofilm effects of zinc oxide nanoparticles (ZnONPs) against these isolates.

**MATERIALS AND METHODS:** This experimental study was conducted on 120 samples collected from the buccal and lingual surfaces of the posterior teeth of elementary school students. *S. mutans* strains were identified using conventional microbiological and biochemical tests, and biofilm formation was assessed using the microtiter plate assay. Antibiotic susceptibility was evaluated by disk diffusion method according to CLSI-2020 guidelines. Antibacterial properties and minimum inhibitory concentration (MIC) of zinc oxide nanoparticles were evaluated by agar well diffusion and broth microdilution assays, respectively

**RESULTS AND DISCUSSION:** The frequency of *S. mutans* was 64.2%, 72% of which could form biofilm. Among 77 *S. mutans* isolates, the highest and lowest antibiotic resistance rates were observed against Amoxicillin (70%) and Cefotaxime (10%). 22 (74%) drug-resistant isolates were eliminated by zinc oxide nanoparticles at a concentration of 150 mg/L. (MIC<sub>90</sub> ≥ 200 mg/L). It has been shown that biofilm formation on the tooth surface leads to dental diseases depending on the major determinants of bacterial pathogenicity. According to various studies results, the antibacterial effect of ZnONPs is correlated to their size and shape. In present and previous studies, ZnONPs with a diameter of 100 nm have been shown to exert the highest antimicrobial effects against gram-positive bacteria. Given the favorable antibacterial effects of zinc oxide nanoparticles in vitro, the clinical application of these nanoparticles in the treatment of tooth decay caused by drug-resistant *S. mutans* could be investigated in future studies.

**Keywords:** *Streptococcus mutans*, Biofilm, Zinc oxide, Drug resistance

# Anti-tumor effects of cytoplasmic extract from *Bifidobacterium breve* on SCC-25 oral cancer cells: potential therapeutic implication

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** Oral squamous cell carcinoma (SCC) is a prevalent malignancy in the oral cavity influenced by various factors. Despite advancements in cancer research, improvements in patient survival for SCC have been modest, highlighting the urgent need for more effective treatments. Bifidobacteria are increasingly recognized for their potential in cancer prevention, particularly in colorectal cancer. Recent studies indicate that cytoplasmic extract from *Bifidobacterium breve* may provide new and effective therapeutic possibilities for treating cancer. This study specifically investigates the extract's cytotoxic and anti-tumor effects on the SCC-25 cell line, aiming to explore its potential as a treatment for oral cancer.

**MATERIALS AND METHODS:** Cytoplasmic extract from *B. breve* was prepared by harvesting bacterial cells through centrifugation, followed by cell disruption using sonication. The resulting extract was filtered to remove cell debris and stored at -80°C until use. SCC-25 oral cancer cells were cultured in DMEM supplemented with FBS and antibiotics in a humidified atmosphere at 37°C and 5% CO<sub>2</sub>. Cells were treated with varying concentrations of the cytoplasmic extract (25, 50, and 100 µg/mL) for 24 hours. Cell viability was assessed using the MTT assay, where absorbance was measured at 570 nm using a microplate reader. For morphological analysis, treated cells were stained with Hoechst 33342 and examined under a fluorescence microscope to observe nuclear changes indicative of apoptosis.

**RESULTS AND DISCUSSION:** The MTT assay demonstrated a significant dose-dependent inhibition of SCC-25 proliferation following treatment with *B. breve* cytoplasmic extract. Specifically, at concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL, cell viability decreased by 20%, 40%, and 60%, respectively, compared to untreated controls ( $p < 0.05$ ). These results were further supported by fluorescence microscopy, which revealed characteristic morphological changes in treated cells, including chromatin condensation and nuclear fragmentation, consistent with apoptosis induction. This study underscores the potent cytotoxic and anti-tumor effects of *B. breve* cytoplasmic extract on SCC-25 oral cancer cells, demonstrating its ability to induce apoptosis and potentially suppress cancer progression. The robust methodology employed, combining MTT assays for viability assessment and fluorescence microscopy for detailed morphological analysis, provides compelling evidence of the extract's efficacy against SCC-25 cells.

**Keywords:** Oral squamous cell carcinoma, SCC-25, *Bifidobacterium breve*, Anti-tumor.

# Cloning and Expression of Interleukin 11 in Bacillus Subtilis

## Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** In patients with cancer who undergo chemotherapy, their blood platelet is lowered. Interleukin 11 is a cytokine that increases blood platelet, we produced this product using recombinant interleukin 11 in Bacillus subtilis. In this study, B.subtilis was used as a good host for gene cloning and protein expression due to non-pathogenicity and the ability to secrete high protein levels. However, the efficacy of transfer of the plasmid DNA attached into the competent B.subtilis cell is low in comparison with the use of calcium competent Escherichia coli, so it is better that the first cloning steps are performed using a shuttle vector in E. coli, and then B.subtilis with a great deal of hybrid vector is transformed.

**MATERIALS AND METHODS:** In this study, interleukin 11 was synthesized in a closed structure with two Bam HI and XbaI cutoffs with a final size of 609 bp by a Chinese company and delivered on a pGH vector. The vector of synthesized gene carrying Interleukin 11 was transferred to DH5 $\alpha$  E. coli, and then the gene was cloned onto a PHT43 shuttle vector and transformed into the B.subtilis WB600. The vector containing the cloned gene was induced within the WB600 by IPTG. The protein expression was evaluated by Bradford Reagent. SDS-PAGE gene was used to confirm the protein.

**RESULTS AND DISCUSSION:** The MFA culture medium sample at the fourth hour and the sample of the culture medium inoculated with plasmid-bearing bacteria without genes were examined for SDS-PAGE and a band of about 22 kD related to the recombinant protein in the positive sample was observed.

**Keywords:** Interleukin-11 (IL-11), Gene expression, Bacillus subtilis, Clone, Shuttle Vector, SDS-PAGE



# Cytotoxic effects of Cell-Free Supernatant of *Lactobacillus paracasei* on Human Breast Cancer Cell Line MCF-7

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** Breast cancer is the most common cancer among women and the second leading cause of cancer-related deaths worldwide. The development of anti-cancer agents with minimal side effects is crucial for improving patient outcomes. This study aimed to investigate the cytotoxic effects of *Lactobacillus paracasei* cellular extract on the human breast cancer cell line MCF-7.

**MATERIALS AND METHODS:** Materials and Methods: In this experimental study, Cell-Free Supernatant of *Lactobacillus paracasei* was prepared and MCF-7 breast cancer cells were treated with different concentrations (5%, 10%, 20%, 40%, and 60%) of *Lactobacillus paracasei* supernatant for 48 hours. Subsequently, the effect of a Cell-Free Supernatant was evaluated by MTT and LDH assay.

**RESULTS AND DISCUSSION:** The results show that higher concentration of the bacterial supernatant caused more cellular damage and cytotoxicity to the MCF-7 breast cancer cells, as evidenced by the increased LDH release into the culture medium (LDH assay) and the percentage of viable cells decreases (MTT assay) (P<0.05). The results showed that *Lactobacillus paracasei* supernatant had concentration-dependent cytotoxic effects on MCF-7 cells, as assessed by the MTT and LDH assays. The findings suggest that the *Lactobacillus paracasei* supernatant have the potential to be used as complementary agents in the treatment of breast cancer. Further research is needed to elucidate the underlying mechanisms of action of these compounds against breast cancer.

**Keywords:** breast cancer, cytotoxicity, Cell-Free Supernatant of *Lactobacillus paracasei*, MCF-7.

# Designing a Novel Fusion Protein from *Streptococcus agalactiae* with apoptosis induction effects on cervical cancer cells

## Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** Cervical malignancy ranks as the fourth most prevalent cancer in females. Rib and  $\alpha$ , two surface proteins belonging to the family of alpha-like proteins (Alps) in *Streptococcus agalactiae*, are presented in *S. agalactiae*. These proteins are involved in binding to host cells and invasion via interacting with glycosaminoglycan on the surface of epithelial cells. These positive charge proteins have become an exciting option for cancer-fighting due to interaction with negatively charged cancer cells. Unprecedented advances in bioinformatics allow the analysis of sequences and access the valuable information in a short.

**MATERIALS AND METHODS:** The fusion gene was constructed in the pET22b (+) vector. The 3D structure was modeled. Molecular dynamics simulation was employed to refine the 3D model. The recombinant plasmid was transferred to *E. coli* competent cells. The recombinant protein was expressed by IPTG. Recombinant proteins were purified by Ni-NTA chromatography. Expressed protein was analysed by SDS and western blot. Anticancer effects on HeLa cells were investigated by, MTT, real-time PCR and Annexin V-FITC/PI-staining. MTT colorimetric assay was applied to assessment the cell viability and IC<sub>50</sub>, defined as the concentration of drug required for inhibition the 50% of the biological process, were determined.

**RESULTS AND DISCUSSION:** Anticancer probability of our protein was 96%. The results of MD analysis showed the stability of fusion protein. SDS PAGE analysis revealed that protein was in soluble form. Percentage of viable cells was reduced in a dose-dependent manner with the IC<sub>50</sub> value of 180  $\mu$ g/ml. Proportion of apoptotic cells increased. The expression level of bax, and caspase 3 genes increased, whereas bcl-2 was down-expressed. The possible anticancer mechanisms were the induction of apoptosis and regulation. The anticancer activity of our protein was related to the sequence. This protein was rich in flexible residues that interact strongly with cancer cell membrane, resulting in increased cellular uptake. High concentration of Ile, Leu, Phe, Trp and aliphatic residue facilitates protein interaction with cancer cells. amino acids such as Ala, Arg, Lys, and Val increase protein hydrophobicity, facilitating protein interaction with cancer cells.

**Keywords:** *Streptococcus agalactiae*; Rib;  $\alpha$ ; Anticancer activity; Apoptosis

# Evaluation of *Klebsiella pneumoniae* bacteria fractions on MCF7 breast cancer cells line

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** Breast cancer has been reported as the most common type of cancer in 2021, accounting for approximately 12% of all new cancer cases worldwide annually. *Klebsiella pneumoniae* is a Gram-negative bacterium that can lead to a wide range of diseases, particularly pneumonia, urinary tract infections, sepsis, meningitis, and diarrhea. This bacterium has the ability to produce a bacteriocin called microcins E492, which is a channel-forming molecule on the cell membrane and can induce apoptosis. In this study, we evaluated the effects of *Klebsiella pneumoniae* bacterial supernatant on MCF7 breast cancer cell line.

**MATERIALS AND METHODS:** In this study, a 24-hour MTT assay was conducted to assess the toxicity of *Klebsiella pneumoniae* supernatant on cancer cells compared to control cells. The MTT assay is a valid method for evaluating cellular health and their response to various compounds in cellular systems. Based on the conversion of surviving samples to purple formazans by live cells, this method measures the activity of the mitochondrial enzyme. It is a fast, sensitive, and reliable method for measuring cellular health and is a useful tool for assessing the toxicity or therapeutic activity of different compounds.

**RESULTS AND DISCUSSION:** Examination of cellular toxicity results in the MCF-7 cell line treated with *Klebsiella pneumoniae* supernatant at concentrations of 42, 21, 10.5, and 5  $\mu\text{g/ml}$  over 24 hours showed no significant difference between treated and control cells in terms of live and dead cell populations. This may indicate the inability of *Klebsiella pneumoniae* supernatant to eliminate MCF-7 cancer cells. The conclusion drawn from the results of this study indicates that the compounds present in *Klebsiella pneumoniae* supernatant not only do not have the ability to eliminate cancer cells but may have reverse effects on them. This suggests the complexity of the effects of different bacteria on cancer cells and the need for further and more detailed research on their relationship.

**Keywords:** *Klebsiella pneumoniae* fractions, cancer, MCF7 cell line

# Investigating the Apoptotic Effects of Lactiplantibacillus pentosus Cytoplasmic Extract on Glioblastoma Cell Line U-87 MG"

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** The utilization of Lactobacillus cytoplasmic extract has garnered considerable attention in recent years due to its diverse biological properties, including antimicrobial, anti-inflammatory, antiviral, immunomodulatory, and anticancer activities. This study aimed to investigate the impact of Lactiplantibacillus pentosus (L. pentosus) cytoplasmic extract on the expression of the apoptosis-related genes bax and bcl-2 in the U-87 MG glioblastoma cell line.

**MATERIALS AND METHODS:** The cytoplasmic extract of L. pentosus was prepared using a sonication method. The concentration and protein content of the extract were evaluated using the Bradford assay and SDS-PAGE, respectively. RNA extraction and cDNA synthesis were performed using a commercial kit (Yektatajhez, Tehran, Iran). The effects of the L. pentosus extract on the expression levels of bax and bcl-2 genes were then determined using real-time quantitative PCR. Data analysis was conducted using SPSS version 21.

**RESULTS AND DISCUSSION:** The cell viability study demonstrated that the L. pentosus extract exerted inhibitory effects on the cellular activity of U-87 MG cells and induced apoptosis. The expression of the pro-apoptotic bax gene was found to be significantly upregulated (P 0.0008), while the expression of the anti-apoptotic bcl-2 gene was significantly downregulated (P 0.0001). The findings of this study confirm the anticancer potential of the L. pentosus cell extract and support its further investigation as a candidate for the development of novel glioblastoma therapeutics.

**Keywords:** Lactiplantibacillus pentosus, Apoptosis, U-87 MG Cell line, Glioblastoma

# Investigating the cytotoxic effects of zinc oxide nanoparticles and analyzing the expression of apoptotic genes in colon cancer cell lines

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** Cancer is a kind of genetic disease caused by DNA mutation. Today, zinc oxide nanoparticles are widely used in therapeutic fields. The aim of this study is to synthesize zinc oxide nanoparticles by *Xanthomonas campestris* and investigate the apoptotic activity of these nanoparticles.

**MATERIALS AND METHODS:** ZnO nanoparticles were synthesized by *Xanthomonas campestris* using zinc nitrate hexa hydrate as substrate, in a shaker incubator at 37 ° C and pH7. The powder produced was then calcined at 600 ° C for 2 hours after drying. morphology and size of ZnO nanoparticles was characterized by scanning electron microscopy. X-ray diffraction pattern indicated that ZnO has hexagonal structure. Formation and purity of the ZnO nanoparticles was confirmed with UV-Vis spectroscopy and Fourier transform infrared spectroscopy. Using the MTT, the minimum inhibitory concentration (IC50) was determined for ZnO nanoparticles against the HT-29 cell line, and this value was used as the basis for the Realtime-PCR. In order to investigate the effect of ZnO nanoparticles in inducing apoptosis, cells were cultured in T25 flasks with the IC50 of ZnO nanoparticles and after 48 hours, the changes in the expression of casp3, casp9, Bax and Bcl-2 genes were evaluated with Realtime-PCR.

**RESULTS AND DISCUSSION:** The synthesized zinc oxide nanoparticles were spherical with an average size of 45 nm. Also, the sample is porous and has a relatively good specific surface. The resulting zinc oxide nanoparticle is almost pure and only a very small impurity of zinc acetate was observed in the infrared spectrum of the sample. The value (IC50) for zinc oxide nanoparticles against colon cancer cell line HT-29 was 77.91 µg/ml. The results of gene expression with the help of Realtime-PCR showed that the synthesized zinc oxide nanoparticles induced apoptosis in HT-29 cells by decreasing the expression of the anti-apoptotic Bcl-2 gene and increasing the expression of the pro-apoptotic casp3, casp9, Bax gene. In general, the results obtained from this study can claim that ZnO nanoparticles have anti-cancer properties and can be introduced after further studies as candidates for cancer treatment.

**Keywords:** zinc oxide nanoparticle, gene expression, apoptosis, *Xanthomonas campestris*

# Investigating the effect of paclitaxel drug attached to biologically produced gold nanoparticles compared to paclitaxel drug alone on MCF7 breast cancer cell line

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** The purpose of this study, the possibility of binding nanoparticles produced by *Fusarium oxysporum* to the cancer drug paclitaxel and their toxicity in MCF7 breast cancer cell culture was investigated.

**MATERIALS AND METHODS:** The supernatant obtained from the culture of *Fusarium oxysporum* was pH adjusted and exposed to a concentration of 1mmol gold salt. Gold nanoparticles were produced and confirmed by the methods of visible light spectrophotometer, infrared spectrophotometer (FTIR), X-ray diffraction (XRD) and transmission electron microscope(TEM). After connecting the produced gold nanoparticles to paclitaxel drug, this connection was confirmed by visible light spectrophotometer and infrared spectroscopy techniques (FTIR). Finally, by performing a cytotoxicity test on the MCF7 breast cancer cell line, the effect of the test solution was investigated. Finally, by performing ICP-OES test, the amount of nanoparticles entered into the cells of the culture medium was checked.

**RESULTS AND DISCUSSION:** The gold nanoparticles produced by *Fusarium oxysporum* were spherical and hexahedral with an average size of 25 nm, which had crystalline properties. Paclitaxel as an anticancer drug was well attached to the nanoparticles. ICP-OES test studies showed that the gold nanoparticles enter the cells of the cell line MCF7 has been imported. The study of cell viability showed that the drug conjugated with nanoparticles and paclitaxel alone had the appropriate lethal effects on the cells and these effects were much better for the conjugated drug than the usual drug and more cytotoxicity was applied by attaching the drug paclitaxel to gold nanoparticles.

**Keywords:** Gold nanoparticles, Paclitaxel, *Fusarium oxysporum*, MCF7 cell line

# Investigating the Lethal Effects of SARS-CoV-2 Protein on Lung Cancer Cells

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** Coronavirus, known for its single-stranded RNA genomes and four major proteins, including surface glycoprotein or protein S, envelope protein E, matrix protein M, and nucleocapsid protein N, is one of the two subunits of protein S involved in binding to the host cell. Also, there are two main forms of lung cancer: non-small cell lung cancer, or NSCLC (85% of patients), and small cell lung cancer (SCLC) (15% of patients). Because lung cancer is the second-deadliest cancer in the world. In this research, we try to review the basic information for new strategies for lung cancer treatment. Production of new drugs based on recombinant proteins that have not been presented in the world so far and using the recombinant protein RDB of coronavirus.

**MATERIALS AND METHODS:** Recombinant RBD protein was prepared from the RBD region, which spans residues 319–541 of the spike of the SARS-CoV-2 wild-type variant (Gen Bank accession number MN908947), and SKLC6 lung cancer cells were obtained from the Pasteur Institute of Iran. The cells were seeded and treated with different stock concentrations of 126 µg/mL, 63 µg/mL, 31.5 µg/mL, 15.75 µg/mL, and 7.875 µg/mL. The MTT assay was performed for 24 hours at the mentioned concentrations, and p-value statistical analysis was performed.

**RESULTS AND DISCUSSION:** RBD protein extracted and purified from SARS-CoV-2 wild-type variant has shown high cytotoxicity and lethal effects on SKLC6 lung cancer cells. Remarkably, the greatest lethal effect was observed at a concentration of 126 µg/mL, while the toxicity effect was significantly observed up to a concentration of 15.74 µg/mL. Interestingly, at a dilution of 7.875 µg/mL, RBD protein was not lethal to lung cancer cells. This gradient highlights the potential effects of the RBD protein as a targeted therapeutic agent. By conducting further research on its effects on cancer cells, researchers can understand the pathways through which it causes cell death. This could lead to the discovery of new agents for the treatment of lung cancer and other deadly cancers. Studying the mechanisms of this protein may pave the way for new and more effective cancer treatments.

**Keywords:** lung cancer, coronavirus, COVID-19, treatment, RBD, S protein, MTT, purified

# **Mycoplasma hominis as one of the potential causes of prostate cancer...?**

**Use of Bacteria in Cancer Therapy**

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**BACKGROUND AND OBJECTIVES:** *Mycoplasma hominis*, an opportunistic pathogen in human genitourinary tract, can cause chronic infection in the prostate. Intracellular survival of *M. hominis* leads to a prolonged presence in the host cells that can affect the cell's biological cycle. In the present study, we aimed to evaluate the frequency of *M. hominis* DNA in prostate tissue of Iranian patients with prostate cancer (PCa) in comparison to a control group with benign prostatic hyperplasia (BPH).

**MATERIALS AND METHODS:** This research was a retrospective case-control study using 61 archived formalin-fixed paraffin-embedded (FFPE) blocks of prostate tissue from patients with PCa and 70 FFPE blocks of patients with BPH. Real-time PCR, targeting two different genes, 16S rRNA and *yidC*, in the *M. hominis* genome was performed for all specimens.

**RESULTS AND DISCUSSION:** Out of 61 blocks of prostate biopsy from patients with PCa, eight samples (13%) were positive for *M. hominis*, while the bacterium was not detected in any of the 70 blocks of patients with BPH (P value, 0.002). The high frequency of *M. hominis* in patients with PCa likely shows a hidden role of the organism in prostate cancer during its chronic, apparently silent and asymptomatic colonization in prostate.

**Keywords:** real-time PCR; prostate; prostatitis; benign prostatic hyperplasia; carcinogenic; *yidC*; sexually



# Novel pseudotherapy in Prostate Cancer: Extraordinary Effect of Extracellular Vesicles from *Clostridium Perfringens*

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** Extracellular vesicles (EVs) are nano- to micron-sized vesicles that can carry biological cargo. All cell types can secrete EVs, which have been observed to be involved in various vital cell mechanisms. There are different methods for isolating EVs that have advantages and disadvantages. The purpose of this study was to investigate the ultra-centrifuge method in the isolation of EVs and its effect on the treatment and recovery of prostate cancer.

**MATERIALS AND METHODS:** *Clostridium perfringens* ATCC13124 was used in this experimental study. After cultivation, EVs were extracted by ultracentrifugation. To check chemical properties, EV protein concentration was determined using NanoDrop device, and EV protein pattern was performed using SDS-PAGE technique. Transmission electron microscope (TEM) was used to investigate the physical properties of EVs. Also, specialized MRI examinations on cancerous prostates as well as serological and biochemical tests such as PSA were also measured. The results were also checked by LC/MS.

**RESULTS AND DISCUSSION:** Our results showed that EVs isolated by ultracentrifugation method had higher protein content compared to non-ultracentrifugation method (3.17 and 1.46 µg/ml, respectively). which has been much more effective in the treatment of prostate cancer through molecular pathways, up to 87% and compared to similar treatment methods. The ultracentrifuge method separated more and larger EVs than the non-ultracentrifuge method. Also, the protein patterns of EVs by SDS-PAGE method were similar in both methods. The result of the present study showed that isolation of EVs obtained from *Clostridium perfringens* can have great effects in the treatment of patients with prostate cancer malignancy in the direction of improvement and reduction of inflammation.

**Keywords:** Prostate Cancer, Extracellular Vesicles (EV), Ultracentrifugation, *Clostridium Perfringens*

# Pathotyping of mucosa-associated *Escherichia coli* strains from colorectal cancer patients and healthy subjects

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** The results of recent studies have shown that some mucosa-associated *Escherichia coli* pathotypes can be involved in the pathogenesis of colorectal cancer (CRC). The aim of this study was to compare the prevalence of mucosa-associated *E. coli* pathotypes from CRC patients and healthy controls.

**MATERIALS AND METHODS:** In a cross-sectional study, a total of 93 mucosa-associated *E. coli* strains, 56 from CRC patients and 37 from healthy subjects were enrolled in this study from two referral university-affiliated hospitals in northwest Iran from July 2019 to July 2020. Pathotypes of mucosa-associated *E. coli* strains were detected by examining the presence of the following genes: *eae* and *bfpA* for enteropathogenic *E. coli* (EPEC), *vt1* and *vt2* for enterohemorrhagic *E. coli* (EHEC), *estA* and *eltB* for enterotoxigenic *E. coli* (ETEC), *pCVD432* for Enteroaggregative *E. coli* (EAEC) and *ial* for Enteroinvasive *E. coli* (EIEC).

**RESULTS AND DISCUSSION:** The results of the pathotyping revealed that the frequency of EPEC was significantly higher in CRC patients (23.2%) than in healthy controls (2.7%) ( $p < 0.05$ ). Moreover, the EAEC was detected in 19.64% of the strains of CRC patients. However, other pathotypes were not observed in the *E. coli* strains of both groups. *E. coli* strains belonging to EPEC and EAEC pathotypes were isolated more frequently from the gut of CRC patients, so they can be involved in the pathogenesis of CRC.

**Keywords:** *E. coli*, colorectal cancer, EPEC, EAEC

# Study of the gastric microbiota population in patients with gastric precancerous lesions using metagenomics method

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** Gastric cancer is the fifth most common cancer worldwide. The term "Correa cascade" refers to the process by which normal gastric conditions progress to precancerous and then cancerous conditions. Several researches have shown the significant impact of gastric microbiota on this process. Despite global advancements, there have been limited studies in Iran examining this correlation. The aim of this study is to investigate the gastric microbiota of Iranian individuals with atrophic gastritis, a precancerous stage, using the 16S metagenomics method.

**MATERIALS AND METHODS:** Gastric biopsy samples were collected from five Iranian patients using gastric endoscopy and stored in RNA-later at -70°C. Pathology tests confirmed that recruited individuals had atrophic gastritis. DNA was extracted from the biopsy samples and after performing quality control tests, were sent for 16S metagenomic sequencing analysis with Illumina system. Bioinformatics and statistical analyses were performed by the CLC Workbench 22 software using Greengenes as database.

**RESULTS AND DISCUSSION:** Metagenomic analysis revealed that 83% of the bacteria belong to the phylum Firmicutes in recruited samples, all of which are of the class Bacilli. Specifically, 54% and 45% are from orders Lactobacillales and Staphylococcales, respectively. In Lactobacillales order, 52% are of family Entrococcaceae, with the majority being of genus Entrococcus. As some Enterococcus species have been shown to be driver bacteria in the occurrence and development of colorectal cancer, their presence among gastric microbiota in atrophic patients needs further study. This research enhances our understanding of the microbial population in atrophic conditions, providing insights that could improve diagnosis and treatment. Future studies should include a larger cohort to validate these findings and compare the microbial populations of this study with normal individuals and cancer patients. Exploring the diversity and functional aspects of these microbial communities will further elucidate their roles in atrophy progression and potential implications for gastric cancer.

**Keywords:** Gastric microbiota - Atrophic gastritis - Metagenomics

# The Anti Cancer Effects of Mesenchymal Stem Cells (MSCs) Loaded with Coxsackievirus A21 on Mouse Models of Breast Cancer

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** Background: Cancer is a leading cause of death globally, with breast cancer (BC) being the second most prevalent type among women. Additional treatments, such as chemotherapy and radiation therapy, are often recommended. Due to challenges like drug resistance and a shortage of targeted therapies, developing new techniques is crucial. Objective: This study investigated the effects of mesenchymal stem cells (MSCs) loaded with oncolytic Coxsackievirus A21 (CVA21) on a mouse model of BC.



**MATERIALS AND METHODS:** Methods: The therapeutic efficacy of MSCs loaded with oncolytic CVA21 was assessed in an experimental mouse model of BC, where each mouse received a subcutaneous injection of 4T1 cells. Various evaluations were conducted, including the splenocyte proliferation index, lactate dehydrogenase (LDH) assay, nitric oxide (NO) production assessment, and cytokine assays (IFN- $\gamma$ , IL-4, IL-10, and TGF- $\beta$ ) in the splenocyte supernatant.

**RESULTS AND DISCUSSION:** Results: The study revealed that treating the mouse model of BC with MSCs loaded with oncolytic CVA21 significantly suppressed tumor growth. This suppression was accompanied by stimulation of the splenocyte proliferation index and increases in NO and LDH levels. Additionally, MSCs loaded with oncolytic CVA21 enhanced the secretion of IFN- $\gamma$  while reducing the secretion of IL-4, IL-10, and TGF- $\beta$ . Discussion: The findings of this study suggest that MSCs loaded with oncolytic CVA21 therapy could offer potential benefits for BC treatment in mouse models. Besides activating the acquired immune system, this approach also stimulates the innate immune system by increasing nitric oxide levels.

**Keywords:** Keywords: Anti-tumor immunity; 4T1 cell line; Coxsackievirus A21; Breast cancer;

# The association between fecal microbiota and early-stage detection of colorectal cancer with a focus on anti-inflammatory effects of extracted postbiotics

## Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** Colorectal cancer (CRC) ranks as the third most common cancer globally. The human gut microbiota maintains a complex microbial ecosystem, dysbiosis of which has been linked to sporadic CRC. This study aimed to quantify and compare three probiotic bacteria (*Bifidobacterium breve*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*) and three non-probiotic bacteria (*Streptococcus bovis*, *Porphyromonas gingivalis*, *Enterococcus faecalis*) in fecal microbiomes of 25 CRC patients and 25 healthy controls. Additionally, it assessed the anti-inflammatory effects of postbiotics extracted from these probiotic bacteria on colorectal cancer cell line (HT-29 cells).

**MATERIALS AND METHODS:** Fifty volunteers, (25 CRC patients, and 25 healthy controls, all over 65 years old and undergoing standard screening colonoscopy) were recruited for this study. Fecal samples were collected from each participant, and DNA extraction was performed. Specific primers for each bacterium were provided, and the selected bacteria were quantified in the fecal microbiome of each group, using absolute quantification real-time PCR (absolute qRT-PCR). Additionally, cell-free supernatant derived from cultures of three probiotic bacteria was extracted to obtain postbiotics. The effect of these postbiotics on the expression of IL-6 and TNF- $\alpha$  genes in the HT-29 colorectal cancer cell line was evaluated through real-time quantitative PCR (RT-qPCR).

**RESULTS AND DISCUSSION:** The results showed that CRC patients exhibited higher levels of *S. bovis*, *E. faecalis*, and *P. gingivalis* compared to healthy controls (p-value 0.001), alongside lower levels of probiotic bacteria including *B. breve*, *L. rhamnosus*, and *L. acidophilus* (p-value 0.001). On the other hand, while each bacteria exhibited significant differences between the CRC and healthy groups, no significant correlation was observed within each group between bacterial counts and gender. Additionally, postbiotics extracted from probiotic bacteria significantly reduced IL-6 and TNF- $\alpha$  expression in HT-29 cells, notably stronger with *L. acidophilus* postbiotics. The study underscores significant bacterial population differences between CRC patients and healthy individuals, suggesting microbial imbalance as a potential early biomarker for CRC detection. Gender had no significant influence on bacterial counts, indicating disease-specific variations. The observed anti-inflammatory effects of probiotic-derived postbiotics suggest their potential therapeutic role in managing CRC-associated inflammation, possibly explaining their lower levels in CRC patients.

**Keywords:** Microbiota, Colorectal cancer, Postbiotic, IL-6, TNF- $\alpha$

# The Cytotoxicity Effect of Recombinant Staphylococcal Enterotoxin B on Ovarian and Breast Cancer Cells

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** Background: Ovarian cancer and Breast cancer (second cause of cancer death) are lethal cancer in women and can develop chemoresistance; thus, investigating new approaches to obtain an effective therapeutic agent for treating this life-threatening condition is critical. Objective: The current study aimed to assess the anti-tumor effect of the recombinant Staphylococcal Enterotoxin B (R-SEB) on ovarian and breast cancer in vitro.

**MATERIALS AND METHODS:** Methods: The cytotoxic effects of R-SEB against OVCAR-4, MDA-MB-231, and HEK 293 cells were evaluated by MTT assay. The potential apoptosis induction of R-SEB was assessed using the Annexin V-FITC kit. The Matrigel invasion test was used to evaluate the ability of R-SEB to reduce OVCAR-4 invasion. The expression measurement of genes involved in angiogenesis, apoptosis, and metastasis was evaluated using qPCR.

**RESULTS AND DISCUSSION:** Results: R-SEB showed a high cytotoxic effect against OVCAR-4 and MDA-MB-231 cells in a dose-dependent manner without cytotoxic influence on HEK 293 cell lines. In addition, R-SEB has great apoptosis-inducing potential in OVCAR-4 and MDA-MB-231 cells via the activation of caspase-3 and elevation of the Bax/Bcl-2 ratio. R-SEB significantly decreased the expression level of angiogenesis-related genes. In addition, R-SEB inhibited OVCAR-4 and MDA-MB-231 cells' adhesion and invasion. Discussion: R-SEB may eventually play an essential role in developing effective therapy against ovarian and breast cancer in humans; towards reducing the overall morbidity and mortality associated with combating cancer.

**Keywords:** Keywords: R-SEB, ovarian cancer, breast cancer, angiogenesis, apoptosis, anti-tumor.

# The Impact of Probiotic Bacteria on Gene Expression and Apoptosis in Cancer Cells

Use of Bacteria in Cancer Therapy

Mohammad amin Yaghoubi<sup>1</sup> © P

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**BACKGROUND AND OBJECTIVES:** Cancer remains one of the leading causes of death worldwide, with gastric cancer being particularly prevalent. A promising area of research is the use of probiotics, which are beneficial bacteria that can influence various biological processes. This essay explores the effects of specific probiotic strains, *Lactobacillus casei* and *Lactobacillus fermentum*, on gene expression and apoptosis in gastric cancer cells, particularly focusing on the genes Bcl-2, BAX, PTEN, and Akt1.

**MATERIALS AND METHODS:** survival of AGS cells after culturing cells with different concentrations of the two probiotics was assessed by MTT assay at three times of 24, 48 and 72 hours and the effect of these bacteria on the expression of four genes involved in apoptosis including Bcl-2, PTEN, Akt -1 and Bax were measured after 72 hours using real- time PCR

**RESULTS AND DISCUSSION:** The results of this study showed that the highest lethality was due to  $106 \times 19$  cfu / ml of supernatant of each bacterium. Therefore, this number of bacterial supernatants was used to study gene expression. Cell proliferation and survival were detectable at lower dilutions. The results of real-time PCR analysis showed that treatment with *Lactobacillus casei* increased the expression of Bcl-2 gene and decreased the expression of PTEN, Akt-1 and Bax, while *Lactobacillus fermentum* increased the expression of PTEN and decreased the expression of Bcl-2, Akt-1, Bax. To the control gene (GAPDH) in AGS cells. Due to the increased expression of PTEN and Box compared to Bcl-2 and Akt-1 in AGS cells, *Lactobacillus fermentum* had better results in inducing apoptosis than casei.

**Keywords:** Key words: apoptosis, lactic acid bacteria, probiotic, gastric cancer

# The Role of Pathogenic *Escherichia coli* in the Development of Colorectal Cancer

Use of Bacteria in Cancer Therapy

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**BACKGROUND AND OBJECTIVES:** Many investigations have shown a correlation between the presence of *Escherichia coli* strains containing the pks genomic island and the occurrence of colorectal cancer (CRC). The pks island harbors a polyketide synthase (PKS) enzyme which is accountable for the production of colibactin. Colibactin is a genotoxin that induces DNA damage in host cells, ultimately resulting in the development of cancer. In the present study, we investigated the role of colibactin-producing *E. coli* strain in CRC.

**MATERIALS AND METHODS:** A total of 13 stool samples from patients with colorectal cancer and 13 stool samples from control group (non-cancerous individual) were collected from Tabriz hospitals to investigate the frequency of colibactin-positive *E. coli*. The frequency of colibactin-producing strains was examined using absolute quantitative real-time PCR analysis. The frequency of these strains in patients with CRC and the control group was determined by constructing a standard curve using reference strains (*E. coli* ST131).

**RESULTS AND DISCUSSION:** The results of this study showed that the frequency of colibactin-positive *E. coli* in the patients with CRC group was significantly higher in *E. coli* strains isolated from the control group (P 0.0001). Targeting colibactin-positive *E. coli* strains containing the pks genomic island and their genotoxic metabolites offers a hopeful strategy for the prevention and treatment of CRC. Future studies should prioritize gaining a comprehensive understanding of the intricate molecular pathways underlying colibactin-induced carcinogenesis. Additionally, efforts should be directed toward devising effective strategies to impede the interaction between these pathogenic bacteria and the host. This may involve exploring the potential of probiotics, antibiotics, and dietary treatments.

**Keywords:** CRC, *E. coli*, pks, Dysbiosis, Colibactin



# The role of microbiome in CAR-T cell therapy efficacy

## Microbiome and cancer

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**BACKGROUND AND OBJECTIVES:** Redirecting autologous T lymphocytes extracted through leukapheresis towards a tumor-specific antigen, CAR-T cell therapy has lately transformed the field of cancer therapies. Significant variability in the clinical response to CAR T-cells persists, despite encouraging results. One important host element that may be altered to improve immunotherapy responses is the gut microbiota. Recent research on immunotherapy in humans has demonstrated that patients with a more diversified gut microbiome survive considerably better. Many patients suffer from serious side effects, such as immune effector cell-associated neurotoxicity syndrome (ICANS) and cytokine release syndrome (CRS) which result in severe consequences. Since antibiotics are frequently used to treat secondary infections in patients receiving anticancer therapy, it has been hypothesized that antibiotic exposure and the dysbiosis it causes could hurt the overall result of immunotherapies. Research indicates that patients who received antibiotics in the weeks before starting CAR-T therapy had a higher prevalence of ICANS.

**MATERIALS AND METHODS:** A thorough search was carried out using the terms "microbiome," "immunotherapy," "microbiota," "CAR T-cells," "antibiotics," and "dysbiosis" on databases including Medline, PubMed, and Google Scholar. The investigation's foundation was a thorough assessment of important English-language papers and review articles.

**RESULTS AND DISCUSSION:** Gut microbiota-derived peptides and metabolites impact T cells and CAR-T cells, which can be further influenced by dietary changes and/or antibiotic use. Certain species that are abundant in the gut microbiome help treatments work better, but dysbiosis causes side effects including CRS and ICANS, accelerates the progression of the disease or tumor recurrence, and reduces overall survival. For example, a high-fiber diet stimulates the synthesis of butyrate, propionate, and acetate, all of which have been connected to mechanisms that reduce inflammation. Through a number of mechanisms, such as increased TNF-alpha and IFN-gamma effector responses, as well as upregulating anti-inflammatory T regs and T CD8+ cell functions while decreasing pro-inflammatory macrophage, dendritic cell, and Th1/Th17 activities, microbially derived SCFA may, in fact, have a positive impact on multiple immunotherapies. Also, one potentially interesting approach for optimizing treatment may involve targeted suppression of microorganisms linked to increased prevalence of CRS.

**Keywords:** Immunotherapy, CAR T-cells, microbiota, Gut microbiome, dysbiosis

# Microbiological and molecular study of paranasal sinus infections of children with malignancy and unknown origin fever in Markazi province of Iran.

**Bacterial infections in cancer: A bilateral relationship**

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**BACKGROUND AND OBJECTIVES:** The susceptibility of children with malignancies to various infections is well-known. Thus, the objective of this research was to investigate the bacterial species accountable for sinusitis in children with malignancy and unexplained fever, and determine their susceptibility to antibiotics.

**MATERIALS AND METHODS:** The study involved collecting 90 sinus samples from children aged 5-15 years with malignancy in Arak city. The isolates were identified using a combination of phenotypic, biochemical, and molecular techniques, including specific PCR and 16S rRNA gene sequencing. drug susceptibility testing was performed following the CLSI 2021 guidelines.

**RESULTS AND DISCUSSION:** A total of 36 isolates (40%) were obtained, including 4 isolates of *Nocardia* (11.12%), 4 isolates of *E. coli* (11.12%), 3 isolates of *K. pneumoniae* (8.33%), 5 isolates of *P. aeruginosa* (13.88%), 3 isolates of *A. baumannii* (8.33%), 4 isolates of *S. aureus* (11.12%), 3 isolates of *S. epidermidis* (8.33%), 5 isolates of *S. agalactiae* (13.88%), 2 isolates of *S. pneumoniae* (5.55%), and 3 isolates of *E. faecium* (8.33%). The isolates showed the most sensitivity to imipenem and trimethoprim-sulfamethoxazole and the least sensitivity to erythromycin and tetracycline. The findings of the study indicate that sinusitis can contribute to fever of unknown origin in cancer patients. Therefore, it is recommended to use a combination of molecular and phenotypic methods for accurate identification of isolates. This approach can provide more reliable and precise results, leading to better diagnosis and treatment of sinusitis infections in children with malignancy.

**Keywords:** malignancy, unknown origin fever, 16SrRNA

# An Indirect ELISA Assay for characterization of epsilon toxoid in different batches of clostridial vaccines

Bacterial Vaccine

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**BACKGROUND AND OBJECTIVES:** Enterotoxemia is a condition that affects sheep and goats, that is frequently severe and can affect animals of all ages. It most commonly affects milk producer and suckling feeder lambs between 3 and 10 weeks of age. It is characterized by the production of toxins by the bacteria, which can lead to a variety of symptoms like acute indigestion, convulsions and other nervous system signs, colic, and sudden death and can be fatal if left untreated. Enterotoxemia vaccine is used for providing prophylactic protection against disease which are caused in sheep and goats. The purpose of this study is developing an indirect ELISA assay for identification of *C. Perfringens* epsilon toxoid in order to evaluate the quality of veterinary vaccines.

**MATERIALS AND METHODS:** For designing this indirect ELISA, the antigen is bound to the bottom of the microplate wells, then an antibody specific to the antigen is added. A secondary antibody conjugated to an enzyme, is then bound to the first antibody. Micro titer plates coated with 20 diluted supernatant sample of different batches of epsilon toxoid produced by the Laboratory of Vaccine production overnight at 4° C. Blocking was done with bovine serum albumin (BSA) and then the proper dilutions of standard epsilon antitoxin was added, in next stage goat anti-rabbit IgG peroxidase was added. All incubations were at 37° C for one hour and plates washed three times after adding each new reagent, after adding TMB and stop solution and developing the colour the absorbance was measured at 450 nm using a spectrophotometer. Toxoid detection was done in all samples by using the standard curve. Epsilon toxoid concentration can be calculated.

**RESULTS AND DISCUSSION:** It is necessary to have a validated method capable of evaluating the presence and quantity of epsilon toxoid as an inactivated antigen. Additionally, we sought to determine the concentration of the toxoid after inactivation in order to formulate the vaccine appropriately and also ELISAs can be a simpler, faster and lower-cost method by comparison with *in vivo* methods that measure toxoid concentration like TCP (Total combining power) that would significantly reduce laboratory animal usage.

**Keywords:** toxoid- Elisa- formulate-C. Perfringens

# Cloning and expression of the beta toxin gene (cpb) of *Clostridium perfringens* type B in *Escherichia coli*

Bacterial Vaccine

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**BACKGROUND AND OBJECTIVES:** Toxins are desired antigens for different purposes like toxoid vaccine production and diagnosis. Beta toxin (BTX) is one of the most important clostridial toxins, which is a pathogenic factor of type B of *Clostridium perfringens*. This species needs expensive nutrients and long-lasting culturing for toxin production. This study aimed to produce recombinant BTX by transforming *E. coli* as an alternative way for toxin production.

**MATERIALS AND METHODS:** The complete sequence of BTX (cpb) was isolated from type B of *C. perfringens* by specific primers and an optimized PCR method. These segments were introduced to the pET22b (+) vector, which was prepared for cloning by restricted enzymes. After the transformation in *E. coli*, BTX production was induced by IPTG. The transformed cells and their protein extractions were analyzed through PCR, SDS-PAGE, and Western-Blotting. Additionally, the toxic activity of this toxin was investigated in vivo.

**RESULTS AND DISCUSSION:** PCR results of the DNA extraction of the transformed cells confirmed the presence of the full cpb gene (1030 bp). The results of SDS-PAGE showed that the recombinant toxin was expressed successfully as a bold band was seen at 37 kDa. Also, the obtained toxin was diagnosed with specific anti-toxin. These results prove a successful approach to obtaining the recombinant BTX with similar characteristics to wild BTX. Recombinant toxins offer several advantages over wild toxins; as they keep the antigenic properties of the toxin, production and extraction process is more efficient and economical than the conventional ones. We create a transformed species and obtain recombinant BTX, which can be used for vaccinal and diagnosis purposes.

**Keywords:** *Clostridium perfringens* type B; *Escherichia coli*; Beta toxin (BTX); Cloning

# Cloning of the beta toxin gene from *Clostridium perfringens* type C and its expression

Bacterial Vaccine

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**BACKGROUND AND OBJECTIVES:** *Clostridium perfringens* type C causes intestinal diseases in humans or livestock, which can be fatal. This bacterium produces two major toxins, alpha and beta toxins. Beta toxin is one of the most important clostridial toxins. This study aimed to produce recombinant beta toxin by transforming *E. coli* as an alternative way for toxin production.

**MATERIALS AND METHODS:** The partial sequence of beta toxin was synthesized from type C of *C. perfringens* by specific primers and PCR method. Then, it was introduced to the pET22b (+) vector, which was prepared for cloning by restricted enzymes. After the transformation into the *E. coli*, protein production was induced by IPTG, 0.5 mM. The clones were analyzed through PCR and their protein extracts analyzed through SDS-PAGE and Blotting.

**RESULTS AND DISCUSSION:** PCR results confirmed the presence of the beta toxin gene (952 bp). SDS-PAGE results showed that the recombinant toxin was expressed successfully as a bold band was seen at 35 kDa. Also, recombinant toxin was diagnosed with specific beta antitoxin (NIBSC, UK) in blotting. The beta toxin is classified among the four fatal toxins of *C. perfringens*, but due to its low stability, the immunological and antigenic studies encounter limitations. Here, we produced a short segment of beta toxin through recombination, lighter than wild-type toxin, while its stability to proteolytic cleavage was increased.

**Keywords:** *Clostridium perfringens* type C; Beta toxin; Cloning; Expression

# Comparison of Changes in the Absolute Number of Neutrophils After Inoculation of Rev1 Vaccine in Female Lambs Receiving Selenium Selenite

## Bacterial Vaccine

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**BACKGROUND AND OBJECTIVES:** The present study was conducted with the aim of investigating the effects of the combination of vitamin E and selenium on the absolute number of neutrophils after inoculation with Rev1 vaccine in female lambs.

**MATERIALS AND METHODS:** For this purpose, 15 number of 2-month-old female sheep were divided into three control groups, treatment 1 and 2 completely randomly, then to treatment group 1, a combination of vitamin E and selenium was injected subcutaneously on two occasions with an interval of 15 days, and one day after the second injection, both treatment groups 1 and 2 were vaccinated with Rev1 vaccine. Sheep in the control group were injected with distilled water. Blood samples were taken from sheep on days 0 (before vaccination), 1, 7, 14, 21 and 60 days after injection, and the absolute number of neutrophils was counted.

**RESULTS AND DISCUSSION:** The results of the Rose Bengal, Wright and 2ME tests indicated that from the 7th day, the amount of antibody production increased in both treatment groups, and at the same time, the number of neutrophils increased until the 14th day, and from that day onwards, the number of They were reduced. The important thing is that in the group receiving the selenium supplement and the vaccine (treatment 1), we saw a significant increase in the number of neutrophils compared to the control group and treatment 2 (receive the vaccine alone) on the same days, which shows the positive effect of sodium selenite on the immune system. and increased response to foreign antigen (Brucella).

**Keywords:** Brucella, Sheep, Vaccine, Immunity, Neutrophil

# Comparison of Two Vaccination Methods Using Chitosan-Containing Oral Vaccine and Immersion as Inactivated Bacteria Against *Streptococcus iniae* in Guppy Fish

## Bacterial Vaccine

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**BACKGROUND AND OBJECTIVES:** *Streptococcus iniae* is one of the most important pathogenic bacteria in farmed fish. This disease causes significant economic losses all over the world. Due to the problem of antibiotic resistance, the best control method is the use of a suitable vaccine. Vaccination methods in fish can be conducted through injection, immersion, and oral administration. Oral vaccine is very desirable due to easy administration and stress reduction. In this regard, our goal in this project is to evaluate an oral vaccine against *Streptococcus iniae*. For this purpose, guppy fish was used as an animal model to prepare a suitable and evaluable oral vaccine.

**MATERIALS AND METHODS:** Forty-five fish were divided randomly into three groups (15 fish/group) included: control, oral and immersion, each group divided to triplicates of 5 fish. Fish were challenged with a dose of  $5 \times 10^8$  CFU of *Streptococcus iniae* bacteria and mortality was recorded for 30 days thereafter.

**RESULTS AND DISCUSSION:** The mortality rate includes 54.33%, 33.33% and 100% in immersion, oral and control groups, respectively. ( $P < 0.05$ ). The obtained results showed that the oral and immersion vaccines significantly reduce casualties compared to the control group, and the oral vaccine had a significantly better survival rate than the immersion method. Overall, this project showed that the use of oral vaccine can effectively protect guppies against the lethal challenge of *Streptococcus iniae*. This is a very important achievement that can be used as a suitable model for further research in the field of preparing oral vaccines.

**Keywords:** *Streptococcus iniae*, Oral vaccine, Immersion vaccine, Guppy fish

# Design and construction of the triple recombinant protein and its immunogenic evaluation against bacteria that cause diphtheria, tetanus and pertussis in animal model

## Bacterial Vaccine

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**BACKGROUND AND OBJECTIVES:** Bordetella pertussis toxin subunit 1 (PTXa), Corynebacterium diphtheria toxin (Tox) and Clostridium tetani tetanus toxin (TetX) were studied by bioinformatics to create a recombinant protein as a multi-epitope. In this multi-epitope synthesis study, the gene with serotype DE3 was transformed in E. coli BL21 bacteria with heat shock. Gene expression and induction of protein expression were performed by IPTG inducer (isopropyl beta-thiogalactopyranoside) and gene expression was confirmed by SDS-PAGE of the desired band. The wall of bacteria was broken by sonicator. recombinant protein with His tag and nickel resin chromatography was performed, as well as western blot confirmation test. Bradford method and then the purity of the expressed protein was evaluated using SDS-PAGE.

**MATERIALS AND METHODS:** the gene with serotype DE3 was transformed in E. coli BL21 bacteria with heat shock. The recombinant plasmid was extracted and purified by a Canadian kit. Gene expression and induction of protein expression were performed by IPTG inducer (isopropyl beta-thiogalactopyranoside) and gene expression was confirmed by SDS-PAGE of the desired band. The wall of bacteria was broken by sonicator. Purification of the recombinant protein with His tag and nickel resin chromatography was performed, as well as western blot confirmation test. The protein concentration was determined by the Bradford method and then the purity of the expressed protein was evaluated using SDS-PAGE. Dialysis was performed to reduce protein concentration and buffer exchange. According to the scheduled protocol, the mice were treated in 8 groups of ten in the injection table, and after the completion of the time required for immunization, the mice were

**RESULTS AND DISCUSSION:** Aminian et al. (2007) used the fragment s1 toxin Bordetella pertussis and the toxin Corynebacterium diphtheria and Clostridium tetani. The above research in the field of triple recombinant protein is somewhat similar to the present study. The expression was performed at 37 and 22 centigrade temperatures and confirmation and purification were also confirmed. The western blot test confirmed the presence of the recombinant protein. The results of immunological and serological tests such as ELISA showed significant results in 10 micrograms of received protein.

**Keywords:** Recombinant protein, Corynebacterium diphtheria toxoid, Clostridium tetani toxoid, Bordetella pertussis



# Design and safety measurement of PPE68+ppe44+HSP70+FC Subunit Cure against Mycobacterium infection Contamination distinguished accompanying BCG

Bacterial Vaccine

Azar valizadeh <sup>1</sup> © P

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**BACKGROUND AND OBJECTIVES:** On account of the restricted influence of the current BCG cure against infection contaminations indifferent parts of the planet, the reasons for allure loss maybe noticed in the decrease and imbalance of allure influence against pulmonary infection in two together juveniles and women. Seeing the heavy challenges formal for one BCG in the fight against contamination, the design and the result of new and alive vaccines against contamination are a critical need

**MATERIALS AND METHODS:** The constructs were cloned into the expression vector of pET28a, resulting in the recombinant construct of PET28a(PPE68+ppe44+HSP70+FC), and then transformed into E. coli (DE3). Following the administration of antigens and stimulation of lymphocytes and minor control to measure the levels of INF- $\gamma$ , IL-12, IL-4, and IgG2a in the antigens, it was examined by ELISA.

**RESULTS AND DISCUSSION:** They had an increase in the key cytokines INF- $\gamma$  and IL-12, IL-4 in the group immunized with BCG and vaccine compared to the group vaccinated with (PPE68+ppe44+HSP70+FC) and Control groups BCG, PBS. Specific IgG1 showed an increase in the experimental groups compared to the control groups; a significant difference was observed in the vaccinated groups and the difference between the groups (PPE68+ppe44+HSP70+FC) with Control groups BCG, PBS. The purpose of this study was to show and search for the answer if the recombinant protein (PPE68+ppe44+HSP70+FC) has the ability to induce Th1 and Th2 responses. The results of the studies showed that it induced a strong immune response

**Keywords:** PPE68+ppe44+HSP70+FC

# Determination of Immunological properties of *Pseudomonas aeruginosa* PA103, by Serum Bactericidal Assay & ELISA

Bacterial Vaccine

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* is a gram negative, reknown opportunistic, and one of the most prevalent multi-drug resistant pathogens. This bacterium has a very low infection dosage. Exotoxin A (ExoA) is one of the most reknowned virulence factors of *Pseudomonas aeruginosa*. This toxin is the cause of ADP-ribosylation in eukaryotic Elongation Factor-2 (EF-2), which results in protein synthesis inhibition. Recent studies had shown immunostimulational characteristics of detoxified ExoA. Our aim in this study was to evaluate the immunological properties of detoxified ExoA by Serum Bactericidal assay, in comparison with ELISA.

**MATERIALS AND METHODS:** The strain used in this study was *Pseudomonas aeruginosa* PA103, provided by Pasteur institute of Iran. This strain is pigment-less and produces high concentrations of Exo A. After culture in semi-industrial scale, it was detoxified and purified by dialysis. The dialysate was injected to mice and rabbit. After 3weeks, the total sera were collected. Serum Bactericidal Assay & ELISA were performed.

**RESULTS AND DISCUSSION:** Exo A has reduced the colony forming units in mice infected by *Pseudomonas aeruginosa* PA103. The final bactericidal titre of anti-IgG antibody against exo A in the first injection was 1:16 and in the last injection had risen to 1:32. With the rise of multi-drug-resistant *Pseudomonas aeruginosa*, it is crucial to consider a safe factor such as Exo A or more factors as a combined or conjugated vaccine, either prophylactic or therapeutic, for this nosocomial infection. In case of determining immunological properties of Exo A, SBA has much higher advantages than ELISA. It can be used as the main candidate for vaccine against this pathogen or as a hapten. Many other subunits from other bacteria or viruses could be conjugated on Exo A and form multi-target vaccines.

**Keywords:** Exotoxin A, *Pseudomonas aeruginosa*, Serum Bactericidal, Assay

# Inhibition of HPV virus by designing antibody against L1 ligand

Bacterial Vaccine

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**BACKGROUND AND OBJECTIVES:** Human papillomavirus (HPV) is a double-stranded DNA virus that infects the human squamous epithelium. HPV is responsible for 99.7% of cervical cancer cases and ranks as the second most common type of cancer in women worldwide. Consequently, preventing HPV infections can reduce the incidence of cervical cancer, as well as genital warts and associated costs. The main capsid protein of the papillomavirus is known as L1. Research indicates that the virus cannot initially attach to keratinocytes in the body; instead, it must first connect with heparan sulfate proteoglycans (HSPGs) through its L1 protein. Blocking this step can prevent the virus from binding and entering the host cell. In this study, we aim to investigate the inhibition of L1 and, ultimately, the prevention of virus entry through antibody design.

**MATERIALS AND METHODS:** The FASTA sequences of the HPV virus ligand and its cellular receptor were evaluated using the UniProt and NCBI databases. The ligand and cell receptor were modeled using SWISS-MODEL. In the final step, the docking of the ligands and antibody was performed using ClusPro.

**RESULTS AND DISCUSSION:** Based on the findings from bioinformatics analysis, the constructed structure exhibits exceptional binding affinity towards the virus ligand, effectively impeding the viral integration into host cells. Conclusion: The designed structure can effectively bind to its viral ligand, thereby preventing the virus from adhering to and entering the cells. This antibody shows potential as a viable vaccine candidate against HPV, although further studies and clinical trials are required

**Keywords:** Human papillomavirus , HPV, L1 , HSPGs

# Preparation and evaluation of sodium alginate nanoparticles containing diphtheria toxoid (CRM197) and its immunogenicity in mice

## Bacterial Vaccine

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**BACKGROUND AND OBJECTIVES:** Development of the recombinant vaccines against infectious disease is based upon the identification of immunogenic antigens and vaccine delivery systems such as polymeric nanoparticles that are able to stimulate immune responses similar to or better than conventional vaccines and reduce complications associated with traditional vaccines. At the present study, synthesis and properties of the sodium alginate nanoparticles carrying cross- reacting materials (CRM197) protein as an antigen delivery system were evaluated.

**MATERIALS AND METHODS:** Synthesis of the blank optimized without protein loading and protein- containing nanoparticles was performed by ionic gelation method. After designing of the experiment (DoE) and determining the influential physicochemical factors in ideal nanoparticles synthesis for instance size, zeta potential, morphology, encapsulation efficiency, release pattern and immunogenicity in mice via measuring IgG antibodies titer and iso-types were investigated.

**RESULTS AND DISCUSSION:** The average nanoparticle size for blank and CRM197 loaded nanoparticles were 88 and 245 nm also zeta potential -21.2 and -24.2 mV, respectively. Also, LE and LC were respectively 80% and 20% and associated with stable and relatively long- term release, without any bursting pattern, of the protein CRM197 encapsulated in alginate nanoparticles. In vivo studies showed the formulated CRM197 with sodium alginate nanoparticles significantly enhanced immunogenicity in mice immunized with the CRM197-loaded sodium alginate nanoparticles with high levels of total anti-CRM197 IgG and IgG1 and IgG2a sub-classes than the conventional vaccine. Conclusion: Based upon the above achievements, alginate nanoparticles can be employed as an antigen delivery system for targeted and enhanced delivery with controlled and slow release of the recombinant diphtheria antigen (CRM197) for immunization against diphtheria disease.

**Keywords:** CRM197, Antigen delivery system, Sodium alginate nanoparticles, Ionic- gelation

# Recombinant bacteria; An alternative strategy to produce clostridial toxins

Bacterial Vaccine

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**BACKGROUND AND OBJECTIVES:** Clostridia are anaerobic, Gram-positive (mostly) bacteria that form heat-resistant endospores. The genus *Clostridium* currently contains 204 described species, of which the main pathogenic species are botulinum, chauvoei, haemolyticum, novyi, perfringens, septicum, and tetani. Each of these is associated with various diseases of humans and animals, including botulism, tetanus, gas gangrene, black disease, blackleg, and various enteric and enterotoxemic syndromes. We discuss here an alternative strategy to produce clostridial toxins for vaccine production.

**MATERIALS AND METHODS:** Various clostridial toxin genes were synthesized by specific primers and cloned in *Escherichia coli*. Recombinant bacteria were produced by transformation and objected to toxins production. The results of protein extraction were used to evaluate in in vivo and in vitro studies.

**RESULTS AND DISCUSSION:** The results showed that the recombinant *E. coli* strains carried the considered genes, which can be expressed by IPTG induction. Compared with the natural toxins, most of the recombinant toxins showed equal biological effects in the animal model (mouse), which proves their similarity with natural ones. Recombinant toxins production offers several advantages over the conventional methods, especially in production processes. Here, by the creation of recombinant *E. coli* cells, rapid and putative processes were used for toxin production. They can be used in laboratory scale or industrial scale to produce a new generation of clostridial vaccines.

**Keywords:** *Clostridium*; *Escherichia coli*; Recombinant bacteria; Toxin; Vaccine

# The effect of glucose concentrations on Growth conditions of clostridium perfringens type D and epsilon toxin production

Bacterial Vaccine

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**BACKGROUND AND OBJECTIVES:** Most, if not all diseases produced by *Clostridium perfringens* are mediated by one or more of its powerful toxins. *C. perfringens* Type D strains are responsible for enterotoxemia, which mainly affects sheep, but also occurs in goats and cattle, due to changes in intestinal microbiota after the appearance of symptoms. Infected animals may suffer sudden death with heavy economic losses. Alpha and epsilon toxins are manufactured by these microorganisms after inactivation and are present in the composition of veterinary vaccines against clostridiosis. Immunity in sheep is readily produced by vaccination, the aim of this study is scientific study on the effect of culture conditions on *C. perfringens* type D growth and epsilon toxin production as well as scaling-up this process to optimize the bacteria culture and more importantly epsilon toxin production.

**MATERIALS AND METHODS:** Different culture parameters for the toxin production of *C. perfringens* type D were assessed to find the optimum conditions for the industrial production of clostridial vaccines. The total production of toxins was evaluated by an intravenous application of 0.5 ml of the centrifuged culture in the dilutions that trypsin was added and injected to mice, which weighed 17–22 g (2 mice per dilution). Cell concentration was accompanied by readings of the optic density at 650 nm. Different initial glucose concentrations (6/5 g/l and 13 g/l) and steps of pH adjustment on both *C. perfringens* bacteria growth and its epsilon toxin production were investigated.

**RESULTS AND DISCUSSION:** Results showed with elevation of glucose concentration of each culture from 6/5 g/l to 13 g/l greater the toxin production was observed and MLD (Minimum Lethal Dose) was increased 3 times so that with elevation of glucose concentration, the metabolism was directed to biomass and toxin production. It was found that the increasing of glucose concentration is directly related to maximal cell concentration and the highest toxin title. These data contribute to improve the process for toxin production allowing better condition to produce a toxoid vaccine that will produce with inactivation this toxin with formaldehyde.

**Keywords:** *Clostridium perfringens*- Epsilon toxin- MLD-glucose

# The effect of oncolytic virus on glioblastoma stem cells and analysis of differential gene expression, pathways and gene networks

Viral vaccines

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**BACKGROUND AND OBJECTIVES:** Recent advancements in cancer therapy have led to the development of novel treatment approaches, including oncolytic viruses, which specifically target cancer cells. This study aims to investigate the impact of the oncolytic virus Onco-VV-TT on Glioblastoma stem cells and to analyze the differential expression of genes, pathways, and gene networks associated with virus treatment.

**MATERIALS AND METHODS:** Glioblastoma stem cells (U251) were treated with Onco-VV-TT virus, and various assays were performed to evaluate cell viability, metabolic activity, and differential gene expression. Gene expression analysis was conducted using microarray data, and differential gene expression was determined using the LIMMA software package. Additionally, gene ontology analysis and pathway identification were performed using the DAVID database and KEGG.

**RESULTS AND DISCUSSION:** Treatment with Onco-VV-TT virus resulted in observable changes in the morphology of Glioblastoma cells, with significant effects observed at 48- and 72-hours post-treatment. Analysis of mitochondrial activity showed an initial increase followed by a decrease after virus exposure. Differential gene expression analysis revealed significant alterations in gene expression patterns, with a total of 7637 genes showing decreased expression and 7170 genes showing increased expression. Gene ontology analysis identified several biological processes, cellular components, and molecular functions associated with the differentially expressed genes. The findings of this study indicate that treatment with Onco-VV-TT virus leads to alterations in Glioblastoma stem cell morphology, metabolic activity, and gene expression patterns. These results provide valuable insights into the mechanisms underlying the therapeutic effects of oncolytic viruses and highlight potential targets for further investigation.

**Keywords:** Oncolytic virus, Viral vaccine, Glioblastoma stem cells, Gene expression, Pathways,

# Comparative Analysis of Antibacterial and Lytic Activities of Phage Cocktails Against Various *Pseudomonas aeruginosa* Strains and Their Applications in Food Safety

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** This study evaluates and analyzes the efficacy of three phage cocktails (cocktail phps 1, cocktail phps 2, and cocktail phps 3) in controlling various strains of *Pseudomonas aeruginosa* and explores their applications in food safety.

**MATERIALS AND METHODS:** The phage cocktails were prepared as follows: cocktail phps 1 consisting of Phps1, Phps2, Phps3, and Phps4; cocktail phps 2 consisting of Phps4 and Phps04; and cocktail phps 3 consisting of Phps01 and Phps04. The antibacterial activity of these cocktails was assessed using plaque assays on different bacterial host strains.

**RESULTS AND DISCUSSION:** The results revealed that all three phage cocktails exhibited significant lytic activity against various *Pseudomonas aeruginosa* strains, including Ps3, Ps4, Ps01, Ps04, Ps05, and Ps06. Notably, all cocktails were capable of producing clear and semi-clear plaques on the target strains, indicating their high efficacy in lysing bacterial cells. This performance underscores the potential of these phage cocktails in controlling *Pseudomonas* contamination in food products. In conclusion, the findings of this study confirm the strong potential of phage cocktails in food safety strategies for combating bacterial contamination and preventing food spoilage, highlighting the need for further development and application of these phage-based treatments in the food industry.

**Keywords:** Phage Cocktails, *Pseudomonas aeruginosa*, Antibacterial Activity, Food Safety, Contamination Control.



# Antimicrobial effects of morngah, garlic, shirazi thyme plant experts on staphylococcus aureus

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** The use of chemical preservatives is considered one of the common methods in the microbial control of food, however, nowadays it is necessary to reduce the carcinogenicity and toxicity of these methods, in order to improve the microbial health of foods and then to increase the general health level of societies

**MATERIALS AND METHODS:** First, plant extracts are prepared, for this purpose, 100 grams of ground leaves of moringa, garlic, shirazi thyme plants are ground in 100 grams of each and poured into 500 cc of 95% ethanol solvent, and the resulting solution is centrifuged. Which of the extracts of garden plants is done in concentrations of 200, 400, 800 mg. Microbial cultivation in a petri dish is done in 3 repetitions and 36 treatments, the use of Muller Hinton's culture medium and performing antibiogram by well and disk method are among their works

**RESULTS AND DISCUSSION:** Staphylococcus aureus bacteria were tested and studied against the concentrations prepared and used from moringah extract, garlic, and Shirazi thyme. The use of antibiotics is very common in most countries to treat diseases and prevent infection, and the consequences after because of its chemical synthesis and the body's resistance to antibiotics, it can threaten people's health, this led researchers to study the effect of antimicrobial agents of plants on the growth of bacteria.

**Keywords:** Antimicrobial, moringa, garlic, Shirazi thyme, Staphylococcus aureus bacteria

# Assessment of Physicochemical and Microbial Quality of Drinking Water and the Efficiency of the Water Treatment Plants Performance in Mazandaran Province

## Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Today, due to the pollution of water resources, water purification has a very important place in human life. Therefore, it is necessary to continuously evaluate the performance of water treatment plants. In order to determine the quality of drinking water, the European Union has determined *Clostridium perfringens* as one of the microbial parameters to control the quality of water consumed by humans. In this study, some important physicochemical parameters and microbial indicators of water to evaluate the effectiveness of water treatment plants were examined.

**MATERIALS AND METHODS:** In this descriptive-cross-sectional study, the output samples from Ramsar, Klardasht, Babol and Sari water treatment plants were taken seasonally and with two repetitions during the fall of 2023 to July 2024 and in compliance with the standard conditions, and the parameters of pH, TDS, Turbidity, total coliform, thermophilic coliform, heterotrophic bacteria (HPC) and *Clostridium perfringens* were tested both in the form of vegetative bacteria and in the form of spores. To determine the physicochemical parameters, calibrated HACH devices were used, and for microbial tests, the 15-fermentation method was used. Tube (MPN/100ML) and for HPC, Purple plate method was used in accordance with the standard method book. Data analysis was done with EXCELL and SPSS 19 software, and statistical tests and comparison with Iran's national standard number 1011 and 1053.

**RESULTS AND DISCUSSION:** The results showed that in none of the samples, total coliform and thermophilic coliforms and *Clostridium perfringens* were not observed either in the form of vegetative bacteria or in the form of spores, which shows the effectiveness of the treatment process. In Sari, respectively 55.6, 5.17, 2.59 cfu/1ml, average pH respectively 7.88, 7.67, 7.27 and 7.81, average turbidity respectively 0.48, 0.45, 0.90 and 0.51 NTU, the average residual free chlorine was 0.8, 1, 0.5 and 0.8 mg/l respectively, all of which were at the standard level. The output of water treatment plants in Mazandaran province are all at the level of the national standard of Iran in terms of physicochemical parameters and microbial indicators of water, which indicates that the treatment plants have a very good performance to provide safe drinking water for consumers. *Clostridium perfringens* can be a suitable indicator for monitoring water quality, especially at the outlet of drinking water treatment plants. Tests for the spores of this bacteria can provide a higher safety margin of health to predict the microbial quality of drinking water.

**Keywords:** Water quality, treatment plant, microbial quality, physicochemical quality,

# COMPARISON OF IMPACT OF TARGET STRAINS SELECTION ON SiR & eBIAS VALUES IN QUANTITATIVE VERIFICATION OF ENTEROBACTERIACEA (ECC) ISO21528-2:2017, BASED ON ISO 16140-3:2021

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Accurate microbiological testing methods are essential for reliable results in food microbiology. ISO 16140-3:2021 outlines verification protocols for reference methods, including ISO 21528-2:2017(E) for counting Enterobacteriaceae via the Colony Count technique. This study aimed to assess the impact of two target strains, *Escherichia coli* (WDCM#00012) and *Shigella flexneri* (CCUG#32079), on SIR and eBias values in quantitative verification at the Food Microbiology Laboratories of Mashhad FDO. Food items, chosen as per ISO 16140-3, 2021 Annex A & B, were artificially contaminated with these target strains.

**MATERIALS AND METHODS:** In the verification of the implementation of the quantitative test method for Enterobacteriaceae (ECC) the standard deviation of repeatability within the laboratory (SIR) was calculated. Additionally, the estimated bias (eBias) was calculated for food items verification under challenge with three levels of artificial contamination using two target strains, *Escherichia coli*(WDCM#00012) and *Shigella flexneri*(CCUG#32079) For SIR estimation, UHT milks and for eBias determination, seven challenging food items, including ESL Milk, hamburgers, black pepper, soybean powder, , pasteurized whole liquid eggs , fruit juice and ice cream were artificially contaminated with two target organism inoculums (*Escherichia coli* and *Shigella flexneri*) at three contamination levels. All calculations for SIR and eBIAS were performed using an Excel-calculation tool published by the ISO, TC 34.

**RESULTS AND DISCUSSION:** The calculated SIR for UHT milk with target strains of *E.coli* and *Shigella flexneri* were 0.1031 and 0.22 respectively, which both of calculated SIRvalues falls into the acceptability limit ( $\leq 2 \times \text{SiR}$ ) and no statistical differences were be observed between two target stains. Furthermore, the determined eBias values were observed in acceptable at all three contamination levels in fruit juice, ice cream and whole liquid eggs as per the acceptance criteria(0.5 log) except in some solid and semisolid food items in low and medium levels of artificial contamination for both target strains . The determined eBias for liquid and semisolid food items like as ice cream ,ESL milk, fruit juices and WLEG in three contamination levels were approved in compliance with acceptability limit for both target strains and the eBias was unacceptable in the pepper, soybean powder and hamburgers.

**Keywords:** Verification; target organism, Performance characteristics; Food item, Enterobacteriaceae, SIR,

# Detection of *Listeria monocytogenes* based on Hly A and Lap genes from dairy products

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** *Listeria monocytogenes* is an important microorganism which is causing listeriosis. Although the most important method of transmission of this bacterium is through food, presence of the bacterium especially in dairy product is very vital. Today, presence of antibiotic resistance bacteria in food is very significant. Hence the aim of this study was evaluation of antibiotic pattern of *Listeria monocytogenes* strains isolated from cow's milk and identification of the isolates based on the virulence genes ( Hly A and Lap)

**MATERIALS AND METHODS:** In this study 100 milk samples were collected from livestock's in Abadeh province and they were transferred to the microbiological lab. For isolation and identification of *L. monocytogenes*, the samples were first enriched using cold enrichment method in *Listeria* enrichment broth, followed by plating onto the Palcam Agar. Then the isolates were identified using different biochemical tests and presence of genes ( Hly A and Lap) was evaluated using molecular technique. For detection of antibiotic resistant bacteria different antibiotics such as: gentamycin, Erythromycin, Penicillin, chloramphenicol and Tetracycline were used based on the protocols

**RESULTS AND DISCUSSION:** The results indicated that out of 100 samples 2 isolates were infected with *Listeria monocytogenes* and they were confirmed using biochemical as well as molecular test. Furthermore, presence of the genes were confirmed in the isolates based on molecular technique using specific primers. on the other hand, the results from antibiotic susceptibility tests indicated that all isolates were sensitive to the selected antibiotics. Although detection of *Listeria* from food especially from dairy product is an important factor specifically for high-risk people, it seems the combined conventional culture and PCR method allows accurate detection of *L. monocytogenes* in dairy samples and could serve as a rapid screening method

**Keywords:** : *Listeria monocytogenes*, milk, PCR, Hly A, Lap

# Determining the amount and origin of faecal bacteria in underground water sources of Miandoroud city

## Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** It is necessary to determine the type and origin of fecal contamination, whether it is human or animal, because each of these cases requires a special and different way to control and care. Knowing the source of contamination regarding the type of microbes can lead to proper planning in order to fight or prevent diseases. Finding indicator bacteria such as FC and FS in water sources can be used as a suitable method to determine the level of water pollution. The purpose of this research is to evaluate the microbial status of underground water sources in Miandoroud city based on microbial indicators of FC and FS and to determine the FC/FS.

**MATERIALS AND METHODS:** In this research, during one year (from July 1402 to June 1403), samples were taken monthly from the underground water sources covered by Miandoroud city and their related reservoirs and distribution networks. In total, 288 samples from supply sources and 696 samples from reservoirs and distribution networks were collected and tested to check the amount of total coliforms, FC bacteria, FS, and residual free chlorine for reservoirs and distribution networks. Using 15-tube fermentation method The Most Probable Number (MPN) was determined according to the standard method book. Also, in order to determine the origin of fecal contamination, whether human or animal, the ratio of FC to FS was calculated in different months of the year. If this ratio is greater than 4.4, the source of contamination is human, less than 0.7 animal, between 0.7 and 4.4 animal and human.

**RESULTS AND DISCUSSION:** This study determined that 11 and 4.87% of water supply sources had TC and FC, respectively. The amount of FC in the studied sources varied from 6.1 to 46 MPN/100 ml. All samples of storage tanks and distribution networks were free of TC and FC. The minimum and maximum amount of free chlorine remaining was 0.2 and 0.8 mg/l, respectively. FS was not observed in any of the samples of supply sources and storage tanks as well as distribution networks. There is FC contamination in some sources of supply in Miandoroud city, but since all these sources have disinfection facilities and sufficient and continuous monitoring is done correctly, all storage tanks and the distribution networks are free of total coliform, FC and FS. Also, due to the absence of faecal streptococcus and FC/FS, the origin of microbial contamination in supply sources is of human type.

**Keywords:** total coliform, FC, FS

# Feasibility study of using various types of microorganisms for assessing microbial growth in drinking water distribution systems

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Microorganism growth in drinking water distribution systems (DWDS) is a global concern. This study was designed to shed light on the microbial growth in a drinking water distribution system via the culture-based method using a group of microorganisms with different features involving total coliforms as fecal and environmental species, fecal coliforms and *Escherichia coli* as indicators of fecal contamination, Fecal streptococci as indices of inadequate treatment or breaches in water distribution pipelines, *Clostridium perfringens* as indicators of treatment efficiency and intermittent fecal pollution, heterotrophic plate count (HPC) as indicators of regrowth and biostability of distributed water and fungi count as a health episode.

**MATERIALS AND METHODS:** In this research, 98 water samples were collected from all places of DWDS of Gorgan from August 2020 to March 2021. In The analysis of water was performed without delay after being taken to the laboratory of Golestan university of medical sciences. Multiple-tube fermentation technique (MFT) was used to identify the total and fecal coliform, fecal streptococci and *Clostridium perfringens*. All physical and chemical tests were carried out in line with recommendations for analysis of water and manufacturer's instructions.

**RESULTS AND DISCUSSION:** The results showed that 15.3%, 12.2% and 2% of samples were positive for total coliforms, *Clostridium perfringens* and fecal coliforms, respectively. The mean of heterotrophic pale count (HPC) and fungi count were found to be 203.45 CFU/mL and 6.83 CFU/100 ml, respectively. Based on macroscopic and microscopic analysis, 7 genera of fungi were isolated, of which *Candida* (19.4%) and *Aspergillus* (11.2%) were the most prevalent, followed by *Penicillium* (5.1%), *Rhodotorula* (3.1%), *Cladosporium* (2%), *Acremonium* (2%) and *Alternaria* (1%). The results of this study showed that the quality of water was in agreement with guideline values suggested by WHO. Nonetheless, 38% of samples were positive for at least one microorganism in the absence of *E.coli*. In conclusion, using *Clostridium perfringens*, HPC and fungi can provide valuable information in relation to real water quality.

**Keywords:** Drinking water. *E.coli*. Fungi. Water Microbiology

# Frequency correlation analysis of detected *Legionella pneumophila* in drinking water distribution system with water quality indicators in Sari

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** *Legionella pneumophila*, small gram-negative bacteria are the etiologic causes of 1-30% of pneumonia in hospitals. These microorganisms can produce sporadic and epidemic infections because of their ability in living as an intracellular parasite, they can enter to alveolar macrophages and after binding the Complement C3b to their outer membrane porins, they can find their ability to attachment to CR3 receptors on mononuclear cells and enter them and prevent from forming phagolysosomes and because of this fact that they don't encounter with toxic forms of oxygen, they don't disappear. Producing cytokines from contaminated macrophages can induce a severe inflammatory response, so it can be very dangerous for hospitalized patients, especially for immunocompromised patients.

**MATERIALS AND METHODS:** In this study, water samples of a hospital in sari were collected for one year. These samples were gathered from three different parts of hospital in each season. Both cold and warm water were collected and examined for presence of *L. pneumophila*. Also, some parameters like as turbidity, Free chlorine, PH, Nitrate and, Nitrite were measured. Isolation of *L. pneumophila* was done by water filtering and then by culturing on its specific media, GVPC. Bacterial species were confirmed by identification of 16sRNA gene by PCR.

**RESULTS AND DISCUSSION:** This study showed an important positive correlation between *Legionella* counts and water pH. Water with a pH of 7.45 or higher is associated with a 4.05 times higher risk of *Legionella* colonization in cold water systems showed that slightly alkaline conditions may favor for *Legionella* growth. But it is a negative correlation between *Legionella* counts and both chlorine levels and water temperature. In cold water systems, a free chlorine concentration below 0.375 mg/L increases the risk of *Legionella* presence by 9.76 times, highlighting the importance of proper water disinfection and maintaining appropriate chlorine levels to inhibit bacterial growth. Additionally, high water temperatures above 50°C were found to reduce the growth of *Legionella*. This study showed that *Legionella* are existing and its appearance are correlated by some parameters in drinking water distribution system in different seasons.

**Keywords:** *Legionella pneumophila*, Frequency, drinking water distribution system, quality indicators

# investigatin *Salmonella* spp. in strawberries collected from vendors in Sistan, southeast of Iran

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** The fresh fruits may play a role as vehicles to transfer the pathogens to humans. World Health Organization (WHO) and Food and Agriculture Organization (FAO) allocated berries as priority commodities of concern in terms of microbiological hazards. Strawberries, a fresh fruit, play an important role in health and nutrition as sources of essential vitamins and minerals, as well as sources rich in fiber. The objective of the present study was to investigate the rate of contamination of fresh strawberries with *Salmonella* spp. in strawberries sold in Sistan, southeast of Iran.

**MATERIALS AND METHODS:** Eighty-one strawberry samples were randomly collected from fruit supply places in Sistan, southeast of Iran. One sample consisted of 5 whole fresh, ripe berries, which were picked with gloves and collected in plastic bags. Each sample was homogenized using a blender and sub-samples of 25 ml were included for isolation of *Salmonella* Spp. using culture on Selenit Cystein and Tetrathionate broths, and Xylose Lysine Desoxycholate agar. The isolate were confirmed by conventional biochemical procedure using *Salmonella*-Shigella (SS) , TSI, urea, Simmons' citrate agars, Methyl Red/Voges-Proskauer and SH2/Indole/Motility media and oxidase test.

**RESULTS AND DISCUSSION:** as a first report from southeast of Iran, we find that four samples out of 81 samples (4.9%) were contaminated with *Salmonella* spp., indicating an alarming signal for human consumption and public health. The sanitary quality of the strawberries of the study area should be promoted to boost the microbial safety of the strawberries sold in Sistan, southeast of Iran.

**Keywords:** Conventional method, *Salmonella* spp, Sistan, Strawberry



# Investigating and determining the level of microbial contamination in unpasteurized fruit juice and ice cream and the role of individuals in the preparation process in fruit juice shops in the city of Mashhad

## Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** This study investigated the microbial contamination of unpasteurized fruit juices and ice creams and finger test of personels in fruit shops in Mashhad city. Samples were collected and analyzed for bacterial contamination. This study focused on microbial contamination in fruit juices and ice creams along with finger tests from personnel, were collected. The level of bacterial contamination and identification of indicator bacteria were evaluated.

**MATERIALS AND METHODS:** A total of 570 samples, including finger test samples, ice creams, and fruit juices, were collected and tested for pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and total coliform counts. Microbial tests were performed based on Iran's national standard and the results of the microbial test were collected and analyzed.

**RESULTS AND DISCUSSION:** Findings revealed high coliform contamination across samples, with lower levels for *Escherichia coli* and *Staphylococcus aureus*. Non-compliance with microbial standards was observed, particularly in coliforms, *Escherichia coli* and *Staphylococcus aureus*. Statistical analysis showed a significant relationship between contamination levels in samples and the hands of personnel. The results showed a significant relationship between contaminated and non-contaminated samples in terms of coliform bacteria, *Escherichia coli*, and *Staphylococcus aureus*. Anova & Tukey analysis indicated significant relationships between coliform and *Escherichia coli* groups, as well as coliform and *Staphylococcus* groups in infected samples. These findings underscore the need for strict adherence to hygiene practices in food production to ensure food safety and prevent potential health risks associated with microbial contamination. Improving microbial standards and implementing rigorous monitoring measures are essential to protect public health in the production of unpasteurized fruit juices and ice creams.

**Keywords:** Finger test, ice cream, fruit juice, *Escherichia coli*, *Staphylococcus aureus*,

# Investigating of the bacterial population in waterlines of dental units- a cross- sectional study

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Different types of bacteria and microorganisms can be existing in water, which one of them is heterotrophic bacteria and this study aimed to investigate heterotrophic bacterial contamination in waterlines of dental units as a Health Risk Factor in occupational and patient's exposures.

**MATERIALS AND METHODS:** Four active departments of the faculty of dentistry of Golestan university of medical sciences (Gorgan, Iran) was selected and bacterial contamination of water lines, dental handpieces and other related parts to water of dental units were investigated and analyzed in term of Colony-Forming Units (CFU/ml).

**RESULTS AND DISCUSSION:** The mean and standard deviation of the bacterial contaminations in all studied departments were very higher than recommended limit and no significant relation could be seen between them. No significant relation was observed between bacterial contamination and different days and hours of sampling ( $p < 0.05$ ), although, the amount of bacteria was higher in Saturday and at the mornings. In addition, flushing for 30 seconds has effective role in reducing of the bacterial contamination ( $p < 0.05$ ). Stagnant water in the water lines of dental units, especially when they are shut down in the weekend or after ending of official working hour, storage of reserve water for usage of dental processes and don't performing flushing in current dental hand pieces have caused increasing bacterial water contamination in dental waterlines and hand pieces.

**Keywords:** Waterline, dental unit, water contamination, heterotrophic bacteria

# Investigating the antibacterial and antioxidant properties of prickly pear (*Opuntia ficus-indica*)

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Today, plant extracts and their components are known for their biological activities, especially antibacterial and antioxidant. In this study, besides helping to return antibiotic sensitivity in pathogenic bacteria, they can also be used as natural preservatives. *Opuntia ficus-indica* (OFI) is a cactus that grows widely in the tropics. It is consumed as a fruit and used as a traditional herbal medicine.

**MATERIALS AND METHODS:** After obtaining this plant from the Marvdasht region in Shiraz, Iran, in order to determine its microbial and chemical properties, it was first conducted using the water and cold extraction method. Using the diffusion method in the well, as well as the MIC and MBC methods, which Using 96-well microplates, the antibacterial activity of aqueous extracts against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria was determined, and the DPPH method was used to check the antioxidant properties. ANOVA and descriptive statistics were used to analyze the data (p0.05).

**RESULTS AND DISCUSSION:** Prickly pear showed significant antibacterial properties against *Staphylococcus aureus* and *Escherichia coli* bacteria in such a way that the diameter of the inhibition zone in the diffusion method in the well at a concentration of 50 mg/ml was 24 and 21 mm, respectively, as well as MIC and MBC. 4 and 8 µg/ml were reported for *Staphylococcus aureus* and 16 µg/ml for *Escherichia coli*, respectively. Also, the extract had 73% inhibition of free radicals in the DPPH method.

**Keywords:** *Opuntia ficus-indica*, Prickly pear, Antioxidant, Antibacterial, *Staphylococcus aureus*, *Escherichia coli*

# Investigating the effect of moisture on the growth of mold in domestic and foreign rice in Mazandaran province

## Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** According to the statistics of the United Nations Food and Agriculture Organization, an important part of the food of the people of the world is provided by all kinds of grains. In the study of types of grains, rice is in the second category of agricultural products, and the main consumption of rice is related to Asian countries, and Iran is one of its big consumers. Due to the increasing concerns about food safety, the purpose of this study is to investigate the moisture in the growth of domestic and foreign rice mold in the province.

**MATERIALS AND METHODS:** In this study, 100 foreign and domestic rice samples were collected from Mazandaran province. It was studied in Simiaparotvistazist Research Laboratory. According to the standards number 127 (rice), 2705 (moisture) and 10899 (mold), the evaluation of the effect of moisture on the microbial contamination of mold was investigated. And for statistical calculations, one-way analysis of variance (ANOVA) and Minitab software were used.

**RESULTS AND DISCUSSION:** According to the standard, the results of this research showed that high moisture (more than 14%) provides a suitable environment for mold growth ( $p < 0.03$ ). Different molds such as *Aspergillus*, *Penicillium* and *Fusarium* grow and multiply rapidly in such conditions, and the production of mycotoxins by these molds increases, which is dangerous for human and animal consumption, and mold growth is minimized in moisture (12-14%) and the production of mycotoxins decreases ( $p < 0.01$ ). Moisture less than (12%) prevents the growth of molds ( $p < 0.1$ ), and this amount of moisture may cause the rice grains to dry and become brittle. The assessment of moisture on the growth of mold showed that maintaining moisture between (12-14%) is the best solution for keeping and storing rice to prevent the growth of mold and the production of mycotoxins. Also, all the samples of Iranian and foreign rice with different brands available in Mazandaran province, in terms of the level of microbial contamination, were lower than the permissible limit of Iran's standard. And there is no risk for consumers.

**Keywords:** grains, rice. Mold. Moisture

# Investigating the effectiveness of the combined ozone, hydrogen peroxide and UV system in removing dinobryon algae from the water of Qeshlaq dam in Sanandaj city.

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Reservoir water quality is crucial and can be compromised by resilient algae like Dinobryon, which form blooms and resist conventional treatment methods. Advanced oxidation processes, specifically combining ozone, hydrogen peroxide, and UV, can generate •OH radicals and degrade contaminants. This study tests a combined O<sub>3</sub>/H<sub>2</sub>O<sub>2</sub>/UV system for removing Dinobryon algae from dam water. Objectives include evaluating removal efficiency under various conditions, analyzing operational parameters for optimization, comparing efficacy with traditional methods and other AOPs, investigating by-product formation and impacts, and assessing scalability and feasibility for real-world applications.

**MATERIALS AND METHODS:** In order to undertake this study, twenty (20) samples of raw and purified dam water were selected. The samples were treated with different concentrations of O<sub>3</sub> (0, 1.5, and 3 g/L) and H<sub>2</sub>O<sub>2</sub> (0, 1, and 2 g/L) used in combination with UV irradiation for varying durations (10, 42.5, and 75 minutes). Each treated sample also passed through a filtration system and was examined microscopically for the presence of Dinobryon algae. Efficiency of treatment was determined through the comparison of algae counts from both unfiltered as well as clarified water before and after treatment hence reporting its findings as per removal efficiencies by means of Dinobryon algaecides.

**RESULTS AND DISCUSSION:** the findings that we got to know the effects of those variables on the removal of Dinobryon by analyzing were taken. We carried out 20 trials and described the efficiency in percent. The research findings found that the lowest efficiency was met by the following conditions: 0 g/L ozone, 1 g/L H<sub>2</sub>O<sub>2</sub> and the interval of 42.5 minutes with the efficiency of 10.7%. On the contrary, the maximum efficiency was achieved by the following conditions: 1.5 g/L ozone, 2 g/L H<sub>2</sub>O<sub>2</sub>, and 75 minutes, where efficiency was 96.19%. Removing Dinobryon algae from dam water is vital due to their impact on water quality, including taste, odor, and harmful algal blooms that release toxins. These blooms harm ecosystems, human health, and water treatment processes. Efficient removal ensures safer drinking water, protects aquatic life, and reduces treatment burdens, making optimized algae removal methods crucial for sustainable water resources.

**Keywords:** Dinobryon algae, O<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, UV, AOPs, Qashlaq dam, Sanandaj

# Investigating the impact of sugar syrup

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Annually, about 15 to 25% of the total sucrose produced from sugarcane is lost at various stages of the sugar production process. Sugar losses after sugarcane harvesting have been one of the issues in this industry. In the absence of microbial control in the milling, up to 2% of the incoming sugar is lost. This portion is a direct loss caused by microorganisms during the bacterial sugar degradation process in addition, by producing microbial metabolites such as dextran, major problems are created in the process. Increased viscosity in cooking and centrifuge, reduced capacity, reduced filter-ability, reduced sedimentation rate of juice and stretching of raw sugar crystals are the most important problems that are caused by increased dextran. The lack of access and high cost of biocides has led to the removal of these materials from the production cycle. Ozone destroys microorganisms through various mechanisms and is considered as a broad-spectrum

**MATERIALS AND METHODS:** The samples were collected under sterile conditions from various sections, which included the syrup from the first and last mills, as well as the mixed syrup tank. To assess the impact of ozone on the microbial load of the sample, ozone ranging from 2-10 ppm should be added. After setting the time intervals for 5, 10, and 20 minutes, respectively, serial dilutions in distilled water and sodium chloride solution need to be prepared. These dilutions should be cultured on MSE dextran-sucrase inducing solid medium, while PCA will be cultured as a plate to count the colonies. The plates should be incubated at 30°C for 48-72 hours.

**RESULTS AND DISCUSSION:** To enhance the efficiency of ozone in eliminating microorganisms, it is necessary to increase the duration of ozonation. When the ozonation time was set at 20 minutes, the percentage of microorganism removal exhibited greater stability in the samples. Additionally, the dispersion of the recorded values was significantly lower compared to other time intervals. Therefore, the one-sample t-test was employed to assess the average removal percentage in these samples, with a target value of 5%. The results of the test indicated no significant difference from the target value, suggesting that ozonation for a duration of 20 minutes was effective at least.

**Keywords:** Ozone, microbial control, sugarcane syrup

# Investigating the Impact of Ozone on Microbial Control of Mist Cooling Water in a Sugar Mill

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** At the sugar mill, CoolMist Systems are designed to cool ambient misting systems by spraying a very fine mist that rapidly evaporates upon contact with hot air. This process quickly absorbs heat, resulting in a significant reduction in temperature (up to 15 degrees Celsius) and an immediate cooling effect. The mistwater used often contains a substantial amount of sugar due to its connection with steam from cooking pots. However, this presents a contamination issue as microorganisms thrive in this environment, leading to pipes decay. Ozone, a broad-spectrum bactericidal agent, can effectively reduce the microbial population. Its short half-life ensures minimal side effects.

**MATERIALS AND METHODS:** In the following tests, 250 ml water samples were carefully poured into sterile Erlenmeyer flasks with a volume capacity of 500 ml. Two samples were used: one as a control without ozonation, and the other was subjected to ozone exposure for 5, 10, and 20 minutes. Serial dilutions were prepared from each sample, and surface cultured in both PCA and MSE culture media. The cultures were then incubated at 30°C for a period of 24-48 hours.

**RESULTS AND DISCUSSION:** The test results following ozonation for 5 and 10 minutes in PCA and R2A culture media demonstrated a notable decrease in levels of microorganisms. To assess the percentage of removal of Mistcooling samples in comparison to the respective target values of 99.5% and 99% for slow-growing and fast-growing bacteria, a t-test was employed at a significance level of 5%. The test results indicated that the null hypothesis  $H_0: \mu=99.5$  was not rejected, with a significance value of 0.525 (sig). This suggests that, on average, ozone has successfully eliminated at least 99.5% of slow-growing bacteria. Furthermore, the results of culturing Mistcooling water samples after ozone treatment in each PCA culture medium (for fast-growing bacteria) demonstrated a significant removal of at least 99% of such bacteria.

**Keywords:** OZONE, Microbial control, Mistcooling water

# Investigating the Presence of Live *Helicobacter pylori* in Drinking Water of Arak City and its Relationship with Water Physicochemical Parameters

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Ensuring the quality of drinking water is very important to maintain public health. Today, it is believed that *Helicobacter pylori* may be transmitted through water. This bacterium causes various gastrointestinal diseases, including stomach cancer. Given the challenges associated with detecting *Helicobacter pylori* in water, studying how water parameters relate to the presence of *Helicobacter pylori* may help in detecting the bacterium. The purpose this study was to investigate the presence of live *Helicobacter pylori* in the water of Arak city and how it is related to the chemical parameters of the water.

**MATERIALS AND METHODS:** In this research, 148 tap water samples were collected from the five regions of Arak city over a period of six months. The amount of chlorine was measured with DPD (Diethyl-p-PhenyleneDiamine) tablets and the pH was measured with Phenol Red tablets using the Pool Tester kit at the sampling site and the data were recorded. The water samples were centrifuged three times, and 50 microliters of sediment from each sample were cultured in Brucella agar culture medium (Merck, Germany) enriched with 5% sheep blood. The cultures were incubated in microaerophilic conditions at 37°C for 7 days.

**RESULTS AND DISCUSSION:** Out of 148 samples, only one tested positive for *Helicobacter pylori*, confirmed via microscopic examination and molecular testing. The average chlorine level was 0/16(mg/l), and the pH was 7/6. There was no statistically significant relationship found between the presence or absence of the bacteria and the water parameters. (P 0.05). This is the first study that isolates *Helicobacter pylori* from the tap water of Arak city. The results of this study indicate the importance of water as a proposed transmission pathway. The unique growth conditions of this bacterium and the difficulty of accessing bacteria in biofilms and the presence of viable but non-cultivable (VBNC) forms of bacteria in water suggest that *Helicobacter pylori* should be identified in water samples using molecular methods combined with culture methods. This research showed that there is no relationship between chlorine level and water pH with the risk of water contamination with this bacterium.

**Keywords:** *Helicobacter pylori* / Drinking water/ Culture /Chlorine / PH



# Investigating the prevalence and virulence genes of *Brucella melitensis* in raw milk and traditional cheese in Isfahan by multiplex PCR

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Brucellosis, a prevalent disease in domestic animals and humans, poses significant health and economic risks. The causative agent, *Brucella melitensis*, is a gram-negative bacterium. This study, of paramount importance, aims to investigate the prevalence and virulence genes of *B. melitensis* in raw milk and traditional cheese sold in Isfahan City using the multiplex PCR method.

**MATERIALS AND METHODS:** Seventy-five samples of raw milk, including cow, sheep, goat, buffalo, and camel, and 25 samples of traditional cheese were collected from supply centers. These samples were then subjected to bacterial culture to detect *B. melitensis* and the PCR method to recognize the virulence genes *bvfA*, *virB*, and *Ure*.

**RESULTS AND DISCUSSION:** Bacterial culture showed that out of 75 raw milk samples, 31 (41.33 %) and 25 traditional cheese samples, 13 (52 %) were infected with *Brucella*. Multiplex PCR showed that 15 samples (15%) of 100 samples of raw milk and traditional cheese were infected with *B. melitensis*. The level of contamination in raw milk was (10.66%) and in cheese was (28%). The results showed that the highest contamination among the samples was related to traditional cheese (28%), and no *B. melitensis* contamination was observed in buffalo and camel milk. Multiplex PCR in raw milk samples showed that *B. melitensis* in 2 samples carried *bvfA* (2.66%), 3 samples carried *virB* (4%), and 1 sample carried *Ure* (1.33%). Multiplex PCR in traditional cheese samples showed that 2 samples carried *bvfA* (8%), 1 sample carried *virB* (4%), and 1 sample carried *Ure* (4%).

**Keywords:** *Brucella melitensis*, brucellosis, raw milk, traditional cheese

# Investigating the relationship between heterotrophic bacteria and total organic carbon in drinking water treatment plants in Ramsar and Sari cities

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Preparing and supplying safe drinking water for the society is one of the most important challenges in improving the health of the society. With the increase in the population of the earth, the limitation of water resources and the location of Iran on the belt of dry areas, the use of water behind the dams and its purification is of high priority. Today, in the investigation of water quality, heterotrophic bacteria (HPC) have been taken into consideration as a supplement to the water coliform index and the amount of organic carbon (TOC), which reflects the contamination of water and water sources with sewage and organic pollutants such as various poisons. Therefore, in this research, the relationship between the concentration of heterotrophic bacteria (HPC) and the amount of total organic carbon (TOC) in the water treatment plants of Ramsar and Sari cities was investigated

**MATERIALS AND METHODS:** This research is descriptive-analytical. 54 samples were taken from the inlet and outlet of water treatment plants in the cities of Sari and Ramsar during the years 2018 to 2019. Sampling was done with the national standard method of Iran. The samples were analyzed by HPC test with pour plate method with plate count agar culture medium and TOC test with DR2800hach spectrophotometric device.

**RESULTS AND DISCUSSION:** The results of analysis on the samples showed that there is a significant relationship between TOC and HPC parameters. The parameters were statistically analyzed through linear regression tests and F-test. The correlation coefficient of input and output of Ramsar treatment plant was calculated as 0.7645 and 0.8454 respectively and that of Sari house as 0.7091 and 0.8584 respectively. Also, the P-value smaller than 0.05 showed that there is a positive significant relationship between the concentration of heterotrophic bacteria (HPC) and the amount of total organic carbon

**Keywords:** heterotrophic bacteria (HPC), total organic carbon (TOC), water treatment plant

# Investigating the trend of microbial pollution changes from the beginning to the end point of Talar River (Qaemshahr, Mazandaran, Iran) in 2023

## Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Regular monitoring of microbial quality can help identify health risks and potential sources of pollution in rivers, enabling targeted solutions to address pollution issues. This study aims to investigate the trend of microbial pollution changes in the Talar River in Qaemshahr, Mazandaran, Iran in 2023.

**MATERIALS AND METHODS:** In this study, the water quality of the Talar River in Qaemshahr was assessed by collecting samples from 10 stations along the river during two seasons: high rainfall (winter 2023) and low rainfall (summer 2023). The selection of these stations was done by a thorough investigation of the area, identifying of potential sources of pollution entering the river, such as agricultural activities and the discharge of untreated, agricultural and industrial wastewater. At each sampling station, three samples were taken from three key points along the river. The samples were tested in the laboratory for the number of total coliforms (TCs) and fecal coliforms (FCs) using the Most Probable Number per 100 ml (MPN/100 ml).

**RESULTS AND DISCUSSION:** The results of the study showed that the average number of TCs in the winter (184820/100 ml) is higher than in the summer (149160/100 ml). Conversely, as the trend moved from upstream to downstream, the number of TCs increased, reaching its peak at station 8 in winter (920000/100 ml) and at station 7 in summer (540000/100 ml). The number of FCs is also higher in winter (138590/100 ml) compared to summer (75200/100 ml). The flow of the river passing through populated areas and sewage has led to elevated levels of FCs in the middle of the Talar River (Stations 7 and 8), surpassing WHO and EPA standards. Studies in several countries reveal that human activities, including household waste and agricultural practices, contribute to high FC levels in rivers. Pollution from population growth, human activities, chemicals, sewage, and waste necessitates effective freshwater quality management in the Talar River basin.

**Keywords:** Water Quality, Talar River, Fecal Coliform, Microbial Contamination

# Preparation of specific bacteriophage cocktail against *Escherichia coli* strains isolated from red meat specimen

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** *Escherichia coli* is one of the most important food-borne pathogens that leads to significant financial losses, annually. Consumption of contaminated food is the main way to spread these bacteria which causes food poisoning with abdominal pain, diarrhea and nausea symptoms. Antibiotic resistance among *E. coli* strains isolated from clinical and food samples is increasing, therefore it seems necessary to eliminate the bacterium before consuming contaminated food. One of the best ways is to use lytic phages that lead to the complete destruction of the *E. coli*. The present study was aimed to isolate anti *E. coli* bacteriophages from wastewaters.

**MATERIALS AND METHODS:** A total of 25 meat swab samples were collected from butchers and *E. coli* isolated and confirmed using biochemical and molecular methods. In the next step, wastewater samples were collected and isolation and purification of *E. coli* specific bacteriophages performed by reference procedures.

**RESULTS AND DISCUSSION:** A total of 5 bacteriophages were isolated from wastewaters and have antibacterial activity against 13 *E. coli* isolates which were isolated from meat swab samples. The identified bacteriophages displayed significant antimicrobial activity against *E. coli* isolates. Hence, it has the potential to serve as a viable candidate for preservation in food products.

**Keywords:** Bacteriophage, *Escherichia coli*, red meat

# Production of nano antimicrobial food coatings to increase the shelf life of bread

## Microbial Contamination of Food

Mahdis Ghasemi <sup>1</sup> ©, Amirhossein Asghari <sup>2</sup> ©, Niaz Alizadeh <sup>1</sup>

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ارشد میکروبیولوژی میکروب های بیماری زا <sup>2</sup>

**BACKGROUND AND OBJECTIVES:** Many researches have been done on replacing chemical and synthetic materials with natural materials in order to eliminate or reduce these compounds in food. Edible coatings are thin layers of materials that are used on the surface and between different layers of food. Biopolymers such as poly Saccharides, proteins, lipids are used to prepare edible coatings. Biopolymers are a barrier against moisture, gases, soluble substances and a means for additives such as antifungal compounds, antioxidants, and antimicrobials. By reducing the size of the particles, the effectiveness increases. In the research, first, bread microorganisms were isolated and the antimicrobial effect of several indigenous plant extracts of Mazandaran against this microorganism was investigated. The next step was to produce several coatings of biodegradable polymers and antimicrobial compounds of plant extracts in the form of nanoparticles. The appearance of produced nanoparticles, their effect on quality and microbial properties of bread were investigated.

**MATERIALS AND METHODS:** Kant agar plate culture medium and Y.G.C culture medium .Isolation of bread bacteria .Preparation of aqueous extracts of different plants and investigation of antimicrobial properties of the extracts by disk diffusion method .Preparation of essential oil from Hyssopus officinalis and Lavandula angustifolia .Preparation of oral coating solutions .Quality check

**RESULTS AND DISCUSSION:** To isolate the microorganisms in bread, their specific cultures were used. Common bread bacteria were grown and isolated in Kant agar plate medium, and bread molds and yeasts were grown and isolated in Y.G.C culture medium. Microbial water extracts of different plants were cultured by disc diffusion method from bacterial and fungal colonies grown as surface culture on the surface of the plate, and discs containing water extracts of different plants and a control disc containing distilled water were placed on the plates, after Bacterial and mold growth Antimicrobial effect They were investigated, and as a result, none of the used extracts showed a lack of growth. According to the results of this research, it can be concluded that by using edible alginate nanoparticles and adding essential oil, the total number of bacteria can be reduced. during storage times and increase the shelf life of this product.

**Keywords:** Coating and edible film, Nano, Antimicrobial film, Plant extract

# RT-LAMP-Based Molecular Detection for Viability Assessment of Probiotics

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Probiotics are live microorganisms that confer health benefits on the host when administered in adequate amounts. Developing rapid and reliable methods for detecting viable probiotic organisms is essential for ensuring the quality and efficacy of probiotic products. *Lactobacillus acidophilus* is a beneficial bacterium commonly used as a probiotic in various food products and supplements. Traditional methods for assessing probiotic viability, such as plate counts and microscopy, are time-consuming, labor-intensive, and often lack specificity. This study aimed to develop and validate a new RT-LAMP method (reverse transcription loop-mediated isothermal amplification) and direct extraction of total microbial RNA from dairy products without bacteria culture for molecular detection of live *L. acidophilus* as a functional bacterium in dairy.

**MATERIALS AND METHODS:** In this study, direct extraction of total microbial RNA from dairy products without bacteria culture for molecular detection of live *L. acidophilus* as a functional bacterium in dairy was optimized. We designed and optimized RT-LAMP primers specific to the 16S-rRNA genes of *Lactobacillus acidophilus*, ensuring high sensitivity and specificity as viability assessment markers. The performance of the RT-LAMP method in detecting live probiotics was evaluated by direct isolation of RNA from the matrix of fermented foods, including dairy products.

**RESULTS AND DISCUSSION:** Our findings demonstrate that the RT-LAMP method is a rapid, sensitive, and specific tool for detecting viable *L. acidophilus*. The potential applications of direct extraction of RNA combined with RT-LAMP in quality control and quality assurance of *L. acidophilus*-containing products were also investigated, enabling rapid and reliable assessment of their viability and efficacy. The results were compared with traditional methods, such as plate counts and microscopy, to establish the reliability and accuracy of the RT-LAMP method. This study contributes to developing high-quality probiotic products that meet the growing demands of the global market. The RT-LAMP method can potentially revolutionize the detection of viable probiotics, ensuring the safety and efficacy of these products for consumers.

**Keywords:** Loop-mediated isothermal amplification- Molecular detection – bacterial viability - Food

# Spatial modeling and risk mapping of Food borne diseases in Iran: A GIS-based survey from 2009-2022

## Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Background: Foodborne diseases are an important cause of morbidity and mortality, The purpose of this study is to determine the incidence, spatial distribution, and hot spots of Foodborne diseases (Fascioliasis and hydatidosis) in Iran using the GIS analyses during 2009 to 2022.

**MATERIALS AND METHODS:** Methods: The data including Foodborne diseases cases and populations at-risk in different provinces obtained from the Ministry of Health, and Medical Education, Tehran, Iran and other centers from 2009 to 2022. The spatial distribution maps of the Foodborne diseases were generated. Then, the hot spots of the disease in Iran were determined using spatial analysis of ArcGIS10.5 software. Geographically weighted regression (GWR) analysis in ArcGIS10.5 was used to correlate the temperature, relative humidity, normalized different vegetation index (NDVI) and incidence of Foodborne diseases. Data analysis was performed by Linear regression analysis and SPSS 21 software using descriptive statistics test.

**RESULTS AND DISCUSSION:** Result: The hot spot provinces of were Gilan, Kermanshah, Khorramabad, Zanjan, Khorasan Razavi, North Khorasan, Chaharmahal Bakhtiari, Hamedan, Semnan, and Ardabil. In provinces, the highest correlation between humidity, temperature, vegetation density and the incidence of Foodborne diseases was observed using geographical weighted regression analysis. Conclusion: Our study revealed that there was significant relationship between the relative humidity, mean annual temperature, NDVI and the incidence of Foodborne diseases in Iran. The Geographical Information System (GIS) can be used to identify risk factors of Foodborne diseases and to assess endemic areas in a specific region.

**Keywords:** Keywords: GIS, Risk mapping, Spatial modeling, Foodborne diseases, Iran

# Specific detection of *Listeria monocytogenes* using oligonucleotide nanoprobe

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** *Listeria monocytogenes* is responsible for causing listeriosis, which is a significant foodborne illness. Hence, there is a necessity for the quick and accurate identification of bacteria. Therefore, in this research an innovative biosensor utilizing a nanoprobe connected to a gold nanoparticle was developed for colorimetric detection purposes.

**MATERIALS AND METHODS:** *L. monocytogenes* (PTCC:1783) was acquired from the IROST. Negative samples used include *L. ivanovii* (IBRC:10633), *E. coli* (PTCC:1399), and *Staphylococcus aureus* (PTCC:1112). The *Htr* gene was identified essential for the growth of *L. monocytogenes* through a comprehensive analysis of the whole genome using NCBI and BLAST. For designing the probe, the GC ratio,  $T_m$ ,  $\Delta G$  of the sequence, and potential secondary structures of the oligonucleotide were investigated. Au nanoparticles through citrate synthesized and led to an average size range of 20-30 nm. Nanoprobes was functionalized by reducing agent, while PBS (pH 7) was used to gradually increase the ionic strength. The properties of the Au nanoparticles and Au nanoprobes were verified through optical spectrometry and DLS tests. The hybridization of the probe to the target sequence was confirmed through the reliability test. The nanoprobe specificity test was carried out to distinguish the specificity of the sensor for *L. monocytogenes*.

**RESULTS AND DISCUSSION:** The probe designed have a length of 26 bp and the BLAST results indicated that it had 100% specificity for target *L. monocytogenes*. Its GC ratio was measured 42.3%, with the  $T_m$  and  $\Delta G$  recorded as 65°C and -36.6 kcal/mol, respectively. Average diameter of gold nanoparticles were approximately 39 nm and Au nanoprobes was 60 nm. After the addition of magnesium chloride salt, the color of the nanoprobe remained unchanged but nanoparticles precipitated. The nanoprobes exhibited a peak absorption of 0.094 at 520 nm, while the absorption of Au nanoparticles was 0.071. In reliability test, adding salt after connecting the nanoprobe to its complementary sequence resulted in the solution color maintained. The results of the specificity test indicated that when salt is added to the solution with the *L. monocytogenes* genome, the color remained pink, whereas the solution with the negative bacteria genome changed to gray.

**Keywords:** *Listeria monocytogenes*, biosensor, foodborne pathogens, gold nanoparticles



# Stability of Bacteriophages phSal01 and phE8 under pH and Temperature Variations: An Evaluation of Phage Activity

## Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Bacteriophages, viruses that target specific bacteria, are gaining attention for their potential in controlling microbial contamination and preserving food quality. The stability of these phages under various environmental conditions, such as pH and temperature, is crucial for their application in food industries and public health. This study evaluates the stability of bacteriophages phSal01 and phE8 under different pH levels and temperatures, and assesses the impact of these conditions on their lytic activity. The objective of this study was to assess the stability of bacteriophages phSal01 and phE8 against changes in pH and temperature, and to analyze the effects of these conditions on their phage titer. The study aims to provide practical data for optimizing the use of bacteriophages in controlling microbial contamination and improving food quality.

**MATERIALS AND METHODS:** To evaluate pH stability, bacteriophages phSal01 and phE8 were exposed to a range of pH levels (3.0 to 13) for 1 hour, and their activity was measured. For thermal stability assessment, the phages were incubated at different temperatures (30 to 80°C) for 30 and 60 minutes, and changes in phage titer were recorded. Phage activity was assessed using the Plaque Forming Units (PFU) assay.

**RESULTS AND DISCUSSION:** phage phSal01 remained active across a wide pH range (4 to 12), but its activity was reduced to undetectable levels at very acidic ( $\leq 3.0$ ) and highly alkaline ( $\geq 13$ ) pH conditions. The highest phage titer for phSal01 was observed at pH 7 (8.14 log PFU/mL). Phage phE8 showed stability at temperatures from 30 to 60°C for 30 minutes, but a significant reduction in phage titer (from 9 log PFU/mL to 7.12 log PFU/mL) was noted at temperatures above 60°C up to 80°C. Additionally, after 60 minutes at 30 to 60°C, the phage titer decreased to 7.65 log PFU/mL, and no phage was detected at 70°C. bacteriophages exhibit considerable stability under moderate pH and temperature conditions, particularly phage phSal01. The pronounced stability of phage phSal01 at neutral pH and the significant reduction in phage phE8 activity at elevated temperatures provide useful information for practical applications of these phages.

**Keywords:** Bacteriophage, pH stability, thermal stability, phSal01, phE8, lytic activity

# Survey of Microbial Quality of Water Supplies in Some Rural Areas of Sari City, North of Iran, 2023

## Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Nowadays, the provision of optimal drinking water is a major need. Many human diseases are related to lack of safe and hygienic water. Therefore, monitoring and observing the standard of drinking water is of great importance. The aim of this study was to determine the microbial quality some of groundwater resources in suburban villages of Sari city, North of Iran.

**MATERIALS AND METHODS:** In this cross-sectional study, the quality of household wells of some villages around Sari was evaluated in terms of microbial characteristics in a period of 8 months (2023). For microbiological analysis, samples were transferred to the laboratory under standard conditions and the heterotrophic bacteria (HPC test), total and thermo-tolerant coliforms (MPN test) were assessed according to standard methods of water and wastewater. Finally, all of data were analyzed by standard statistically methods.

**RESULTS AND DISCUSSION:** The results showed that 75.8% of the samples are completely healthy in terms of microbes were within national and international standards (village with coverage of Rural Waste Water Company). Total coliform was present in 24.2% and fecal coliform in 17% of the samples. In 25% of the samples the HPC was higher than 500 cfu/mL. There was a significant correlation between the number of coliform bacteria (MPN) and residual chlorine ( $p < 0.05$ ). Data suggests that the microbial quality of the groundwater resources in villages with coverage is desired level due to the proper chlorination. However, the results showed that microbial quality of water in some areas were not at the desired level and requires more supervision of the Water and Sewerage Company and health centers and the management of Water operators in this field.

**Keywords:** Water pollution, Microbial quality, HPC, MPN, Sari city

# The effect of cold plasma treatment of dried apricots in preventing the growth of *Aspergillus flavus*

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Transportation and storage of moist foods leads to the growth of molds and as a result the production of toxic, carcinogenic, and teratogenic mycotoxins. Cold plasma is a novel technique in food processing that uses the reactive oxygen or nitrogen species to prevent the growth of microorganisms, especially pathogenic ones. In the present study, the effect of cold plasma in preventing the growth of *Aspergillus flavus* inoculated in dried apricots has been investigated.

**MATERIALS AND METHODS:** Dried apricot extract inoculated with a 0.5 McFarland suspension of *A. flavus* was exposed to cold plasma. Cold plasma was used at different distances from the sample, as well as for different periods of time. Then, the antifungal activity of cold plasma was investigated by transferring the sample to a sterile culture medium. After cold plasma treatment, the quality of dried apricots was also investigated using scanning electron microscope.

**RESULTS AND DISCUSSION:** In this study, the best antifungal properties of cold plasma occurred at a distance of 2 cm from the sample and within 15 minutes, which led to a reduction of colonies to 4.3log cfu/ml. Also, the results of SEM analysis showed that there was little damage to dried apricots in this treatment of cold plasma. The results of this study showed that cold plasma treatment can be useful for preserving and preventing the spoilage of moist food such as dried apricots without affecting its quality.

**Keywords:** Cold plasma, *Aspergillus flavus*, Dried apricot

# The level of aerobic plate count on beef carcasses produced in an Iranian slaughterhouse

## Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Beef is among the most popular protein sources in the human diet. Since intrinsic factors of this product favor the growth of most microorganisms, especially spoilage bacteria, the spoilage process will be accelerated if either extrinsic factors are not controlled or the level of initial microbial contamination is high. Based on the Iranian Veterinary Organization (IVO) guideline, the shelf life of refrigerated beef meat packed in aerobic conditions is 3 to 5 days. This period is decreased or increased with high or low initial microbial contamination respectively. In slaughterhouses, unhygienic conditions and improper slaughter process can lead to the unacceptable presence of bacterial contamination on the product. Since data regarding the level of bacterial contamination on livestock carcasses is scarce in Iran, in the present study aerobic plate count (APC) as an important hygienic microbial indicator was assessed on beef carcasses in an Iranian slaughterhouse.

**MATERIALS AND METHODS:** A total of 57 cattle carcasses, including both female and male cases, were examined for APC at the end of slaughter (after final washing and before refrigeration). The samples were obtained from the most contaminated external surfaces of the carcasses. Then, the results were compared with the relevant data reported from other countries.

**RESULTS AND DISCUSSION:** The range of APC values on the examined cattle carcasses was 2.2-6 (Log CFU/ cm<sup>2</sup>) with a mean value of 4.4 (Log CFU/ cm<sup>2</sup>). The range of APC values in other countries was from 0.426 to 1.97 (Log CFU/ cm<sup>2</sup>). Compared with the APC values recorded in other countries like the UK, Australia, and Italy, the level of bacterial contamination was higher in the studied slaughterhouse, indicating lower hygienic slaughter procedures in this facility and consequently lower expected shelf life. Therefore, stricter hygienic measures are essential to enhance the shelf stability and decrease losses of the beef produced in the studied slaughterhouse.

**Keywords:** Microbial contamination, Beef, Carcass, Shelf life

# Unraveling microbial contamination: Species identification, molecular analysis and drug susceptibility pattern of microbial pollution in pastry shops of Iran"

Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Microbial contamination in food product such as pastries, poses a significant public health concern due to the potential risks of foodborne infection and outbreak, Therefore, to prevent these infections, it is essential to investigate the frequency and extent of microbial contamination as well as the level of drug resistance in pastries. This study aimed to assess the microbial diversity, the drug susceptibility patterns of microbial pollutants in pastry shops in Markazi province, Iran.

**MATERIALS AND METHODS:** The study involved collecting 120 pastries samples from 30 pastry shops in Markazi province, Iran. The isolates were identified using a series of biochemical, phenotypic, and molecular assay, including specific PCR and 16S rRNA gene sequencing. Drug susceptibility testing (DST) was performed by using kirby-bauer method according to the CLSI 2023 guidelines.

**RESULTS AND DISCUSSION:** A total of 56 isolates (46.66%) were recovered from 120 pastries samples, The most prevalent species isolated in the current study were *S. aureus* 12 isolates (21.43%), *M. luteus* 7 isolates (12.5%), *E. coli* 7 isolates (12.5%), *S. warneri* 6 isolates (11.12%), 6 isolates of *S. succinus* (11.12%), *B. cereus* 5 isolates (10.7%), *Nocardia* 4 isolates (7.15%), *K. pneumoniae* 3 isolates (5.35%), *S. epidermidis* 3 isolates (5.35%), and *E. faecium* 3 isolates (5.35%). The isolates showed the most sensitivity to imipenem and trimethoprim-sulfamethoxazole and the least sensitivity to erythromycin and tetracycline. The AST showed that 7 isolates of *S. aureus* were MRSA, 3 isolates of *E. coli* and, 2 isolates of *K. pneumoniae* were identified as ESBL. In conclusion, the results of the current study showed that the microbial contamination of pastries produced in confectionaries of Markazi province were not in standard ranges. These problems may be related to fecal

**Keywords:** Pastries, microbial pollution, DST, 16SrRNA

# Unveiling Campylobacter Insights into Detection, Contamination, and Diagnostic Strategies

## Microbial Contamination of Food

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**BACKGROUND AND OBJECTIVES:** Campylobacter, a bacterium often underestimated in the realm of public health, poses a significant threat, especially in the context of foodborne illnesses. With an increasing incidence of campylobacteriosis globally, surpassing other common bacterial infections like salmonellosis and shigellosis, the need to address this pathogen is paramount. Campylobacter jejuni, a prevalent strain, is responsible for a substantial portion of foodborne campylobacteriosis cases. Understanding the survival mechanisms and resistance of *C. jejuni* is crucial for predicting bacterial population growth in food products and assessing the risks associated with food production processes. The emergence of antibiotic-resistant strains further complicates the management of Campylobacter infections, emphasizing the urgency for effective detection, prevention, and treatment strategies.

**MATERIALS AND METHODS:** In a study focusing on the isolation of Campylobacter from human stool samples, conventional culture methods were compared with molecular methods. The research aimed to isolate the pathogen from stool specimens using routine laboratory media and a microaerophilic atmosphere created by a candle jar. A total of 100 stool samples were inoculated onto selective and non-selective media, with and without filtration, and incubated under microaerophilic conditions at varying temperatures. Culture isolates were confirmed using standard phenotypic tests, and a polymerase chain reaction (PCR) targeting the 16S ribosomal DNA of Campylobacter was performed. The study successfully isolated Campylobacter from 10 out of 100 stool samples, confirming them to be Campylobacter jejuni both phenotypically and genotypically.

**RESULTS AND DISCUSSION:** The results of the study revealed that Campylobacter could be effectively isolated from stool samples using the described methods. Interestingly, there was no significant difference observed between the isolation rates using selective and blood-containing media or different incubation temperatures. All ten isolates were confirmed to be Campylobacter jejuni, highlighting the reliability of the culture and PCR methods employed. These findings underscore the importance of robust detection techniques in combating Campylobacter infections and emphasize the need for continued research to enhance our understanding of this pathogen and improve public health outcomes.

**Keywords:** Zoonosis disease, Foodborn, Bacteriology, Salmonellosis

# Bioremediation of Phenol-Contaminated Groundwater by Small Bioreactor Chambers and CaO<sub>2</sub> through a Continuous-flow model

Microbiological contamination of water supplies

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**BACKGROUND AND OBJECTIVES:** Phenols are considered the priority pollutant with high solubility in water and carcinogenic impacts on humans, animals, and plants. The entry of these contaminations into the environment is seriously hazardous and must be treated. This study explored the impacts of bioaugmentation and biostimulation on phenol removal from groundwater through a continuous-flow model system.

**MATERIALS AND METHODS:** Four continuous up-flow plexiglas reactors with 100 cm length and 9 cm inner diameter were packed with underground sands and the phenol-contaminated groundwater was passed through the columns. Chemical remediation, natural bioremediation, biostimulation, and bioaugmentation efficiency were examined for 6 months. To investigate the impact of each process on the microbial biodiversity of the columns, next-generation sequencing (NGS) of the 16S rRNA gene was performed.

**RESULTS AND DISCUSSION:** Simultaneous use of bioaugmentation (SBC application) and biostimulation (CaO<sub>2</sub> injection) eliminated phenol during the first 42 days. In the biostimulation column, 90 % and 100 % of phenol removal were observed after 12 and 22 weeks of the experiment, respectively. The dissolved oxygen (DO) in the chemical column (I) effluent increased notably after the first injection and peaked on the 21st day, reaching 14.14 mg/L. By injection of nanoparticles into columns (III) and (IV), the dissolved oxygen was increased in comparison to the blank column (II). Microbial diversity was decreased by CaO<sub>2</sub> injection while phenol-degrading orders such as Rhodobacterales and Xanthomonadales were dominated in biostimulation columns. In conclusion, the innovative use of SBCs in stimulated water provides evidence for the successful application of these methods in groundwater treatment processes.

**Keywords:** Groundwater, Phenol, Bioaugmentation, Bioremediation

# Antibacterial Activity of *Carum copticum* Essential Oil Against *Escherichia Coli* O157:H7 in Meat: Stx Genes Expression

Control of microorganisms in food

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**BACKGROUND AND OBJECTIVES:** This work aimed to (a) determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Carum copticum* essential oil (EO) against *Escherichia coli* O157:H7

**MATERIALS AND METHODS:** in vitro Trypticase Soy Broth (TSB) and in ground beef; (b) evaluation of the effect of sub-inhibitory concentrations (sub-MICs) of EO on the growth of the bacterium in TSB over 72 h (at 35 \_\_\_\_\_ C) and ground beef over 9 days (at 4 \_\_\_\_\_ C); and (c) investigation of gene expression involved in Shiga toxins production using relative quantitative real-time PCR method.

**RESULTS AND DISCUSSION:** The MIC in broth and ground beef medium was determined to be 0.05 (v/v) and 1.75 % (v/w), respectively. In comparison with control cultures, the EO concentration of 0.03 % in broth caused the reduction of colony counting as 1.93, 1.79, and 2.62 log<sub>10</sub> CFU ml<sup>-1</sup> after 24, 48, and 72 h at 35 \_\_\_\_\_ C, and similarly, EO (0.75 %) in ground beef reduced colony counting as 1.03, 0.92, 1.48, and 2.12 log<sub>10</sub> CFU g<sup>-1</sup> after 2, 5, 7, and 9 days at 4 \_\_\_\_\_ C, respectively. An increase and decrease in gene expression were observed as a result of EO addition (0.03 %) to broth and (0.5 %) to ground beef was noticed, respectively

**Keywords:** *Carum copticum*, Essential Oil, Stx Genes Expression.



# Identifying new bacterial species and strengthening the antioxidant properties of kombucha tea enriched with medicinal plants

Role of microorganisms in food production and preservation

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**BACKGROUND AND OBJECTIVES:** The present study was conducted with the aim of identifying bacterial species in kombucha tea enriched with medicinal plants and evaluating the antioxidant effect of tea

**MATERIALS AND METHODS:** Identification of bacteria from kombucha solution was done using molecular method. Black tea was used as a substrate and white sugar as a carbon source. Kombucha tea was prepared enriched with Kakuti medicinal herbs, marshmallow, marjoram and chamomile. The antioxidant activity of the samples was measured using the Cuprac method

**RESULTS AND DISCUSSION:** Kumagataeibacter and Lactobacillus spp. were the abundant isolated bacteria. Kumagataeibacter xilinus kombu NKJ1, Kumagataeibacter saccharivorans kombu NKJ2, Lactobacillus rhamnosus kombu, Lactobacillus acidophilus kombu NKJ7, and Lactobacillus acidophilus kombu NKJ8 were novel isolates which were reported in kombucha solution in the present study. The antioxidant activity in most of the samples increased with the addition of medicinal plants; so that the average antioxidant activity measured in the solutions during fermentation had a range of 0.99-224.86 nM trolox equivalent per ml. By adding medicinal plants to kombucha in the present study, we were able to increase the antioxidant properties of this beneficial product.

**Keywords:** Key words: Kombucha, Kumagataeibacter, Lactobacillus, Antioxidant activity

# Assessment of the ability of *Lactobacillus plantarum* to reduce aflatoxin M1

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Aflatoxin M1 (AFM1) is known to be a potent carcinogen and continues to pose a public health concern through consumption of contaminated dairy products. The role of Probiotics in the reduction or removal of aflatoxin has been predicted by binding. These bacteria can reduce the risk of AFM1 on human health to a certain extent. In the present study, the detoxification activity of *Lactobacillus plantarum* (L.p) ATCC14917 was investigated with the aim of reducing aflatoxin M1 using high performance liquid chromatography. Scanning electron microscope (SEM) was used to observe the morphology of L.p bacteria. The present findings show that L.p can play a role in reducing aflatoxin M1.

**MATERIALS AND METHODS:** L.p (ATCC14917) was purchased from the Iranian Biological Resource Center (IBRC). methanol (99.5%) and AFM1 (soluble in methanol) were purchased from Sigma-Aldrich (USA). NaCl, De Man, Rogosa and Sharp broth (MRS broth) and bacteriological agar were from Merck (Germany). L.p was cultured in 5 mL of MRS broth at 37°C. After 18 h, the culture medium was centrifuge at 5000 rpm for 10 min at 4°C to pellet the bacteria. After, discarding the supernatant, the pellet was resuspended in 10 mL of 0.15 M NaCl (pH 5.6) and centrifuged again. Aflatoxin M1 with a concentration of 0.1 mg/L was added to water and methanol (75:25) to prepare 10 ng/ml. 300µl of L.p suspension was added to 3 ml of AFM1 solution. Then it was vortexed and incubated at 37°C. After 24 hours, the tubes were centrifuged and after collecting the supernatant, they were transferred to clean tubes for HPLC analysis.

**RESULTS AND DISCUSSION:** SEM images show rod shape of our applied bacteria L.p in present study. the average size of bacteria was about 1 µm. In the present study, our obtained results showed that the highest level of detoxification by L.p was ±43.76% (10 µg/liter to 5.6 µg/liter). Previously (Jebali et al., 2015) in a study reported that *Lactobacillus plantarum* MON03 has significant binding ability to AFB1 and AFM1 in PBS (82% and 89%, respectively) within 24 hours of incubation.

**Keywords:** *Lactobacillus plantarum*, Aflatoxin M1, Detoxification

# Detoxification of AFB1 by Yeasts Isolates from Kefir and Traditional Kefir-Like Products

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Aflatoxin B1 (AFB1) is the most toxic aflatoxin produced by a large number of *Aspergillus* species. Successful detoxification of this toxin is an important attempt to improve community health. The aim of this study was to evaluate reducing effects of yeasts isolates from kefir and traditional kefir-like fermented beverages on AFB1 in a broth medium.

**MATERIALS AND METHODS:** Polymerase chain reaction-sequencing was carried out to identify the yeast isolates from kefir and kefir-like beverages. Effects of the isolates on AFB1 adsorption and biotransformation in peptone dextrose broth medium were evaluated by using high performance liquid chromatography.

**RESULTS AND DISCUSSION:** *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* were isolated from kefir and kefir-like beverages and resulted in 46% and 53% AFB1 adsorption, respectively. The isolates 27Y and 2Y caused 7% toxin biotransformation, while 10% toxin biotransformation was achieved by the isolate 18Y. Our results indicate that the yeast isolates from kefir and traditional kefir-like products can bind to and detoxify AFB1, thereby reducing its harmful effects.

**Keywords:** Kefir , Yeasts , Aflatoxin B1 , Adsorption , Biotransformation

# Development of a novel colon-specific oral delivery system for *Lactobacillus plantarum* by spray drying

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** *Lactobacillus plantarum* strains are well-known probiotics for gut health promotion, colonic cancer prevention, immune system modulation, and psychobiotic effects. However, like other probiotics, maintaining viability in storage and gastrointestinal conditions faces obstacles. This study aimed to investigate the application of hypromellose phthalate (HPMCP) together with hypromellose (HPMC) to produce a suitable encapsulation system for colon delivery of *Lactobacillus plantarum* following oral administration.

**MATERIALS AND METHODS:** Microparticles based on HPMCP encapsulating *Lactobacillus plantarum* bacteria were prepared using the spray drying method and also HPMC was applied due to its protecting effects against thermal and osmotic stresses. The viability of the encapsulated bacteria was evaluated under storage conditions and simulated gastrointestinal fluids via the plate count method. Physicochemical characterization of microparticles was carried out to investigate the morphology, particle size and size distribution, residual moisture, solubility, and flowability of the microparticles.

**RESULTS AND DISCUSSION:** The results showed that usage of HPMCP together with HPMC increases the survivability ratio of *Lactobacillus plantarum* in the spray drying process from 0.35% to 25.40% and maintains the viability of 7.27 log CFU.g<sup>-1</sup> after 6 h incubation in the simulated gastrointestinal fluids. *Lactobacillus plantarum* cells in the microparticles remained viable above 9 log CFU.g<sup>-1</sup> for 12 weeks in storage conditions. The results of this study demonstrated the great potential of HPMCP-based formulations in the microencapsulation of probiotics. Therefore, it can be a promising strategy for producing probiotic products and requires further investigations to expand the applications of probiotics.

**Keywords:** Probiotics, *Lactobacillus plantarum*, Microencapsulation, Spray Drying, HPMCP.

# Effect of Dietary Spirulina Cyanobacterial Supplementation on Hemolymph Immune Indices of Pacific White Shrimp (*Litopenaeus vannamei*) under Low Temperature Stress

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** The present study aimed to investigate the effect of dietary spirulina cyanobacterial powder on the improvement of hemolymph immune indices of Pacific white shrimp (*Litopenaeus vannamei*) under low temperature stress.

**MATERIALS AND METHODS:** 300 shrimp weighing 30-35 g were obtained from the Shrimp Research Center and acclimated to the experimental conditions. After weighing, the shrimp were divided into five groups and fed diets containing different levels of spirulina powder (0, 2, 5, and 10%) for 90 days. The temperature was gradually reduced from 24°C to 2-3°C over six hours. After 24 hours of exposure to low temperature, survival rate was calculated and hemolymph was collected from three live shrimp from each treatment for blood parameter assessment.

**RESULTS AND DISCUSSION:** The results showed that the total hemocyte count (THC) was significantly higher in the 10% treatment compared to the other treatments (P0.05). In the differential hemocyte count, the number of semi-granular cells was significantly higher in shrimp fed with 10% spirulina powder compared to the control treatment (P0.05). Large and hyaline granular cells were significantly higher in the control treatment compared to the 2% and 5% treatments (P0.05), but did not differ significantly from the 10% treatment. Overall, feeding with different levels of spirulina cyanobacterial powder, especially at 10%, improved the immune indices and survival of Pacific white shrimp (*Litopenaeus vannamei*) under low temperature stress.

**Keywords:** Pacific white shrimp, Cyanobacteria, Spirulina, Immune indices

# Evaluating the effect of *Lactobacillus plantarum* probiotic in limiting the growth of breast and liver cancer cells and normal cells by MTT assay

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Conventional cancer treatment often fails. Breast and liver cancer are two common types of cancer. In this study, we will examine the effects of *Lactobacillus planetarium* probiotic on the growth of cancer cells as well as normal cells using the MTT assay method.

**MATERIALS AND METHODS:** In this research, probiotic *Lactobacillus plantarum* (ATCC 14028) was prepared from Pasteur Institute, Tehran. Breast cancer cells (MCF-7), liver (HepG2) and normal cells (NIH3T3) were cultured in DMEM medium. Cells were treated with different concentrations of bacteria. The survival rate of cells after treatment with probiotics was measured by MTT assay. Statistical calculations for the comparison of different concentrations of bacteria (IC50) have been done using ANOVA and the corresponding post test (Tukey-Krame multiple comprehension test).

**RESULTS AND DISCUSSION:** There is a significant difference between the data of probiotic suspension and cisplatin drug on breast cancer, liver, and normal cell lines. The investigation of bacterial toxicity in breast cancer cell line showed that there is a significant difference with the control group at concentrations of 109, 1010, 1011 mg/ml (P0.001), and concentrations of 107 and 108 mg/ml have a significant difference with the cisplatin group (P0.0001). The rate of bacterial toxicity in the liver cancer cell line, the concentrations of 1010 and 1011 have a significant difference with the control group (P0.0001) and the concentrations of 107 and 108 mg/ml have a significant difference with cisplatin (P0.05). The amount of probiotic suspension (IC50) on MCF7, HepG2 and normal skin fibroblast cell lines is 39.79, 40.06 and 33.169 mg/ml, respectively. In this research, the probiotic *Lactobacillus plantarum* showed that it can be useful as a therapeutic agent. It is also suggested to use probiotic yogurt as a preventive agent in the treatment of breast cancer in the food industry.

**Keywords:** probiotic-cisplatin-cancer cell-normal cell-MTT method

# Evaluation of the Use of Microorganisms in the Production of Drugs Containing Beneficial Bacteria or Fungi for Gut Health

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Introduction The microbiome, comprising trillions of microorganisms inhabiting the human gastrointestinal tract, plays a pivotal role in maintaining gut health and overall well-being. Among these microorganisms, certain strains of bacteria and fungi, such as Lactobacillus and Bifidobacterium species, have garnered significant attention for their potential therapeutic effects. Harnessing the beneficial properties of these microorganisms, researchers are exploring their use in the production of drugs aimed at enhancing gut health. This study contributes to this emerging field by evaluating the effects of probiotic supplementation on mRNA expression levels of key gut health markers in a diverse demographic sample.

**MATERIALS AND METHODS:** Materials and Methods The study involved 200 participants (100 males and 100 females) aged 20-60 years, randomly selected from urban and rural areas. Participants were assigned to receive either a probiotic supplement or a placebo for a duration of 12 weeks. Blood samples were collected at baseline and post-intervention to extract RNA, and the mRNA expression levels of specific gut health markers, including Lactobacillus rhamnosus and Bifidobacterium longum, were quantified using quantitative real-time PCR.

**RESULTS AND DISCUSSION:** Results The demographic distribution exhibited a balanced representation across different age groups and geographic locations. Participants in the probiotic group demonstrated a significant increase in the mRNA expression levels of Lactobacillus rhamnosus (mean mRNA copies: 500 to 1250; p0.001) and Bifidobacterium longum (mean mRNA copies: 400 to 1280; p0.001) compared to the placebo group, which showed no significant changes (p0.05). Conclusion The findings of this study underscore the potential of probiotic supplementation in modulating the expression of beneficial gut bacteria, thereby promoting gut health. Incorporating microorganisms into drug formulations holds promise for the development of novel therapeutics targeting gut-related disorders. This research contributes valuable insights to the burgeoning field of microbiome-based interventions for enhancing human health.

**Keywords:** Microbiome Gut health Probiotics Lactobacillus rhamnosus Bifidobacterium longum Therapeutic effects

# In vitro detoxification of Aflatoxin B1 by *Lactiplantibacillus plantarum* isolated from the north of Iran: A pioneering insights into the origin of fermented beverages

Probiotics: application in food industry and medicine

Abdoljalil Eiri <sup>1</sup> © ©, Hami Kaboosi <sup>1</sup>, Abdollah Ardebili <sup>2</sup>, Farhad Niknejad <sup>3</sup>, Hamid Reza Joshaghani <sup>4</sup>

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**BACKGROUND AND OBJECTIVES:** Abstract The contamination of food and animal feeds with mycotoxins, particularly aflatoxin B1 (AFB1), poses significant risks to human health and causes economic losses. This study investigated bacteria from various fermented milk products to assess their ability to detoxify AFB1.

**MATERIALS AND METHODS:** A variety of household fermented kefir milk, kefir-like beverages, and kefir grains were collected from rural areas and subjected to microbial analysis. Gram-positive bacterial isolates were further identified based on the 16S rRNA gene homology analysis.

**RESULTS AND DISCUSSION:** Seven bacterial isolates that were initially identified as lactic acid bacteria were selected for their potential to detoxify AFB1. Effects of environmental factors and bacterial components were evaluated on AFB1 detoxification. The most frequent isolates belonged to the new genus *Lentilactobacillus* and *Lactiplantibacillus*, of which three strains were identified as *L. kefiri*, *L. diolivorans*, and *L. plantarum*. The selected *L. plantarum* isolate demonstrated optimal AFB1 detoxification at pH 4, a 4-hour exposure time, and a cell concentration of  $1.0 \times 10^{16}$  CFU/mL. Significant differences were observed in toxin removal between fermentation supernatant and cells ( $p < 0.05$ ), while temperature showed no significant effect on toxin detoxification. This study demonstrated the high ability of *L. plantarum* for AFB1 detoxification, suggesting potential applications for food and feed safety enhancement. Further research is warranted to optimize its effectiveness and explore broader applications.

**Keywords:** Aflatoxin B1, Detoxification, Fermented milk products, Lactic acid bacteria, *Lactiplantibacillus*



# Investigating the crucial role of selected bifidobacterium probiotic strains in preventing or reducing inflammation by affecting the autophagy pathway

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Several studies have shown that probiotics can prevent and reduce inflammation in inflammation-related diseases. However, few studies have focused on the interaction between host and probiotics in modulating the immune system through autophagy. Therefore, we aimed to investigate the preventive and/or therapeutic effects of native potential probiotics including three strains of Bifidobacterium (i.e., *B. bifidum*, *B. longum*, and *B. infantis*) on the inflammatory cascade by affecting autophagy gene expression 24 and 48 hours after treatment.

**MATERIALS AND METHODS:** Autophagy genes involved in different stages of the autophagy process were evaluated by quantitative polymerase chain reaction (qPCR). Gene expression investigation was accomplished by exposing the human colorectal adenocarcinoma cell line (HT-29) to sonicated pathogens ( $1.5 \times 10^8$  bacterial CFU/ml) and adding Bifidobacterium spp. (MOI10) before, after, and simultaneously with the induction of inflammation. An equal volume of RPMI medium was used as a control.

**RESULTS AND DISCUSSION:** Generally, our native potential probiotic Bifidobacterium spp. can increase the autophagy gene expression in comparison with the pathogen. Moreover, an increase in gene expression was observed with our probiotic strains' consumption in all stages of autophagy. Totally, our selected Bifidobacterium spp. can increase autophagy gene expression before, simultaneously, and after the inflammation induction, so they can prevent and reduce inflammation in an in vitro model of inflammation.

**Keywords:** Bifidobacterium spp.; autophagy; inflammation; gut microbiota; probiotics

# Investigating the Effects of *Lactobacillus Delbrueckii* on Blood Sugar and Insulin Level in Diabetic Mouse

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Lactobacilli are gram positive microorganisms that are beneficial for human health. They are often found in materials like probiotic dairy or supplements. According to some studies, Lactobacilli play a significant role in improving symptoms of diabetes and reducing blood sugar level. The present study probes into the effects of oral administration of *Lactobacillus Delbrueckii* on reducing blood sugar level in diabetic mice.

**MATERIALS AND METHODS:** In this study, 28 Syrian female mice were supplied. Then, 14 of them (weighing 180 mg/kg) were made diabetic through intraperitoneal injection of streptozocin. After 7 days, a blood sugar test was applied to verify their being diabetic. One group of the mice was fed with *Lactobacillus Delbrueckii* and carrot juice. The second diabetic group, on a daily basis, was treated with only carrot juice with no *Lactobacillus Delbrueckii*. The blood sugar level and signs of improvements in all of the groups were considered weekly. After four weeks, the mean of blood sugar and insulin serum levels in different groups were compared.

**RESULTS AND DISCUSSION:** The obtained results showed that in comparison with the control group, the symptoms of diabetes (Polyuria and Polydipsia) and blood sugar level in diabetic mice fed with *Lactobacillus Delbrueckii* improved significantly. In addition to it, investigation of insulin level in the mice treated with *Lactobacillus Delbrueckii* showed that they increased significantly in comparison with that of the control group.

**Keywords:** *Lactobacillus Delbrueckii*, diabetes, blood sugar, insulin

# Investigating the Effects of Synbiotic Intervention on Working Memory in Healthy Young Women

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** The gut-brain axis is increasingly recognized for its influence on the interaction between the central nervous system (CNS) and enteric nervous system (ENS), impacting various cognitive processes. While extensive research has explored these effects in disease contexts, the impact of gut microbiota modulation on working memory in healthy humans remains underexplored.

**MATERIALS AND METHODS:** We conducted a study to investigate the effects of synbiotic intervention on working memory among healthy young adults using a between-subjects pretest-posttest design with a control group. Thirty participants aged 19–35 were enrolled. Over a 15-day intervention period, the synbiotic group received daily doses of synbiotics containing live bacteria, including *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, *Lactobacillus reuteri*, *Bifidobacterium lactis*, *Bifidobacterium longum*, and *Bifidobacterium bifidum*, along with fructooligosaccharides (FOS) as a prebiotic, in enteric-coated capsules ( $10^9$  colony-forming units per capsule). Working memory was assessed using a parametric auditory working memory task. A generalized linear model (GLM) was employed to analyze the relationship between pretest and posttest sensitivity and threshold.

**RESULTS AND DISCUSSION:** In the synbiotic intervention group, significant correlations were found between pretest and posttest sensitivity ( $r = 0.79$ ,  $p = 0.007$ ) and threshold ( $r = 0.79$ ,  $p = 0.008$ ). Participants with higher pretest scores tended to exhibit higher posttest scores following the intervention. GLM analysis confirmed the significant effect of the synbiotic intervention on both sensitivity and threshold. In contrast, the control group showed weaker correlations in sensitivity ( $r = 0.12$ ,  $p = 0.67$ ) and threshold ( $r = 0.41$ ,  $p = 0.14$ ), indicating minimal improvement without intervention. Our findings demonstrate that synbiotic intervention effectively enhances both sensitivity and threshold among healthy young adults. These results underscore the potential benefits of synbiotic treatments not only in improving sensory outcomes but also in enhancing cognitive function. This suggests promising applications for synbiotic treatments as cognitive enhancers, warranting further investigation in clinical settings and populations with cognitive impairments.

**Keywords:** Gut-brain axis Synbiotics Probiotics Working memory cognitive function

# Investigating the stability of vitamin D3 and Bifidobacterium lactis nanoparticles coated with polycaprolactone-polyethylene glycol polycaprolactone triblock copolymer in Iranian white cheese and determining its physicochemical and sensory properties

Probiotics: application in food industry and medicine

Faeze Bayat<sup>1</sup> ©, Mehran Mohseni<sup>1</sup> P

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**BACKGROUND AND OBJECTIVES:** To investigate the stability and efficacy of vitamin D3 and Bifidobacterium lactis nanoparticles coated with polycaprolactone-polyethylene glycol-polycaprolactone (PCL-PEG-PCL) in enhancing the nutritional value of Iranian white cheese and to assess their impact on the cheese's physicochemical and sensory properties.

**MATERIALS AND METHODS:** Bifidobacterium lactis and vitamin D3 were encapsulated using PCL-PEG-PCL. The particle size was analyzed using Dynamic Light Scattering (DLS). Five different cheese groups were produced and analyzed over two months. This included assessing microbial viability in simulated gastric and intestinal environments, quantifying vitamin D3 content, and evaluating physicochemical and sensory characteristics using a 9-point hedonic scale.

**RESULTS AND DISCUSSION:** The Cheese with Bifidobacterium lactis coated with PCL-PEG-PCL and vitamin D3 nanoparticles (BCND3), showed a marked improvement in the stability of both Bifidobacterium lactis and vitamin D3. Specifically, the coated bacteria maintained a concentration of 10<sup>6</sup> cfu/g until day 45 in the intestinal model, demonstrating effective protection against the acidic gastric environment. The vitamin D3 nanoparticles displayed enhanced stability within the cheese matrix, contributing to the nutritional value and potentially extending the shelf life. Physicochemically, initial pH reduction due to lactic acid production stabilized by day 60, aligning with other groups. Sensory evaluation revealed a preference for the softer texture of cheese with vitamin D3 nanoparticles, suggesting a positive impact on consumer acceptability. The application of PCL-PEG-PCL coating significantly improved the stability of probiotics and vitamin D3 in cheese. This method proved effective in protecting Bifidobacterium lactis from harsh gastrointestinal conditions and in sustaining vitamin D3 content.

**Keywords:** Bifidobacterium lactis, Enrichment, Cheese, Vitamin D, Polycaprolactone-polyethylene glycol-polycaprolactone.

# Investigating the synergistic effect of *Lactobacillus casei* supernatant with citric acid on some of food pathogens

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Food consumers are concerned about the adverse effects that chemical food additives may have on people's health. Food spoilage and food poisoning caused by microorganisms can be prevented by a variety of food preservation methods. There are certain organic acids that are used to control the spread of food pathogens. The growth of pathogenic bacteria in foods is inhibited due to competition in food and the presence of inhibitory substances (obtained from lactic acid bacteria) such as lactic acid, hydrogen peroxide and bacteriocins. Natural preservatives can be used in food systems and in this Research Supernatant obtained from *Lactobacillus casei* was investigated as a natural preservative.

**MATERIALS AND METHODS:** *L. casei* was cultured in two environments: whey and MRS broth. After comparing the kinetics of bacterial growth in two environments and obtaining supernatants of different hours of growth of *L. casei* in two environments, the resulting supernatants on *E. coli* O157:H7 was investigated as a sample of food pathogens. The amount of  $10^6$  cfu/ml of *Escherichia coli* was added to the supernatant of *Lactobacillus casei* and after 24 hours of heating, the amount of one ml of the desired supernatant along with different amounts of citric acid (0.1, 0.2 and 0.3%) in separate steps with the porplate method to count. The number of *Escherichia coli* colonies was investigated. The supernatant obtained from 24 hours of growth of *L. casei* in whey medium and also the supernatant obtained from 24 hours of growth of *L. casei* in MRS broth medium had the highest antimicrobial effect among other supernatants.

**RESULTS AND DISCUSSION:** *L. casei* was cultured in whey and MRS broth, and after comparing the growth kinetics of bacteria in two media and obtaining the supernatant at different growth times, the supernatant was analyzed on *E. coli* O157:H7. The supernatant obtained from 24 hours of growth of *L. casei* in both environments had the highest antimicrobial effect among other hours and decrease the initial value of  $10^6$  cfu ml<sup>-1</sup> to about  $4 \times 10^2$  cfu ml<sup>-1</sup>. Although that supernatant was able to reduce the number of *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus* to a desirable and good extent, except for *E. coli* O157:H7 in the case of other 3 pathogens, no enhancement effect was observed between 24-hour supernatant and citric acid.

**Keywords:** Supernatant , Preservative, citric acid

# Isolation and Molecular Identification of Bifidobacterium in Industrial and Traditional Yogurt from Razavi Khorasan

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Bifidobacterium, a crucial bacterial group in the human gastrointestinal tract, is widely used in the food industry for its health benefits. Yogurts, both traditional and industrial, are key dietary components. However, there is a notable lack of studies isolating probiotics, particularly bifidobacterium, from industrial and traditional yogurts produced in specific towns of Razavi Khorasan. This study aims to isolate and identify bifidobacterium in these products using culture and PCR methods.

**MATERIALS AND METHODS:** Nine samples of probiotic industrial yogurt and eleven samples of traditional yogurt were collected from Mashhad, Neyshabour, and Gonabad in Razavi Khorasan province, Iran. Each sample was diluted, homogenized, and cultured on TOS, Bif, and APT media. Based on cell morphology and gram staining, bifidobacteria were identified using the colony count technique per ISO 29981(IDF 220): 2010. Final confirmation of isolates was performed using PCR methods.

**RESULTS AND DISCUSSION:** Bifidobacteria with irregular, curved, rod-shaped, or branched morphology were observed in most traditional and industrial samples after anaerobic culturing. The frequency of bifidobacterium in TOS, Bif, and APT media for probiotic industrial yogurt was 33.33%, 88.88%, and 88.88%, respectively. In traditional yogurt, the frequencies were 9.09%, 90.9%, and 90.9% for the same media. These isolates were further identified using PCR techniques. This study supports these products' claims that not all industrial probiotic yogurts contain bifidobacterium and may contain other probiotics. Additionally, bifidobacterium is present in most traditional yogurts, indicating their potential as equivalent to industrial yogurts in nutritional value. However, species determination requires sequencing and further investigation.

**Keywords:** Yogurt, Bifidobacterium, PCR, Bacterial Identification, Probiotics

# Probiotic *Bifidobacterium animalis* subsp. *lactis* BB-12 Reduces Inflammation in HT-29 Cells Infected by Some Foodborne Pathogens

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Foodborne illness is a serious public health concern. Among the various strategies to prevent foodborne illness, probiotics are found to be effective. *Bifidobacterium animalis* BB-12 have generated a lot of interest due to its potential health benefits, which include the ability to reduce the spread of food-borne diseases. In this study, we examined the anti-inflammatory effects of the BB-12 strain and its cell-free supernatant (CFS) on *Listeria monocytogenes* and *Salmonella enterica* subsp. *enterica* Serovar Typhimurium.

**MATERIALS AND METHODS:** This study assessed the apoptosis and cytotoxicity induced by pathogens, both individually and in co-culture with probiotic or its CFS, using Trypan blue staining and acridine orange (AO) staining and MTT assay. Additionally, the protein content in the supernatant was quantified using a BCA protein assay. We also investigated the expression levels of TGF- $\beta$  and TNF- $\alpha$  through qRT-PCR in both pathogens and co-cultured groups within HT-29 cells.

**RESULTS AND DISCUSSION:** Based on the MTT test results, the 8% concentration of probiotic supernatant showed the least cytotoxic effect and the highest survival rate in HT-29 cell. It was reported that the 24-hour supernatant had a protein percentage of 78/47 by using BCA protein assay. The best anti-inflammatory results of probiotics, by examining the results of Real-Time PCR and the expression of TNF $\alpha$  and TGF- $\beta$  genes, were the use of supernatant at 4 hours of treatment. The amount of apoptosis for treating the cell line with *S. Typhimurium* that was done by AO staining, was reported as 87/69%, which decreased to 18/27% after probiotic treatment, 6/42% after treatment with 8% CFS. Also, the amount of apoptosis for treating the cell line with *L. monocytogenes* was reported as 76/71%, which decreased to 19/79% after probiotic treatment, 8/67% after treatment with 8% CFS. Using the supernatant of this probiotic bacteria as a medicinal supplement or

**Keywords:** Probiotics, *Bifidobacterium lactis* BB-12, Anti-inflammatory, food-borne pathogens

# Production of synbiotic anti-cancer supplement containing probiotic bacteria and native prebiotic compounds

Probiotics: application in food industry and medicine

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ارشد میکروبیولوژی میکروب های بیماری زا<sup>۲</sup>

**BACKGROUND AND OBJECTIVES:** prebiotics are non-digestible but fermentable compounds that improve health by stimulating and activating probiotic bacteria in the end parts of the digestive system, including the colon and rectum. When prebiotic compounds and probiotic bacteria are used in a product at the same time, the resulting product is called synbiotic, which are known today as a factor for preventing many infectious and cancerous diseases. Therefore, in this research, the effect of prebiotics extracted from several local plants (*Cynara cardunculus* var. *scolymus*, *Aloe vera*, *Cichorium intybus* L., *Helianthus tuberosus* and *Quercus*) on the viability of the probiotic *Lactobacillus casei* was investigated. The results indicated that the prebiotic extracted from the *Cichorium intybus* L. plant was more effective in increasing the survival of *Lactobacillus casei* and therefore a synbiotic supplement containing the probiotic *Lactobacillus casei* and the prebiotic compound extracted from the *Cichorium intybus* L. plant was produced as a synbiotic anti-cancer supplement.

**MATERIALS AND METHODS:** MATERIALS: *Lactobacillus casei* bacteria, M.R.S broth culture medium, agar. METHODS: 1.Extraction from plants and extraction of prebiotic compounds 2.Activation and preparation of *Lactobacillus casei* bacteria 3.Investigating the fermentability of indigenous prebiotic compounds

**RESULTS AND DISCUSSION:** The results of the growth and survival rate of *Lactobacillus casei* bacteria in the environment prebiotics extracted from local plants (*conifer*, *aloe vera*, *Cichorium intybus* L., *Helianthus tuberosus*, *Quercus*) According to the results, the highest increase in (OD) was related to prebiotics from *cichorium intybus* L. and *aloe vera*, followed by *helianthus tuberosus* and finally *cynara cardunculus* var. *scolymus* and *quercus*, which is due to the property of creating gel in water. The metabolism of polysaccharides causes a decrease in the PH of the culture medium, which was the largest decrease in prebiotic *cichorium intybus* L., followed by *aloe vera* and *helianthus tuberosus*, and the lowest decrease was in *conifer* and *Quercus*. In the results obtained, the prebiotic from *cichorium intybus* L. plant is effective in increasing the survival of *Lactobacillus casei*. and a synbiotic supplement containing probiotic bacteria and prebiotic composition from *cichorium intybus* L. plant was produced as an anti-cancer synbiotic supplement.

**Keywords:** Probiotic, Prebiotic, Synbiotic, Cancer, Supplement, *Lactobacillus casei* bacteria



# Sensory evaluation and investigation of bacterial viability in probiotic yogurt enriched with rice bran oil

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Rice bran oil is an excellent source of natural antioxidants such as tocopherol, tocotrienol, and oryzanol. Adding rice bran oil emulsion to many products, including yogurt, improves the nutritional profile. Therefore, this study aimed to determine the effect of rice bran oil on the sensory characteristics and viability of probiotic yogurt bacteria.

**MATERIALS AND METHODS:** The rice bran oil was hydraulically extracted, and after preparing the emulsion, probiotic yogurt containing bifidobacterium bifidum was enriched in 5 groups (control, yogurt + free bran oil, yogurt + bran oil emulsion 2, 4, and 6%).

**RESULTS AND DISCUSSION:** Rice bran oil used in probiotic yogurt significantly increased the viability of probiotic bacteria (p0.05). Improvement of organoleptic properties (taste, aroma) of probiotic yogurt enriched with rice bran oil (p0.05). This increasing slope was more significant with the increase of emulsion percentage from 2 to 6%. In general, it can be concluded that fortified probiotic yogurt with 6% bran oil emulsion, a product with unique marketing potential, can be created, which preserves the beneficial properties of a probiotic product for 14 days.

**Keywords:** rice bran oil, yogurt, probiotic, emulsion, bacterial viability

# The advantageous anti-inflammatory impacts of native probiotic and paraprobiotic on kidney via gut-organ axis

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Background: The anti-inflammatory characteristics of probiotics and paraprobiotics can mitigate the inflammation in the kidney by modulating the gut-kidney axis by influencing the autophagy pathway. Our aim was to evaluate the anti-inflammatory effect of probiotics and paraprobiotics on the gut-kidney axis by targeting the autophagy pathway in the reduction of kidney inflammation.

**MATERIALS AND METHODS:** Methods: After a seven-day acclimation period, male C57Bl/6 mice were orally administered with either PBS, PBS combined with 2% DSS, a cocktail of probiotics consisting of native *Lactobacillus* spp. and *Bifidobacterium* spp. alongside 2% DSS, or a cocktail of paraprobiotics with 2% DSS for two weeks. Evaluation encompassed disease severity, colonic pathology, and the hepatic expression of autophagy-related genes.

**RESULTS AND DISCUSSION:** Results: An increase in colitis indices was observed in the DSS group. However, both treatments with probiotics and paraprobiotic cocktails were equally effective in reducing these colitis symptoms. Moreover, all autophagy-related genes were downregulated after exposure to DSS, while the paraprobiotic and probiotic were able to increase the expression of these genes. Conclusion: According to the current study, both our native probiotic strains and paraprobiotic could have anti-inflammatory effects by affecting the gut-kidney axis and through affecting the autophagy signaling pathway.

**Keywords:** Autophagy; Gut-kidney axis; Inflammation; Paraprobiotic; Probiotic

# The combined effect of fish oil containing Omega-3 fatty acids and *Lactobacillus plantarum* on colorectal cancer

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Colorectal cancer (CRC) is one of the deadliest malignancies. Recent attempts have indicated the role of diet in the etiology of CRC. Natural dietary compounds such as probiotics and Omega-3 fatty acids that act synergistically can be beneficial in finding a tremendous solution against CRC. To date, the combined effect of fish oil containing Omega-3 fatty acids (Omega-3) and *Lactobacillus plantarum* (*L. plantarum*) on CRC has been left behind. We here evaluated the effects of co-encapsulation of Omega-3 and probiotic bacteria on CRC cell lines compared to normal cells.

**MATERIALS AND METHODS:** Omega-3 and *L. plantarum* bacteria were co-encapsulated in three ways, including gelatin–gum Arabic, gelatin–chitosan, and chitosan–gum Arabic complex coacervate microcapsules. After treatment of cells (Normal [L929] and colorectal [C26]) by *L. plantarum*, Omega-3, and microcapsules, viability and growth capacity of cell lines were measured using the MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay. Isolated total RNA was used to evaluate the expression profile of BCL2-associated X protein (BAX), B-cell lymphoma 2 (BCL-2), and Caspase-3 (CASP3) genes by real-time polymerase chain reaction (PCR). Statistical analysis was performed with SPSS 25 software. A value of *p* .05 was considered statistically significant.

**RESULTS AND DISCUSSION:** The results indicated a significant reduction in cell viability of C26 in a concentration-dependent manner in the treated cells with all treatments, except gelatin–gum Arabic microcapsules. The messenger RNA (mRNA) expression level of the BAX and CASP3 genes in C26 cells being treated with all treatments significantly increased than in untreated cells, and the expression level of the anti-apoptotic factor of the BCL-2 gene decreased in C26 cells simultaneously (*p* .05). Although, the combined effect of Omega-3 and *L. plantarum* and microcapsulated treatments had no more effect on viability and apoptosis gene expression of cancer cells compared to Omega-3 or *L. plantarum*. In conclusion, combination therapy with fish oil containing Omega-3 and *L. plantarum* does not improve the anticancer effect of each alone.

**Keywords:** co-encapsulation, colorectal cancer, *Lactobacillus plantarum*, omega-3 fatty acids

# The effect of *Lactobacillus reuteri* bacteria on the levels of lipid peroxidation products such as malondialdehyde in adult Wistar rats

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Lipid peroxidation is one of the most important destructive effects of free radicals, leading to the destruction of cellular membranes and intracellular organelle membranes such as mitochondrial membranes. In this process, free radicals produce substances such as ketones, ethers, and aldehydes. The major aldehyde produced is malondialdehyde (MDA), which is an indicator of lipid peroxidation and a measure of oxidative stress conditions that underlie many diseases such as Parkinson's, heart failure, cancer, and Alzheimer's disease. On the other hand, there is evidence confirming the antioxidant properties of probiotics such as *Lactobacillus*. Their health-promoting properties, such as anti-cancer activity, anti-mutagenic activity, and hypocholesterolemia, lead to an increased use of these probiotics. The aim of this study was to investigate the effect of *Lactobacillus reuteri* bacteria on the concentration of MDA as the most important parameter of oxidative stress.

**MATERIALS AND METHODS:** In this study, 20 adult Wistar rats weighing  $200 \pm 20$  grams and aged 5 weeks were used after obtaining approval from the ethics committee. The animals were divided into two groups, control and intervention, receiving normal saline and  $1 \times 10^9$  CFU/g of *L. reuteri* bacteria solution in raw culture medium via gavage for one month. The level of MDA concentration was measured by blood sampling from the heart and Lipid Peroxidation Assay Kit. The mean and standard deviation of MDA concentration were calculated using SPSS version 26 software, and one-way analysis of variance was used to assess the significant relationship.

**RESULTS AND DISCUSSION:** Daily gavage administration of *L. reuteri* for one month resulted in a significant decrease in the mean MDA levels in the intervention group compared to the control group ( $P \leq 0.05$ ). (Control:  $0.030 \pm 0.005 \text{ M} \times 10^{-5}$  and Intervention:  $0.010 \pm 0.005 \text{ M} \times 10^{-5}$ ). Based on the findings of this study, *L. reuteri* significantly reduced the production of MDA due to the intervention of *L. reuteri*, likely leading to a reduction in free radicals through its antioxidant effect and decreasing the risk of diseases associated with oxidative stress. It can be inferred from these results that the consumption of *Lactobacillus* strains as antioxidants has a significant impact on reducing oxidative stress.

**Keywords:** *Lactobacillus reuteri*, malondialdehyde, lipid peroxidation, oxidative stress

# The Effect of Prebiotic Consumption on Calcium Absorption and Bone Density in Postmenopausal Women

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Postmenopausal women often experience decreased bone density due to lower estrogen levels, which can lead to osteoporosis and increased fracture risk. This study investigates the impact of prebiotic consumption on calcium absorption and bone density in this demographic.

**MATERIALS AND METHODS:** A total of 120 postmenopausal women, aged between 55 and 70 years (mean age: 62 years), participated in this randomized controlled trial. Participants were divided into two groups: the intervention group (n=60) received a daily prebiotic supplement, while the control group (n=60) did not receive any supplementation. Bone mineral density (BMD) was measured using dual-energy X-ray absorptiometry (DEXA) at the lumbar spine and hip at baseline and after six months. Serum calcium levels were also assessed at these time points

**RESULTS AND DISCUSSION:** The intervention group showed a significant increase in serum calcium levels, with an average rise from 9.1 mg/dL to 9.6 mg/dL after six months. In contrast, the control group showed no significant change in serum calcium levels (9.0 mg/dL at baseline and 9.1 mg/dL after six months). Additionally, the intervention group experienced a notable improvement in BMD at both the lumbar spine (an increase from 0.85 g/cm<sup>2</sup> to 0.88 g/cm<sup>2</sup>) and hip (an increase from 0.78 g/cm<sup>2</sup> to 0.81 g/cm<sup>2</sup>), while the control group showed a slight decrease in BMD at these sites (lumbar spine: 0.85 g/cm<sup>2</sup> to 0.84 g/cm<sup>2</sup>; hip: 0.78 g/cm<sup>2</sup> to 0.77 g/cm<sup>2</sup>). Conclusion The consumption of prebiotics significantly enhances calcium absorption and positively influences bone density in postmenopausal women. This suggests that prebiotics could be a beneficial dietary supplement for improving bone health and reducing the risk of osteoporosis in this population.

**Keywords:** Prebiotics, Calcium Absorption, Bone Density, Postmenopausal Women, Osteoporosis, Dual-energy X-ray

# the effect of probiotic drugs in the treatment of vaginitis in women suffering from antibiotic resistance

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** In recent years, antibiotics have played a vital role in controlling infections. However, self-administration of drugs without a doctor's prescription increases drug resistance. Antibiotics have the highest per capita consumption among drugs. Therefore, we see an increase in antibiotic resistance among people, especially women. Since women with active erectile dysfunction often suffer from various types of vaginitis, antibiotics are the most commonly used among these people. Also, drug side effects such as digestive disorders are more common in these people. The purpose of this study is to investigate probiotics in the treatment of vaginitis and prevent recurrent infections as well as prevent antibiotic resistance.

**MATERIALS AND METHODS:** In this study, half of a small group of women with vaginitis were given antibiotics for 7 days, while the other half were given antibiotics plus a probiotic for 7 days. The recovery rate in 7 days was nearly 90% in the antibiotic plus probiotic group, compared to 40% in the antibiotic alone group. In another study conducted on 42 healthy women, taking just one probiotic supplement was enough to treat vaginitis and maintain a healthy level of bacteria in the vagina. Also, in another study, we examined the effects of using probiotic vaginal suppositories for the treatment of vaginitis. 57% of women who used Lactobacillus vaginal suppositories were able to cure their vaginitis and maintain a healthy balance of vaginal bacteria after treatment.

**RESULTS AND DISCUSSION:** More than fifty different species of tiny organisms called the microbiome live inside the vagina. Many of these microbes are a type of bacteria called Lactobacillus. These bacteria help maintain vaginal health and prevent infections. Lack of lactobacilli and excessive growth of some other microbes can cause imbalance in the vagina. Suffering from vaginal imbalance may increase the possibility of getting urinary tract and vaginal infections. One of the things that causes the destruction of lactobacilli is the excessive use of antibiotics

**Keywords:** Antibiotic resistance, vaginitis, women's diseases

# The effects of *Limosilactobacillus reuteri* and *Pediococcus acidilactici* probiotics in ulcerative colitis in wistar rat model.

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Ulcerative colitis (UC) is a widely recognized form of inflammatory bowel disease characterized by recurring inflammation in the rectum and colon.

**MATERIALS AND METHODS:** , thirty-two male Wistar rats were categorized into four separate groups to evaluate the protective effects of *Limosilactobacillus reuteri* and *Pediococcus acidilactici* probiotics against acetic acid-induced ulcerative colitis. The normal group, Group 1, was given only PBS, while Group 2 was induced with acetic acid to trigger ulcerative colitis. Group 3, post-UC induction, was provided with probiotics at a daily dose of 30mg/kg, and Group 4, post-UC induction, was treated with prednisolone at a daily dose of 4mg/kg. After ten consecutive days, the rats were euthanized, and their intestinal tissue and blood serum were thoroughly examined to evaluate inflammatory mediators and oxidative stress indices. After histopathological assessments, biochemical enzyme assays, survival rate evaluations, and real-time PCR analyses for cytokines gene expression

**RESULTS AND DISCUSSION:** it was observed that rats treated with probiotics displayed increased survival rates. Levels of inflammatory enzymes such as MPO and NO decreased, and improvements were noted in the liver and intestinal tissues. Pathologically, the intestinal tissue returned to its normal state, and there was a reduction in the release of inflammatory cytokines. The results were superior to those of the prednisolone group and were statistically comparable (p0.05). These findings suggest that *Limosilactobacillus reuteri* and *Pediococcus acidilactici* probiotics possess anti-inflammatory properties that effectively manage UC. Moreover, enhancing intestinal microflora can aid in controlling UC.

**Keywords:** Ulcerative Colitis, probiotic, lactobacillus, inflammation, immunomodulator.

# The preventive and therapeutic role of native probiotic *Lactobacillus* species in controlling inflammation

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Inflammatory bowel disease is a chronic condition that leads to inflammation and damage to the gastrointestinal tract. Since dysregulation of the immune system is one of the triggers for IBD, taking probiotics as an immunomodulator in the gut could help control inflammation and IBD by influencing signaling pathways. The present research applied in vitro models to investigate the effectiveness of our native probiotic *Lactobacillus* spp. in modulating MAPK and MYD88 independent inflammatory signaling pathways.

**MATERIALS AND METHODS:** HT-29 cells were exposed to Gram-negative bacteria to assess alterations in pathways associated with inflammation activities before, after, and simultaneously treatment with *Lactobacillus* spp. cocktail (*Lactiplantibacillus plantarum* PR 365, *Lactiplantibacillus plantarum* PR 42, *Lactocaseibacillus rhamnosus* PR 195, *Levilactobacillus brevis* PR 205, *Limosilactobacillus reuteri* PR 100). This assessment was conducted using the real-time PCR technique.

**RESULTS AND DISCUSSION:** Our findings indicated that the expression of MAPK and MYD88- independent signaling pathways genes increased after exposure to Gram-negative components, however, using our probiotic strains led to a significant reduction in the expression level of MAPK genes in all treatments and phases. The expression levels of MYD88- independent genes were different after our selected *Lactobacillus* strain treatment. The *Lactobacillus* spp. strains demonstrated anti-inflammatory effects on HT-29 cells by modulating MAPK and MYD88-independent signaling pathways. Our results indicate that these native potential probiotic strains exhibited beneficial effects across all three treatment phases. Therefore, it can be inferred that these probiotic strains, as beneficial agents, may be considered for the prevention and treatment of inflammation-related diseases such as inflammatory bowel disease.

**Keywords:** IBD, inflammation, *Lactobacillus* spp., MAPK, MYD88-independent, signaling pathways



# The therapeutic potential and preventive effect of calcium alginate and magnesium hydroxide microcapsule probiotics in ulcerative colitis animal model

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** Inflammatory Bowel Disease (IBD), is classified in chronic and inflammatory diseases of the gastrointestinal tract categories, which includes ulcerative colitis and Crohn's disease. Symptoms associated with this disease include diarrhea, abdominal pain, rectal bleeding, and weight loss. probiotics have shown a favorable protective feature against the occurrence of this digestive inflammatory disease. In fact, probiotic compounds through regulation of pro-inflammatory to anti-inflammatory cytokines ratio, maintaining homeostasis in the Intestinal microbial flora. Encapsulation of probiotics in a gel matrix (micron scale) with the feature of maintaining the viability of purified bacteria, known as bulk encapsulation, is a common method or using probiotic cells. The aim of our study is to encapsulate isolated probiotic bacteria in a calcium alginate and magnesium hydroxide hydrogel coating, as a protective strategy against adverse conditions of the gastrointestinal tract, and especially to evaluate its preventive and therapeutic potential in an animal model of ulcerative colitis

**MATERIALS AND METHODS:** 30male Wistar rats were divided into five groups: 1) control 2) colitis 3) Colitis receiving plantarum probiotic 4) Mesalazine 5) Colitis receiving Plantarum probiotic + mesalazine. In order to evaluating the preventive potential of plantarum encapsulated, Groups 3 to 5 were treated for 7 days with Plantarum probiotic, by dose of  $10^{10}$  cfu/ml by gavage before creating the colitis model. After 7 days, groups except the control, our colitis model was established by intrarectal injection of 4% acetic acid and then treatments were continued for more five days. During this period, subjects were evaluated for Disease activity index (DAI): weight loss, diarrhea, rectal bleeding and the consistency of stool tissue

**RESULTS AND DISCUSSION:** The results showing the encapsulated plantarum consumption, especially in combination with mesalazine, significantly improved DAI index including rectal-bleeding, stool-consistency, diarrhea, and weight loss. Histological examination of the colon tissue indicated that consumption of plantarum encapsulated could reducing the incidence of inflammation and fibrosis in colon tissue compared to colitis model. so, its able to improves the colon structure: crypts and goblet cells. results demonstrating that, plantarum encapsulated probiotics using in combination with standard drugs, can improve clinical signs and histological changes.

**Keywords:** Ulcerative-colitis, Lactobacillus-plantarum, probiotics, micro-encapsulation, calcium-alginate-magnesium-hydroxide

# The unknown potential of microorganisms: Bacillus and the production of probiotic microcapsules from them

Probiotics: application in food industry and medicine

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**BACKGROUND AND OBJECTIVES:** The background and the objective of probiotic is about the alive microbes that when added to food, improves the flord microbes in digestion system and it has positive effect for the host body. Study shows that the bacteria producing lactic acid specially bacillus in high temperature remain stable because of having endospore, and keep the probiotic features at cooking temperatures.

**MATERIALS AND METHODS:** In this research first, we took five sample from jamshidieh park randomly, then by serial dilution and thermal agitation to separate exclusively bacillus in this research. Next purification of grown colonies and coloring the spore's catalase test, oxidase test verifies the first material of bacillus was conducted. Next, some test such as acid resistance, salt test, bile salt test, and antibiotics resistance test were done to check the probiotics being of the bacillus which were purified, in order to clearly specify the material and species of the stronger one's probiotic molecular sequencing (16srRNA) were conducted. Next stage, the production of microcapsules, the bacillus probiotic using sodium alginate were done. The analyses to verify production of microcapsules were done.

**RESULTS AND DISCUSSION:** in this study 32 strain of bacillus were separated in which 7 strain has probiotic features and finally (ZK1) was the specific strain with all the feature of probiotics was selected. After sequencing (16srRNA) the special strain had similar characteristic of bacillus mojavensis up to 99.8% percent was identified. Microcapsuling result with sodium alginate in the mentioned process microcapsules with the size of 12-5 micrometer with uniform dispersion were verified. By the result of this study, we can say that the local soil in this country and the sample bacillus separated from it, is a good source to produce and identification of local valuable bacteria and non-pathogenic as new source of probiotic bacteria. In this study we found that bacillus whit spores which have probiotic feature for great maintenance can be use in food and pharma logical industries.

**Keywords:** Bacillus, Probiotic, microcapsules

# A deep learning-based model for detecting *Leishmania amastigotes* in microscopic slides: a new approach to telemedicine

Applications of Artificial Intelligence in Microbial Diagnosis

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**BACKGROUND AND OBJECTIVES:** Leishmaniasis, an illness caused by protozoa, accounts for a substantial number of human fatalities globally, thereby emerging as one of the most fatal parasitic diseases. The conventional methods employed for detecting the leishmania parasite through microscopy are not only time-consuming but also susceptible to errors. Therefore, the main objective of this study is to develop a model based on deep learning, a subfield of artificial intelligence that could facilitate automated diagnosis of leishmaniasis.

**MATERIALS AND METHODS:** In this research, we introduce LeishFuNet, a deep learning framework designed for detecting *Leishmania* parasites in microscopic images. To enhance the performance of our model through same-domain transfer learning, we initially train four distinct models: VGG19, ResNet50, MobileNetV2, and DenseNet 169 on a dataset related to another infectious disease, COVID-19. These trained models are then utilized as new pre-trained models and fine-tuned on a set of 292 self-collected high-resolution microscopic images, consisting of 138 positive cases and 154 negative cases. The final prediction is generated through the fusion of information analyzed by these pre-trained models. Grad-CAM, an explainable artificial intelligence technique, is implemented to demonstrate the model's interpretability.

**RESULTS AND DISCUSSION:** The final results of utilizing our model for detecting amastigotes in microscopic images are as follows: accuracy of  $98.95 \pm 1.4\%$ , specificity of  $98 \pm 2.67\%$ , sensitivity of 100%, precision of  $97.91 \pm 2.77\%$ , F1-score of  $98.92 \pm 1.43\%$ , and Area Under Receiver Operating Characteristic Curve of  $99 \pm 1.33$ . The newly devised system is precise, swift, user-friendly, and economical, thus indicating the potential of deep learning as a substitute for the prevailing leishmanial diagnostic techniques.

**Keywords:** Leishmania, Deep Learning, Transfer Learning, Image processing, Machine Learning, Artificial

# Artificial intelligence KNN algorithm with blood test on the killing ability of neutrophils in mouse model for diagnosis and treatment of infectious diseases

Applications of Artificial Intelligence in Microbial Diagnosis

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**BACKGROUND AND OBJECTIVES:** In today's world, intelligent systems based on a series of artificial intelligence algorithms and methods learn various problems and perform tasks automatically. KNN (k-Nearest Neighbors) is a supervised machine learning algorithm that is used to predict the label of data points by observing the majority in the nearest neighbors. A practical application of it in the field of medicine includes a complete blood cell count, which is an important diagnostic test to assess the general state of health in the diagnosis of various diseases. The purpose of this research, a machine learning approach for the identification and counting automatically of three types of blood cells including red blood cells, white blood cells, and platelets are presented using a recognition algorithm and classification objects with helping of images YOLO (you only look once) and supervised machine learning for doing diagnose and treat infectious diseases.

**MATERIALS AND METHODS:** In this study, the free dataset CBC (Complete Blood Count) was used. It contains 360 blood smear images of 45 adult female mice along with their annotation files, which are divided into training, test and cross validation sets. YOLO predicts five values along with class probabilities for each box. We apply the KNN algorithm to each neutrophil and we determine the nearest neutrophil and then using the intersection of union (IOU) between two neutrophils, also we calculate their overlap Level.

**RESULTS AND DISCUSSION:** The diagnostic method of KNN algorithm of blood parameters showed that the total number of white blood cells in all 4 experimental groups has a significant increase compared to the control group (P 0.05). The number of neutrophils and lymphocytes in all 4 experimental groups showed a significant increase compared to the control group. But no significant difference was observed in eosinophils and monocytes. The therapeutic performance of the KNN algorithm by analyzing clinical and laboratory data shows that the extract of the *Abolus sembacus* plant can probably strengthen the immune system by increasing the number of white blood cells with a significant level of p 0.001.

**Keywords:** artificial intelligence algorithm, YOLO, KNN, infectious diseases, blood cells

# Differential Diagnosis of Bacterial Blood Infections Using AI-Based Expert Systems: A Case-Control Study

Applications of Artificial Intelligence in Microbial Diagnosis

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**BACKGROUND AND OBJECTIVES:** Bacterial blood infections, or bacteremia, present significant diagnostic challenges due to their varied clinical manifestations. Rapid and accurate differentiation between types of bacterial infections is crucial for effective treatment. Traditional diagnostic methods can be time-consuming and sometimes lack the precision needed for timely intervention. This study investigates the efficacy of artificial intelligence (AI)-based expert systems in the differential diagnosis of bacterial blood infections, aiming to enhance diagnostic accuracy and speed.

**MATERIALS AND METHODS:** A case-control study was conducted involving 300 participants, divided equally into cases (those diagnosed with bacterial blood infections) and controls (healthy individuals). The sample comprised 160 males and 140 females, with a mean age of 45 years. Data were collected from patient records, including demographic information, clinical symptoms, laboratory results, and microbiological findings. The AI-based expert system was trained on a dataset comprising these variables and then used to analyze the data for diagnostic accuracy. The system's performance was evaluated based on its sensitivity, specificity, and overall accuracy.

**RESULTS AND DISCUSSION:** The AI-based expert system correctly identified bacterial blood infections in 138 out of 150 cases, resulting in a sensitivity of 92%. For the control group, 142 out of 150 individuals were correctly identified as not having an infection, resulting in a specificity of 95%. The overall accuracy of the system was 93.5%, demonstrating its potential for reliable diagnostic support. Additionally, the system significantly reduced the time required for diagnosis compared to traditional methods, providing results within minutes. Conclusion: AI-based expert systems show high accuracy in the differential diagnosis of bacterial blood infections, providing a valuable tool for clinicians. Their implementation could enhance diagnostic efficiency, leading to improved patient outcomes and optimized use of medical resources. These systems offer a promising solution to the challenges of diagnosing bacteremia, especially in settings where rapid decision-making is critical.

**Keywords:** Bacterial blood infections, differential diagnosis, artificial intelligence, expert systems, case-control

# The application of Artificial Intelligence (AI) in diagnosing Dengue disease

Applications of Artificial Intelligence in Microbial Diagnosis

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**BACKGROUND AND OBJECTIVES:** Dengue fever is a viral disease transmitted by mosquito bites, presenting symptoms from mild flu-like signs to severe complications. It predominantly affects tropical and subtropical regions, especially in developing countries, posing a threat to over half of the global population. Although vaccines and other treatments have been discovered, early detection remains the most effective way to reduce the mortality rates and severe complications of the disease. Artificial intelligence (AI) encompasses technologies that enable computers to perform advanced functions such as understanding and translating language, analyzing data, and making recommendations. AI has demonstrated significant potential in various medical fields, including as a predictive tool for diseases. The aim of this study is to investigate the applications of artificial intelligence in the prediction and diagnosis of dengue disease.

**MATERIALS AND METHODS:** The study, conducted in 2024, used three global scientific databases, including ISI, PubMed and Scopus. The present paper is a review study. In this study, 11 articles published from 2015 to 2024, which were in the form of quantitative studies, meta-analysis and original research and systematic review were examined. Entry criteria included: Availability of full text and articles published between 2015 and 2024, and exit criteria included: Case Report studies. The study used the keywords Artificial Intelligence, Dengue, Diagnosis.

**RESULTS AND DISCUSSION:** Over the past decade, the global incidence of dengue has risen sharply, becoming a significant public health concern. Early detection and medical intervention can reduce the mortality rate of severe dengue from 50% to 2%. AI models can analyze various factors, including climate data, population density, and historical outbreak patterns, to predict future dengue outbreaks, aiding in preventive measures. Additionally, AI can assist in diagnosing dengue by analyzing blood smear images. Machine learning algorithms can detect the presence of the dengue virus in blood samples, supporting clinicians in making accurate diagnoses. The use of AI in diagnosing Dengue can lead to earlier detection, which is crucial for effective treatment and management of the disease. AI-based models can enhance the accuracy and speed of diagnosis by analyzing complex data patterns that are often missed by traditional methods.

**Keywords:** Artificial Intelligence, Dengue, Diagnosis.

# Computational Screening for Staphylococcus aureus Aureolysin Inhibitors: A Molecular Docking Approach

Role of Microorganism in Pharmaceutical Industry

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**BACKGROUND AND OBJECTIVES:** Staphylococcus aureus can cause a wide range of diseases, from relatively mild skin infections to potentially life-threatening conditions such as pneumonia and sepsis. Aureolysin, a significant virulence factor of the bacterium, is a secreted metallopeptidase that enhances hypervirulence and facilitates the transition from a sessile biofilm-forming lifestyle to a mobile invasive phenotype. To date, no specific inhibitors of Aureolysin have been discovered, making it a vital target for the development of novel antimicrobial drugs amid rising antibiotic resistance. Consequently, in this study, we evaluated twenty selected Aureolysin inhibitory ligands and identified the most effective candidate using a molecular docking method, thereby providing a foundation for future drug design to combat Staphylococcus aureus infections.

**MATERIALS AND METHODS:** The 3D structure of Aureolysin (PDB ID: 7skL) was obtained from the RCSB PDB database. The inhibitory ligands of Aureolysin including Phenanthroline, Ethylenediaminetetraacetic acid (EDTA), Cu, Zn, and a total of 5 analogs for each ligand were chosen from the zinc database in SDF format. After preparing the protein and ligands, molecular docking was performed using Virtual Docker Molegro ver. 6.0. Subsequently, the best interaction with the lowest energy binding was analyzed using Molegro Molecular Viewer software. Finally, pharmacokinetic properties such as the distribution, absorption, metabolism, and excretion of the ligand were investigated using the SwissADME server.

**RESULTS AND DISCUSSION:** Among all the inhibitory ligands and their analogs, the best ligand for Aureolysin was identified as an EDTA analog with a molecular weight of 305.38 g/mol and a binding energy of -108.5754 kcal/mol. This ligand formed five steric interactions with the Aureolysin residues Thr362, Glu361, Asp318, Gln317, and His313, one hydrogen bond with Glu361, and an electrostatic interaction with Glu361 of Aureolysin. Moreover, the ADME results indicated that this ligand had a logS water solubility score of -1.72, lipophilicity (XLoGP3) of 0.18, polarity (TPSA) of 99.52, and hydrogen bond donors and acceptors of 2 and 4, respectively. As a result, compared to other ligands, the EDTA analog may be used as an important candidate to inhibit the targeting of the Aureolysin protein of Staphylococcus aureus after conducting further in vitro and in vivo analyses.

**Keywords:** Aureolysin, EDTA, Molecular Docking, Staphylococcus aureus

# Effect of LT Fishmeal Supplementation on Immune Response and Survival of Pacific White Shrimp under Low Temperature Stress

Role of Microorganism in Pharmaceutical Industry

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**BACKGROUND AND OBJECTIVES:** The present study aimed to investigate the effect of LT fishmeal dietary supplementation on the improvement of hemolymph immune indices of Pacific white shrimp (*Litopenaeus vannamei*) under low temperature stress. LT fishmeal is a special type of fishmeal produced by drying fish at low temperatures (around 70°C) for a longer period of time, which results in a high-quality protein powder with minimal protein degradation.

**MATERIALS AND METHODS:** Pacific white shrimp were fed with diets containing different levels of LT fishmeal (0%, 25%, 50%, and 100%) for 60 days. After 60 days of feeding with different levels of LT fishmeal the temperature was gradually reduced from 24°C to 2-3°C over six hours. After 24 hours of exposure to low temperature, survival rate was calculated and hemolymph was collected from three live shrimp from each treatment for blood parameter assessment.

**RESULTS AND DISCUSSION:** The results showed that the total hemocyte count (THC) was significantly higher in the 50% treatment compared to the other treatments (P<0.05). In the differential hemocyte count, the number of semi-granular cells was significantly higher in shrimp fed with LT fishmeal compared to the control treatment (P<0.05). Large and hyaline granular cells were significantly higher in the control treatment compared to the 25 and 100% treatments (P<0.05), but did not differ significantly from the 50% treatment. Overall, feeding with different levels of LT fishmeal, especially at 50%, improved the immune indices and survival of Pacific white shrimp (*Litopenaeus vannamei*) under low temperature stress.

**Keywords:** Pacific white shrimp, LT fishmeal, Immune indices



# Evaluation of molecular docking between the pertussis toxin from *Bordetella pertussis* and its inhibitory ligands

Role of Microorganism in Pharmaceutical Industry

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**BACKGROUND AND OBJECTIVES:** *Bordetella pertussis* is a gram-negative, aerobic, pathogenic, and encapsulated coccobacillus responsible for causing pertussis or whooping cough. The rising number of cases in recent years demonstrates that pertussis is not being effectively managed and is resurging. This, coupled with the absence of effective treatments for whooping cough, underscores the importance of developing novel pharmacological methods for combating toxin-related illnesses. As the major virulence factor of pertussis is pertussis toxin (PT), our objective was to identify the most potent ligand for inhibiting toxin as a potential drug candidate through molecular docking analysis.

**MATERIALS AND METHODS:** The 3D structure of pertussis toxin (PDB ID: 1PRT) and its inhibitor (7-Nitro-4-(1-oxidopyridin-1-ium-2-yl) sulfanyl-2,1,3-benzoxadiazole (Pubchem CID: 313619), along with 17 its available analogs, were retrieved from RCSB PDB and the PubChem databases, respectively. After preparing the protein and ligands by lowering the energy level, and adding electric charges and hydrogen atoms, molecular docking was performed using Molegro Virtual Docker software v. 6, and the best interaction with the lowest energy binding was analyzed in Molegro Molecular Viewer software. Using the SwissADME database, the pharmacokinetic properties of ligands (ADME) were evaluated.

**RESULTS AND DISCUSSION:** Among all the ligands studied, 1,7-Bis(but-2-en-2-yl)-3,9-dihydroxy-4,10-dimethylbenzo[b][1,4]benzodioxepin-6-one (PubChem CID: 132267) with a molecular weight of 290.24 g/mol, exhibited the most negative  $\Delta G_{bind}$  (-130.095 Kcal/mol). This ligand formed three hydrogen bonds with the PT residues Tyr20, Tyr29, and Asp81, as well as six steric interactions involving the PT residues Leu82, Asp81, Asp81, Tyr40, Tyr20, and Pro3. Moreover, the ADME results indicated that this ligand had a LogS water solubility score of -2.84, a lipophilicity (XLogP3) of 1.34, a polarity (TPSA) of 135.5, and hydrogen bond donors and acceptors of 3 and 6, respectively. Our findings provide a foundation for future experimental validation and potential drug development efforts aimed at mitigating the impact of whooping cough through targeted inhibition of pertussis toxin. Further analyses are needed to prove this finding.

**Keywords:** Molecular Docking, *Bordetella pertussis*, pertussis toxin, ADME.

# Expression of Ribosome-Inactivating Protein ebulinI Isolated from Dwarf elder in *E. coli* and evaluation of Cytotoxicity of Recombinant Protein on HT-29 Colon Cancer Cell Line

Role of Microorganism in Pharmaceutical Industry

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**BACKGROUND AND OBJECTIVES:** Ribosome-inactivating proteins (RIPs) are a family of enzymes with N-glycosidase activity on the 28s rRNA in eukaryotic cells that irreversibly prevent protein synthesis. These protein toxins are mostly present in higher plants, but with the different tissue distribution and abundance depending on the species. *Sambucus ebulus* L. (Dwarf elder) is one of the medicinal and valuable species of the North of Iran which because of having a complex mixture of diverse types of RIPs and related lectins, it is a suitable plant model for studying these proteins.

**MATERIALS AND METHODS:** In this study, encoding sequence of type 2 RIP ebulinI was isolated from *Sambucus ebulus* L. The nucleotide sequence of ebulinI was ligated to the pET-28a (+) expression plasmid and cloned into the *E. coli* strain BL21 (DE3) in order to express heterologously of recombinant protein. In order to increase the expression of recombinant protein in soluble form, co-expression of the target protein with pG-Tf2 chaperone plasmid and reduction of growth temperature after induction were used to increase protein solubility and function. Finally, the recombinant protein was purified by Ni-NTA affinity purification and the anti-cancer activities of the purified protein were examined by MTT assay.

**RESULTS AND DISCUSSION:** The results of cytotoxicity assay showed that ebulinI considerably inhibited the proliferation of cancer cell lines HT-29 in a time- and dose-dependent manner. The inhibition of cell viability enhanced with increasing concentration of ebulinI. The IC<sub>50</sub> values, estimated after 24, 48, and 72 h incubation, were 0.5, 0.009, and 0.006 µg/mL for HT-29 cells. Based on our findings, it can be considered that ebulinI possesses a high potential to use in medicine especially in producing RIP-based immunotoxins and antitumor drugs.

**Keywords:** Type 2 Ribosome-inactivating protein, Anti-cancer activity, HT-29 Colon Cancer Cell

# Investigation of Potent Inhibitors to Control Mycobacterium abscessus by Targeting Its Dihydrofolate Reductase Protein: A Molecular Docking Study

Role of Microorganism in Pharmaceutical Industry

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**BACKGROUND AND OBJECTIVES:** Mycobacterium abscessus is a non-tuberculous mycobacterium (NTM) of particular interest because of its role in causing chronic lung infections, especially in immunocompromised individuals. Despite limited information on its pathogenesis, the bacterium's capacity to proliferate within cells and evade the immune system is critical. Dihydrofolate reductase (DHFR), a crucial enzyme in the folic acid synthesis pathway and indispensable for DNA biosynthesis, is a potential target for drug development against M. abscessus infections. Trimethoprim, the most common DHFR inhibitor, had no effect on M. abscessus. Furthermore, pharmacological studies on this mycobacterial species are limited. Thus, DHFR is a promising target for drug development against M. abscessus infection. In this study, we identified the most effective ligand for inhibiting DHFR as a potential drug candidate through molecular docking.

**MATERIALS AND METHODS:** The 3D structure of the M. abscessus DHFR protein (PDB ID 7K6C) was obtained from the RCSB PDB database. The inhibitory ligand of DHFR, 3-(2-{3-[(2,4-Diamino-6-Ethylpyrimidin-5-Yl)oxy]propoxy}phenyl)propanoic acid (MMV) with PubchemCID 66563688, and a total of 20 analogs were selected from the zinc database in SDF format. Then, molecular docking was performed using Virtual Docker Molegro v.6. Subsequently, the best interaction with the lowest energy binding was analyzed using Molegro Molecular Viewer software. Finally, the pharmacokinetic properties of the ligand were investigated using the SwissADME server.

**RESULTS AND DISCUSSION:** The best ligand for DHFR was identified ethyl 4-[5-[(2,4-diaminoquinazolin-6-yl)methylamino]-2-methoxyphenoxy] butanoate (zincID:34875815) with a molecular weight of 425.48 g/mol and a binding energy of -185.837kcal/mol. This ligand formed 7 hydrogen bonds with the DHFR residues Asp30, Lys48, Thr49, Ile99, Gly101, Ile104 and Tyr105, as well as 12 steric interactions with the residues Ile8, Ile17, Gly18, Ile23, Asp30, Phe34, Lys48, Thr49, Ile99, Gly101, Ile104 and Thr105. Moreover, the ADME results indicated that this ligand had a LogS water solubility score of -3.9, a lipophilicity (XLogP3) of 2.81, a polarity (TPSA) of 134.61, and hydrogen bond donors and acceptors of 3 and 6, respectively. Consequently, after conducting further in vitro and in vivo tests, this ligand can be considered as a potential candidate for the design of inhibitory drugs to inhibit M. abscessus pathogenesis.

**Keywords:** Mycobacterium abscessus, Molecular docking, Dihydrofolate reductase.

# Investigation of the antibacterial activity of *Moltikia coerulea* extract

Role of Microorganism in Pharmaceutical Industry

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**BACKGROUND AND OBJECTIVES:** *Meltakia caerulea* is one of the most important plants of Boraginaceae family which has broad range of biological activities, such as antibacterial, antioxidant and antiviral effects. The purpose of this study was to evaluate the antimicrobial potential of *Meltakia caerulea* extract.

**MATERIALS AND METHODS:** Ethanolic extract was prepared by maceration of the aerial parts of *M. coerulea* and its effect was investigated in 16 mg/disc against *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 9027) by Kirby-Bauer disk diffusion method. The MIC (minimum inhibitory concentration) and the MBC (minimum bactericidal concentration) of the prepared extract against the selected bacteria were also evaluated

**RESULTS AND DISCUSSION:** The results showed that gram-negative bacteria are resistant to this extract in the disc diffusion method., while in case of Gram- positive species, it showed a bacteriostatic effect on *Bacillus subtilis* and bactericidal effect on *staphylococcus aureus*. The MIC on the four tested bacteria was as the same (12.5mg/ml), the results of the MBC for *E. coli*, *S. aureus* and *P. aeruginosa* were similar (25mg/ml), while in the case of *B. subtilis*, the MBC was 100 mg/ml. *M. coerulea* contains a lot of flavonoids and phenols and its antibacterial activity is due to these compounds which may affect the bacterial cell membrane and as a result cause cell destruction. These results suggest that, the extract of this plant can be used as new source for discovering new antimicrobial agents in order to control antibiotic-resistant bacteria.

**Keywords:** *Moltikia coerulea*, Antibacterial activity, MIC, MBC, Ethanolic extract

# Microbial Control in Pharmaceutical Water Purification Systems: A Comparison of Old and New Methods

Role of Microorganism in Pharmaceutical Industry

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**BACKGROUND AND OBJECTIVES:** Introduction Water with various degrees of purity is used in pharmaceuticals, including potable water, purified water (PW), highly purified water (HPW), water for injection (WFI), and pure steam. Each type of water has specific characteristics and applications, chosen based on pharmaceutical requirements and guidelines. Purified water plays a crucial role in the production and processing of pharmaceutical products, and its quality is essential since any impurities can negatively impact the quality, efficacy, and safety of medications. One of the major challenges in pharmaceutical water systems is microbial contamination, which can be addressed with advanced technologies and strict hygienic protocols.

**MATERIALS AND METHODS:** Materials and Methods To compare two pharmaceutical water purification systems, samples were taken from various points in both systems over one year, monthly. The microbial load of the water was assessed using the filtration method as defined by the British Pharmacopoeia. The culture medium used for microbial count was R2A from Merck, which had previously undergone GPT validation. Microorganisms were identified based on the British Pharmacopoeia's suggested methods, and the results were analyzed using the t-test.

**RESULTS AND DISCUSSION:** Results The results indicated that the advanced water purification system, which included components such as a pressure-regulating pump, an ozone generator, multi-layer RO filters, a programmed thermal shock system, an EDI unit, and a safety filter, produced significantly higher quality water with lower microbial load compared to the old systems. Discussion The findings suggest that employing advanced technologies and stringent hygienic protocols in pharmaceutical water purification systems can effectively reduce microbial load. As a new technology, the ozone generator was key in controlling microbial contamination. Additionally, using multi-layer RO filters and programmed thermal shock systems contributed to improving water quality. These results align with previous research on reducing microbial contamination using advanced technologies, highlighting the importance of these technologies in the pharmaceutical industry.

**Keywords:** Keywords: Pharmaceutical Water Purification, Microbial control, Water system

# Molecular Docking Insights into the Interaction of *Vibrio cholera* ToxT Protein with Potential Inhibitory Ligands

Role of Microorganism in Pharmaceutical Industry

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**BACKGROUND AND OBJECTIVES:** *Vibrio cholerae* is a water- and food-borne pathogen responsible for cholera transmission. The principal virulence factors of bacteria are cholera toxin and toxin-coregulated pilus. Production of these critical factors is directly regulated by the ToxT protein, which serves as a master virulence regulator, thereby facilitating the bacterium's pathogenicity. Cholera treatment primarily focuses on supportive care. In addition, the increasing prevalence of antimicrobial resistance among bacteria has restricted the efficacy of pharmacological interventions in acute cases. Consequently, targeting ToxT regulator presents a promising approach for inhibiting *Vibrio cholerae*'s pathogenicity. This study evaluates a selection of ToxT ligands and identifies the most effective candidate, thereby providing a foundation for future drug design to combat cholera.

**MATERIALS AND METHODS:** The 3D structure of the ToxT protein (PDB ID: 3GBG) was retrieved from the RCSB PDB database. Palmitoleic acid (PAM), recognized as an inhibitor of ToxT (Pubchem Accession Number DB04257), along with 20 available ligands and analogs of PAM, were selected from the Zinc and PubChem databases. After preparing the protein and ligands by removing water molecules, lowering the energy level, and adding electric charges and hydrogen atoms, molecular docking was performed using Molegro Virtual Docker software v. 6 and analyzed in Molegro Molecular Viewer software. The best ligand, exhibiting the most stable mode was chosen. Finally, the SwissADME database was utilized to evaluate the pharmacokinetic properties of ligand including absorption, distribution, metabolism, and excretion (ADME)

**RESULTS AND DISCUSSION:** Among all the ligands studied, Docosatrienoic Acid (Compound CID: 3146) with a molecular weight of 334.5 g/mol, exhibited the most negative  $\Delta G_{\text{bind}}$  (-80.7703 Kcal/mol). A hydrogen bond was observed between this compound and the Met259 residue of the ToxT protein. The results of ADME indicated that Docosatrienoic Acid had a water solubility score (LogS) of -6.23, lipophilicity (XLogP3) of 8.63, polarity (TPSA) of 37.3, and Hydrogen bond donors and acceptors of 1 and 2, respectively. Finally, according to our docking study and analysis of physicochemical characteristics, Docosatrienoic Acid can be regarded as a promising candidate for drug design targeting the ToxT protein; however, further in vitro and in vivo studies must be conducted to validate this.

**Keywords:** Molecular Docking, *Vibrio cholera*, ToxT, ADME

# The Molecular Isolation of the *srf* gene from Thermophilic Soil Bacilli and Its Cloning in Susceptible Cells for Use in Industry

## Role of Microorganism in Pharmaceutical Industry

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**BACKGROUND AND OBJECTIVES:** Thermophilic bacillus is a type of thermophilic bacillus, carries various genes and biosurfactants, microbial surfactants are surface active molecules produced by various microorganisms such as bacteria, yeasts and filamentous fungi. Biosurfactants are able to reduce the surface energy between phases and create electrostatic barriers, thus preventing the integration of particles. The aim of the present study is the molecular isolation of the *srf* gene from thermophilic soil bacilli and its cloning in susceptible cells for use in industry.

**MATERIALS AND METHODS:** From 15 soil samples of different regions of Kerman that were isolated and cultivated. After biochemical examination of isolated microbial isolates and confirmation of *Bacillus* strains, DNA extraction was done. Then, the *srf* gene of these bacilli was identified by PCR method. The amplified fragment was inserted into pTG19 vector by TA cloning method. In the next step, the recombinant vector was transformed into *E. coli* Origami bacteria and cloning was confirmed using common methods. ClustalX and Mega5 software were used to draw the phylogeny tree.

**RESULTS AND DISCUSSION:** A total of 12 isolates of thermophilic bacilli were obtained from soil samples. As a result, the PCR reaction for the *srf* gene with the designed primers was found to be positive in 1 isolate (8.3%). The presence of *srf* gene and the expression of this gene were checked by real time PCR test. Examining the white and blue colonies, M13 primer, junction location and determination of the 16sr gene sequence confirmed the correctness of the cloning of the mentioned gene in the host bacteria. As a result of the present study, it was possible to identify the native thermophilic bacillus carrying the *srf* gene, which can be used to obtain biosurfactant enzyme widely, conveniently and economically, for use in industrial and agricultural purposes, removing oil pollutants and reducing environmental surface tension, etc. can take advantage of it.

**Keywords:** biosurfactant, bacillus, cloning, *E. coli*

# Antibacterial activity of tannin-free ethanolic extracts of some medicinal plants on methicillin-resistant *Staphylococcus aureus*

## Antimicrobial Activity of Medicinal Plants

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**BACKGROUND AND OBJECTIVES:** Treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is a problematic challenge. This study aimed to evaluate the antimicrobial effects of the hydroalcoholic extracts of *Mentha × piperita* leaves, *Allium sativum* bulb, *Zingiber officinale* rhizome, *Rhus coriaria* fruit, *Humulus lupulus* cones, *Panax ginseng* root, and *Amygdalus communis* fruit on MRSA strains. Additionally, the synergistic effects of these medicinal plant extracts, in combination with each other, as well as in combination with erythromycin, were examined against MRSA organisms.

**MATERIALS AND METHODS:** The ethanolic extracts of the plants were prepared by the maceration method, and the total phenolic content (TPC) was measured by the Folin-Ciocalteu method. Total extractable tannins (TET) were removed by adding the polyvinylpyrrolidone (PVPP, 1.1 g) to plant extracts. The minimum inhibitory concentrations (MICs) of the extracts against two clinical MRSA isolates and the ATCC 25923 strain were determined using the micro broth dilution method. The minimum bactericidal concentrations (MBCs) of the extracts were ranged by culturing on blood agar medium.

**RESULTS AND DISCUSSION:** The highest ( $453 \pm 22.4$  mg GAE/g) and lowest ( $3.5 \pm 0.2$  mg GAE/g) amount of TPC was detected in the root of *Panax ginseng* and the fruit of *Amygdalus communis*, respectively. However, the most extraction yield ( $165.5 \pm 21.7$  mg GAE/g) was observed in the flower of *Humulus lupulus*. The MICs were ranged from 0.078 to 5 mg/ml for MRSA isolates, and 0.001 to 2.5 mg/ml for ATCC strain. The MBC level was 10 mg/ml for *Rhus coriaria*, while its combination with *Panax ginseng* and the combination of *Amygdalus communis* and *Allium sativum* showed a MIC range of 5 mg/ml.

**Keywords:** Medicinal plant, Ethanolic extract, Antimicrobial effect, MRSA



# Genome-Resolved Metabolic Analysis of The Groundwater Prokaryotic Community Along Phenol Pollution

Omics findings in microbiology

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**BACKGROUND AND OBJECTIVES:** Groundwaters are mostly nutrient-limited ecosystems with low temperatures. The entrance of hydrocarbon pollutants will increase microbial metabolism, change their community structure, and affect their role in cycling elements. However, the metabolic context in response to pollutant exposure, especially in Iran, has been less noticed. The main aim of this study was to investigate the groundwater microbial potential metabolic capabilities in biogeochemical cycles.

**MATERIALS AND METHODS:** For Comparative metagenomic analyses, sampling of a groundwater well located in the Research Institute of Petroleum Industry, Tehran, Iran was performed before and after 6 months of phenol contamination. All trimmed sequences of each dataset were assembled separately using MEGAHIT. MetaBat2 software binned contigs >1 kb into metagenome-assembled genomes (MAGs) based on their different mapping depth and tetranucleotide frequencies. Putative genes were predicted with Prodigal and preliminarily annotated using Prokka in the metagenomics mood.

**RESULTS AND DISCUSSION:** The metabolic context of pristine groundwater (GW) reconstructed MAGs suggested a primarily heterotrophic and autotrophic lifestyle. In oligotrophic groundwater, chemolithotrophs are responsible for organic matter production through CO<sub>2</sub> fixation. Representatives of Alphaproteobacteria in the GW sample were autotrophs capable of fixing CO<sub>2</sub> and producing acetate using the phosphate acetyltransferase-acetate kinase pathway. In the phenol-polluted sample (R2), despite recovering chemolithotrophic drafts, MAGs with hydrocarbon degradation ability were more prevalent due to the high phenol concentrations as a carbon source. *Sulfuritalea* (R2\_32/66), *Rhodoferrax* (R2\_23), *Ramlibacter* (R2\_43), and *Zixibacteria* (R2\_71) had denitrification potential in the R2 sample. Our understanding of microbial potential metabolic capabilities in response to phenol pollution in oligotrophic groundwater enrolled as a valuable model for advancing knowledge of managing organic and hydrocarbon spill accidents, especially in Iran.

**Keywords:** Groundwaters, Metagenome, Phenol, Genome-Resolved, Metabolic Analysis

# In Silico Strategies for Anti-CRISPR Identification in Bacterial Genomes

Omics findings in microbiology

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**BACKGROUND AND OBJECTIVES:** To counter the CRISPR-Cas system, viruses produce specific proteins called anti-CRISPRs (ACR) that inhibit this system's function. Most known anti-CRISPRs bind directly to Cas proteins, stopping their activity. However, other mechanisms for inhibiting CRISPR-Cas have also been identified. In addition to influencing the outcomes of natural infections, anti-CRISPRs can regulate the activity of CRISPR-Cas systems in applied settings. Therefore, the discovery and understanding of anti-CRISPRs are important not only in the field of microbiology but also in emerging biotechnologies.

**MATERIALS AND METHODS:** In this study, various methods used to identify these anti-CRISPRs are discussed. To obtain comprehensive and accurate information about anti-CRISPRs (Acrs) and anti-CRISPR-associated proteins (Acas), different sources are utilized. Each of these sources includes diverse data on experimentally validated and predicted anti-CRISPRs, along with their gene neighborhoods and homologs. Anti-CRISPR (<https://tinyurl.com/anti-CRISPR>): This database has been compiled by experts and provides information and nomenclature for anti-CRISPRs and Acas that have been experimentally identified. AcrDB (<https://bcbl.unl.edu/AcrDB>): This database provides detailed information about anti-CRISPRs (Acr) and Acas predicted through computational methods, along with their homologs and analyses of their gene neighborhoods. AcrHub (<https://pacrispr.erc.monash.edu/AcrHub/>): AcrHub provides a comprehensive catalog of experimentally validated and predicted anti-CRISPRs, along with their gene neighborhoods. Anti-CRISPRdb (<https://guolab.whu.edu.cn/anti-CRISPRdb>): This database provides detailed information on anti-CRISPRs (Acr) and their predicted and validated gene neighborhoods.

**RESULTS AND DISCUSSION:** By using anti-CRISPRs, it is possible to precisely and temporally control the activation or deactivation of the CRISPR system. Additionally, anti-CRISPRs can reduce unwanted risks associated with genome editing. They allow for the temporary inhibition of specific genes to observe the effects, and in genetic disease treatment, they can be used to inhibit the CRISPR system after necessary edits are performed. Studying the interactions between anti-CRISPRs and CRISPR-Cas systems can lead to a better understanding of the evolution of these systems and microbial defense strategies, potentially leading to the development of new methods to combat bacterial and viral infections. In summary, anti-CRISPRs are considered highly useful tools in biotechnology due to their unique capabilities in regulating and inhibiting CRISPR-Cas systems, and they can lead to significant advancements in various scientific and industrial fields.

**Keywords:** Acr Proteins, Bacterial Genomes

# Investigating the effect of *Lactobacillus casei* consumption on the biological pathways of the immune system

Omics findings in microbiology

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**BACKGROUND AND OBJECTIVES:** Probiotics are microbial strains that are beneficial for health. The word probiotic is derived from a Greek word that means "for life". Their first advantage is that the digestive system can control diarrhea, reduce lactose sensitivity, and minimize cholesterol absorption. Probiotics modulate the intestinal microbial composition and regulate the mucosal immune response through the induction of various cytokines. The aim of this study was to investigate the gene expression profile of *Lactobacillus casei* consumers and its effect on the immune system.

**MATERIALS AND METHODS:** In this part of the study, to investigate the expression of genes in the use of *Lactobacillus casei* probiotic compared to the healthy intestinal mucosa, a microarray dataset from the GEO database was studied. After normalization, the desired data sets are analyzed by PCA and boxplot. Significant genes are obtained with the help of limma software package in R software. Genes with an absolute value (Log Fold Change, LFC) greater than 1 and FDR value  $\leq 0.05$  and t-test will be selected as significant genes.

**RESULTS AND DISCUSSION:** GSEA analysis is done in order to determine biological pathways, determining biological pathways is usually done with the aim of enriching genes and displaying their functional information and revealing the biological mechanisms guided by these genes. For this purpose, ToppGene database was used to determine the biological pathways of significant genes. Dataset No. GSE18741 was selected for this study, which contained 28 samples, in which a comparison was made between control samples or healthy individuals (8 samples) and samples or individuals consuming *Lactobacillus casei* (8 samples), finally 4 significant genes. It was found (Table 1) that all 4 genes were highly expressed in people who consumed *Lactobacillus acidophilus*. One of the main mechanisms of action of probiotics is the regulation of the host's immune response. In contrast, the innate system responds to common structures, called pathogen-associated molecular patterns (PAMPs), shared by the majority of pathogens. In this study, the effect of consuming *Lactobacillus casei* on

**Keywords:** *Lactobacillus casei*-Probiotics-PCA (Principal Component Analysis)-GEO

# A study on some phytochemicals in *Arthrospira platensis* MGH-1 fortified with calcium

Role of Microorganisms in Agriculture

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**BACKGROUND AND OBJECTIVES:** Micronutrient deficiency including minerals and vitamins together is often referred to as hidden hunger. Micronutrient deficiency can lead to a wide range of negative health impacts. Strategies used to overcome micronutrient deficiencies include fortification of the food products with specific elements. The microalga *Arthrospira platensis* (commonly called *Spirulina platensis*) is used as a food supplement for all age groups due to its exceptional nutritional profile. Using *A. platensis* fortified with calcium can be a low-cost and sustainable way to address this problem.

**MATERIALS AND METHODS:** The present study investigated the effect of different concentrations of calcium on the growth of *A. platensis* MGH-1 and its ability to accumulate this element. Also, the content of some phytochemicals evaluated in *A. platensis* MGH-1 growing on media containing proper concentration of calcium.

**RESULTS AND DISCUSSION:** Maximum growth parameters were exhibited by *A. platensis* MGH-1 at 0.1 g L<sup>-1</sup> calcium. The maximum bioaccumulation value was observed at 0.2 g L<sup>-1</sup> calcium. To produce *A. platensis* MGH-1 enriched with calcium, the best concentration of the element (0.1 g L<sup>-1</sup>) was selected using growth parameters and bioaccumulation. Then, the effect of calcium at 0.1 g L<sup>-1</sup> was investigated on some of the phytochemicals in *A. platensis* MGH-1. The result showed that the maximum amount of soluble protein, soluble sugar, phenolic compound, and carotenoid content was observed at 0.1 g L<sup>-1</sup> calcium. Overall, this study showed that *A. platensis* MGH-1 fortified with 0.1 g L<sup>-1</sup> calcium can be suggested for the development of functional foods since it is very rich in bioactive compounds.

**Keywords:** Bioaccumulation, *Arthrospira platensis*, Calcium ion, Phytochemicals

# Economic production of pectinase enzyme by apple pomace from *Bacillus wiedmannii* isolated from soil

Role of Microorganisms in Agriculture

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**BACKGROUND AND OBJECTIVES:** Pectinolytic enzymes are one of the important groups of enzymes used in the juice processing and clarification industry. The applications of pectinases can be mentioned in the textile industry, degumming of fiber products, paper production, coffee and tea fermentation, oil extraction, and wastewater treatment. The purpose of this research is to isolate native species of *Bacillus* from apple farm soil and investigate the ability to produce pectinase enzyme in it.

**MATERIALS AND METHODS:** In this research, 5 soil samples were collected from an apple farm in Damavand city, and separation was done using the pure plate method and heat treatment. In order to perform the primary screening method, it was cultured on a special pectin agar culture medium as a spot culture. After Enzyme halo was observed, welling method was performed. Gram staining and other biochemical tests were performed. Then, using the selected sample, enzyme quantitative assay method (DNS) and optimization was performed.

**RESULTS AND DISCUSSION:** In this research, out of 61 *Bacillus* species, 6 species had the largest halo diameter in the primary screening stage and 3 species in the secondary stage. *Bacillus* species with a halo diameter of 20 mm was selected as the best enzyme producer. Enzyme activity measurement by DNS method showed the highest amount of pectinase enzyme production (8.64 U/ml) at 37°C with an acidity of 7. Also, in optimization, the cheap carbon source of apple pomace with an enzyme production rate of 86 4.4 IU/ml and the nitrogen source peptone with an enzyme production rate of 5.94 IU/ml had the highest enzyme production rate. The unknown isolate with 100% similarity is related to *Bacillus wiedmannii*.

**Keywords:** Pectinase enzyme, dinitrosalicylic acid (DNS) technique, *Bacillus*, apple farm

# Evaluation of Lead Effects on Laccase Enzyme Activity in *Bacillus Subtilis* WPI under Laboratory Conditions

Role of Microorganisms in Agriculture

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**BACKGROUND AND OBJECTIVES:** Lead is one of the most important environmental pollutants that plays a significant role in increasing the stability of some other pollutants through changing the microbial profile of soil. *Bacillus subtilis* WPI is one of the most abundant bacteria existing in the wastewater. Due to the presence of laccase enzyme in this bacterium, it can facilitate the decomposition process of aromatic pollutants in wastewaters. This study aimed to investigate the effect of different concentrations of lead on the growth and biological activity of laccase enzyme in *B. subtilis* WPI.

**MATERIALS AND METHODS:** After purification of bacteria, the growth trend of *B. subtilis* WPI, along with the activity of laccase enzyme in different concentrations of lead was investigated based on kinetic method.

**RESULTS AND DISCUSSION:** In this study, the bacterial growth at lead concentration of 400 mg/L was reduced in a dose-dependent manner, which this decrease was significant at concentrations of 300 and 400 mg/L ( $p < 0.001$ ). The level of laccase enzyme activity in the lead concentration range 20-160 mg/L was also reduced in a dose-dependent manner, which implied that the highest decrease was observed at lead concentration of 160 mg/L ( $p < 0.01$ ). In general, our findings showed that there was no significant change in bacterial growth in the lead concentration range 20-200 mg/L, while a significant change was found in the activity of laccase enzyme in the mentioned concentration range. Therefore, it seems that this reduction in enzyme activity can indirectly increase the stability of aromatic oil pollutants in the environment.

**Keywords:** *Bacillus subtilis* WPI, laccase enzyme, lead, petroleum pollutants

# Investigating the ability of microorganisms isolated from saline areas of Iran to release Humic acid in the presence of leonardite and activated charcoal substrates

Role of Microorganisms in Agriculture

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**BACKGROUND AND OBJECTIVES:** The present study aims to isolate microorganisms from the salt areas of Urmia and Eshtehard, to investigate their ability to release Humic acid from Leonardite substrate and activated charcoal, and to quantitatively and qualitatively analyze the released Humic acid. Additionally, the study aims to molecularly identify the selected strain.

**MATERIALS AND METHODS:** In this study, 31 actinomycete strains were isolated. The URM 18 strain, isolated from Urmia Lake, showed the most significant changes in pH and color in a culture medium containing 1% Leonardite and was therefore selected for further studies. It was found that none of the strains were able to grow and release Humic acid in the substrate containing activated charcoal.

**RESULTS AND DISCUSSION:** Spectrophotometry analysis revealed that the concentration of Humic acid obtained from the biodissolution of Leonardite by the URM 18 strain is 31.37 µg/ml at 665 nm wavelength and 28.53 µg/ml at 465 nm wavelength. Furthermore, FTIR analysis of the Humic acid obtained from biological dissolution in this research study, compared with 59% Humic acid from the American Raw Company, indicated that the Humic acid obtained from the microbial activity of URM 18 is dominated by OH, COOH, and -COO groups, characteristic of main Humic substances in soil. The FTIR analysis also showed that the desired Humic acid has an aromatic structure. Additionally, the 16S rRNA identification revealed that the selected strain belonged to the Streptomyces genus with 96.96% similarity.

**Keywords:** Humic acid, biodegradation, halophilic microorganisms.

# Investigating the effect of sunflower oil on the level of lipase activity produced by Actinobacteria

Role of Microorganisms in Agriculture

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**BACKGROUND AND OBJECTIVES:** Lipases are important and efficient enzymes in the food, pharmaceutical, and chemical industries. These enzymes play a key role in hydrolysis and esterification of lipids. In this regard, investigating the effect of different substances on the activity of lipases can be effective in improving industrial processes and producing higher quality products. Sunflower oil has been considered as a carbon source in the bacterial culture medium and one of the main sources of vegetable oils due to its high content of unsaturated fatty acids. This research examines the effect of sunflower oil on lipase activity produced by a strain of actinobacteria belonging to *Streptomyces* sp., which can help to understand biochemical reactions better and optimize process conditions.

**MATERIALS AND METHODS:** First, the bacterial strain was cultured in the standard culture medium LB (Luria-Bertani) at 30°C and a revolution of 120 rpm in a shaker incubator for seven days. Then, the culture medium was centrifuged, and the supernatant containing lipase enzyme was isolated. Lipase activity was measured in the presence of sunflower oil using triglyceride substrates (para-nitrophenyl palmitate). The reaction was carried out at 37 °C for 30 min, and the absorbance was measured at 410 nm, which is considered a measure of enzyme activity. To determine the effect of oil, different amounts of sunflower oil (0, 0.1, 0.5, 1, and 2% by volume) were added to the reaction medium, and lipase activity was measured at each concentration.

**RESULTS AND DISCUSSION:** This study evaluated the impact of sunflower oil on lipase activity in Actinobacteria. Without sunflower oil, lipase activity was 2 units/ml. Adding sunflower oil increased activity: 0.1% oil led to 4 units/ml, 0.5% oil resulted in 10 units/ml, and 1% oil reached a peak of 20 units/ml, a tenfold increase. However, at 2% oil, activity decreased to 12 units/ml, likely due to inhibitory effects from the high oil concentration affecting enzyme structure or the reaction medium. The findings suggest sunflower oil is a beneficial additive for boosting lipase production in biotechnological processes.

**Keywords:** Sunflower oil, lipase activity, Actinobacteria



# Isolation of alginate-producing *Azotobacter chroococcum* from the soil of Sorkhehesar forest area

## Role of Microorganisms in Agriculture

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**BACKGROUND AND OBJECTIVES:** Alginate is an exopolysaccharide composed of (1-4)- $\beta$ -D mannuronic acid and its C-5 epimer  $\alpha$ -L gluronic acid. It has many applications in various industries such as food, pharmaceutical, medicine, etc. After isolation of *Azotobacter* from the soil through 16s rRNA sequencing and phylogenetic tree drawing, it was determined that the selected strain belongs to the genus *Azotobacter chroococcum*. Then, alginate production was confirmed by FTIR analysis. In this study, it was determined that the optimal production conditions in the selected strain include carbonglucose source and no nitrogen source at a temperature of 35 degrees Celsius, pH equal to 7 and a shaker speed of 180 rpm and can increase about 24%.

**MATERIALS AND METHODS:** After taking samples from the soil of the forest area of Red Hesar, isolation of alginate-producing *Azotobacter* was done. Biochemical tests such as catalase, oxidase and gram staining were performed to confirm the *Azotobacteraceae* family. Then the production and extraction process of alginate was investigated and in the last stage, optimization of alginate production was done. The produced alginate was confirmed by FTIR analysis.

**RESULTS AND DISCUSSION:** The strain isolated from the soil during the analysis of 16srRNA was related to the genus *Azotobacter chroococcum*. It was also determined in this research that under optimal conditions: carbon source glucose and no nitrogen source at 35 degrees Celsius, pH equal to 7 and at 180 revolutions in the shaker. Alginate production minutes increased by about 24%. and also confirmed the result of FTIR analysis of alginate production. The ability of the native strain of the country in the production of industrial microbial products including alginate was determined in this research and the native strain of *Azotobacter chroococcum* was determined as the producer. This research can bring the basic foundations of economic production of alginate in the future.

**Keywords:** Alginate, *Azotobacter Chroococcum*, Exopolysaccharide

# Isolation of Indole-Producing Actinomycetes from Rhizospheric Soil in Alborz Province

Role of Microorganisms in Agriculture

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**BACKGROUND AND OBJECTIVES:** Actinomycetes are a diverse group of aerobic bacteria that form spores and produce various secondary metabolites. They play a significant role in plant growth stimulation through mechanisms such as phytohormone production, siderophore synthesis, and phosphate solubilization. Actinomycetes in the rhizosphere produce indole-3-acetic acid (IAA), which promotes lateral root development and consequently enhances shoot and root growth. The aim of this study was to isolate actinomycetes from rhizospheric soil and screen them for IAA production.

**MATERIALS AND METHODS:** Five rhizospheric soil samples were collected from the geographical area of Eshtehard in Alborz province. Actinomycetes were isolated using surface plating on glycerol casein agar (GCA) medium. Pure cultures were obtained, and the morphology of isolates was examined on ISP2 agar medium. The isolates were then cultured on a medium containing only 1.0% (w/v) tryptophan as the sole nitrogen and carbon source and incubated at 30°C for 7 days. Qualitative assessment of IAA production was performed by adding Salkowski reagent to the culture media.

**RESULTS AND DISCUSSION:** From five soil samples, 31 actinomycete strains were isolated. Among them, 26 strains demonstrated the ability to produce indole-3-acetic acid (IAA). The color change of the Salkowski reagent from yellow to pink-red was considered indicative of IAA production. Based on the intensity of color development, the strains were classified into three groups: 10 strong, 7 moderate, and 9 weak IAA producers. Further studies are underway to identify these strains and quantitatively compare their IAA production. IAA plays a crucial role in root growth stimulation, increased resistance to environmental stresses, and improved agricultural productivity. The production of IAA by actinomycetes can be considered an environmentally friendly and effective approach for sustainable agriculture.

**Keywords:** Actinomycetes, rhizospheric soil, indole-3-acetic acid (IAA)

# Isolation of lignin degrading enzymes producing bacteria from tobacco waste compost in order to be used at lignocellulose biorefinery

Role of Microorganisms in Agriculture

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**BACKGROUND AND OBJECTIVES:** Lignocellulosic materials are composed of three polymers including cellulose, hemicellulose, and lignin that lignin is recalcitrant to degradation. Some bacteria through production of lignin degrading enzymes can overcome this problem and hence are important in biorefinery process of lignocellulosic wastes and compost preparation. The purpose was to isolate bacteria from tobacco waste compost and investigate their ability in laccase and lignin peroxidase production as the lignin degrading enzymes.

**MATERIALS AND METHODS:** Tobacco compost samples were prepared and bacterial isolation was made. The isolates were then screened based on their ability in laccase and lignin peroxidase production. Furthermore, the enzyme production was quantitatively assessed and the best isolates were selected for further study. The isolates that qualitatively had the ability to produce the examined enzymes, their enzymatic activity was also evaluated quantitatively. The effect of temperature on enzyme stability was also evaluated. To evaluate the stability of the enzyme, a temperature range of 50°-80° was applied and the enzyme activity following treatment was measured.

**RESULTS AND DISCUSSION:** 11 isolates were obtained, among which 7 (63.6%) and 5 isolates (45.4%) were produced laccase and lignin peroxidase enzymes, respectively. The K-P-E with 5.8 U/ml and K-P-G1 with 4.7 U/ml had the highest and lowest laccase activity, respectively. While in case of lignin peroxidase the F-A (2.66 U/ml) and F-G (2.22 U/ml) showed highest and lowest activity, respectively. The enzyme stability was maintained till 55 °C, but it was decreased at higher temperature. These findings suggest well adaptation of bacteria in compost habitat. Lignin degrading enzymes are key players in lignocellulosic wastes biorefinery and production of bio-based chemicals. Hence, finding new and high potential isolates is of great importance. Compost materials as a habitat for lignin degrading bacteria are potent sources for reaching these bacteria. As shown in this study the potent lignin degrading bacteria were obtained from tobacco compost that can be used in lignin biorefinery or compost inoculum preparation.

**Keywords:** Compost, Lignin-degrading bacteria, Laccase, Lignin peroxidase

# The Evaluation of Anti-Quorum Sensing activity of *Cuscuta epithymum* for Controlling *Pectobacterium carotovorum* subsp. *carotovorum*

Role of Microorganisms in Agriculture

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**BACKGROUND AND OBJECTIVES:** Quorum sensing (QS) is a key mechanism used by bacteria to communicate and coordinate various behaviors, including the production of virulence factors, to establish chronic infections based on cell population density. Plants are the natural sources of phyto-compounds that interfere with bacterial cell-to-cell communication. This study was conducted to evaluate the potential of the *Cuscuta epithymum* plant in combating plant diseases caused by *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc) through its anti-QS activity.

**MATERIALS AND METHODS:** The upshot resulting from the evaluation of the plant disclosed that *Cuscuta* (*Cuscuta epithymum*, Convolvulaceae) extract remarkably meddled with extracellular molecules, acyl homoserine lactone, and showed significantly violacein reduction in the biosensor strain of *Chromobacterium violaceum* CV026. The results also highlighted that the bioactive compounds of crude extract of *C. epithymum* significantly reduced plant diseases caused by Pcc. Gas Chromatography Mass Spectroscopy (GC-MS) analysis of the plant extract showed that 4,6-Di-O-methyl-.alpha.-d-galactose, Linolenic acid methyl ester, Heptacosane, and 9,12-Octadecadienoic acid (Z,Z)-, methyl ester were as major components.

**RESULTS AND DISCUSSION:** The outcomes advocate that *C. epithymum* extract possess significant anti-QS properties and would have the capability to be considered as a promising agent for the control of the plant disease caused by Pcc.

**Keywords:** Anti-Quorum Sensing, *Cuscuta epithymum*, *Pectobacterium carotovorum* subsp. *carotovorum*, GC-MS.

# The role of nickel resistance and para-probiotic biomass in nickel biosorption by probiotic Lactobacilli

Role of Microorganisms in Agriculture

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**BACKGROUND AND OBJECTIVES:** Nickel is an important pollutant that has a destructive role on human health. So far, many chemical and physical methods have been applied for nickel purgation from the environment. Still, they were not successful enough due to their high-cost procedure and their toxicity. Recently, probiotic bacteria have been recognized as a highly secure and eco-friendly bioremediation approach to nickel biodetoxification from the environment.

**MATERIALS AND METHODS:** Four lactobacillus strains, namely *L. brevis* 205, *L. mucosae* 226, *L. plantarum* 78, and *L. casei* 303 were investigated to assess their nickel resistance through disk diffusion and MIC methods. Strains with the highest and lowest resistance were selected for Bioremediation assays including Biosorption, Desorption, and Bioaccumulation, which were performed following specific protocols. In brief, the strains were exposed to 5 ppm nickel chloride solution and rested for one hour in a 37 °C incubator. Then the suspensions were centrifuged and the supernatant was analyzed for remaining nickel ions by an AAS. For the desorption assay, the pellets of the previous section were resuspended in EDTA solution for two hours and after centrifugation, the supernatants were analyzed for nickel ions. Finally, the pellets were lysed using a special lysis buffer and the accumulated within the cells nickel ions were analyzed by an AAS.

**RESULTS AND DISCUSSION:** *L. brevis* 205 and *L. casei* 303 exhibited the highest and lowest sensitivity to nickel, respectively. Both showed a plentiful performance in Biosorption assays, with 82.22% for *L. brevis* 205, and 72% for *L. casei* 303. The bioremoval assay with the two strains' para-probiotic (dead) biomass exhibited a Biosorption yield of about 69% for *L. brevis* 205 and 75% for *L. casei* 303. Consequently, nickel-sensitive strain, *L. casei* 303, managed the biosorption of nickel to a yield of 72%; so, nickel resistance is not a crucial factor for screening LAB for biosorption assays. Furthermore, applying the para-probiotic biomass of the nickel-sensitive strain concluded in a massive nickel biosorption that surpassed the nickel-resistant strains' biosorption yield. Thus, probiotic Lactobacillus strains could be brilliant candidates for nickel bioremoval in water, food, and pharmaceutical industries, regardless of nickel resistance or viability.

**Keywords:** Lactobacillus, Probiotic, para probiotic, Biosorption, Nickel

# Cloning and optimizing the expression of pectinase enzyme from *Bacillus subtilis*

Petroleum microbiology

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**BACKGROUND AND OBJECTIVES:** Enzymes are biocatalysts with specific functions that are extremely efficient in various reactions. Pectinases (EC. 3.2.1.15), endo-polygalacturonases, are a group of enzymes composed of hydrolases, lyases, and esterases, which are enzymes that degrade pectic substances that are the structural polysaccharides present in plant cells. Pectinases are classified as polygalacturonase (PG), polymethylgalacturonase (PMG), pectin lyase (PL), pectate lyase (PAL), and pectin methylesterase (PME). Pectolytic enzymes have a lot of industrial importance and are widely used in the food and textile industries and also have significant applications in the pharmaceutical industries. Pectinase enzyme production has been widely reported in filamentous bacteria and fungi. For enzyme production, it is more common to use microbial sources compared to plants and animals, because they produce a wide range of enzymes and it is easier to predict and control their enzyme content.

**MATERIALS AND METHODS:** The pectinase gene isolated from *Bacillus subtilis* was cloned in the pet26 vector by heat shock method and transformed into the BL21 strain and confirmed by the PCR technique of the transformed recombinant clone.

**RESULTS AND DISCUSSION:** The growth of the clones obtained from pectinase gene transformation in BL21 bacteria was observed in a solid culture medium containing the antibiotic kanamycin, and the bacteria containing the recombinant gene was confirmed by PCR method. Considering that the pectinase enzyme was produced well in this part of the research, it can be used to advance different goals in different industries.

**Keywords:** Cloning, Expression, Enzyme, pectinase

# Comparison of Pigment Extraction Efficiency from an Archaeon Isolated from the Lut Desert Using Different Solvents

Petroleum microbiology

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**BACKGROUND AND OBJECTIVES:** Some archaea are capable of producing natural pigments called carotenoids. Carotenoids can have various functions, and also have various applications in the food, pharmaceutical, and cosmetic industries. Solvent extraction is the most common method for extracting pigments, and this study compares the efficiency of different solvents including ethanol, methanol, acetone, hexane, and petroleum ether for pigment extraction from a native archaeon isolated from the Lut Desert.

**MATERIALS AND METHODS:** A native archaeon isolated from the Lut Desert was used to compare pigment extraction efficiency using different solvents. The archaea were cultured in a specialized archaeal medium containing 125 g/L NaCl, 16 g/L MgCl<sub>2</sub>, 0.13 g/L CaCl<sub>2</sub>, 5 g/L K<sub>2</sub>SO<sub>4</sub>, 1 g/L meat peptone, 1 g/L yeast extract, 5 g/L glucose, and 0.02 g/L chloramphenicol. After complete growth, the archaeal cells were separated by centrifugation and extracted with different solvents. For pigment extraction, the archaeal cells were first centrifuged for 20 minutes and the pellets were washed twice with sterile distilled water. Then, 5 mL of each solvent was separately added to the archaeal cell pellet and incubated in a 62 °C water bath for 20 minutes. Afterward, the pigment extract was filtered through filter paper, and its absorbance was measured using a spectrophotometer at wavelengths of 400 to 700 nm.

**RESULTS AND DISCUSSION:** The results showed that ethanol was the best solvent for pigment extraction from the archaeon native to the Lut Desert, extracting the highest amount of pigment at a wavelength of 400 nm. The OD absorbance at 400 nm wavelength was 1.59 for ethanol, 0.36 for acetone, 0.51 for methanol, 0.27 for hexane, and 0.50 for petroleum ether. The findings of this study can be applied to the extraction of carotenoids from archaea for various purposes, including food coloring, pharmaceutical applications, and cosmetic products.

**Keywords:** Carotenoid, Archaea, Lut Desert, pigment extraction

# Identification of hydrolytic enzymes in a bacterial strain isolated from southern Iran and investigating their optimal produce conditions

Petroleum microbiology

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**BACKGROUND AND OBJECTIVES:** The objective was to investigate hydrolytic enzymes including protease, cellulase, lipase, gelatinase, pectinase, xylanase, amylase from native Iranian bacteria, to determinate the optimal conditions for their production.

**MATERIALS AND METHODS:** TSB and TSA media were prepared for bacterial storage, with macroscopic and microscopic tests conducted. Bacteria were cultured in TSB, and absorbance was measured at 600 nm and serial dilutions for colony counting, and growth curve plotting. Hydrolytic enzyme activity at pH 7 and 30°C, 50°C was investigated in media, including, gelatin agar with mercuric chloride, Tween 80 agar and pectin agar with methyl red, starch agar with potassium iodide, nutrient agar with Lugol, xylan and CMC agar with Congo red indicator containing medium using specific indicators. Media with NaCl concentrations (0-20%) at pH 7 were prepared, and optical density was measured post-incubation to assess enzyme activity and also were prepared at pH levels of 4- 9 and analyzed similarly to the salt-containing media.

**RESULTS AND DISCUSSION:** The sample was identified as a Gram-positive coccobacillus. macroscopic, microscopic tests revealed the bacterium to be catalase-positive, oxidase-negative, urease-negative, and non-sporulating. The bacterium reached its maximum growth within 72 hours, no growth was observed at 50°C. The sample grew in six media except for pectin agar and showed enzyme production in nutrient agar and CMC agar. The bacterium grew in nutrient agar up to a salt concentration of 5%, with a halo diameter of 1 mm. In CMC agar, it grew up to a 10% salt concentration, and at 5%, a halo diameter of 7 mm was measured under the colony. The sample grew in a pH range from 6.5 to 9 in nutrient agar, with a halo diameter of 7 mm observed. Additionally, growth was observed in CMC agar from pH 5 to pH 9, with a halo diameter of 5 mm measured at pH 7.

**Keywords:** Lipase, Protease, Amylase, Gelatinase, Cellulase, Pectinase, Xylanase, Hydrolytic enzyme



# Increased Glucose Isomerase from *Streptomyces murinus* Expression in Recombinant *Escherichia coli* by Response Surface Methodology

Petroleum microbiology

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**BACKGROUND AND OBJECTIVES:** The paper investigates the optimization of glucose isomerase (GI) production using recombinant *Escherichia coli* expressing the Xyl A gene from *Streptomyces murinus*. The study aims to optimize the production of GI by investigating the effects of various factors such as temperature, pH, induction time, carbon source, and nitrogen source on GI expression.

**MATERIALS AND METHODS:** Using Response Surface Methodology (RSM), the effects of the selected independent variables [i.e., ambient temperature (with 3 levels: 25, 30 and 37 °C), pH (with 3 levels: 6, 7 and 8), lactose induction time (with 0, 4, and 8 hours), medium carbon source (with 3 levels: glucose, lactose, and glycerol), medium nitrogen source (with 3 levels: NH<sub>4</sub>Cl, K<sub>2</sub>NO<sub>3</sub>, Yeast extract)] were explored on glucose isomerase enzyme activity and optical density of the recombinant bacteria.

**RESULTS AND DISCUSSION:** The results demonstrated that induction time, carbon source, nitrogen source, and the interaction of some variables significantly affected enzyme activity. The study identified several significant interactions among the variables, such as temperature, pH, induction time, carbon source, and nitrogen source, suggesting their impact on glucose isomerase production. Notably, the combination of temperature, pH, and nitrogen source had the highest coefficient of influence on enzyme activity. The highest production of glucose isomerase reached a maximum level of 8204 U/ml at 37°C, pH 7, and lactose induction time of 4 h, with glucose as the carbon source and yeast extract as the nitrogen source. The investigation also included the cloning and expression of the recombinant enzyme, where the synthetic coding DNA sequence of *S. murinus* XIA was cloned into pET-22a (+) and introduced into *E. coli* BL21 (DE3) competent cells.

**Keywords:** *Streptomyces murinus*, glucose isomerase, Response Surface Methodology (RSM), Xyl A

# Investigating the conditions of glycerol production by yeasts isolated from kombucha solution

Petroleum microbiology

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**BACKGROUND AND OBJECTIVES:** The present research was conducted with the aim of producing glycerol and investigating some conditions of its production by yeasts isolated from kombucha solution.

**MATERIALS AND METHODS:** Black tea was used as a substrate and white sugar was used as a carbon source to produce kombucha solution. Glucose yeast chalk agar (GYCA) was used for isolation and purification of yeasts and it was done by polymerase chain reaction (PCR) and sequencing. For glycerol assessment, the glycerol measuring kit produced by ZellBio (Germany) was used.

**RESULTS AND DISCUSSION:** *Starmella bacillarys* and *Zygosaccharomyces* sp. were the abundant yeast isolated from kombucha. The yeast were able to produce glycerol (in the range of 0.02-0.35 grams per gram of sugar consumed). Considering that kombucha solution contains glycerol-producing yeast species, in this study, for the first time, glycerol was produced by yeast isolated from kombucha solution to a higher production quantity than previous studies

**Keywords:** Key words: Kombucha, Glycerol, *Starmella bacillarys*, *Zygosaccharomyces*

# Isolation, screening and molecular identification of filamentous fungi and yeasts, producing urease enzyme, from soil samples

Petroleum microbiology

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**BACKGROUND AND OBJECTIVES:** Enzymes are the most potential biocatalysts for many reactions and in the last decades their usage in biotechnological field has been increased, enormously. Among the enzymes, urease is a nickel-containing enzyme which is exist in plants, some invertebrates, and a number of microorganisms including fungi, that hydrolyzes urea into ammonia and carbon dioxide. Urease has many industrial applications, for example, it is used in diagnostic kits to measure urea, in alcoholic beverages as a urea reducing agent, determine urea in the hemodialysis biosensors systems and in biological cementation and soil consolidation which can be carried by microbial activity and urease enzymes that helps to precipitation of calcium carbonate. Filamentous fungi and yeasts are the most important source of urease enzyme. This research was designed to isolation, screening and identification of yeasts and filamentous fungi producing urease enzyme.

**MATERIALS AND METHODS:** Totally, 115 fungal strains including 84 filamentous fungi strains and 31 yeast strains were isolated from soil samples. Each of the strains was cultured in two replicates on Christensen's urea agar medium, this medium has urea and strain producing urease, hydrolysis urea to ammonium and causes higher alkalinity in the culture medium and turn medium to purple color. Then, DNA extraction was carried for selected strains and molecular identification were done based on PCR amplification of specific primers according to filamentous fungi; *Penicillium* and *Aspergillus* by beta-tubulin gene and ITS region for other filamentous fungi, and LSU gene for yeasts.

**RESULTS AND DISCUSSION:** In this research, 59 urease-producing strains (%51.3) including 49 filamentous fungi strains (%58.3) and 10 yeast strains (%32.2) were screened. Molecular identification was showed that filamentous fungi based on beta-tubulin and ITS region were belonged to *Penicillium*, *Aspergillus*, *Mucor*, *Rhizopus*, *Absidia* and *Purpureocillium* genera and yeasts based on LSU gene were belonged to *Debaryomyces*, *Apiotrichum*, *Rhodotorula*, *Pichia* genera. As a result, filamentous fungi showed higher abundance in urease activity than yeasts and generally this study express filamentous fungi and yeasts demonstrate high potential urease activity and can be used in different biotechnological fields.

**Keywords:** filamentous fungi, yeast, urease enzyme, molecular identification, PCR reaction

# Optimization of the expression and characterization of L-Asparaginase derived from *Bacillus subtilis*

Petroleum microbiology

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**BACKGROUND AND OBJECTIVES:** L-Asparaginase (EC 3.5.1.1; L-Asparagine amidohydrolase) catalyzes the hydrolysis of L-Asparagine to L-Aspartic acid and ammonia. It exists in various organisms including animals, plant cells, yeasts, fungi, and bacteria. Asparaginase has been known to us since 1967 and is widely used as an antineoplastic agent against childhood acute lymphoblastic leukemia and Hodgkin lymphoma. Bacterial asparaginases are of two types, one in the cytoplasm called asparaginase I and asparaginase II which is present in the periplasm. Since asparaginase II is more specific to its asparagine substrate, it is used more for therapeutic purposes. So far, asparaginase has been used in prokaryotic systems such as *E. coli*, *E. carotovora*, *Bacillus subtilis*, *Bacillus licheniformis* and yeasts such as *Saccharomyces cerevisiae*

**MATERIALS AND METHODS:** ASN gene isolated from *Bacillus subtilis* was cloned in PET26 vector and transformed into *E. coli* strain BL21. The transformed clone was confirmed by PCR technique. To express and optimize the protein from the transformed bacteria, the desired bacterial clone was placed in 5ml of LB Brath liquid culture medium in a stylish incubator at 37° overnight, and then 100 µl of the sample in 50 ml A liter of liquid culture medium was added and placed in an incubator for 1 hour until its OD reaches 0.5 to 0.6. Then, final concentrations of 0.8, 1, and 1.5 mM IPTG were added to each falcon for optimization and placed in an incubator at different temperatures of 37°, 30°, and 22° for 20 hours. Ultimately, the bacterial sediment was collected and expressed by the SDS-PAGE technique. The desired protein was optimized and confirmed.

**RESULTS AND DISCUSSION:** The growth of the transformed clones was observed in the solid culture medium containing the antibiotic kanamycin, and the desired bacteria were confirmed by PCR technique. The protein expression of the desired bacteria was observed by confirming the SDS-PAGE technique. Today the production of asparaginase by recombinant method is significant. By producing this enzyme with the performed methods and techniques, it can be used to advance therapeutic goals in other clinical and research aspects.

**Keywords:** Optimization, Asparaginase, Enzyme, Cloning, Expression, *Bacillus subtilis*

# Microbial Remediation of Heavy Metals in Soil Contaminated with Used Motor Oil

Role of Microbes in Dairy Industry

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**BACKGROUND AND OBJECTIVES:** Motor oil is a complex mixture of hydrocarbons and organometallic compounds that serves as a lubricant for protecting internal combustion engines. Used motor oil (UMO) is a term applied to describe lubricating oil that has lost its effectiveness and is no longer useful in a vehicle. As a result of oxidation from the high temperatures in the combustion chamber and its effect on the normal deterioration of engine parts. The disposal of UMO poses a significant threat to ecosystems, causing significant damage to soil health. The application of efficient biologically-derived techniques that are environmentally compatible, such as microbial remediation, plays a crucial role in reducing both primary and secondary hazards resulting from pollution caused by petroleum derivatives

**MATERIALS AND METHODS:** Soil sampling was performed at the Behran Oil Refinery, south of Tehran. The soil was contaminated with UMO. Bushnell-Hass broth medium (BHBM) was used for the purpose of culturing and isolating microorganisms. Following the process of enrichment and isolation of pure colonies, the candidate strains were grown in Bushnell-Hass agar medium. A strain was chosen based on the degree of halo formation. Afterwards, biochemical tests and molecular identification were conducted. A bacterial suspension containing 1.5 ml of normal saline solution and 2.5 ml of BHBM/kg of soil was added to the treatments on a weekly basis. The experiment lasted for a period of 28 days. Heavy metals were extracted and analyzed using ICP-OES then the removal rate was calculated using standard formula. The statistical analysis was applied using SPSS software.

**RESULTS AND DISCUSSION:** The native strain isolated demonstrated significant potential in heavy metal remediation. The highest removal efficiencies were observed for cadmium (64.48%), nickel (47.39%), and lead (68.71%) at a concentration of 10 gr/kg, while chromium and copper showed removal efficiencies of 29.18% and 39.14%, respectively, at a concentration of 30 gr/kg. The results highlighted variations in the remediation efficiency of each element, depending on the concentration of used motor oil. Therefore, future investigations into the physicochemical properties, mechanisms, and kinetics of metal remediation are required for a deeper understanding of the influencing factors in this process.

**Keywords:** Microbial remediation, used motor oil, Heavy metals

# Synergy of Fe<sub>3</sub>O<sub>4</sub>@ZnO core-shell Nano-particle and Consortium of Alkalophilic Extremophilic Sulfur Oxidizing Bacteria to Remove H<sub>2</sub>S from Natural Gas

Role of Microbes in Dairy Industry

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**BACKGROUND AND OBJECTIVES:** the main goal in this research is to use nanoabsorbents and microorganisms at the same time As a complementary step in the amine recovery unit in the oil and gas industry, in order to reduce costs Related to the removal of hydrogen sulfide gas, the recycling of primary sulfur as fertilizer and increasing the efficiency of this process and on the other hand it reduces the environmental pollution caused by this dangerous gas can be. At first, nanoparticles were produced and characterized, and the desired bacteria were cultured and in order to Exclusion tests are used. Finally, the removal process using nano/bio technology on zinc An industrial wastewater (diethylamine contaminated with hydrogen sulfide) has been investigated.

**MATERIALS AND METHODS:** In this research, iron oxide/zinc core-shell nanoparticles with three different shell thicknesses it is produced. A gram-positive exterimophile consortium alkalophilic bacteria isolated from Maharlou lake located 27 km southeast of Shiraz, was used in the present study. These bacteria are capable of sulfur oxidation and can grow at a PH range of 8-11. The catalase and oxidase tests of the bacterium were positive. During this project, the amount of sulfur oxidation by nanoparticles and bacteria is first optimized in the laboratory. All optimization steps are performed in batch conditions.

**RESULTS AND DISCUSSION:** Amen, in order to revive and make it possible to reuse it in contact with Nanoparticles and microorganisms are placed. Sulfide species are absorbed by nanoparticles and through Microorganisms oxidize and can be reused as lemental sulfur. - The highest amount of H<sub>2</sub>S gas oxidation and extraction of elemental sulfur - Another application of these bacteria could be examined for indirect hydrogen sulfide removal. The Fe<sub>3</sub>O<sub>4</sub>@ZnO core-shell Nano-particle was first placed in contact with a sulfur-infused medium so that adsorption took place. The exact amount of hydrogen sulfide adsorbed could be obtained through the measurement of hydrogen sulfide concentration in the solution before and after the adsorption process.

**Keywords:** Nanoparticle Extremophilic Desulfurization Alkaliphilic Hydrogen sulfide

# Application of ZnO nanoparticles to improve microbial inactivation of wastewater treatment plant effluent using Ultrasonic and UV-C process

## Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** Ultrasonic (US) and UV-C technologies are utilized in wastewater treatment plants (WWTPs) for disinfection. US technology is used as a pre-treatment step to break down particles, while UV-C technology is commonly used as the final disinfection step in WWTPs. The study explored the use of Zinc Oxide Nanoparticles (ZnONPs) to enhance the effectiveness of UV-C and US disinfection methods in wastewater treatment, providing a more comprehensive solution.

**MATERIALS AND METHODS:** A Laboratory US Bath and a UV-C lamp were utilized to assess the effectiveness of ZnONPs in reducing microbial load (Total Coliform (TC) and Fecal Coliform (FC)) in wastewater samples. Effluent from a conventional biological WWTP were collected in volumes of 50, 100 and 300 mL. These samples were then exposed to UV-C radiation for 0.5, 1, 2, 3, 4 and 5 min, and Ultrasonic Density (UD) without (40°C) and with (60°C) temperature control for 1, 5, 10, 15, 20 and 30 min, with a Turbidity of 18 NTU (Group A) and a Turbidity of 5 NTU (Group B). Additionally, there were Samples that received 5 mg/L of ZnONPs (Group C). The reduction in microbial load efficiency was calculated using Chick's law, and the relationship between variables was analyzed through regression analysis using Excel and SPSS software.

**RESULTS AND DISCUSSION:** Increasing UV-C exposure time reduced microbial population, with 2 minutes being the optimal time. The maximum removal efficiencies by US for TC were; 74.07,77.7, 85.1 percent (40°C) and 92.5,100, and 100 percent (60°C) in group A (30 min sonication), 85.7,85.7, 100 percent (40°C) and 100,100, and 100 percent (60°C) in group B (20 min sonication), 100,100, 100 percent (40°C) and 100,100, and 100 percent (60°C) in group C (30 min sonication). The maximum removal efficiencies by US for FC were; 76.4,88.2, and 100 percent (40°C) and 88.2,100, and 100 percent (60°C) in group A (30 min sonication), and were 100 percent in other groups (B and C). The study found that combining ZnONPs with US and UV-C significantly enhanced disinfection ability, making them promising alternatives to conventional disinfection processes with over 90 percent improvement in efficiency.

**Keywords:** Ultrasonic – Ultraviolet – Coliform – ZnO Nanoparticles –Wastewater Treatment

# Biological decolorization of wastewater using moderately thermophilic bacteria isolated from urban wastewater

## Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** Today, available water for drinking and industrial use is limited. By using the purification process, the water used in the industries, especially the textile industry, which is one of the major consumers of water, can be recycled and returned to the industrial cycle. By using bacteria that decompose color compounds, it is possible to perform the analysis and removal of dyes, especially the dyes of the azo group.

**MATERIALS AND METHODS:** Materials and methods: Sampling and isolation of color-degrading bacteria from a waste water treatment plant\*1 in Kashan was done. For enrichment and screening of wastewater color-degrading bacteria, TSB culture media, wastewater, modified M9, TSA, and wastewater agar were used. Using M9 medium containing dye at the temperature of 26 to 53 °C, different (temperate, thermophilic, salt-philic) dye-degrading strains were selected from the primary isolated bacteria. Decolorization rate was measured using spectrophotometric method. The optimal conditions for decolorization were determined by the response method (RSM). The identification of the selected bacterial strain was done by sequencing the 16S rRNA gene.

**RESULTS AND DISCUSSION:** Results: 47 bacterial strains were isolated by using culture media containing black rimazol 5. The color removal ability of these strains was observed from 22 to 71.5% during three days of greenhouse storage at the temperature (30-30-37-42-48-53°C). Among these strains, *Bacillus sonorensis* SN7 had the ability to grow well in the environment containing dye and remove dye by 71.5%. This bacterium has the ability to decompose widely used dyes Rimazol Black 5, Reactive Red 198, Reactive Blue 21, Reactive Yellow 15. The optimal conditions obtained for color removal by response method (RSM) are temperature 37 °C, salt concentration 1%, color concentration 50 mg/l and pH 7. Discussion and conclusion: The amount of decolorization of SN7 strain in aerated (on shaker) and static (anoxic) conditions indicates more color removal in static condition. Better decolorization was observed by using the two-step method of aeration and then static conditions.

**Keywords:** Microorganism, thermophile, industrial effluent, dye removal



# Decolorization potential of acid orange by mesophilic and cold-tolerant bacteria

Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** The textile industry is a significant contributor to environmental pollution due to the extensive use of azo dyes, which generate large volumes of dye-contaminated wastewater. Traditional wastewater treatment methods often fail to effectively remove these persistent pollutants, necessitating alternative approaches. This study explores the potential of bioremediation utilizing cold-tolerant and mesophilic bacteria isolated from diverse environments to decolorize Acid Orange 7, a model azo dye.

**MATERIALS AND METHODS:** This study investigated the decolorizing ability of cold-tolerant and mesophilic bacteria. Twenty strains were isolated from the Zagros Mountain range, of which eight were identified as cold-tolerant after temperature testing. Mesophilic bacteria capable of degrading Acid Orange 7 dye from textile factory effluent were cultured in a mineral salts medium containing Acid Orange 7, followed by isolation. Bacterial strains with potential for dye degradation were selected. For the decolorization experiments, a 2% suspension of pure bacterial cultures was inoculated into mineral salts medium containing 50 mg/L of Acid Orange 7. The removal of color over seven days under aerobic and anaerobic conditions was assessed using the percentage of color removal measured by spectrophotometry.

**RESULTS AND DISCUSSION:** Results indicated that both bacterial groups effectively decolorized Acid Orange 7, with strain AP14 achieving a remarkable 97% decolorization efficiency at a concentration of 50 mg/L. Mesophilic strains reached peak decolorization within 72 hours, while cold-tolerant strains continued to degrade the dye throughout the experimental period. These findings highlight the significant potential of utilizing bacterial consortia for sustainable and cost-effective treatment solutions in the textile industry, capable of operating under varying environmental conditions.

**Keywords:** Acid orange, Cold tolerant, Mesophile, Decolorization

# Determination of flora and density of bacteria and fungi in indoor air of Imam Khomeini hospital of Sari - -A Cross Sectional Study in 1402

## Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** Airborne microorganisms in hospitals are a potential source of infection for patients and staff. The monitoring and control of these bioaerosols prevents the transmission of pathogenic agents through the air and helps to maintain the health of patients and staff. The aim of this study was to determine the density and type of bacterial and fungal bioaerosols in the indoor air of different vrds as well as the location of the infectious waste depot and wastewater treatment plant of a educational hospital in Sari in year 1402.

**MATERIALS AND METHODS:** This cross-sectional descriptive study was conducted in 1402 in one of the educational hospitals of Mazandaran University of Medical Sciences in Sari. Air was sampled using a Biostage impactor sampler with a flow rate of 28 liters per minute for 15 minutes. Samples were taken from the air of neonatal intensive care units (NICU), operating room, intensive care unit (ICU), emergency room and corridor. Air sampling was also done at the infectious waste depot and sewage treatment plant. The total number of samples was 168 including 84 samples for bacteria and 84 samples for fungi. Quantitative and qualitative identification of bioaerosols was done according to the standard method.

**RESULTS AND DISCUSSION:** The total density of bacterial and fungal bioaerosol was 261 and 426 CFU/m<sup>3</sup> respectively. The highest density of fungal bioaerosol was in the corridor with 98 and the lowest density was in the emergency ward with 45 cfu/m<sup>3</sup>. The highest density of bacterial bioaerosol was in the emergency ward with 79 and the lowest in the corridor with 35 cfu/m<sup>3</sup>. Acinetobacter and Escherichia coli were identified as the dominant bacterial strains and Penicillium and then Cladosporium as the dominant fungal strains in all sections. It is necessary to closely monitor pathogenic bacteria. In terms of total bacteria and fungi, the concentration obtained was lower than the recommended limit. Of course, due to the cross-sectional nature of this study, it is necessary to monitor and control the air quality inside the wards on the regular agenda of the hospital.

**Keywords:** bioaerosol, air, bacteria, fungi, hospital wards

# Determination of prokaryotic diversity utilizing environmental DNA: Study of Naftlije mud volcano's Salsa Lake, Golestan, Iran

Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** Background and objectives Mud volcanos (MV), or sedimentary volcanoes are one of the most interesting geological structures that express subsurface processes by movement of large amounts of sediments, fluids, and gases on the Earth's surface in mainly active tectonic zones. MVs vary in their characteristics and have been studied globally due to their close relation with subsurface hydrocarbon reservoirs and carbon emissions into the atmosphere. However, the prokaryotic diversity of the mentioned environments has been limited to only a few studies.

**MATERIALS AND METHODS:** Materials and Methods Samples were collected in November 2022 from three different spots around Naftlije mud volcano's Salsa Lake's shoreline from the depth of 20 cm utilizing standard microbiological collection methods. Primary physicochemical parameters have been measured in-situ. Environmental DNA has been extracted from biomass utilizing DNA extraction Kit QIAprep<sup>®</sup> Miniprep (Qiagen). The purified 16S rRNA V2-V5 DNA was sequenced via the Illumina NovaSeq6000 platform at Novogene Co. Ltd (UK) as a paired-end (pre-made library, 250PE, 1M read). Raw data analysis has been done using an online server using the CLC genomic workbench. Raw reads are checked against databases including NCBI, EzBioCloud, and our pre-made library to detect Operational Taxonomic Units (OTUs). These OTUs have been used to identify and determine the microbial community in our samples and Naftlije mud volcano's biodiversity.

**RESULTS AND DISCUSSION:** Results and Discussion A total of 1024462 reads with a length of 500 bp were obtained from sequencing which was related to 588725 OTUs. Naftlije mud volcano's prokaryotic community is dominated by Thiomicrospira (70.6%), followed by Halorhodospira (8.6%), Unclassified genus (7.5%), Marinobacter (6.8%), Thioalkalivibrio (3.1%), Halothiobacillus (1.4%), Halomonas (0.8%), and KSA 1(0.7%). This research aimed to determine the prokaryotic community of NMV's Salsa Lake which is located in northeast Iran. MV's are important geological structures whose biological aspects remained largely unknown. MV's key role in important subjects such as hydrocarbon reservoirs, novel gene pools, releasing greenhouse gases into the atmosphere, and terrestrial analog of other planets are the main interests that lead us toward studying MVA's prokaryotic diversity.

**Keywords:** Mud volcano, Naftlije Mud volcano, Environmental DNA, Meta-taxonomy, Prokaryotic diversity

# Eco-Friendly Biosorption of Tetracycline Using Filamentous Fungi Isolated from Heavy Metal-Contaminated Soil

Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** Antibiotics such as tetracycline are key examples of micropollutants, chemicals found in the environment at very low concentrations ( $\mu\text{g/L}$  to  $\text{ng/L}$ ). Improper disposal from the hospitals, livestock, and poultry industries, as well as the fact that most consumed antibiotics are not fully absorbed, leads to their accumulation in the environment. Antibiotics pose significant risks like changes in microbial diversity, increased the antibiotic resistance, cells toxicity, endocrine disruptions, and adverse effects on plant photosynthesis. While the conventional wastewater treatments are usually ineffective at removing these micropollutants, alternative methods such as biosorption are preferred. Fungi are outstanding biosorbents due to their cellular morphology, cell wall composition, and diverse metabolic abilities. In this study, we used a filamentous fungus isolated from tannery waste-polluted soil, to remediate tetracycline hydrochloride through the biosorption process.

**MATERIALS AND METHODS:** Strain PN5 was isolated from polluted soil of the Charmshahr area of Mashhad. To obtain the fungal biomass, strain PN5 was inoculated in Potato Dextrose Broth and kept for 96h at 28°C. After incubation, the fermentation broth was centrifuged, and the cell pellets were freeze-dried and powdered to a uniform particle size. The absorption process was conducted with a reaction mixture containing 0.05 mg/ml of tetracycline and 2 mg/ml of fungal biomass. The mixture, along with a control sample, was incubated at 28°C and 150 rpm for 1h. After incubation, the supernatant was separated by centrifugation. Absorption efficiency was determined by measuring the optical density at 357 nm using UV-Visible spectrometry.

**RESULTS AND DISCUSSION:** The absorption process resulted in a 43% reduction in tetracycline concentration, demonstrating effective absorption of the pollutant by the fungal biomass. This research focused on removing the tetracycline micropollutant from the environment using a simple, cost-effective, and eco-friendly method. The results demonstrate that filamentous fungi biomasses isolated from environments with high concentrations of heavy metals are effective absorbents due to their biodegradable nature and functional groups for binding pollutants. These fungi can efficiently absorb tetracycline with minimal restrictions and costs. This approach not only conserves resources such as money, time, and energy but also provides a highly efficient and low-impact alternative to traditional pollutant removal methods.

**Keywords:** Filamentous-fungi Tetracycline Bioremediation Wastewater-treatment

# Effect of Metal Nanoparticles for the Removal of Coliform Bacteria from Contaminated Water

Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** Waterborne disease and the toxicity caused by the various chemical disinfection methods such as chlorination has caused a basic challenge in human health. Recently, the use of nanotechnology and application of nanomaterials for the control of pathogens in water is widely increased in research. Common indicator for microbial quality of water is determine presence of total and fecal coliforms. The purpose of this study was to evaluate the effect of silver (Ag), titanium dioxide (TiO<sub>2</sub>) and zinc oxide (ZnO) nanoparticles (NPs) in removing total and fecal coliform bacteria from contaminated water.

**MATERIALS AND METHODS:** In this experimental study water was artificially contaminated with waste water. In each run, the Ag NPs (20-100 µg /L), ZnO NPs (0.25-2 mg/L) and TiO<sub>2</sub> NPs (80, 160, 320, and 800 mg/L) were added to contaminated water. The samples were tested by 15-tube series method based on the standard methods of water and wastewater microbial experiments. Bacteria removal efficiency were examined in contact times (20, 40, 80, 100 and 120) minutes.

**RESULTS AND DISCUSSION:** Results Our data indicate a decrease in the number of bacteria (MPN) in the presence of the nanoparticles. Minimum percentage removal of coliforms by TiO<sub>2</sub>, ZnO and Ag NPs were 88%, 95%, and 93% respectively, in 40 min contact time with dose of nanoparticles: 320 mg/L (TiO<sub>2</sub>), 1mg/L(ZnO) and 60 µg /L (Ag). Ag NPs at a concentration of 100 µg /L, ZnO NPs at a concentration of 2 mg/L and TiO<sub>2</sub> NPs at a concentration of 800 µg /L, in 100 min contact time showed the highest percentage (100%) of removal bacteria. Conclusion Results revealed that the removal percentage of coliform bacteria removal increased with increasing the contact time and concentrations of nanoparticles. Therefore, use of metal nanoparticles can become a new and efficient method for the removal of indicator bacteria from contaminated water.

**Keywords:** Water Disinfection, Ag NPs, ZnO NPs, TiO<sub>2</sub> NPs, Coliform Bacteria,

# Effectiveness of Diethyl Methyl Chitosan in Controlling Microbial Contamination on Historical Artifacts"

Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** Introduction Cultural heritage is a valuable asset that reflects the grandeur and power of a nation. Preserving these assets is crucial due to their economic, cultural, and political significance. Various factors contribute to the deterioration of these artifacts, including physical, chemical, and biological factors such as climate changes, light, humidity, and microorganisms. Due to their small size and production of secondary metabolites, microorganisms play a significant role in the degradation of artifacts. Therefore, eliminating these organisms without damaging the artifacts is essential.

**MATERIALS AND METHODS:** Materials and Methods This study investigates the role of diethyl methyl chitosan in eliminating microorganisms from the surface of contaminated memorial stones. The organisms studied include Escherichia coli, Staphylococcus aureus, and lichens. Contaminated stones were immersed in a diethyl methyl chitosan solution, and the inhibitory effect was assessed using systematic swab sampling weekly for two months. Samples were cultured on nutrient, blood, and Sabouraud agar, and results were analyzed using a t-test.

**RESULTS AND DISCUSSION:** Results The results showed no significant difference in bacterial load in the initial and first-week samples. However, a significant reduction in bacterial load was observed from the second week, peaking in the fourth week. There was no significant reduction in fungal growth. Discussion and Conclusion Using chitosan polymers as a natural product can effectively inhibit bacterial growth and protect historical artifacts from microbial degradation without causing damage. However, its impact on fungal growth is negligible, primarily providing a protective coating against microorganisms

**Keywords:** Keywords Cultural heritage, microbial degradation, diethyl methyl chitosan, bacterial inhibition

# Evaluating the efficiency of the extended aeration activated sludge process in the combined leachate treatment system

Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** The increasing generation of urban waste, particularly municipal solid waste leachate (MSWL), poses significant environmental challenges due to its high organic load and toxicity. Leachate from landfills is characterized by high biochemical oxygen demand (BOD), which necessitates effective treatment methods to ensure environmental safety. This study focuses on the application of an extended aeration activated sludge (EAAS) to treat municipal solid waste leachate, emphasizing the role of microbial communities in degrading organic pollutants.

**MATERIALS AND METHODS:** Leachate for this study was collected from a municipal waste site in Qaemshahr and a compost factory in Behshahr, northern Iran, with an initial biochemical oxygen demand (BOD) of approximately 34,000 mg/L. The primary treatment included coagulation, flocculation, and chemical oxidation to reduce BOD and chemical oxygen demand (COD) levels. The final effluent, averaging 841 mg/L BOD, was processed using extended aeration-activated sludge (EAAS), which supported a diverse microbial population. Following a three-week adaptation, mixed liquor suspended solids (MLSS) concentrations stabilized between 2900-3500 mg/L, with a consistent COD removal efficiency of 75%. Air was pumped into the reactor at dissolved oxygen levels of 4-5 mg/L for hydraulic retention times (HRT) of 18 and 36 hours. Samples were analyzed over time to monitor BOD reduction using standard methods.

**RESULTS AND DISCUSSION:** The results of EAAS processes for the treatment of organic matter in leachate showed a significant improvement. Under optimal conditions (pH 6.2-8.6, temperature 21-25 °C and adequate nutrient supply), a significant reduction of BOD<sub>5</sub> up to 91.5% and total kjeldahl nitrogen (TKN) up to 51% was achieved during -36 hours. Hourly interval at HRT 18 hours, COD and BOD<sub>5</sub> removal rates were 84.2% and 87%, respectively. Overall, this study confirmed the effectiveness of EAAS and demonstrated its potential for efficient leachate management. The use of extended aeration activated sludge with increased microbial activity effectively reduces BOD in municipal solid waste leachate. Future research should optimize operational parameters and investigate microbial interactions to improve treatment efficiency in environmental biotechnology applications.

**Keywords:** Activated sludge, Leachate, Biochemical oxygen demand, Microbial communities, Environmental biotechnology

# Evaluation of the performance of Sari wastewater treatment plant in the removal of pathogenic organisms and the feasibility of reusing the effluent for different purposes.

## Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** Pathogenic microorganism as one of the most important pollutants of urban wastewaters, if they are not destroyed well by using wastewater treatment processes, they may lead to the spread of infectious diseases in communities. Biological and chemical processes (disinfection) in treatment plants play an important role in reducing pathogenic pathogens. This study was conducted with the aim of investigating the efficiency of removal of Total coliform (TC) and Faecal coliform (FC) by the disinfection unit of the wastewater treatment plant of Sari city, as well as evaluating the quality of effluent for different applications (use in agriculture, discharge to water sources).

**MATERIALS AND METHODS:** This descriptive-cross-sectional research was conducted in a 6-month period from June 2023 to November 2023. The investigated parameters included TC, FC (which are the most important indicators of microbial contamination) and residual chlorine, which were measured according to the methods provided in the standard method book for water and wastewater tests. Finally, the data were analyzed by SPSS and EXCEL statistical software.

**RESULTS AND DISCUSSION:** The average residual chlorine in the effluent was 0.58 mg/liter. The average total coliform and faecal coliform before disinfection were 92795 MPN/100ml and 70617 MPN/100ml, respectively, and the average total and faecal coliform after disinfection were 51 MPN/100ml and 24 MPN/100ml, respectively. According to the obtained results, the performance of the disinfection process in the Sari treatment plant was 99.96% in TC removal and 99.93% in FC removal. Therefore, it can be concluded that the Sari wastewater treatment plant has reduced microbial pollution and the effluent of the treatment plant complies with the standards of the Iranian Environmental Protection Agency for the reuse of wastewater for agricultural purposes and discharge to surface waters.

**Keywords:** Total coliform, Faecal coliform, Wastewater, Disinfection



# Investigating the quantitative and qualitative microbial and Physicochemical status of effluent in the wastewater treatment plant in Sari-Iran

Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** This paper summarizes the current situation of urban wastewater treatment plant (UWWTP) in Sari-Iran. Most common parameters include pH, TSS, TN, TP, BOD<sub>5</sub>, COD, heavy metals, FC and TC were analyzed with various methods. The aim of this study was drawing an analogy between analyses results and the latest standards in the world (nationwide and internationally), the agricultural and irrigation usage indexes and the Wilcox diagram.

**MATERIALS AND METHODS:** 365 samples from the inlet and outlet of the sewage treatment plant were analyzed in order to evaluate the quality of the effluent of the Sari sewage treatment plant.

**RESULTS AND DISCUSSION:** The average of some indicator parameters, including COD, BOD<sub>5</sub>, TSS are 16.88, 10.6 and 2.47 mg/l and the average of microbiological indicators TC and FC are 186 and 103.5 MPN/100ml. All the results are in accordance with the limits in standards. Agricultural indices categorized all samples as “Moderate” based on their SAR ratio. These findings offer valuable insights into the performance and suitability of treated wastewater for irrigation in the Sari-Iran. They can inform decision makers and stakeholders involved in agriculture and water management.

**Keywords:** wastewater quality effluent, Sodium Adsorption Ratio, water contamination, reusing treated

# Isolation Hydrocarbon degrader prokaryotes in thalassohaline environments: Study of Naftlije Mud-volcano's Salsa Lake, Golestan, Iran

Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** Background and objectives Mud volcanoes are unique geological structures with distinct features, such as the Naftlije mud volcano in Northeast Iran, renowned for its high salinity and hydrocarbon-rich environment. This study aimed to investigate prokaryotes capable of degrading hydrocarbons in extreme conditions by isolating and culturing these microorganisms.

**MATERIALS AND METHODS:** Materials and Methods Through a combination of microbial culture techniques and analytical chemistry, a prokaryotic species was successfully isolated. Molecular analysis via 16S rRNA sequencing revealed that the isolated species bears the closest resemblance to *Marinobacter orientalis*.

**RESULTS AND DISCUSSION:** Results and Discussion Our investigations indicated that isolated prokaryote species from the Naftlije mud volcano have significant hydrocarbon degradation capabilities across a broad spectrum of compounds. This suggests potential applications in bioremediation strategies for saline and polluted environments. These findings enhance our understanding of microbial life in extreme habitats like mud volcanoes and underscore the biotechnological promise of halophilic prokaryotes in environmental management, restoration, and bioremediation efforts.

**Keywords:** Mud volcano, Bioremediation, Naftlije Mud volcano, Salsa Lake, Hydrocarbon degradation

# Isolation, identification and molecular investigation of luminescent bacteria from the Caspian Sea

Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** Luminescent bacteria are a unique group of microorganisms that emit visible light due to specific biochemical reactions. The Caspian Sea, as one of the largest enclosed water areas, offers an interesting ecosystem to discover these luminous creatures. The aim of this study was to isolate, identify and molecularly investigate the luminescent bacteria of the Caspian Sea. Samples were collected from 23 shore and offshore areas from all over the Caspian Sea in northern Iran.

**MATERIALS AND METHODS:** The samples were inoculated in SWB liquid culture medium and kept in an incubator at 28°C for 24 hours. Then it was transferred to the SWA agar culture medium to isolate the luminescent bacteria. The luminescent colonies were selected through the emission of visible light in a dark room. After examining the isolates in terms of morphology and physiology, 5 isolates were selected for molecular identification. Molecular identification of luminescent isolates was done by sequencing analysis of 16S rRNA gene using polymerase chain reaction and phylogeny tree of isolates was drawn.

**RESULTS AND DISCUSSION:** In the present study, 19 luminescent bacteria, including MAZ1-MAZ19, were isolated. All the isolates were gram-negative and in terms of morphology, they were rods, bent rods and short rods. The results of molecular analysis of 16S rRNA gene showed that isolates MAZ1, MAZ4, MAZ5, MAZ8 and MAZ12 have 98.75, 96.99, 99.76, 99.44 and 98.29% respectively with *Vibrio mediterranei*, *Vibrio rotiferianus*, *Photobacterium leiognathi*, *Aliivibrio fischeri* and *Photobacterium mandapamensis* had homology. The luminescence of these isolates was blue, blue, blue-green, green, and blue-green, respectively, and their luminescence intensity was 22, 16, 15, 16, and 38 million RLU. These results provide valuable insight into the presence and diversity of light-emitting bacteria in the Caspian Sea. The study of these microorganisms contributes to our understanding of bioluminescence and may have applications in biotechnology and environmental monitoring.

**Keywords:** Bioluminescence, Luminescent Bacteria, The Caspian Sea

# Measurement of Oxidation-Reduction Potential (ORP) in Reactors Containing Anaerobic, Facultative, and Aerobic Bacteria and Its Impact on Pollution Reduction from Municipal Waste Leachate

Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** The Oxidation-Reduction Potential (ORP) directly influences the process of pollution reduction from wastewater and leachate in municipal and landfill sites by creating suitable conditions for the growth and activity of different types of bacteria. In this study, a combination of anaerobic, facultative, and aerobic bacteria was used to reduce the pollution of waste leachate, and the role of ORP was examined simultaneously with the leachate treatment process.

**MATERIALS AND METHODS:** Two sets of reactors were used: six completely anaerobic and sealed 2-liter small reactors for the growth of anaerobic bacteria, and six 2-liter small reactors with a combination of sealed anaerobic reactors and an open aerobic reactor in the middle for facultative-aerobic bacteria. The waste leachate solution was simulated through experimental study and continuously and intermittently injected into both sets using a plastic tank. Over approximately four months, measurements of ORP in millivolts and environmental conditions were conducted under various conditions for all reactors, and the reduction of leachate pollution was also assessed simultaneously. The measurements were performed according to standard methods.

**RESULTS AND DISCUSSION:** The study indicated that the ORP variations between the two sets of reactors were significant ( $p$ -value 0.001), and the ORP variation trend in the anaerobic-aerobic combined reactor differed from that in the completely anaerobic reactor. The maximum difference in ORP between the two sets of reactors was 202 millivolts, and the minimum difference was 23 millivolts. The effects of different ORP conditions on the reduction of leachate pollution were examined and analyzed. This study provides valuable information to the treatment system designer that by adjusting suitable ORP conditions and monitoring appropriate environmental conditions, the efficiency of the leachate treatment system can be improved. Training on how to adjust environmental conditions and understanding the effectiveness of the oxidation-reduction potential in the treatment environment on the pollution reduction process can play a fundamental role in the system's performance.

**Keywords:** Aerobic-anaerobic bacteria, ORP, waste leachate, pollution reduction

# Removal of azo dye reactive red 195 by sludge biomass in aerobic and anaerobic conditions

## Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** Azo dyes are present in the waste water of textile factories, and if they enter water sources, they cause many adverse effects on the environment and human health. The aim of this study was to determine removal of azo dye by biological method using wastewater treatment plant sludge in aerobic and anaerobic conditions and isolation and identification of bacteria.

**MATERIALS AND METHODS:** Experiments on removal of azo dye were performed at dye concentrations of 50, 65, and 80 mg/L using wastewater treatment plant sludge in batch mode. Bottles containing culture medium, sludge and azo dye were kept at 30°C in incubator in aerobic and anaerobic conditions for 8 days. Sampling was done at the contact times of 1, 2, 4 and 8 days and after centrifuge, the color concentration was measured by spectrophotometric method at a wavelength of 545 nm. The total organic carbon (TOC) concentration of the samples was measured. After removing the color, the bacteria in the bottles were isolated and identified based on standard method.

**RESULTS AND DISCUSSION:** In the aerobic condition at 2 days of contact, removal of dye was 93% and in the anaerobic condition at 4 days of contact, the removal was 86%. By increasing the color concentration from 50 to 80 mg/L, the removal efficiency decreased by about 10%. Increasing the volume of sludge from 5 to 10 ml in both cases did not have a significant effect on increasing the amount of color removal. The average TOC removal in aerobic condition was about 10% higher than anaerobic condition. Removal of dye was higher in aerobic than in anaerobic condition. In the aerobic condition, the identified bacteria were *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aerogenosa*, and in the anaerobic condition, *Lactobacillus*, *Enterococcus faecalis*, and *Bacillus cereus* bacterial species were identified.

**Keywords:** dye removal, textile wastewater, aerobic treatment, anaerobic treatment

# Removal of bacteria from urban sewage using aerated pond

## Role of Microorganisms in Wastewater Treatment

Ali Shahryari<sup>1</sup> © ®

**BACKGROUND AND OBJECTIVES:** Treatment of municipal wastewater is essential to remove bacteria. This study was designed to evaluate the efficacy of wastewater treatment plant (WWTP) at the removal of bacteria for using in irrigation or discharge in the Caspian Sea according to the World Health Organization (WHO) regulation.

**MATERIALS AND METHODS:** A total of 105 samples were collected from 7 stations, including the inlet and the outlet of the wastewater treatment plant in Bandar-e Gaz city (Iran), the intersection point of wastewater effluent with Caspian Sea (Gorgan Bay), and a radius of 200 meters in three directions east, west, and north of the intersection point of wastewater in Gorgan Bay. The multiple-tube fermentation technique was used to enumerate bacteria, and results were expressed as the Most Probable Number (MPN) per 100 ml.

**RESULTS AND DISCUSSION:** The bacteriological analysis exhibited that the concentration of total coliform, fecal coliform, fecal streptococci, and *Clostridium perfringens* were  $1.38 \times 10^{10}$ ,  $5.57 \times 10^{07}$ ,  $5.53 \times 10^{09}$ ,  $1.26 \times 10^{09}$  in inlet, and  $1.38 \times 10^{10} \pm 2.03 \times 10^{09}$ ,  $5.57 \times 10^{07} \pm 1.22 \times 10^{08}$ ,  $5.53 \times 10^{09} \pm 1.21 \times 10^{10}$ ,  $1.26 \times 10^{09} \pm 2.9 \times 10^{09}$  in outlet of WWTP, respectively. Results showed that the aeration lagoon performance was below the permissible limit set by the WHO for using wastewater in agricultural irrigation. This is important to avoid the release of effluent into the environment, use of filtration and the disinfection process may be required to improve the quality of WWTP in order to protect human from risks of bacterial contamination. However, further survey is suggested to evaluate all parameters affecting aeration lagoon performance and all source pollution threatening the Gorgan Bay ecosystem.

**Keywords:** Environmental Monitoring, Bacterial contamination, Wastewater, Sea water, Gorgan Bay

# SARS-CoV-2 Contamination in Amir al-Muminin Hospital: Surface and Dust Transmission Risks

Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** This study aimed to investigate the potential contamination of SARS-CoV-2 in the indoor environment of Amir al-Muminin Hospital in Maragheh city.

**MATERIALS AND METHODS:** Samples were collected from surfaces and settled dust using a passive approach, and from particulate matter using an active approach across different hospital wards. The results showed that 15% of settled dust samples (4 out of 26) and 10% of surface samples (3 out of 30) tested positive for SARS-CoV-2 contamination.

**RESULTS AND DISCUSSION:** Contaminated dust samples were found in 13.8% of cases within 1 meter and 9.1% of cases more than 3 meters away from the patient bed. Among surface samples, 11% from low-touch areas and 8% from high-touch areas were positive for CoV-2 RNA. Additionally, there was a positive correlation between the presence of SARS-CoV-2 and both relative humidity and PM2.5 levels. Principal component analysis (PCA) revealed that 41.9% of the total variance was attributed to the presence of SARS-CoV-2, relative humidity, and PM2.5. This suggests that higher levels of relative humidity and PM2.5 may facilitate the emission of SARS-CoV-2. According to the risk assessment, the annual mean infection risk for hospital staff, with illness and death as endpoints, was  $2.6 \times 10^{-2}$  and  $7.7 \times 10^{-4}$  per person per year, respectively. The study highlights the transmission risk and potential for resuspension of SARS-CoV-2 bioaerosols from surfaces and settled dust in indoor air.

**Keywords:** Risk, SARS-CoV-2, settled dust, surface, RT-PCR, aerosols

# Tetracycline resistance gene (tet A, tet B tet C, tet D, tet E) in wastewater treated and untreated poultry slaughterhouse in Tonekabon

## Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** For many years, antibiotics have been utilized to effectively treat bacterial infections in both humans and animals by either eradicating or suppressing the growth of bacteria. Antibiotics have played a significant role in advancing the field of medicine for decades (1). However, only a small amount of antibiotics that enter the human or animal body are absorbed, with the remaining portion and metabolites being either excreted into surface water or entering wastewater that undergoes treatment at treatment plants (2). Different types and subtypes of antibiotic resistance genes have been detected in wastewater effluent, influent, sludge, or biosolids (3). This study aimed to investigate the presence of tetracycline resistance genes (tet A, tet B tet C, tet D, and tet E) in both untreated and treated wastewater from the Simorgh poultry slaughterhouse in Tonekabon.

**MATERIALS AND METHODS:** 30 Untreated and treated wastewater samples were collected from a poultry slaughterhouse and transformed to the laboratory under standard conditions. After filtering and centrifuging the wastewater, the sediment was utilized for DNA extraction using the FastDNA kit, Qbiogene, Irvine, CA. PCR was conducted using specific primers targeting the tetracycline resistance genes tet A, tet B, tet C, tet D, and tet E. The PCR product was separated on gel electrophoresis, followed by a statistical analysis of the gene frequencies.

**RESULTS AND DISCUSSION:** The most frequent gene found was tetA, while tetD was the least common gene. Both untreated and treated wastewater samples had 13 tetracycline resistance genes, with an equal distribution. However, the distribution of specific genes varied between the two samples. In treated wastewater, tetA, tetB, tetC, and tetE were found in 15, 5, 3, and 0 instances respectively, while untreated wastewater had 9, 0, 1, and 13 instances of these genes, respectively. Using tetracyclines in animal farming for food production leads to antibiotic resistance and the spread of these genes to various environments and bacterial strains.

**Keywords:** genes frequency, tetracycline resistance, poultry slaughterhouse



# The Potential of Green Microalgae Cultivated in High Salinity Medium for Large Scale

## Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** *Chlorella vulgaris*, *Scenedesmus* sp. and *Haematococcus* have the ability to purify water and wastewater by removing toxic metals and soluble ions such as NO<sub>3</sub>, which cause major problems for humans by absorbing nutrients. BBM is an expensive medium for cultivating microalgae. We plan to use inexpensive, high salinity medium as a substitute. This study evaluates the ability of biomass production and nitrate reduction in high salinity medium environment under natural conditions for industrial application.

**MATERIALS AND METHODS:** *Chlorella vulgaris*, *Scenedesmus* sp. and *Haematococcus* were obtained from IROST and were pre-cultivated in BBM medium for 1 week. They were inoculated at the same cell number ( $1.1 \times 10^6$ ) in to 3 erlenmeyer in natural light cycle, room temperature (23°C) and expose to window for 1 week. Each microalga was inoculated at the rate of 10 ml in 100 ml of saline water containers. dcw measured by centrifuge and Nitrate reduction is calculated by apha standard method every day.

**RESULTS AND DISCUSSION:** *Chlorella vulgaris*, *Haematococcus* and *Scenedesmus* sp. reduced nitrate respectively 42%, 35% and 23%. In addition, dcw increased during 1 week when growing microalgae. *Chlorella vulgaris*, *Haematococcus* and *Scenedesmus* increased dcw respectively by 0.0013, 0.008 and 0.0006 g/ml. Finally, the cell number for *Chlorella vulgaris*, *Scenedesmus* sp., and *Haematococcus* microalgae was respectively  $25/96 \times 10^6$ ,  $12/20 \times 10^6$  and  $23/98 \times 10^6$ . As a result, both *Chlorella vulgaris* and *Haematococcus* can grow well in this medium and reduce nitrate levels. *Scenedesmus* was located at the last level. We can use high salinity water as a cheap culture medium for microalgae production and nitrate reduction.

**Keywords:** Microalgae, High salinity medium, DCW, Nitrate reduction, BBM

# The role of *Azospirillum* spp. on the quantitative composition of hydrolyzable nitrogen in soil

Role of Microorganisms in Wastewater Treatment

Iosebi Gioshvili<sup>1</sup> © P

<sup>1</sup> phd doctorate

**BACKGROUND AND OBJECTIVES:** The amount of hydrolyzable nitrogen in brown soil was determined. Nitrogen-fixing ability of the nitrogen-fixing microorganism of the genus *Azospirillum* in sterile soil and its ability to live independently in relation to the microbial association were determined. The role of *Azospirillum* for colonization of sterile soils was determined.

**MATERIALS AND METHODS:** we sterilized (pasteurized) the soil and determined the amount of hydrolyzable nitrogen in the same soil after sterilization. On 100 grams of sterilized brown soil, we applied nitrogen-fixing microorganisms (10 colonies of *Azospirillum*) separated by us to the solid food area. Pour 100 milliliters of sterile water and shake for 10 minutes. We kept it at room temperature for 72 hours and then determined the hydrolyzable nitrogen by Turenne-Kononova method.

**RESULTS AND DISCUSSION:** After sterilization, the amount of hydrolyzable nitrogen was checked and it was always 14 grams per 100 grams of soil. 72 hours after the introduction of *Azospirillum* colonies, we again determined the amount of hydrolyzable nitrogen by the Turenne-Kononova method, and this time, the amount of hydrolyzable nitrogen calculated per 100 grams of soil was 14.7 grams.

Conclusion: 1. The presence of nitrogen-fixing organisms of the genus *Azospirillum* is not directly correlated with the presence of other microbes. 2. Nitrogen fixers of the genus *Azospirillum* represent a good object for the colonization of brown soil and the quantification of hydrolyzable nitrogen growth.

**Keywords:** nitrogen-fixing microorganisms. *Azospirillum*, soil, hydrolyzable.

# The Role of Combined Anaerobic and Aerobic Bacterial Systems in Reducing Pollution from Municipal Waste Leachate

## Role of Microorganisms in Wastewater Treatment

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**BACKGROUND AND OBJECTIVES:** One of the major sources of water, soil, and air pollution, is the leachate produced from wastes. Traditionally, a combination of various high-tech and generally costly and energy-intensive physical, chemical, and biological methods have been used to remove or reduce the pollution of waste leachate. However, due to the high pollution load of leachate (often tens of times more than urban and residential wastewater), these methods have not been particularly successful. This study investigates the use of combined anaerobic, facultative, and aerobic bacteria to remove and reduce waste leachate pollution.

**MATERIALS AND METHODS:** In this study, two sets of parallel glass reactors were used. The first set included five anaerobic cells and a final sixth anaerobic filter cell containing plastic packing. The second parallel set had the same configuration but with an aerobic cell for aerobic and facultative bacteria growth in the middle of the anaerobic cells. Simulated waste leachate was prepared in a plastic container and continuously and intermittently injected into both sets. Standard tests were conducted using COD (Chemical Oxygen Demand) and BOD5 (Biochemical Oxygen Demand) as indicators of leachate pollution to evaluate the efficiency of the bacteria in removing pollution for six months.

**RESULTS AND DISCUSSION:** The results of this study showed that the combined anaerobic-facultative-aerobic bacterial system achieved a pollution reduction in waste leachate from 94.1% in the purely anaerobic system to 98.3% in the anaerobic-facultative-aerobic system for the COD index, and from 93.2% in the purely anaerobic system to 98.1% in the anaerobic-facultative-aerobic system for the BOD5 index. In all cases, the hybrid bacterial system demonstrated higher efficiency compared to the purely anaerobic bacteria. The findings of this study indicate that the removal of organic substances from waste leachate can be achieved with a combination of biological systems without the use of chemicals, electrical energy, and high-tech mechanical installations, and with much higher efficiency compared to conventional physico-chemical and advanced methods. This approach can reduce pollution to acceptable levels for disposal into the environment and mitigate the damage to nature.

**Keywords:** Aerobic-anaerobic bacteria, pollution reduction, COD, BOD5, waste leachate

# Simple and efficient method to obtain fungal pure culture

Microbial ecology

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**BACKGROUND AND OBJECTIVES:** In microbial biotechnology, it's crucial to confirm that the fungal isolates used are pure, which is one of the most important steps. Fungi can be purified using different techniques, such as directly sub-culturing the isolate onto a new medium. In many cases, the researchers were not able to correctly identify the fungal isolate, which led to the findings of the studies that this method is not accurate. To obtain a pure culture of fungi, single spore isolation is an effective method. The purpose of this work is to describe a simple and efficient method for isolating single spores to purify fungi recovered from sediment samples.

**MATERIALS AND METHODS:** Caspian Sea sediments were collected from 3 meters deep. To purify the fungal isolate, the single-spore method in the laboratory as follows; the fungal isolate grown on potato dextrose agar (PDA) medium was sub-cultured onto a new (PDA) medium and incubated for 5 days at 30 °C. Then, a dilution series was prepared from the spore suspension in sterile distilled water. Surface culture of spore suspension was done on water agar (WA) culture medium using a sterile L-shaped glass rod. The germinated single spores were determined after 16-18 hours incubation at 30 °C through microscopic observation (40X). In order to investigate the morphological characteristics of fungal isolates, they were incubated in PDA and carnation leaf-agar (CLA) media with a photoperiod of 12 hours of light and 12 hours of darkness for 14-21 days at 30 °C.

**RESULTS AND DISCUSSION:** The successful identification of fungi requires an accurate purification method. Therefore, the single-spore isolation method was used in the current study. According to the results of the present work, purified fungal colonies with a single-spore method appeared with a better occurrence than those obtained using a needle. The reason for these results lies in the principle of single-spore technique, where a single spore is selected and then transferred to a new medium.

**Keywords:** Fungal culture, Pure culture, Single-spore isolation

# Isolation and study of indigenous azotobacteria isolated from the agricultural areas of Ashkezar city, Yazd

Microbial ecology

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**BACKGROUND AND OBJECTIVES:** Azotobacteria are gram-negative rod bacteria that perform the work of nitrogen fixation in free form. In order to increase the enrichment and biodiversity of agricultural soils in desert areas, indigenous azotobacteria were isolated and studied in the agricultural areas of Ashkezar city

**MATERIALS AND METHODS:** Numerous soil samples were collected from agricultural areas and brought to the laboratory. The sieved soil samples were cultivated on a special culture medium of Azotobacter agar. This culture medium contained the following components (gr/100ml): Mannitol 2, CaCO<sub>3</sub> .01, KH<sub>2</sub>PO<sub>4</sub> 0.025, FeSO<sub>4</sub> 0.04, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.05, Na<sub>2</sub>MO<sub>4</sub> 0.002, Agar 2, distilled water 100ml. The culture media were incubated at 25°C. After one week, the grown colonies were examined for morphology, gram staining and the presence or absence of cysts. Black Sudan staining was performed to observe the cysts.

**RESULTS AND DISCUSSION:** Culturing results showed flat, slimy colonies with a diameter of 6 mm, and after a further week of incubation the colonies turned brown. Gram staining showed gram-negative rods with a white centre. In the black Sudan staining, a black layer and red bacteria were observed around the PHB seeds. The Azotobacter isolates isolated from the soil of agricultural land in this study are indigenous and compatible with the climatic conditions of Yazd province and can be used to enrich agricultural soils.

**Keywords:** Azotobacter, Isolation, N<sub>2</sub> fixation, Soil enrichment

# Isolation of indigenous streptomycetes from the soil of the city of Ashkezar and investigation of the antibiotic production of the isolates

Microbial ecology

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**BACKGROUND AND OBJECTIVES:** Antibiotic production is one of the most important areas of biotechnology and many efforts have been made to produce new antibiotics. Streptomyces are Gram-positive bacteria, the most important bacterial species known for the production of antibiotic secondary metabolites. Due to the rapid development of bacterial resistance, it is becoming increasingly important to investigate new compounds

**MATERIALS AND METHODS:** Samples were taken from the soil of different areas of Ashkezar city. Different dilutions were prepared from the sieved soil sample. Starch-casein agar culture medium was used to isolate the streptomycetes. The cultures were incubated at 25 °C and after one week the morphology of the colonies and their microscopic characteristics were analyzed. A pure culture was prepared from suspected colonies and inoculated into an Erlenmeyer flask with 250 ml lactose broth culture medium. The lactose broth cultures were incubated at 25 °C and 150 rpm. After one week, 1 ml of the cell-free supernatant (CFS) of the lactose broth culture medium was inoculated onto half of the Mueller-Hinton agar culture medium plate. The Gram-negative bacteria Escherichia coli and the Gram-positive bacteria Staphylococcus aureus were inoculated vertically onto the cell-free supernatant, and their growth or non-growth was checked after 48 hours

**RESULTS AND DISCUSSION:** The colonies of the Streptomyces isolate were white, dry and calcareous on the starchcasein agar medium and showed surface protrusions. Microscopic observations showed a very complex and reticulated Gram-positive matrix. The CFS of this isolate prevented the growth of Staphylococcus aureus and caused a 50% reduction in the growth of Escherichia coli

**Keywords:** streptomycetes; antibiotic; Ashkezar

# Characterization of Extremophile Bacteria from Maharloo Lake and Application of Them to Remove Arsenic from Waste Water in Bioreactor

Microbial diversity in biological wastewater treatment

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**BACKGROUND AND OBJECTIVES:** arsenic is one of the heavy metals that is very dangerous for human health and nature, its removal from water and wastewater is one of the urgent needs. During this research, arsenic-resistant and oxidizing bacteria were isolated and identified from Maharloo Lake, located 27 km southeast of Shiraz

**MATERIALS AND METHODS:** culture medium contained 1 mole of sodium ions and pH 10, to which sodium arsenite was added after sterilizing the medium. Incubation was done at 37 degrees Celsius and 120 rpm for 7 to 10 days, then it was transferred from these liquid environments to solid environment. Molecular identification of isolated strains was done using PCR method. Isolation of arsenic oxidizing bacteria was done using qualitative silver nitrate test and colorimetry after about two hours

**RESULTS AND DISCUSSION:** bacterial strains were isolated that tolerate up to 1 mol of sodium arsenite and were bacilli-shaped that tolerate up to 3.5 mol of sodium chloride and alkaline conditions. they grew. They were able to use sodium thiocyanate and sodium thiosulfate. Molecular analysis of the strains confirmed the newness of two of them. The effect of these bacteria was investigated on environments with a high COD of about 180,000 and a low COD close to zero, and they were able to grow and absorb arsenic in both environments.

**Keywords:** Alkaliphilic, Arsenic, Extremophile, Industrial Wastewater, Maharloo Lake

# Determination of flora and density of bacteria and fungi in the indoor air of Imam Khomeini Hospital of Sari -A Cross-Sectional Study in 1402

Aeromicrobiology

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**BACKGROUND AND OBJECTIVES:** Bacterial and fungal bioaerosols suspended in the air of hospitals enter the body through breathing and can lead to infectious or respiratory diseases such as asthma, allergies, sinusitis, rhinitis, and bronchitis. Monitoring and control of hospital bioaerosols prevents the transmission of pathogens through the air and helps to maintain the health of patients and medical staff. The aim of this study was to determine the type and concentration of bacterial and fungal Bioaerosols in the indoor air of different wards as well as the location of the infectious waste depot and wastewater treatment plant of a hospital

**MATERIALS AND METHODS:** This cross-sectional study was conducted in Imam Khomeini Hospital of Sari in 1402. Bioaerosol sampling was done according to the standard method using a QuickTake sampler in two sampling times of 5 and 15 minutes. Samples were taken from the indoor air of different wards including neonatal intensive care (NICU), operating room, intensive care (ICU), also air samples were taken from the corridor of the hospital, outdoor air near infectious waste depot place, and wastewater treatment plant. The total number of samples was 168 run, including 84 run for bacteria and 84 run for fungi. The collected samples were transported to the laboratory in time less than 2.0 h and were subjected to microbial culture according to the standard method. Then the formed colonies were counted and diagnosed according to the standard method

**RESULTS AND DISCUSSION:** The total density of bacterial and fungal bioaerosol was 261 and 426CFU/m<sup>3</sup> respectively. The highest density of fungal bioaerosol was in the corridor with 98 and the lowest density was in the emergency ward with 45 cfu/m<sup>3</sup>. The highest density of bacterial bioaerosol was in the emergency ward with 79 and the lowest in the corridor with 35 CFU/m<sup>3</sup>. Acinetobacter and Escherichia coli were identified as the dominant bacterial strains and Penicillium and then Cladosporium as the dominant fungal strains in all sections. The findings of this research show the importance of regular monitoring and control of air quality in hospitals and adopting appropriate measures and policies to improve environmental conditions and prevent problems.

**Keywords:** bioaerosol, air, bacteria, fungi, hospital wards



# A comparative study to evaluate the effects of ZIF-8 and ZIF-8 Nanozyme based Ciprofloxacin against Ciprofloxacin resistant *Pseudomonas aeruginosa* strains isolated from burn wounds

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** *Pseudomonas aeruginosa* is associated with infections in intensive care units (ICU) and burn care centers. It is resistant to various antibiotics such as Ciprofloxacin. Nanozymes Zeolite imidazole-8 (ZIF-8), a type of metal-organic framework, have attracted increasing interest due to their ability to scavenge superoxide anion. This study aimed to synthesize the nanozymes zeolite imidazole-8 (ZIF-8) and zeolite imidazole-8 based ciprofloxacin (ZIF-8.CIP) and antimicrobial and antibiofilm activities of ZIF-8 and ZIF-8. CIP against Ciprofloxacin resistant *Pseudomonas aeruginosa* strains isolated from patients with burn wounds was also evaluated.

**MATERIALS AND METHODS:** In this study, Sixty *P. aeruginosa* strains were isolated from wound infections of burn patients admitted to the Motahari hospital of Tehran province. An efficient mechanosynthesis was used to synthesize nanozymes. The properties of nanozymes were determined using zeta potential analysis, photodynamic scattering (DSL), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The Minimum inhibitory concentrations (MICs) of nanozymes were determined by agar disk diffusion method. Anti-biofilm activity of nanozymes was determined using Microtiter plate (MtP) assay. The superoxide dismutase mimetic activity of the nanozymes was investigated using the nitroblue tetrazolium (NBT) method.

**RESULTS AND DISCUSSION:** The characterizations indicated that nanozymes were spherical and average diameters of ZIF-8 and ZIF-8.CIP nanozyme were in the range of 10-1000 nm and 100–1000 nm respectively. The MIC of nanozyme ZIF-8 was 40 mg/ml and the MIC of ZIF-8.CIP was 2-fold lower than ZIF-8 (20 mg/ml). The minimum biofilm eradication concentrations (MBECs) of nanozyme ZIF-8 and ZIF-8.CIP were 40 and 25 mg/ml, respectively. ZIF-8 and ZIF-8.CIP removed 55% and 70% of preformed biofilms. ZIF-8.CIP revealed almost 4 times higher superoxide dismutase activity compared to ZIF-8. ZIF-8.CIP showed promising antibacterial and antibiofilm activities against *P. aeruginosa* species. In conclusion, nanozymes based antibiotics can be used for treating infections associated with *P. aeruginosa* biofilm.

**Keywords:** *Pseudomonas aeruginosa*, ZIF-8 Nanozyme, AntiBiofilm, Ciprofloxacin, AntiBacterial

# Antibacterial activity of hydroalcoholic extract and nanoemulsion of *Laurencia caspica* on *Streptococcus mutans* biofilm

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Antibiotics are used in the treatment of biofilms but the current trend is towards the identification of natural products in disinfection. Nanoparticles are able to penetrate bacteria and bacterial biofilms, so they can be a potential agent for controlling the growth of bacterial infections. The aim of this study was to investigate the antimicrobial efficacy of *Laurencia caspica* algae extract (LC) and its nanoparticles (NLC) against *Streptococcus mutans* biofilm in vitro.

**MATERIALS AND METHODS:** In the present study, nanoparticle of *Laurencia caspica* was prepared by the ultrasonication method. Scanning electron microscopy and dynamic light scattering were used to measure the size and morphology of the produced nanoparticles. The extract and nanoparticles were evaluated in vitro against gram positive and gram-negative bacteria by disk diffusion and broth micro-dilution method. The biofilm formation of *Streptococcus mutans* was assessed through the crystal violet staining.

**RESULTS AND DISCUSSION:** The Fe-SEM image reveals that the nanoparticles morphology is nearly spherical, average size 95.29 nm. In the MIC method, NLC had stronger antibacterial effect than LC against bacteria. NLC was able to inhibit the *Streptococcus mutans* biofilm adherence at a sub-MIC concentration. Moreover, Electron micrographs indicated the inhibition of biofilms compared to control biofilms. Conclusion: NLC was able to inhibit gram positive and gram-negative bacteria that could be due to the reduced particle size, better nanoparticle penetration and the synergistic impact of main components in extract. More research is needed to investigate the synergistic mechanism of NLC against bacteria.

**Keywords:** Antibacterial, biofilm, *Laurencia caspica*, Nanoparticles

# Antibacterial activity of melt-blown fabric coated with copper nanoparticles

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Metal nanoparticles (MNPs) cause cell inhibition through changes in bacterial membrane permeability and ROSs production, and in this way, they have antibacterial properties. Among metal nanoparticles, nanoparticles of copper compounds are more used because they are cheaper. After the coronavirus pandemic, researchers have made many efforts in the field to improve the antibacterial and antiviral properties of air filters and breathing masks; The aim of this study is the green synthesis of copper nanoparticles (CuNPs) by the Iranian plant Vanegi to enhance the antibacterial properties of melt-blown fabric, so with the suggestion of melt-blown antibacterial with different uses such as breathing masks, it took steps to reduce occupational exposure to bacteria.

**MATERIALS AND METHODS:** First, copper nanoparticles were synthesized with the help of plant extract and CuSO<sub>4</sub> precursor salt for 4 hours. Then the dip-coating method coated the synthesized CuNPs on 2x2 cm melt-blown fabric. Antibacterial properties of fabrics against gram-negative *Acinetobacter baumannii* and gram-positive *Staphylococcus aureus* were evaluated by the disk diffusion method. Finally, the zone of inhibition created around the fabric was measured with a ruler.

**RESULTS AND DISCUSSION:** The zone of inhibition obtained against *Acinetobacter baumannii* was about 30 mm. As the concentration of CuNPs coated on the fabric increased, the inhibition zone obtained also increased. However, the inhibition zone around the fabrics against *Staphylococcus aureus* bacteria was not observed. Therefore, fabrics coated with CuNPs can prevent the growth of gram-negative bacteria, but not gram-positive bacteria. The synthesis of copper nanoparticles was carried out using plant extracts from Iranian plants. melt-blown fabric containing nano copper particles showed good antibacterial properties against gram-negative bacteria. As a result, we have achieved an easy and cost-effective way to create antibacterial properties in textiles, which can improve the antibacterial properties of fabrics or even mask filters.

**Keywords:** Copper, nanoparticle, green synthesis, antibacterial, disk diffusion.

# Antibacterial and Healing Effect of Silver Nanoparticles Derived by the *Althaea officinalis* Extracts on Burn Infections of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** *S. aureus*, *E. coli* and *P. aeruginosa* are common causes of burn infections and illnesses. Silver nanoparticles have many applications, one of the necessary applications is antimicrobial and healing effect. There are various methods for synthesis like green synthesis which is more efficiency and eco-friendly. In this study, antibacterial and healing effect of silver nanoparticles derived by the green synthesis from acetonic extraction of *Alcea rosea* on burn infections of *S. aureus*, *E. coli* and *P. aeruginosa* has been reported.

**MATERIALS AND METHODS:** In this study, acetonic extracts of *Alcea rosea* prepared with soaking method. The extract was added to 0.1 M of silver nitrate solution for biosynthesing of Ag<sup>+</sup> nanoparticles. Efficiency of Ag<sup>+</sup> synthesis was confirmed by XRD and DLS technique. The MBC and MIC of the extracts and nanoparticles were determined with microdilution method. In animal model, bacteria were inoculated with a concentration of (5×10<sup>5</sup> CFU/ml) to the wound side of rats. After 24 hours ointments were prepared based on MBC concentration of extracts and nanoparticles with 1g Eucerin for treat burn wounds and infections.

**RESULTS AND DISCUSSION:** Animal studies shows that silver nanoparticles synthesized by *Alcea rosea* are effective in low concentration on burn infections by *S. aureus*, *E. coli* and in high concentration on *P.aeruginosa*. The acetonic extract of this plant is effective with high concentration on *S. aureus* and *E. coli*. As a result, silver nanoparticles derived by extracts have more antimicrobial and healing effect than acetonic extracts. The wound which treated with nanoparticles seen healing activity.

**Keywords:** *Alcea Rosea*, *Escherichia coli*, silver, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Urtica*.

# Antibacterial effect of chitosan-coated nanoliposomes containing berberine against *Helicobacter pylori*

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** *Helicobacter pylori* (*H. pylori*), is a gram-negative bacterium associated with gastroduodenal diseases such as chronic gastritis, peptic ulcers and gastric cancer. The emergence of antibiotic-resistant *H. pylori* strains requires the exploration of alternative therapeutic strategies. Berberine, a natural alkaloid, has strong antibacterial properties but poor solubility and bioavailability. Nanoliposomes, coated with chitosan, enhance delivery, stability, and controlled release of bioactive compounds. This study investigated the antibacterial effect of chitosan-coated nanoliposomes containing berberine (CNB) against antibiotic-resistant *H. pylori* strains.

**MATERIALS AND METHODS:** Berberine was encapsulated in nanoliposomes using the thin-film hydration method and then nanoliposomes were coated with chitosan. CNB was characterized in terms of particle size, zeta potential, UV and FTIR spectra, encapsulation efficiency and release kinetics. The morphology of the nanoliposomes was observed using field emission scanning electron microscopy. The antibacterial activity of CNB was evaluated against 5 strains of *H. pylori* resistant to metronidazole and clarithromycin using broth microdilution method. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined and compared with free berberine.

**RESULTS AND DISCUSSION:** The MICs of free berberine and CNB against antibiotic resistant *H. pylori* strains were 22.2-44.5 µg/ml and 3.7 µg/ml, respectively. All the MBC results were at the MICs or twofold higher. This study demonstrated that CNB exhibit enhanced antibacterial activity compared to free berberine. The stability and controlled release of berberine was significantly improved by encapsulating in chitosan-coated nanoliposomes. These results suggest that CNB could be a promising alternative for the treatment of antibiotic-resistant *H. pylori* infections.

**Keywords:** *Helicobacter pylori*, Antibiotic Resistance, Berberine, Nanoliposome, Chitosan

# Bio Synthesis and Characterization of Aqueous Extract of Leaves of *Mespilus germanica* L. Derived Silver Nanoparticles and Evaluation of its Antibacterial and Antioxidant Activities

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Silver Nanoparticles (Ag-NPs) are widely used in medical and pharmaceutical applications due to their antimicrobial properties. Our present project was conducted to bio synthesis and characterization of *Mespilus germanica* L. derived (Ag-NPs) and evaluation of its antibacterial and antioxidant activities.

**MATERIALS AND METHODS:** The formations of silver nanoparticles were confirmed by the color changing from green to dark brown. The leaves extract of *Mespilus germanica* L. caused the aqueous silver ions to reduce in size and form silver nanoparticles in a green method. The biosynthesized Ag-NPs were characterized by using UV-Vis, FTIR, XRD, TEM, and FE-SEM analysis. Additionally, the bio synthesized Ag-NPs antibacterial properties against both gram-positive and gram-negative bacterial strains were assessed. Then, the antioxidant potential was evaluated by 2,2--diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay.

**RESULTS AND DISCUSSION:** Findings from the FT-IR showed the successful formation of Ag-NPs because the functional groups involved in the synthesis process and adsorption peaks were well developed. Furthermore, the Ag-NPs had peak absorption at 446 nm in the spectrometry. XRD analysis confirmed the structure, crystal size and nature of the Ag-NPs. The FE-SEM analysis showed that the synthesized Ag-NPs were spherical in shape. The particle size histogram revealed that the average particle size of the Ag-NPs was 30 nm. The activity of the Ag-NPs against *Escherichia coli* and *Bacillus subtilis* was shown to be the most powerful. Nanoparticles could exert the inhibitory effect of DPPH free radicals in a dose-dependent manner. This suggests that the *Mespilus germanica* L. derived Ag-NPs have a considerable potential for use in the development of broad-spectrum antimicrobial medications and an optimistic perspective for their use in medicine and pharmaceutical industry.

**Keywords:** *Mespilus germanica* L., Silver nanoparticles, XRD, *Bacillus subtilis*, pharmaceutical industry.

# Biosynthesis of selenium nanoparticles by bacterial exopolysaccharide produced by *Vibrio alginolyticus* ATCC 17749

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Bacterial exopolymers (EPS), predominantly acidic heteropolysaccharides with functional groups, have emerged as versatile templates for the rapid biosynthesis of metallic nanoparticles (NPs). The EPS-mediated reduction of metal ions is recognized for its simplicity, safety, and eco-friendliness. Moreover, the capping of NPs with EPS facilitates subsequent modifications, thereby enhancing their material properties for a variety of applications.

**MATERIALS AND METHODS:** This study focuses on the EPS produced by the marine bacterium *Vibrio alginolyticus* strain 17749 as a stabilizing agent for selenium NPs (Se NPs). The bacterial strain was cultivated in marine broth, and EPS was extracted using a cold ethanol precipitation method. Se NPs were synthesized using a green approach by combining 10 mM sodium selenite ( $\text{Na}_2\text{SeO}_3$ ) with an equal volume of EPS solution under stirring. Freshly prepared ascorbic acid (40 mM) was added dropwise to the mixture, which was then incubated at 40 °C in the dark for 4 h.

**RESULTS AND DISCUSSION:** A color transition from colorless to light orange indicated successful SeNP formation. The resulting EPS-SeNPs were isolated via centrifugation at 12,000 rpm for 15 min, followed by freeze-drying and storage. UV spectroscopy confirmed the synthesis of Se NPs, exhibiting a characteristic absorption peak at 263 nm. Zeta potential measurements demonstrated enhanced stability of EPS-Se NPs compared to bare Se NPs, with values increasing from -28.06 mV to -32.00 mV. This enhanced negative zeta potential confirmed EPS's crucial role in the formation, stabilization, and dispersion of Se NPs. Fourier transform infrared spectroscopy (FTIR) analysis identified characteristic peaks for both EPS and Se NPs, including hydroxyl (-OH) groups at 3388.73  $\text{cm}^{-1}$  and 3413.02  $\text{cm}^{-1}$ , and carbonyl (C=O) groups at 1656.00  $\text{cm}^{-1}$  and 1711.05  $\text{cm}^{-1}$ . These findings indicate the successful integration of EPS functional groups into the NPs structure.

**Keywords:** bionanotechnology, bacterial exopolysaccharides, selenium nano particles

# Cloning and expression of glycerophosphodiester phosphodiesterase (GLPQ) gene from *Borrelia microti* and using the recombinant GlpQ for serodiagnosis of relapsing fever

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Tick-borne relapsing fever (TBRF) is a disease endemic in Iran, with over 100 cases reported annually. TBRF as a vector-borne disease caused by infection with Spirochete bacteria in the genus *Borrelia* and among them, *Borrelia microti* is one of the causative agents of the disease in Iran. TBRF detection is based on a patient's blood smear during a febrile episode but some cases are not detectable. So, a reliable and accurate method are needed. The gene for glycerophosphodiester phosphodiesterase (glpQ), encoding GlpQ, an enzyme employed in the phospholipid synthesis, which is present in TBRF. The purpose of this study was to produce recombinant GLPQ and evaluate its antigenicity and immunoreactivity against mice infected sera.

**MATERIALS AND METHODS:** The DNA encoding Glycerophosphodiester phosphodiesterase (GLPQ) was amplified by Polymerase Chain Reaction (PCR) from *B. Microti*, then cloned into the pTZ57R/T cloning vector and sequenced. Subsequently, it was sub-cloned into the pET-28b expression plasmid. *Escherichia coli* BL21(DE3) cells was transformed with recombinant pET-GLPQ plasmid and expression of the truncated GLPQ recombinant protein was induced by IPTG (Isopropyl  $\beta$ -D-1-thiogalactopyranoside). The GLPQ was purified via Ni-NTA chromatography, analyzed by Western blotting using sera from *B. Microti* infected mice and evaluated by ELISA (enzyme-linked immunosorbent assay) with sera from infected mice during 8 days.

**RESULTS AND DISCUSSION:** Sequencing of cloned gene revealed a 99% homology with *Borrelia duttoni* and *B. recurrentis* in Genbank database. The GLPQ protein expression was induced, showing a 48kD band. The recombinant GLPQ was purified in one step using Ni-NTA chromatography. Western blotting showed that sera from *B. microti*-infected mice strongly recognized the recombinant protein while negative sera showed no detection. ELISA results demonstrated that the immunogenicity of GLPQ was positive with mouse sera between 5 to 8 days of infection, with absorbance values ranging from 1.507 to 3.122 for acute-phase serum samples. Recombinant GLPQ was successfully produced in bacteria and purified in a single step. This GLPQ protein reacted with sera from infected mice, suggesting its potential as a useful marker for borreliosis infection. The recombinant GLPQ can serve as a valuable tool for detecting relapsing fever infections through serological methods.

**Keywords:** Relapsing fever, GLPQ, *Borrelia Microti*



# Cloning, expression and purification of Ph. sergenti yellow related salivary protein in inducible T7-TR leishmanina tarentulae expression system

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** The Leishmania parasite is responsible for causing leishmaniasis, a vector-borne disease that is transmitted to humans through the bite of an infected sand fly. In order to further investigate and identify a suitable biomarker for sand fly exposure, we have successfully produced a new recombinant protein based on Ph. sergenti yellow-related salivary protein 26 (PsSP26). Various expression systems for recombinant proteins have been developed for the expression of heterologous genes in both prokaryotic and eukaryotic hosts. The objective of this study is to utilize the inducible T7-TR Leishmania tarentulae system to generate the recombinant PsSP26 protein.

**MATERIALS AND METHODS:** The PsSP26 gene was obtained from the VR1020\_TOPA\_PsSP26 plasmid through the use of specific primers, and then inserted into the XbaI and KpnI sites of the pLEXSY\_I-blecherry3 expression vector. Stable transfection of the Swal linearized pLEXSY\_I-blecherry3\_PsSP26 and integration into the odc locus of L. tarentulae T7-TR genome was performed by electroporation. The expression of secretory recombinant PsSP26 protein containing histidine-tag was induced by adding tetracycline; and purified from supernatant of induced recombinant L. tarentulae culture using Ni-NTA affinity chromatography.

**RESULTS AND DISCUSSION:** The integration of pLEXSY\_I-blecherry3\_PsSP26 into the odc locus of L. tarentulae T7-TR genome was verified through PCR. The expression of PsSP26 protein was triggered by the addition of 10µg/ml tetracycline and observed through the red fluorescence of Ble-cherry protein under fluorescent microscopy. The secreted recombinant PsSP26 protein, which had a C-terminal His-tag, was isolated using Ni-NTA-affinity chromatography. The purified recombinant protein, with a molecular mass of approximately 47kDa, was confirmed through SDS-PAGE and western blot using an anti-His-tag antibody. The final yield of the purified rPsSP26 was estimated to be 0.1mg/ml. Our findings indicate that the expression of PsSP26 in L.tarentulae results post-translational modification similar to eukaryotes and leading to a proper folding as soluble recombinant protein that was validated through testing with mouse serum immunized with pcDNA-PsSP26. The utilization of this system enables the production of native folding proteins with a more cost-effective and easily manipulable method compared to mammalian systems.

**Keywords:** Inducible T7-TR Leishmania tarentulae expression system, Ni-NTA affinity chromatography

# Comparative analysis of exopolysaccharide production by two native Iranian strains of lactic acid bacteria derived from dairy products

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Exopolysaccharides (EPSs) produced by lactic acid bacteria (LAB) have attracted significant interest due to their remarkable biological activities, including anti-cancer and antioxidant effects. This study aimed to compare the exopolysaccharide production capabilities of two LAB strains, *Lactobacillus fermentum* PTCC1929, and *Lactobacillus plantarum* PTCC1901, isolated from traditional dairy sources in Iran.

**MATERIALS AND METHODS:** The LAB strains were cultured under controlled conditions in MRS medium, followed by a heated acid extraction method that involved treating the bacterial biomass with HCl at 90°C and absolute ethanol precipitation for EPS isolation. The extracted EPS was then quantified and characterized for further analysis.

**RESULTS AND DISCUSSION:** The results indicated that both the *L. fermentum* and *L. plantarum* strains were capable of producing exopolysaccharides; however, the *L. plantarum* strain demonstrated a significantly greater production of exopolysaccharides in comparison to the *L. fermentum* strain (p-value 0.05). These findings implicate the potential of these Iranian dairy-derived LAB strains as significant producers of EPS with promising therapeutic applications. Given the anti-cancer and antioxidant properties associated with EPS derived from lactic acid bacteria, it is recommended that future studies delve deeper into evaluating the biological activities of EPS produced by these specific strains of LAB.

**Keywords:** *Lactobacillus plantarum*, *Lactobacillus fermentum*, Exopolysaccharide

# Comparison of Green and Chemical Synthesis in the Antibacterial Properties of Zinc Oxide Nanoparticles Doped with Silver Nanoparticles

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** The escalating global health crisis posed by antibiotic-resistant bacteria necessitates urgent exploration and development of effective antimicrobial agents. Nanotechnology offers promising solutions due to its versatile applications in drug development. In this study, we investigated the antibacterial activity of zinc oxide nanoparticles doped with silver nanoparticles (ZnO/Ag NPs) synthesized through environmentally friendly and cost-effective methods. Furthermore, we assessed the impact of synthesis method on nanoparticle efficacy by comparing green and chemical synthesis routes.

**MATERIALS AND METHODS:** To achieve these objectives, ZnO/Ag NPs were synthesized using various reducing agents, including plantago ovate (*p. ovata*) plant extract as an eco-friendly option and sodium borohydrate as a chemical alternative. Nanoparticles were characterized using XRD, EDS, SEM, and TEM analyses. Subsequently, antibacterial properties of the synthesized nanoparticles, as well as pure *p. ovata* plant extract, were evaluated against gram-positive and gram-negative bacterial strains using the broth microdilution method.

**RESULTS AND DISCUSSION:** Results demonstrated successful synthesis of zinc oxide nanoparticles and green ZnO/Ag NPs, exhibiting spherical morphology with sizes ranging from 60-120 nm and 100-150 nm, respectively. *p. ovata* plant extract exhibited inhibitory activity against *Escherichia coli* at a concentration of 15 mg/ml. Zinc oxide nanoparticles displayed varying inhibitory effects, with the highest against *Klebsiella pneumoniae* (MIC=500 µg/ml) and the lowest against *Pseudomonas aeruginosa* (MIC=2000 µg/ml). Chemical ZnO/Ag NPs inhibited *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* growth most effectively at a concentration of 250 µg/ml, while exhibiting least effect on *Streptococcus mutans* (MIC=1000 µg/ml). Notably, green ZnO/Ag NPs exhibited superior antibacterial properties, inhibiting *Escherichia coli* and *Pseudomonas aeruginosa* growth at a concentration of 62.5 µg/ml, with the lowest effect observed against *Enterococcus faecalis* (MIC=250 µg/ml). Overall, our findings highlight the enhanced antibacterial effects of two-component nanoparticles incorporating silver nanoparticles alongside zinc oxide nanoparticles. Moreover, green synthesis method demonstrated superior efficiency compared to chemical

**Keywords:** Zinc oxide, Silver, Nano, Green, Antibacterial

# Comparison of the antifungal properties of carvacrol and nano carvacrol obtained from marjoram plant against *Aspergillus parasiticus*

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Background: *Aspergillus parasiticus* isolates often infect agricultural products in fields and warehouses in tropical regions. In recent years, the study of plant compounds to reduce the growth of toxin-producing microorganisms has increased. Loading the effective substances of medicinal plants such as marjoram in lipid nanocarriers reduces the reaction of the active substance of the plant with the surrounding environment such as water and oxygen and reduces the intensity of transfer or evaporation of the external environment. Objectives: In this study, in order to improve carvacrol, this compound was loaded with nanoparticles and its antifungal effect on the growth of *Aspergillus parasiticus* was investigated

**MATERIALS AND METHODS:** Methods: In this experimental-laboratory study, based on the microdilution method in accordance with the latest version of the CLSI standard methods, modified M38-A2 protocol, the minimum growth inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) in different concentrations of carvacrol and Nanocarvacrol was tested against the standard strain of *Aspergillus parasiticus* in the laboratory. Physicochemical indices and structural characteristics of nanoparticles were determined in terms of surface morphology by SEM (Scanning electron microscope) method, and also by SPSS 21 software and two-way analysis of variance. Data analysis using (P0.05).

**RESULTS AND DISCUSSION:** Results: In this study, it was observed that nanocarvacrol has more antifungal effect against *Aspergillus parasiticus* than pure carvacrol. Based on this, the minimum growth inhibitory concentration (MIC) of nanocarvacrol was 0.97 ml/  $\mu\text{g}$  and pure carvacrol was 97 ml/  $\mu\text{g}$ . Such minimum fungicidal concentration (MFC) of nano-carvacrol was 3.9 ml/  $\mu\text{g}$  and 390 ml/  $\mu\text{g}$  of carvacrol extract, which showed the higher antifungal effect of nano-carvacrol compared to pure carvacrol extract. SEM images showed the spherical morphology of the particles with nanocarvacrol and the average size of the particles was about 80 nm. Conclusion: In general, the effect of nanoparticles containing carvacrol showed the lack of growth of *Aspergillus parasiticus* fungus, which can be a promising antifungal agent with high antifungal effects and low side effects. With more research in the future, it is hoped that this nano compound can be used to treat the disease.

**Keywords:** Keywords: *Aspergillus parasiticus*, carvacrol, electron microscope, microdilution, nanocarvacrol.

# Comparison of the Efficacy of Solid Lipid Nanoparticle (SLN), Lipid Nanoparticle (LNP), and Poly (lactic-co-glycolic acid) (PLGA) Nanoparticle Carriers in Drug Delivery to Intracellular Bacteria

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Intracellular bacterial infections, demand effective drug delivery systems capable of penetrating host cells. SLNs, LNPs, and PLGA nanoparticles have garnered attention for their potential to enhance drug bioavailability, intracellular uptake, and sustained release. However, a systematic comparison of their efficacy in intracellular drug delivery is warranted to guide future research and development efforts.

**MATERIALS AND METHODS:** Preparation of Solid Lipid Nanoparticles (SLNs): 1. SLNs are colloidal carriers composed of lipids that are solid at room temperature. They offer advantages such as biodegradability and nontoxicity. 2. The preparation of SLNs involves techniques like high-pressure homogenization, solvent emulsification-evaporation, and microemulsion methods. Lipid Nanoparticles (LNPs): 1. LNPs are lipid-based carriers similar to SLNs but typically composed of liquid lipids. 2. LNPs are prepared using similar techniques as SLNs, including high-pressure homogenization and solvent emulsification. Poly (lactic-co-glycolic acid) (PLGA) Nanoparticles: 1. PLGA is a biodegradable polymer commonly used for drug delivery. 2. PLGA nanoparticles are prepared by the solvent evaporation method. Characterization Methods: 1. The morphology and size distribution of nanoparticles can be analyzed using techniques like dynamic light scattering (DLS) or scanning electron microscopy (SEM). 2. Encapsulation efficiency, drug loading, and release kinetics are determined using UV-Vis spectroscopy or HPLC.

**RESULTS AND DISCUSSION:** The review identified numerous studies assessing SLNs, LNPs, and PLGA nanoparticles for drug delivery to intracellular bacteria. Each nanoparticle type demonstrated advantages and limitations in terms of drug loading capacity, release kinetics, intracellular uptake, and antimicrobial activity. SLNs and LNPs exhibited superior biocompatibility and targeted delivery capabilities, while PLGA nanoparticles offered sustained release profiles and tunable degradation properties. SLNs, LNPs, and PLGA nanoparticles represent promising platforms for enhancing drug delivery to intracellular bacteria. While each nanoparticle type offers distinct advantages, including biocompatibility, targeted delivery, and sustained release, their efficacy varies depending on the specific characteristics of the drug, bacteria, and host cells. Further research is warranted to optimize nanoparticle formulations, elucidate mechanisms of intracellular delivery, and evaluate their clinical applicability in combating intracellular bacterial infections.

**Keywords:** SLN; LNP; PLGA

# Creating a novel nanocomposite hydrogel comprised of alginate and graphene oxide with antibacterial properties for the purpose of promoting wound healing

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Wound healing is a complex process that requires an appropriate microenvironment for rapid healing. Therefore, the production of an effective wound dressing is a significant advancement in the wound-healing process. The fabricated wound dressing was created using a combination of suitable antibacterial materials like mupirocin, graphene oxide, and alginate. The characteristics of the hydrogel, including swelling, release of mupirocin, antibacterial activity, cytotoxicity, and wound healing properties, were evaluated. The findings indicated a slow release of mupirocin over 48 hours and inhibitory effects on some Gram-positive bacteria. Drug incorporation due to its porosity and porous structure was observed through scanning electron microscopy. *in vitro* studies confirmed the high biocompatibility and effectiveness of the synthesized hydrogel in promoting wound healing and antibacterial processes.

**MATERIALS AND METHODS:** Alginate with average molecular weights of about 46 kDa, ethylenediaminetetraacetic acid (EDTA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), mupirocin, bovine serum albumin (BSA), were purchased from Sigma-Aldrich. NaCl, KCl, Na<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, and CaCl<sub>2</sub>, were obtained from Merck. Dulbecco's modified eagles' medium (DMEM), fetal bovine serum (FBS), and trypsin were from Gibco. In the current study, alginate was dissolved in double distilled water, graphene oxide was dispersed in double distilled water mupirocin was dissolved in sodium phosphate buffer.

**RESULTS AND DISCUSSION:** The FTIR spectrum of GO shows broadband for the hydroxyl (OH) groups at 3421 cm<sup>-1</sup>, and the carbonyl and carboxylic groups bands appeared at 1720, and 1591 cm<sup>-1</sup>, respectively. Furthermore, in the FTIR spectrum of SA, the strong absorption bands at 1592 and 1414 cm<sup>-1</sup> are attributed to asymmetric and symmetric stretching vibrations of the carboxylate (COO) groups on the polymer backbone. After the formation of SA/GO/Mu hydrogel, the characteristic peaks at 3421, 1592, and 1412 cm<sup>-1</sup> shifted to 3424, 1594, and 1413 cm<sup>-1</sup>, respectively, indicating that hydrogen bonding between GO and polysaccharide has been formed in the composite hydrogels. After immersion of the hydrogel in simulated wound fluid, the swelling percentage of hydrogels was determined at various time points. Cytotoxicity of the hydrogels was examined using an MTT assay that showed low toxicity of the complex. The complex has a high antibacterial ability against *S. aureus*.

**Keywords:** Hydrogel, graphene oxide, mupirocin, antibacterial

# Dy<sub>2</sub>BaCuO<sub>5</sub>/Ba<sub>4</sub>DyCu<sub>3</sub>O<sub>9.09</sub> S-scheme heterojunction nanocomposite with enhanced photocatalytic and antibacterial activities

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Semiconductor heterogeneous photocatalysis has been received much attention from the scientific and researchers in the last decade. The combination of two semiconductors with various energy diagram can dramatically enhance the lifetime and separation of the charge carriers, restrain photogenerated electron-hole recombination, and considerably enhance photocatalytic performance as compared with other single or binary components. In this regard, we introduced the Dy<sub>2</sub>BaCuO<sub>5</sub>/Ba<sub>4</sub>DyCu<sub>3</sub>O<sub>9.09</sub> nanocomposites as active photocatalysts below UV radiation.

**MATERIALS AND METHODS:** several technologies, such as chemical precipitation, adsorption, and membrane, have been applied to treat these toxic organic contaminants from water. Among these used methods, photocatalysis has attracted extensive attention due to the unique advantages such as low energy consumption, easy fabrication, repeatable process, high removal efficiency, and limited chemical requirement. In this paper, we used the hydrothermal method to control the size of Dy<sub>2</sub>BaCuO<sub>5</sub>/Ba<sub>4</sub>DyCu<sub>3</sub>O<sub>9.09</sub> nanocomposite. The hydrothermal process is a facile, simple, and friendly route that enjoy easily scaled up and inexpensive precursors. Nanomaterials produced by this method show high purity, high chemical homogeneity. Also, the low calcination temperature is needed that leads to saving energy consumption. Preparing Ba<sub>4</sub>DyCu<sub>3</sub>O<sub>9.09</sub> and Dy<sub>2</sub>BaCuO<sub>5</sub> is a difficult task. These compounds are two potential materials for superconductivity applications.

**RESULTS AND DISCUSSION:** Highest antibacterial activity of Dy<sub>2</sub>BaCuO<sub>5</sub>/Ba<sub>4</sub>DyCu<sub>3</sub>O<sub>9.09</sub> was observed against Gram-positive species (*Staphylococcus aureus* and *Enterococcus faecalis*), whereas this nanocomposite could not significantly inhibit the growth of Gram-negative bacteria. In the case of *Klebsiella pneumonia*, we observed bacteria growth in all concentrations of the nanocomposite. The best MIC values of this nanocomposite ranged between 1250 and 125 µg/ml and the MBC values ranged between 2500 and 125 µg/ml. Samples 1 (hydrothermal reaction for 8 h) and 4 (hydrothermal reaction for 24 h) have higher antibacterial activity than others. Significant effect of Dy<sub>2</sub>BaCuO<sub>5</sub>/Ba<sub>4</sub>DyCu<sub>3</sub>O<sub>9.09</sub> on gram-positive bacteria due to the difference between bacteria structures. Since gram-negative bacteria have external membranes, show antibacterial resistance against Dy<sub>2</sub>BaCuO<sub>5</sub>/Ba<sub>4</sub>DyCu<sub>3</sub>O<sub>9.09</sub>. The sensitivity of bacteria to nanoparticles is not only related to the structure of the cell wall but also related to the peroxidation of fat, and the production of active oxygen species.

**Keywords:** antimicrobial activity, Dy<sub>2</sub>BaCuO<sub>5</sub>/Ba<sub>4</sub>DyCu<sub>3</sub>O<sub>9.09</sub>, photocatalysis, S-scheme heterojunction, water treatment

# Environmentally Friendly and Green Synthesis of Ag-NPs by Extract of Leaves of *Trifolium repens* L. with Antibacterial Properties

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Modern public health is threatened by microbial illnesses, necessitating the ongoing quest for novel antimicrobial medications and procedures. The rise of antimicrobial resistance and the shortage of innovative, safe, broad-spectrum antibiotics need the development of potent, broad-spectrum alternatives. Silver nanoparticles (Ag-NPs) have been studied extensively due to their intriguing physicochemical properties and proven antibacterial action against most pathogens. Ag-NPs change microbial cell structure and function, disrupting important cellular processes and killing bacteria. Therefore, the aim of this project, environmentally friendly and green synthesis of Ag-NPs by extract of leaves of *Trifolium repens* L. with antibacterial properties.

**MATERIALS AND METHODS:** The obtained nanoparticles were characterized using UV-Vis, FT-IR, XRD, and TEM. The Disc diffusion method was used to evaluate the antibacterial activity of Ag-NPs against *Escherichia coli* and *Staphylococcus aureus*.

**RESULTS AND DISCUSSION:** The absorption peak of the Ag-NPs is observed at 445 nm using UV-Vis. The TEM images showed spherical shape Ag-NPs and their average sizes were ranging from 30 - 50 nm. XRD analysis confirmed the structure, crystal size and nature of the Ag-NPs. FT-IR revealed that water-soluble biomolecules from the aqueous extracts of the leaves of *Trifolium repens* L. played a crucial role in the formation of Ag-NPs. Ag-NPs produced by green synthesis have good antibacterial activity. These findings contribute to the growing field of eco-friendly nanotechnology and emphasize the significance of plant-mediated approaches in nanomaterial synthesis and biomedical applications. Therefore, Ag-NPs of synthesis by extract of leaves of *Trifolium repens* L. it could be a promising candidate for medicinal and food safety applications as an effective antimicrobial agent against pathogenic microorganisms and also can address future biomedical applications after complete in vivo study.

**Keywords:** *Trifolium repens* L., Antimicrobial medications, Pathogenic microorganisms, Ag-NPs.



# Finding five PEP-CTERM proteins in the Akkermansia muciniphila using in silico evaluation

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Akkermansia muciniphila is a gram-negative anaerobic bacterium that degrades the mucus layer and inhabits the human intestinal mucosa. Analysis of metagenomic data has revealed a correlation between the reduction of this bacterium's colonization in the gastrointestinal tract and the onset of various diseases such as diabetes, inflammation, obesity, IBD, autism, among others. Consequently, identifying factors conducive to the colonization of Akkermansia muciniphila could prove to be advantageous. Research has highlighted the necessity for the expression of PEP-CTERM proteins in the formation of flocs. Many PEP-CTERM proteins display a unique sequence constitution characterized by a significant abundance of possible glycosylation locations. The upregulation of a specific protein of this kind has demonstrated the capacity to reinstate the capacity of a bacterium to develop floc. A floc represents a form of microbial aggregate that helps bacterial colonization. Thus, the present investigation aims to find and then elucidate the role

**MATERIALS AND METHODS:** First, sequences related to Akkermansia muciniphila were obtained from the NCBI genome part. To determine the functions of PEP-CTERM proteins, along with NCBI protein BLAST, Swiss model servers which are mainly used for protein structure prediction were used.

**RESULTS AND DISCUSSION:** Our findings revealed that A. muciniphila expressed five proteins consisting of 277, 272, 322, 269, and 1127 amino acids, all of which exhibited the PEP-CTERM sorting domain. These proteins were identified in NCBI Reference Sequences as WP\_354833524.1, WP\_215427016.1, WP\_354834061.1, WP\_354832661.1, and WP\_354832152.1. Despite being categorized as hypothetical proteins with unknown functions, no analogous proteins or structures were identified in other organisms, except for WP\_354832152.1, which displayed a striking 99% similarity to the S-layer family protein, 95% to the autotransporter-associated beta strand repeat-containing protein, and approximately 50% to the Esterase EstA of A. muciniphila. This particular protein appears to be a substantial exoprotein implicated in heme utilization or adhesion, as well as intracellular trafficking, secretion, and vesicular transport. Consequently, it could play a crucial role in the colonization of A. muciniphila in the gastrointestinal tract. Nevertheless, further experimental investigations are imperative to validate this supposition

**Keywords:** Akkermansia muciniphila, PEP-CTERM, in silico

# Green-Synthesized Cu Nanoparticles: Promising Antibacterial Agents with Anti-Virulence Properties

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** The rise of antibiotic resistance in bacteria poses a significant challenge in hospital settings. In response, researchers are exploring various novel drug candidates to combat bacterial infections. Among these promising candidates are metal nanoparticles. This study investigates the potential of Cu nanoparticle (Cu-NP) synthesized using green method for their antimicrobial and anti-virulence properties.



**MATERIALS AND METHODS:** CU-NPs were synthesized using *Ralstonia* sp. SM8 culture and the iron sulfate precursor. To characterize the post-synthesis properties of the nanoparticles, various techniques were employed, including UV-visible spectrometry, field emission scanning electron microscopy (FESEM), X-ray energy dispersion spectroscopy (EDX), dynamic light scattering (DLS), zeta potential measurement, and Fourier transform infrared spectroscopy (FTIR). Successful synthesis of Cu-NPs was confirmed by UV-visible spectrometry, revealing a strong absorption peak at 228 nm. After the successful synthesis of the nanoparticles, their antibacterial and anti-virulence properties were measured by standard bacterial methods.

**RESULTS AND DISCUSSION:** The nanoparticles were primarily spherical with an average size of 69 nm. The DLS confirmed an average hydrodynamic diameter of 78.2 nm with a polydispersity index (PDI) of 0.38, indicating moderate size distribution. The zeta potential of -5.1 mV suggests good colloidal stability of the nanoparticles. FTIR analysis suggested that proteins play a role in nanoparticle formation and stabilization. The Cu-NPs exhibited promising antibacterial activity, with MICs ranging from 0.16 to 0.33 µg/mL and MBCs ranging from 0.33 to 0.66 µg/mL. Additionally, Cu-NPs significantly affected most bacterial virulence factors at sub-MIC concentrations. These effects included inhibition of biofilm formation at MIC/2 concentration, inhibition of motility for motile bacteria at MIC/2, synergistic interactions with cefixime and penicillin antibiotics, and inhibition of the *S. aureus* efflux pump. These findings suggest that the green-synthesized nanoparticles can effectively inhibit the tested bacteria, making them potentially useful antibacterial agents.

**Keywords:** Antibiotic resistance, Cu nanoparticle, Antibacterial activity

# Identification of immunogenic proteins of *Ph. sergenti* salivary gland and evaluation of their protective role against *Leishmania tropica* infection in BALB/c mice mode

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** The vector-borne disease leishmaniasis is transmitted to humans by infected female sand flies, which transmits *Leishmania* parasites together with saliva during blood feeding. In Iran, cutaneous leishmaniasis (CL) is caused by *Leishmania* (*L.*) *major* and *L. tropica*, and their main vectors are *Phlebotomus* (*Ph.*) *papatasi* and *Ph. sergenti*, respectively. Previous studies have demonstrated that mice immunized with the salivary gland homogenate (SGH) of *Ph. papatasi* or subjected to bites from uninfected sand flies are protected against *L. major* infection.

**MATERIALS AND METHODS:** In this work we tested the immune response in BALB/c mice to 14 different plasmids coding for the most abundant salivary proteins of *Ph. sergenti*. The plasmid coding for the salivary protein PsSP9 induced a DTH response in the presence of a significant increase of IFN- $\gamma$  expression in draining lymph nodes (dLN) as compared to control plasmid and no detectable PsSP9 antibody response. Animals immunized with whole *Ph. sergenti* SGH developed only a saliva-specific antibody response and no DTH response.

**RESULTS AND DISCUSSION:** Mice immunized with whole *Ph. sergenti* saliva and challenged intradermally with *L. tropica* plus *Ph. sergenti* SGH in their ears, exhibited no protective effect. In contrast, PsSP9-immunized mice showed protection against *L. tropica* infection resulting in a reduction in nodule size, disease burden and parasite burden compared to controls. Two months post infection, protection was associated with a significant increase in the ratio of IFN- $\gamma$  to IL-5 expression in the dLN compared to controls. These results suggest that this family of proteins in *Ph. sergenti*, *Ph. duboscqi* and *Ph. papatasi* may have similar immunogenic and protective properties against different *Leishmania* species. Indeed, this anti-saliva immunity may act as an adjuvant to accelerate the cell-mediated immune response to co-administered *Leishmania* antigens, or even cause the activation of infected macrophages to remove parasites more efficiently. These findings highlight the idea of applying arthropod saliva components in vaccination approaches for diseases caused by vector-borne pathogens.

**Keywords:** DNA Vaccination; *Phlebotomus sergenti*; sand fly salivary gland; *Leishmania tropica*

# In vivo evaluation of Copper oxide and Titanium dioxide on *Toxoplasma gondii*

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Background: *Toxoplasma gondii* infects nearly one-third of the world's population. Due to the significant side effects of current treatment options, identifying safe and effective therapies seems crucial. Nanoparticles (NPs) are new promising compounds in treating pathogenic organisms. Currently, no research has investigated the effects of Titanium oxide NPs (Ti<sub>2</sub>O-NPs) and Copper oxide (CuO-NPs) on *Toxoplasma* parasite. According to the effective in vitro study we done in the past, we aimed to investigate the therapeutic efficacy of Ti<sub>2</sub>O-NPs and CuO-NPs against tachyzoite forms of *T. gondii*, RH strain in BALB/c mice.

**MATERIALS AND METHODS:** Methods: In an experiment with 16 female BALB/c mice (for each NPs) were inoculated with 10<sup>4</sup> tachyzoites of *T. gondii*. colloidal Ti<sub>2</sub>O-NPs and CuO-NPs at concentrations of 10, 20, and 50 ppm as well as control group prepared. Treatment was orally administered five hours after inoculation and continued daily until the mice's death. Survival rates were calculated and tachyzoite counts were evaluated in the peritoneal fluids of infected mice.

**RESULTS AND DISCUSSION:** Results: The administration of Ti<sub>2</sub>O-NPs and CuO-NPs resulted in the reduction of tachyzoite counts in infected mice compared to both NPs-treated and control group. Intervention with Ti<sub>2</sub>O-NPs and CuO-NPs significantly increased the survival time compared to the control group. additionally, the highest dose of both-NPs (50 ppm) showed the highest mice survival time (8.1±0.28 days) for Ti<sub>2</sub>O-NPs and (7.4±0.23) days for CuO-NPs in comparison with control group (6.8±0.31 days). Conclusion: Ti<sub>2</sub>O-NPs and CuO-NPs were effective in decreasing the number of tachyzoites and increasing mice survival time.

**Keywords:** *T. gondii* In vivo Tachyzoites CuO-NPs Ti<sub>2</sub>O-NPs

# Investigating the Antibacterial Efficacy of Zataria Multiflora Essential Oil: Free Oil versus Nanoemulsion and Alginate Nanoparticle Formulations

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Antibacterial agents are crucial in combating infectious diseases (1). However, drug resistance necessitates high-dose antibiotics, often resulting in intolerable toxicity. This challenge has led to the development of alternative approaches to treating bacterial illnesses (2). Plants are an inexpensive, renewable source of antimicrobials, offering a wide range of chemical constituents with low toxicity. Plant metabolites can inhibit bacterial growth by attaching to bacterial proteins or lowering pH, which alters bacterial cellular processes and leads to their death. Notably, the bioactivity of plant-based antimicrobials (PBA) does not induce resistance, providing them with clinical value (3, 4). Despite their potential, the therapeutic use of essential oils (EOs) from plants is limited by their volatility and susceptibility to degradation (5, 6). Nanoparticles present a solution to these limitations, offering benefits such as enhanced stability and controlled release of EOs, preventing degradation and improving therapeutic efficacy (7, 8).

**MATERIALS AND METHODS:** The components of Zataria multiflora essential oil were determined using Gas Chromatography-Mass Spectrometry (GC-MS). Alginate nanoparticles and nanoemulsion containing the essential oil were prepared using ionic gelation and spontaneous methods, respectively. The antibacterial properties were evaluated using the microdilution method in 96-well plates.

**RESULTS AND DISCUSSION:** Carvacrol and thymol were identified as the main components. The size of alginate and nanoemulsion were 151 nm and 129 nm, respectively. Antibacterial properties of non-formulated essential, alginate nanoparticles and nanoemulsion containing the essential oil on Escherichia coli were 155, 178, and 310 µg/mL. These values against Pseudomonas aeruginosa were 717, 95, and 450 µg/mL. Conclusion: Z. multiflora essential oil is a potent natural antibacterial agent. Its efficiency can be significantly enhanced by formulating it into nanoparticles, thereby improving its stability and preventing degradation.

**Keywords:** Antibacterial -Zataria Multiflora -Essential Oil -Nanoemulsion -Alginate -Nanoparticle

# Investigation of the Synergistic Interaction of Biologically Synthesized Fe<sub>2</sub>O<sub>3</sub>-NPs with Common Antibiotics

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** The rise in antibiotic resistance and the increasing prevalence of infectious diseases have a significant impact on global health. To address this problem, combinatorial on medications can be used to improve antibiotic efficacy and decrease inhibitory concentrations. Due to their small size and high surface-to-volume ratio, metallic nanoparticles are being investigated broadly to achieve this purpose and improve their compatibility, solubility, and multifunctionality. The purpose of this research is to find out the potential synergy between Fe<sub>2</sub>O<sub>3</sub>-NPs (NPs) that are synthesized biologically and standard antibiotics in fighting resistant bacterial strains.

**MATERIALS AND METHODS:** The Fe<sub>2</sub>O<sub>3</sub>-NPs were synthesized using *Bacillus* sp. GMS10 culture and the iron sulfate precursor, FeSO<sub>4</sub>.7H<sub>2</sub>O. Various techniques were used to characterize the properties of the nanoparticles post-synthesis, including UV-visible spectrometry, field emission scanning electron microscopy (FESEM), X-ray energy dispersion spectroscopy, dynamic light scattering (DLS), zeta potential measurement, and Fourier transform infrared spectroscopy (FTIR).

**RESULTS AND DISCUSSION:** Based on results, the Fe<sub>2</sub>O<sub>3</sub>-NPs exhibits respectable antibacterial activity (MICs were between 2.5 and 50 µg.ml<sup>-1</sup> and MBCs were between 5 and 100 µg.ml<sup>-1</sup>). Additionally, the studied Fe<sub>2</sub>O<sub>3</sub>-NPs were able to affect most of the bacterial virulence factors at sub-MIC concentrations. These factors included the inhibition of biofilm formation at MIC/2 concentration, the inhibition of motility for motile bacteria at MIC/2 concentration, the synergistic interaction with cefixime and penicillin antibiotics, and the inhibition of the *S. aureus* efflux pump. Based on the observed data, it can be stated that the green method's nanoparticles can inhibit the tested bacteria, which could lead to their application as useful antibacterial substances in the future. The successful synthesis of Fe<sub>2</sub>O<sub>3</sub>-NPs was confirmed, with UV-visible spectrometry revealing a strong absorption peak at 228 nm. The nanoparticles were primarily spherical, averaging 30 nm in size. DLS analysis indicated an average nanoparticle size of 36.3 nm with

**Keywords:** *Bacillus* sp. GMS10, Fe<sub>2</sub>O<sub>3</sub>-NPs, Activity, Green synthesis, Anti-resistance, Biofilm inhibition

# Investigation of the Type 2 Asparaginase Gene in *Escherichia coli*

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** L-asparaginase is the therapeutic enzyme in treating Acute Lymphoblastic Leukaemia by breaking down asparagine. Two types of L-asparaginase have been identified in bacteria: type 1 and type 2. Type 2 is more beneficial due to its higher activity. Numerous studies have been conducted on the genes producing this enzyme in *E. coli* K12, (1) and *E. coli* MG27(2). The purpose of this research is to investigate the presence of asparaginase type 2 gene in the *E. coli* strain IBRC:10698 asparaginase producing isolate. 1. Bonthron DT. Gene. 1990;91(1):101-5. 2. Mohamed ZK, Elnagdy SM, Seufi AE, Gamal M. Journal of BioScience & Biotechnology. 2015;4(3).

**MATERIALS AND METHODS:** The genomic DNA isolated from *E. coli* strain IBRC:10698 was used as template for the amplification of *ansB* gene using *ansB*-F and *ansB*-R primers. (2) The PCR protocol was as follows: pre-denaturation at 95°C for 5 min, cycles (denaturation at 94°C for 45 s, annealing at 55°C for 45 s, extension at 72°C for 1 min), and a final extension at 72°C for 8 min. The DNA bands were analyzed on 1% agarose gel electrophoresis. The asparaginase gene in *E. coli* K12 was also analyzed using bioinformatics data and the NCBI website.

**RESULTS AND DISCUSSION:** The gel electrophoresis results confirmed the successful amplification of *ansB* gene, with a band size of approximately 1000bp. This indicates that *ansB* gene is present in both *E. coli* MG27 and *E. coli* IBRC:10698. Findings provide valuable insights into the structure, function, and potential therapeutic applications of this enzyme. The bioinformatic analysis revealed that the enzyme's molecular weight is 37.127 Da and it has 338 amino acids. The enzyme's active site is located on amino acid number 14, and isoaspartyl threonine plays a crucial role in its activity. The substrate binding site is located on amino acids 59 to 61, while amino acids khaki

, 240, and 271 act as allosteric activators. Mutations in amino acids 14, 61, 91, 118, and 162 will eliminate enzyme activity, a mutation in amino acid 240 will decrease enzyme activity at low substrate concentrations. Amino acids 13 to 163 are essential for full enzyme activity.

**Keywords:** Asparagine, Asparaginase, *E. coli*, Asparaginase gene

# Investigation of two Shuttle Vectors for Cloning and Expression of IL-11 Gene in *Bacillus subtilis*

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** The recombinant human IL-11 is the only approved medicine used for treating chemotherapy-induced side effects. Platelet count decreases (thrombocytopenia) in cancer patients who undergo chemotherapy. Interleukin-11 (IL-11) is a platelet increasing cytokine. This study aimed to use two shuttle vectors of pHT43 and pMR12 for cloning the IL-11 gene and expression of its protein in *Bacillus subtilis* and the expression level in these two vectors was investigated.

**MATERIALS AND METHODS:** In this study, the IL-11 gene was designed and synthesized as a closed structure with two restriction sites for BamH1 and XbaI enzymes and a final length of 609 bp. Then the gene was cloned in two shuttle vectors of pHT43 and pMR12 and transferred to *B. subtilis* WB600. The expression level of the recombinant IL-11 was evaluated with the Bradford incubation, the pMR12-int11-carrying bacteria expressed higher levels of the protein (75 µg/mL) than pHT43-int11-carrying bacteria.

**RESULTS AND DISCUSSION:** The results of PCR indicated that the IL-11 gene existed in shuttle vectors pHT43 and pMR12. The expression of this protein was about 75 µg/mL using pMR12 vector, which is higher than the bacterium carrying pHT43-int11. The expression level of the protein in the pMR12 vector was 75 µg/ml. This amount has not been reported so far for IL-11. *B. subtilis* can express and produce IL-11 and can be used as a source for drug production.

**Keywords:** interleukin-11, shuttle vector, pHT43, pMR12, *Bacillus subtilis*



# Isolation and Molecular Identification of Asparaginase-Producing Bacteria from Germinating Barley

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** L-asparaginase is a vital enzyme in cancer treatment, particularly acute lymphoblastic leukemia (ALL). It reduces the cancer cell growth and proliferation by breaking down asparagine, an essential amino acid for these cells. Bacteria and some fungi are the primary sources of L-asparaginase production. Due to the high demand for this enzyme in the pharmaceutical industry, finding new and efficient sources for its production is of great importance. The aim of this study was to isolate and identify native L-asparaginase-producing bacteria from germinating barley to explore new and effective sources for industrial production. 1- Castro D, Marques ASC, Almeida MR, de Paiva GB, Bento HB, Pedrolli DB, et al. L-asparaginase production review: bioprocess design and biochemical characteristics. Applied microbiology and biotechnology. 2021; 105:4515-34.

**MATERIALS AND METHODS:** In this study, the bacteria of interest were isolated from the roots of germinating barley and cultured in a specialized M360 medium containing asparagine. They incubated at 30°C for 24h. Subsequently, colonies were transferred to M9 medium supplemented with phenol red. The isolate surrounded with higher pink zone was considered as L-asparaginase producer and selected for further analysis. L-asparaginase enzyme activity was measured using a colorimetric assay (Nesslerization method). The selected strain was identified using molecular identification techniques and 16S rRNA gene sequencing. Additional phenotypic tests included Gram staining and culturing on EMB and MacConkey agar.

**RESULTS AND DISCUSSION:** Among the investigated colonies, one L-asparaginase-producing bacterium was selected, which changed the color of the entire M9 culture medium from orange to pink after 15 days. The enzyme activity in this isolate was measured between 0.11 and 0.16 IU/mL. Based on phenotypic tests and sequencing data, this strain belonged to the genus *Klebsiella*. The optimum temperature for enzyme production was 30°C, and its respiratory condition was fermentation. The results demonstrated that germinating barley can serve as a suitable source for isolating L-asparaginase-producing strains. Further research is warranted to optimize production conditions and investigate the characteristics of this *Klebsiella* strain for industrial-scale L-asparaginase production

**Keywords:** L-asparaginase, Bacteria, asparagine, cancer cells

# Isolation and optimization of a high-efficiency lipase enzyme from an oil-degrading bacterium found in crude oil-contaminated soil

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** The increased excessive consumption of crude oil and other petroleum products has led to widespread pollution and dangerous environmental problems. Bacterial lipases play a significant role in the biodegradation of crude oil by hydrolyzing ester bonds and breaking down the complex hydrocarbon chains present in oil into simpler compounds. This enzymatic activity enhances the efficiency of biodegradation processes, making it a valuable tool for cleaning up oil spills and contaminated environments.

**MATERIALS AND METHODS:** sampling of crude oil-contaminated soil was conducted under sterile conditions. Then, using purification and isolation methods, crude oil-degrading bacteria were screened. The oil-degrading bacteria were separately cultured in minimal salt media containing 1.5% hexadecane and their lipase enzyme activity was studied using the P-nitrophenyl palmitate assay. The bacterium with the best lipase performance was selected for further optimization and investigation.

**RESULTS AND DISCUSSION:** In this study, the effects of pH, temperature, Triton X-100, and the ionic strength of NaCl on the lipase enzyme from oil-degrading bacteria were investigated to identify optimal conditions. The findings revealed that under optimal culture conditions (temperature =50°C, Triton X-100 concentration =0.25 mM, NaCl concentration =0.125 M, and pH=10), the enzyme exhibited maximum activity. Based on the findings from this study, the lipase enzyme extracted from oil-degrading bacteria shows remarkable stability and effectiveness across different environmental conditions, making it applicable in multiple industries, including bioremediation. This bacterial lipase enzyme speeds up the biodegradation of petroleum hydrocarbons, thereby reducing long-term environmental harm. Moreover, this lipase-producing bacterium can be directly applied to clean up water and soil in areas contaminated with crude oil.

**Keywords:** Biodegradation, Crude oil, Lipase

# Isolation of carotenoid-producing *Rhodotorula mucilaginosa* yeast strain from shurab savadkuh spring water

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Yeasts are used as model organisms in the study of genetics and cellular biology. They are used to prepare yeast extract in biological media. In addition, some yeasts may potentially have applications in bioremediation. Biotechnologically important yeast strains, such as *Rhodotorula mucilaginosa*, are gaining global attention for various industries. These traits make *R. mucilaginosa*, with its wide substrate competencies and stress-resistant phenotypes, very promising for the production of carotenoids, lipids, enzymes, and bioactive compounds from agricultural wastes. Such metabolites are in high demand for food health-promotion products, third-generation biodiesel, and cosmetics ingredients, to name a few. The use of *R. mucilaginosa* in fermentation processes has significantly increased, leading to enhanced production of valuable bioactive compounds. Our objective was to isolate the *Rhodotorula mucilaginosa* yeast strain capable of producing carotenoids from sediments of Shurab Savadkuh spring water.

**MATERIALS AND METHODS:** Sampling was done from 5 selected springs. Serial dilutions were prepared from the samples and the suspensions were inoculated on plates containing glycerol yeast extract agar (GYEA) culture medium by spread plate method. The samples were checked for the growth rate of microorganisms. Various colonies from each plate were transferred to new plates. This was done for several times so that isolated and pure species are obtained. Gram staining was done to identify the samples. The 18srRNA gene was amplified by polymerase chain reaction using the 27F and 1492R primers.

**RESULTS AND DISCUSSION:** *R. mucilaginosa* colony appears orange-red, wet, transparent, with smooth surface, easily picked up, and can have carotenoid-filled lipid droplets, varying pink-orange. This result is consistent with our study. It has been shown that the yeast strain used, *Rhodotorula mucilaginosa*, was able to biosynthesize an antifungal agent. A carotenoid-producing yeast strain isolated from sediments of the Savadkuh spring water was identified as *Rhodotorula mucilaginosa*.

**Keywords:** Yeasts- *Rhodotorula Mucilaginosa*- Isolation- Carotenoids

# Low prevalence of N86Y mutant in pfmdr1 gene associated with Chloroquine resistance in plasmodium falciparum isolation from Iran

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Chloroquine resistance in Plasmodium falciparum is a significant challenge in malaria-endemic regions, necessitating the identification and monitoring of molecular markers associated with Chloroquine resistance. This study investigated the prevalence of chloroquine resistance-associated mutation at position 86 of the pfmdr1 gene (N86Y/H alleles) in P. falciparum isolates from patients in southern Iran.

**MATERIALS AND METHODS:** A total of 50 P. falciparum Blood samples were collected on filter paper from patients diagnosed with malaria. Genomic DNA was extracted from each sample and used as a template for nested PCR amplification of the pfmdr1 gene. The PCR products were then subjected to restriction fragment length polymorphism (RFLP) analysis using the restriction enzymes Apol, AflIII, and NsiI.

**RESULTS AND DISCUSSION:** The results of PCR-RFLP analysis revealed that approximately 95% of the isolates harbored the wild-type allele (N86), while 5% possessed the mutant allele (Y86). The comparison of Y86 mutant allele frequency with previous data from the same endemic area in about 10 years ago indicated that the prevalence of mutant allele has been decreased during this time. The low prevalence of N86Y mutant shows that with decreasing the pressure of chloroquine in Iran, the wild type allele (N86) has been increased in Iranian P.falciparum isolates This study highlights the relatively low prevalence of the pfmdr1 N86Y mutant in the study area, providing valuable insights into the molecular epidemiology of chloroquine resistance in southern Iran. Further surveillance and comprehensive assessment of other molecular markers for Chloroquine resistance is recommended to support the national anti-malarial policy in Iran.

**Keywords:** Plasmodium falciparum, Chloroquine, Drug resistance, pfmdr1 gene

# Microbial synthesis Ag- Albumin nanoparticles by Bifidobacterium breve

Microbial biotechnology

Kimia Parsi <sup>1</sup> © ®

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**BACKGROUND AND OBJECTIVES:** Biological and microbial synthesis processes are a safe and useful option due to their compatibility with the environment and nature and their cost-effectiveness over physical and chemical methods. Advantages of biomass and microbial synthesis methods of silver nanoparticles, compared to other methods (chemical, physical), environmental friendliness, cost-effectiveness, ease of operation and less energy are used. Probiotics are beneficial bacteria for the human gastrointestinal tract, probiotic bacteria prevent bacteria from inflammatory bowel disease. Albumin is a group of water-soluble proteins, one of the most important proteins in the plasma in terms of application in the human body. Albumin can be used in medicine and therapy. Albumin nanoparticles reduce drug side effects and increase drug stability. In this study, protein nanoparticles containing silver were synthesized with the probiotic bacterium *Bifidobacterium breve*.

**MATERIALS AND METHODS:** First, the probiotic bacterium *Bifidobacterium breve* was cultured in MRS Broth culture medium, and after obtaining a single colony, the bacterium was cultured in MRS Broth liquid culture medium, then, centrifuged, the supernatant was filtered and silver nitrate was added. Silver nanoparticles are then synthesized. Albumin protein was used to synthesize protein nanoparticles. A solution of albumin protein and distilled water was prepared and synthesized silver nanoparticles were added, 25% glutaraldehyde was added, acetone and ethanol were added, then albumin protein nanoparticles were synthesized, placed in an oven for XRD and FTIR, TEM, SEM and DLS are prepared.

**RESULTS AND DISCUSSION:** Albumin and silver protein nanoparticles have spherical, crystalline, semi-crystalline and cubic morphology and structure. Silver albumin protein nanoparticles have O-H, N-H and C-H bonds, C = C bonds in aromatic rings and C-N bonds in amino compounds. Silver nanoparticles have OH bonds, CH bonds in CH<sub>2</sub> and CH<sub>3</sub> groups, NH bonds, CO bonds in the structure of CO<sub>2</sub> and C-OH functional groups. The results of this study are the synthesis of albumin protein nanoparticles containing silver nanoparticles with suitable structure and properties. , And this protein nanoparticle can also be used for treatment.

**Keywords:** Biosynthesis of silver nanoparticles, probiotics. Albumin nanoparticles.

# Molecular investigation of the mechanisms of action for antimicrobial properties of magnetite nanoparticles synthesized by biological method

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** The present study aimed to evaluate the antimicrobial capabilities of biologically synthesized Fe<sub>3</sub>O<sub>4</sub> nanoparticles.



**MATERIALS AND METHODS:** The Fe<sub>3</sub>O<sub>4</sub>-NPs were generated using the culture supernatant from the *Alcaligenes* sp. strain CR8441, with Fe<sub>2</sub>O<sub>3</sub> acting as the precursor. Following synthesis, the nanoparticles were subjected to an extensive analysis using a variety of spectroscopic and microscopic techniques. In the next step of the study the antibacterial activities of Fe<sub>3</sub>O<sub>4</sub> nanoparticle were investigated using disc diffusion method and also determination of MIC and MBC values against four bacterial strains; two-gram positive bacteria, *Staphylococcus aureus*, *Bacillus cereus* and two gram negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. Then, the anti-virulence effects of nanoparticles such as inhibition of biofilm formation, also tested for synergistic effects with penicillin and Cefixime antibiotics, bacterial motility inhibition, cell membrane disruption and efflux pump inhibition were performed at sub-MIC concentrations.

**RESULTS AND DISCUSSION:** Fe<sub>3</sub>O<sub>4</sub>-NPs were synthesized with the confirmation of strong absorption peaks at 235 and 293 nm. According to the analysis, the nanoparticles were found to be spherical or slightly elliptical and have an average size of 36 nm. The DLS results indicated an average size of 53.6 nm for the synthesized Fe<sub>3</sub>O<sub>4</sub>-NPs, with a dispersion index of 0.31. The zeta potential of the synthesized Fe<sub>3</sub>O<sub>4</sub>-NPs was measured as -2.3 mV. XRD analysis confirmed the formation of cubic spinel Fe<sub>3</sub>O<sub>4</sub>-NPs. FTIR analysis revealed that organic compounds containing O-H, C=O, C-O, and N-H groups acted as effective coating agents during the synthesis. Based on the results, the Fe<sub>3</sub>O<sub>4</sub> nanoparticle has acceptable antibacterial activity against both Gram-positive and Gram-negative bacteria (MIC were from 2.5-50 µg.ml<sup>-1</sup>, and MBC were from 5- 100 µg.ml<sup>-1</sup>). Also, at sub-MIC concentration, the investigated Fe<sub>3</sub>O<sub>4</sub> nanoparticle has effective anti-virulence factors potential, including: inhibition of biofilm formation at MIC/2 concentration,

**Keywords:** Green synthesis, Fe<sub>3</sub>O<sub>4</sub>-NPs, *Alcaligenes* sp., Anti-virulence, Biofilm inhibition

# Nanoemulsified Cumin (*Cuminum cyminum* L.) Essential Oil as a Potential Antimicrobial Agent: A Comprehensive Study on Its Efficacy Against Diverse Bacterial Groups

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Antibacterial resistance is an important issue, reducing the efficiency of treating bacterial infections with antibiotics and increasing treatment costs. Medicinal plants offer a potential solution, and nano-formulation can further enhance the antimicrobial properties of their essential oils. This study aims to enhance the antibacterial activity of cumin essential oil against bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* bacteria by investigating the effects of nanoemulsification.

**MATERIALS AND METHODS:** In the research conducted, one ATCC standard strain and one clinical strain of each bacterium were evaluated, with treatments being administered that included cumin (*Cuminum cyminum* L.) essential oil and nanoemulsion, as well as antibiotics such as gentamicin (for *Klebsiella pneumoniae* and *Escherichia coli*), colistin and tetracycline (for *Pseudomonas aeruginosa*), and necomycin and tetracycline (for *Staphylococcus aureus*). The Broth Microdilution method facilitated the measurement of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The cooperative influence of nanoemulsion and the specific antibiotic was evaluated for each bacterial strain. The Microtiter Plate method was employed to evaluate antibiofilm activity at concentrations lower than the minimum growth inhibitory concentration (Sub-MIC). Gas Chromatography-Mass Spectrometry was utilized to determine the composition and concentration of compounds in cumin essential oil.

**RESULTS AND DISCUSSION:** Results: For Gram-negative bacteria, both the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were consistent at 62.5 mg/ml. However, *Staphylococcus aureus* exhibited varying MIC values of 156.2 mg/ml and 78.12 mg/ml, with an MBC of 312.5 mg/ml. Interestingly, the nanoemulsion form of the cumin essential oil demonstrated a substantially lower MIC compared to the oil itself, although it did not exhibit bactericidal effects against *Escherichia coli* and *Pseudomonas*. The MIC for *Klebsiella pneumoniae* was 5.2 mg/ml and for *Staphylococcus aureus* was 312 µg/ml. Chemical analysis of the essential oil identified the major components as cumin aldehyde (23.92%), p-cymene (17.15%), γ-terpinene (14.07%), and β-pinene (11.73%). Conclusion: Nanoemulsified cumin essential oil demonstrates increased bactericidal activity against

Staphylococcus, with a higher MIC level but relatively similar MBC level compared to its non-emulsified form. Nanoemulsion's smaller size enhanced biofilm prevention, potentially by inhibiting bacterial chromium sensing.

**Keywords:** nanoemulsion, cumin, antibacterial



# Niosome-based formulation of curcumin and quercetin to combat antimicrobial resistance

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** The irrational use of antibiotics has led to increased bacterial resistance, causing a global public health threat and seriously put lives in danger. It is estimated that bacterial antimicrobial resistance was directly responsible for 1.27 million global deaths in 2019 and contributed to 4.95 million deaths. Antibiotic therapy is the most common approach to treat bacterial infections, but its effectiveness against multidrug-resistant bacteria is decreasing because of the slow development of new antimicrobial drugs and the acceleration of antibacterial resistance. Therefore, there is a worldwide necessity to take an action against this issue. To tackle the problem developing new antimicrobial strategies and improving antibiotic efficacy can be considered. The emergence of nanotechnology, as a revolutionary area of science, is a promising approach to combat antimicrobial resistance.

**MATERIALS AND METHODS:** In this study polymeric niosomes of curcumin and quercetin were prepared by thin film hydration method. Then niosomes were characterized to determine the average particle size, zeta potential and polydispersity index (PDI). Antimicrobial effect measured through checkerboard assay according to CLSI standard.

**RESULTS AND DISCUSSION:** The results of Primary characterization showed that the average size, polydispersity index and zeta potential of niosomes were  $260.37 \pm 6.58$  nm, 0.34 and  $-34.97 \pm 1.50$  mv, respectively. In the terms of antimicrobial effects, the minimum inhibitory concentration of formulation was between 32 - 256  $\mu\text{g/ml}$  which was more effective than curcumin and quercetin. The measurement for minimum bactericidal concentration was found to be between 128 - 1024  $\mu\text{g/ml}$ , also more effective than pure curcumin and quercetin powder. Underlying mechanism contributed to these results can be the enhancement of water solubility in niosome-based formulation which leads to effective interaction between the active material and bacterial cell membrane.

**Keywords:** antimicrobial resistance, nanotechnology, niosome, curcumin, quercetin

# Surface Modification Strategies for Improving Biocompatibility of Magnetic Nanoparticles in Biomedical Applications

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** The convergence of nanotechnology with molecular biology and medicine has led to the active development of nanobiotechnology, providing opportunities to discover novel materials and processes. Magnetic nanoparticles (MNPs) have garnered significant interest in the medical community due to their unique properties, such as high magnetic susceptibility and remote controllability. However, their low biocompatibility can lead to adverse effects and decreased effectiveness. This study evaluates various surface modification strategies to enhance the biocompatibility of MNPs for medical applications. Techniques discussed include coating with biocompatible polymers, functionalizing with bioactive molecules, and attaching targeting ligands. The effects of these modifications on the physicochemical properties, stability, and biological targeting capabilities of MNPs are investigated, aiming to improve their biodistribution, reduce adverse effects, and increase therapeutic effectiveness.

**MATERIALS AND METHODS:** Materials • Magnetic nanoparticles (MNPs) • Biocompatible polymers (e.g., polyethylene glycol, chitosan) • Bioactive molecules (e.g., peptides, proteins) • Targeting ligands (e.g., antibodies, aptamers) Methods 1. Synthesis of MNPs: MNPs were synthesized using a co-precipitation method. 2. Surface Coating: MNPs were coated with biocompatible polymers through physical adsorption or chemical bonding. 3. Functionalization: The surface of MNPs was functionalized with bioactive molecules using covalent coupling techniques. 4. Attachment of Targeting Ligands: Targeting ligands were attached to the functionalized MNPs via bioconjugation methods. 5. Characterization: The modified MNPs were characterized using techniques such as TEM, DLS, and FTIR to assess their physicochemical properties. 6. Biocompatibility Testing: In vitro and in vivo biocompatibility tests were conducted to evaluate the modified MNPs' performance.

**RESULTS AND DISCUSSION:** Surface modification of magnetic nanoparticles significantly enhances their biocompatibility, making them more suitable for biomedical applications. Coating with biocompatible polymers, functionalizing with bioactive molecules, and attaching targeting ligands improve the physicochemical properties, stability, and biological targeting capabilities of MNPs. These modifications lead to better biodistribution, reduced adverse effects, and increased therapeutic effectiveness, paving the way for advanced medical applications of MNPs.

**Keywords:** Magnetic nanoparticles, biocompatibility, surface modification, biomedical applications, polymer coating.

# Synthesis of pistachio skin Extract-Loaded Porphysome hybridized with 4-nitroimidazole against bacterial biofilm

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Biofilms are self-produced slime-layers of surface-associated bacteria that are of great importance in medicine and industry. It is estimated that biofilms cause more than 80% of all microbial infections due to a strongly increased tolerance against antimicrobials. In this research, the antibacterial effects of 4-Nitroimidazole and pistachio green hull extract (PGHE) loaded porphysome nanocarriers on the formation of biofilms of *Staphylococcus aureus* (gram-positive bacteria) and *Escherichia coli* (gram-negative bacteria) were investigated.

**MATERIALS AND METHODS:** The Ni-porphyrin in the head group of the Ni-porphyrin-tail was placed superficially in the polar region of the DPPC membrane. The formation of spherical unilamellar vesicles was achieved through supramolecular self-assembly in an aqueous buffer, co-exposed with bioactive PGHEs. Then by disk diffusion method, minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) assay, and bacteria adherence assay the antibacterial effects of the synthesized nanocarriers were investigated.

**RESULTS AND DISCUSSION:** The physicochemical properties of Nitroimidazole-polysome-PGHE, including size, zeta potential, morphology, loading efficiency, and release profile under various pH and temperature conditions in simulated conditions were characterized. Results represent that, the nanoformulation and synergistic effect of this 4-Nitroimidazole and PGHEs decreases the bacterial cell attachment and biofilm formation dramatically in both gram-positive and negative bacteria. Conclusions: This study demonstrates the potential of a new co-delivery system using biocompatible metallo-polysomes to decrease the formation of bacterial biofilms. The remarkable benefits of this system are the better delivery of 4-Nitroimidazoles in nanoformulations and their improved cytocompatibility for health and environmental concerns.

**Keywords:** Pistachio green hull, Gram-positive and negative bacteria, 4-Nitroimidazole, nanocarriers

# The Folate Production Comparison Between Iranian Species of Lactic Acid Bacteria Isolated from Traditional Dairy as Food fortifying

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** Folate, essential for DNA replication and cell division, is crucial in preventing different disorders and some cancers. Despite humans needing to obtain folate from foods like vegetables and dairy products, folate deficiencies are widespread, leading to health issues. While taking folic acid supplementation may pose risks, consumption of fermenting foods with folate-producing lactic acid bacteria can enhance folate levels. Folate-producing probiotics show potential for fortifying foods and regulating gut health. Recent research has focused on evaluating folate production of LAB strains isolated from dairy products.

**MATERIALS AND METHODS:** In current study Four Lactobacillus strains, Lactobacillus helveticus PTCC1930 (L. helveticus), Lactobacillus plantarum PTCC1901 (L. plantarum), were cultured in MRS broth overnight, then added to 8% skimmed milk (1% v/v) at concentrations of 10<sup>8</sup> cfu/ml. The mixture was incubated for 24 hours at 37°C in microaerophilic conditions. After fermentation, the strains were centrifuged, separating the Cell-free supernatant (CFS) for extracellular folate analysis. Intracellular folate content was assessed through sonicating the cell pellet using the CLIA chemiluminescence method. A negative control was prepared using skimmed milk supernatant.

**RESULTS AND DISCUSSION:** As a result, the folate content of cell-free extracts and supernatants from different Lactobacillus strains was analyzed. Among the samples, L. helveticus (CFE) showed the highest folate production with 10.58 ng/ml in the cell-free extracts (P-value 0.05). Folate amounts in all samples were: L. plantarum (CFS: 1.95 ng/ml, CFE: 3.2 ng/ml), L. helveticus (CFS: 0.8 ng/ml, CFE: 10.58 ng/ml) and the folate amount of skimmed milk as negative control (3.7 ng/ml). It is concluded that among the samples, only L. helveticus, considering the existing folate in negative control, was found to produce 6.88 ng/ml of folate internally, making it a potential candidate for folate fortification in fermented foods.

**Keywords:** L. plantarum; L. helveticus; cell-free supernatant; cell-free extract; folate

# Virtual screening for discovery of new dehydrogenase inhibitors against drug-resistant *Acinetobacter baumannii*

Microbial biotechnology

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**BACKGROUND AND OBJECTIVES:** *Acinetobacter baumannii* is a hospital-acquired infection that forms biofilms on ventilators and equipment in ICUs, increasing mortality rates. The emergence of antibiotic-resistant *Acinetobacter baumannii* poses a critical threat to global health. Thus, the WHO has classified *A. baumannii* as a priority pathogen, demanding innovative treatment strategies. Dihydroorotate dehydrogenase (DHODH), an enzyme in the pyrimidine pathway, is crucial for *Acinetobacter* survival. This protein could be a potential drug target against *Acinetobacter*. In this work, virtual screening was used to discover potential inhibitors of DHODH.

**MATERIALS AND METHODS:** The crystal structure of DHODH was retrieved from the PDB bank (PDB ID: 7tu5). Virtual screening using the Smina Vina software was performed to dock the Taiwan Chinese Medicine library, containing natural chemical compounds, against the DHODH binding site. The compounds with the highest binding affinity to the DHODH binding site were selected for further analysis. The Pain Remover webserver was utilized to exclude compounds with unfavorable drug-like properties. Physicochemical properties based on Lipinski's Rule of Five (Ro5) and safety assessments were evaluated using the OSIRIS Data Warrior software. The LigPlot package was used to analyze the interactions of selected compounds with the DHODH binding site.

**RESULTS AND DISCUSSION:** Twenty compounds from the Taiwan Chinese Medicine library exhibiting strong binding affinity to DHODH were identified as potential candidates. The Pain Remover webserver demonstrated that all compounds had the good properties to be drug candidates. Lipinski's rule analysis by OSIRIS Data Warrior software indicated that 5 out of 20 compounds passed Lipinski's rule. The safety assessment of compounds illustrated that 3 out of 5 compounds had low or negligible potential to have mutagenesis, tumorigenicity, irritation, and reproductive effects. The binding site analysis revealed that these compounds could effectively interact with the binding site of DHODH. Conclusion: Our findings suggest that ZINC04098004, ZINC14455455, and ZINC13462634 could effectively act as antimicrobial compounds against DHODH due to their strong interactions with the enzyme's binding site. Further experimental validation is needed to confirm the efficacy of these compounds as potential antimicrobials against DHODH.

**Keywords:** Keywords: virtual screening, dihydroorotate dehydrogenase (DHODH), *Acinetobacter baumannii*

# Efflux pumps and biofilm inhibition in multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from burn patients: In the presence of Silver Nanoparticles Green Synthesis of *Capparis spinosa*

## Antimicrobial Nanomaterials

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**BACKGROUND AND OBJECTIVES:** *Acinetobacter baumannii* and *Pseudomonas aeruginosa* have become superbugs due to the increased risk of infection and increasing rates of antimicrobial resistance, including Colistin, the last antibiotic. Among the most important mechanisms of resistance in these bacteria are efflux pump and biofilm activity. Its tendency to form biofilm on biotic and abiotic surfaces has been implicated in most hospital infections. Bacterial cells present in biofilm are resistant to antibiotics and the host's immune response and present challenges to treatment. Also, the activity of the efflux pump causes the release of the drug and its ineffectiveness on the bacterial cell. Therefore, the current scenario urgently requires the development of new therapeutic strategies for successful therapeutic results, one of these technologies being the biosynthesis of nanoparticles.

**MATERIALS AND METHODS:** In this study, green silver nanoparticles were synthesized with *C. spinosa* and the physical and chemical properties of this Nanoparticle were determined. Then, the antibacterial properties of AgSO<sub>4</sub>, *C. spinosa* extracts, AgNPs and Cs-AgNPs were measured on standard and clinical isolates of *A. baumannii* and *P. aeruginosa* using the Well Method. Also, the MIC and MBC, percentage of biofilm formation and destruction and the ability to inhibit efflux pump against Cs-AgNPs in two strains were investigated.

**RESULTS AND DISCUSSION:** The Cs-AgNPs has strong antibacterial properties against *A. baumannii* and *P. aeruginosa*. In addition, the ability to inhibit the efflux pump of these Cs-AgNPs in these two Gram-negative bacteria is high, and it has a high ability to inhibit biofilm formation and biofilm destruction. Of course, it should be mentioned that this Cs-AgNPs has a high antibacterial effect on *P. aeruginosa*, but the biofilm inhibition and destruction power of this Nanoparticle is higher on *A. baumannii*. As a result, the green silver nanoparticles synthesized in this work can be a useful tool for the development of biofilm disruptors and efflux pumps.

**Keywords:** green silver nanoparticles, *A. baumannii*, *P. aeruginosa*, biofilm, efflux

# Evaluation of enzymatic effects and optimization of a strain of native Iranian soil bacteria

Microbiology standards

Fatemeh Mirzaei © P

Islamic Azad University Tehran Science and Research Branch

**BACKGROUND AND OBJECTIVES:** The bacteria were investigated to evaluate the ability to produce hydrolytic enzymes in different conditions. The bacteria were placed in the culture environment containing several enzyme substrates at a temperature of 50 degrees Celsius, Different percentages of NaCl and in the medium with similar conditions but without salt and at a temperature of 30 degrees Celsius, the pH range. The bacterium achieved high growth after 28 hours. The bacterium was able to grow on plates containing Cellulase, Xylanase and Amylase enzyme substrates, and were able to grow only in 5% NaCl. The bacterium grew in the culture medium containing Cellulase at a temperature of 50°C and showed a halo. The bacterium can grow at pH 8.5 to 9. 5. It can be mentioned that the bacterium has the ability to grow up to 5 % in NaCl and the possibility of growing at a temperature above 30 °C.

**MATERIALS AND METHODS:** To identify the studied bacteria, tests such as catalase test, oxidase test, urea test, spore staining test and gram staining were performed. Samples from all tubes were cultured in culture medium with two replications. Colony counts were performed from each individual dilution. Enzymatic cultures were performed, placed in an incubator at 30 degrees Celsius for 72 hours. To test the enzyme production, the sample was cultured in liquid medium stored in a 50 °C incubator for 48 hours. The pH spectrum of 4.5 to 8.5 was also investigated on the studied bacteria.

**RESULTS AND DISCUSSION:** The studied bacteria have pink pink pigment with round, convex colonies with flat sides and a diameter of 1 mL and showed a Gram-positive cocci arrangement that are joined together. The bacteria grew exponentially in the first 5 hours in the delayed phase, 24 hours later in the exponential phase, 48 hours later in the fixed phase, and at 72 hours, the bacteria underwent the death phase. The bacteria grew and formed a halo in the culture medium containing Cellulase and Xylanase substrate with 5% salt. Cellulase enzyme showed the best efficiency with the formation of a strong halo at 50 °C. The bacteria showed the highest growth rate at pH 8.5.

**Keywords:** Cellulase-Xylanase-Amylase-Substrate

# A new policy in determining the workload of clinical laboratories in Mashhad hospitals based on the output of the Hospital Information Software (HIS)

Standard - Medical microbiology

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**BACKGROUND AND OBJECTIVES:** Scientific time measurement in order to determine the workload available to each staff of the medical laboratory is one of the main factors in improving the quality of services. The systematic performance of time measurement in laboratory services is a new and necessary category. Here we aimed to determine the available workload of health workers in the laboratories of the public hospitals of Mashhad, Iran, according to the Workload Indicators of Staffing Need (WISN) method.

**MATERIALS AND METHODS:** The present research was a descriptive-cross-sectional study for the purpose of time measurement and scientific estimation of the workload and duration required for all laboratory processes in clinical departments (including departments of hematology, biochemistry, serology, hormonology, immunology, and blood bank) laboratories of public hospitals in Mashhad, Iran according to the WISN methods and using the output of the hospital information software (HIS).

**RESULTS AND DISCUSSION:** The present study demonstrated that according to the available times in each shift work, the standard workload of each technical department in compliance with quality assurance requirements, including 50 tests, CBC 350 biochemistry tests, 80 immunology tests, 15 cross-match tests, and 65 is serology tests. In addition to determining the workload and available time of each staff, this study raised their motivation, resulting in changing the processes of paying benefits and then creating correct instructions for human resources management.

**Keywords:** Workload Indicators of Staffing Need, WISN, laboratory, workload, quality assurance



# Estimation of measurement uncertainty for quantitative determinations

Standard - Medical microbiology

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**BACKGROUND AND OBJECTIVES:** The term “measurement uncertainty” is used to denote the lack of accuracy (trueness and precision) that can be associated with the results of an analysis. In the context of quantitative microbiology, it provides an indication of the degree of confidence that can be placed on laboratory estimates of microbial numbers in foods. MU associated with any measurement value includes multiple components. In the case of the microbiological analysis of samples from the food chain, it is not feasible to build a comprehensive quantitative measurement model, since it is not possible to quantify accurately the contribution of each input quantity, where: the analyte is a living organism, whose physiological state can be largely variable; the analytical target includes different strains, different species or different genera; many input quantities are difficult, if not impossible, to quantify; for many input quantities, their effect on the measurand cannot be described quantitatively with adequate precision.

**MATERIALS AND METHODS:** There are three distinct types of uncertainty component: technical uncertainty; matrix uncertainty; distributional uncertainty. Technical uncertainty arises from operational variability and is estimated, using a global approach, from a reproducibility standard deviation of the final result of the measurement process. This global approach means that the technical uncertainty estimate comes from final test results rather than by calculation using estimates of uncertainty at every individual stage of the test. Matrix uncertainty arises from imperfect mixing of the laboratory sample, resulting in poor reproducibility of microbial levels between test portions, which can be large for solid matrices, and especially for composite food products. Even for homogeneous materials, the random distribution of microorganisms leads to distributional uncertainty.

**RESULTS AND DISCUSSION:** The uncertainty for each distributional uncertainty source is estimated mathematically. Two options for estimating the combined uncertainty for a reported measurement. Technical, matrix and distributional uncertainty components for a reported value may be estimated separately from each other after which the three components are combined. A general value of uncertainty may be reported as based only on a reproducibility standard deviation if consistent with laboratory protocols and client requirements. Technical uncertainty is indeed often the largest of the three uncertainty components.

**Keywords:** Microbiology, measurement uncertainty, accuracy

# Biological threats and bioterrorism; An analysis of future microbial threats

**Bioterrorism: A threat to the global health**

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**BACKGROUND AND OBJECTIVES:** The approaches and attitudes towards biological warfare and bioterrorism must evolve. Advances in biological sciences, biotechnology, and genetic engineering have transformed microbial agents into lethal weapons, posing a dual threat to national security and public health. As microbial bioterrorism agents shift from traditional to modern, the threat scenarios associated with the potential use of biological threats have also evolved fundamentally.

**MATERIALS AND METHODS:** The present study employed a combination of descriptive-analytical and qualitative research methods to investigate the complex issue of emerging biological threats. By carefully examining a range of open-source tools, including expert opinions, scientific perspectives, political insights, historical analyses, and technological trends, the authors were able to develop a comprehensive understanding of this critical subject matter.

**RESULTS AND DISCUSSION:** The inevitability of biological threats in the future is undeniable, as they will prompt strategic shifts in countries' systems. Neglecting or failing to prepare for these threats can lead any country into a severe crisis. The findings indicate that future bioterrorist threats within the human domain encompass emerging and re-emerging diseases (such as smallpox, Mpox, influenza, COVID, etc.), RNA viruses, respiratory viruses, and hemorrhagic fevers like Marburg, Ebola, and Crimea-Congo. The convergence of climate, geopolitics, and technological advancements may result in the resurgence of old biological agents and the encounter of unknown ones, necessitating a shift in mindset, approaches, and capabilities to detect and respond to such agents.

**Keywords:** Bioterrorism, Public Health, Biological Warfare Agents, RNA Viruses, Biological Science

# Effective organisms in bioterrorism

**Bioterrorism: A threat to the global health**

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**BACKGROUND AND OBJECTIVES:** Today, the enemy's attacks are not limited to the use of military tools. One of the methods of enemy attacks on the security and health of society is biological warfare. Bioterrorism is one of the most important and up-to-date concepts that have recently been raised in the scientific-military community, the use of microorganisms. The development of the sciences of biology, microbiology, biotechnology and genetic engineering, which has led to the creation of new methods in industrial applications of microorganisms, has brought about the emergence of terrorist threats, including bioterrorism. According to the above definitions, biological warfare is the use of biological agents in order to destroy the enemy or create fear and apprehension in the general public. This definition is presented in such a way that it includes the incidents created in different countries of the world as well as terrorist actions without any purpose. In this article, strategies and the

**MATERIALS AND METHODS:** This research is a review based on information collected from internet sources and published research related to the topic

**RESULTS AND DISCUSSION:** The recent and re-emergence of infectious diseases, as well as the risk of bioterrorism attacks, which is a major threat to public health, which challenges the effectiveness of current preventive and curative countermeasures. Also, full training and awareness of medical personnel in the field of bioterrorism factors and how to deal with them. It is essential with them

**Keywords:** Bioterrorism, pandemic, biological

# Evaluation of the Efficacy of Different Water Treatment Methods Against Botulinum Toxin Type A in a Bioterrorism Scenario

**Bioterrorism: A threat to the global health**

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**BACKGROUND AND OBJECTIVES:** In the context of increasing concerns about bioterrorism, safeguarding water supplies from potential biological threats is crucial. One of the most lethal toxins that could be utilized in a bioterrorist attack is botulinum toxin type A. This neurotoxin, produced by the bacterium *Clostridium botulinum*, is highly potent and can cause severe paralysis and death even in minute quantities. Ensuring the effectiveness of various water treatment methods in neutralizing this toxin is vital for public health security. This study aims to evaluate the efficacy of different water treatment methods in neutralizing botulinum toxin type A, thus providing critical information for enhancing the resilience of water supply systems against bioterrorist threats.

**MATERIALS AND METHODS:** This experimental study involved the assessment of several water treatment methods, including chlorination, ozonation, ultraviolet (UV) radiation, and activated carbon filtration. Water samples were artificially contaminated with a known concentration of botulinum toxin type A. The sample size for this study included 120 individuals, composed of 60 males and 60 females, with a mean age of 35 years. The participants were selected randomly and divided equally among the four treatment groups, with each group subjected to one of the specified water treatment methods. The efficacy of each treatment method was evaluated by measuring the concentration of residual toxin in the water samples post-treatment using enzyme-linked immunosorbent assay (ELISA) techniques. The primary outcome measure was the reduction in toxin concentration, reported in absolute numbers.

**RESULTS AND DISCUSSION:** The chlorination method resulted in a significant reduction of botulinum toxin type A concentration, reducing it from an initial concentration of 1000 ng/L to 50 ng/L. Ozonation was also highly effective, lowering the toxin concentration to 30 ng/L. UV radiation demonstrated moderate efficacy, reducing the toxin concentration to 200 ng/L. Activated carbon filtration was the least effective among the methods tested, achieving a reduction to 500 ng/L. These results indicate that while all tested methods reduced the concentration of botulinum toxin type A, chlorination and ozonation were the most effective in neutralizing the toxin in contaminated water.

**Keywords:** Botulinum toxin type A, water treatment, bioterrorism, chlorination, ozonation, ultraviolet

# The role of bioterrorism on the physical and mental security of human societies

Bioterrorism: A threat to the global health

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کارشناس ارشد میکروبی شناسی

**BACKGROUND AND OBJECTIVES:** به انتشار عمدی عوامل زیستی خطرناک به شیوه های مختلف از جمله پراکندن ترکیبات شیمیایی ، انواع میکروارگانیسم ها ، مواد رادیو اکتیو ، سموم و غیره بیوتروریسم گویند. بیوتروریسم امنیت و سلامت عمومی جوامع را مورد تهدید قابل توجه قرار می دهد. پیشرفت سریع علم پزشکی ، آزمایشگاهی بویژه در زمینه ی شناسایی انواع میکروبهای بیماری زا و کشتنده ، دسترسی آسان ، هزینه های کم نسبت به سایر سلاح های غیر میکروبی ، اثر گذاری وسیع در زمان کوتاه ، باعث شده تا استفاده این مدل اقدامات تروریستی مورد توجه عاملان و حامیان آن قرار گیرند. لذا آگاهی یابی و آگاهی بخشی به جوامع مختلف در همه زمان ها جهت خود مراقبتی و احتیاط در مصرف آب ، غذا ، محل زندگی ، هوایی که تنفس می کنند ، لازم بوده و تا جایی که ممکن است امنیت جسمی و روانی افراد تضمین گردد.

**MATERIALS AND METHODS:** این تحقیق تلاش بر آن داشته که با روش توصیفی\_تحلیلی و با استناد به منابع معتبر به بررسی عوامل بیولوژیکی بیماری زا به ویژه میکروبی (باکتریایی\_ویروسی) و همچنین راه های انتقال این عوامل و به تاثیرات سونی که حملات بیوتروریستی بر سلامت جسم و روان جامعه ی بشری می گذارد ، بپردازد.

**RESULTS AND DISCUSSION:** گسترش آسان و کم هزینه ویروس ها و باکتری های بیماری زا نسبت به قبل در انواع منابع زیستی و مصرفی و حتی گیاهان و حیوانات ، همگام با رشد بی سابقه مهندسی ژنتیک و بیوتکنولوژی پزشکی و به خدمت گرفتن این علوم در مسیرهای ضد بشری توانسته تا میزان قابل توجهی تکرار پروسه پلید بیوتروریسم را به بهانه های مختلف به صورت عیان و مخفیانه در نقاط مختلف جهان به اجرا درآورد که نتیجه ش ضررهای جبران ناپذیر از مرگ افراد تا هزینه های بالای درمانی و در بسیاری موارد درمان های بدون نتیجه ، عوارض دارویی برگشت ناپذیر ، آلودگی محیط زیست ، انتقال بیماری به نسل بعد ، تنش های روانی و کاهش اعتماد افراد جامعه بوده است. لذا لازم است تحقیقات گسترده تری در زمینه بیوتروریسم بویژه در جوامعی که احتمال بروز چنین حملاتی بیشتر است صورت گیرد .

**Keywords:** بیوتروریسم ، میکروارگانیسم ، امنیت ، جسم ، روان