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خدمات بهداشتی درمانی کرمان
معاونت تحقیقات و فناوری

بیستمین کنگره بین المللی میکروب شناسی ایران

Iran's 20th International Congress of Microbiology

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August 27-29, 2019, Kerman, Iran

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◆ دارای امتیاز بازآموزی

◆ Main Topics :

Antimicrobial Resistance and Nosocomial Infections
Anaerobic Infections
Bacterial Pathogenesis and Virulence
Challenges in Microbial Research
Food , Industrial and Applied Microbiology
Global Trends in Emerging Infectious Diseases
Infectious Disease: Diagnosis and Treatment
Microbiota and Probiotics
Mycobacteria and Acid Fast Bacteria
Parasitic , Fungal and Viral Infections
Prevention and Control of Infectious Diseases
Zoonotic Diseases

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In the Name of GOD

20th International Congress of Microbiology

27-29 August 2019

Kerman, Iran

Organized by:

Kerman University of Medical Sciences

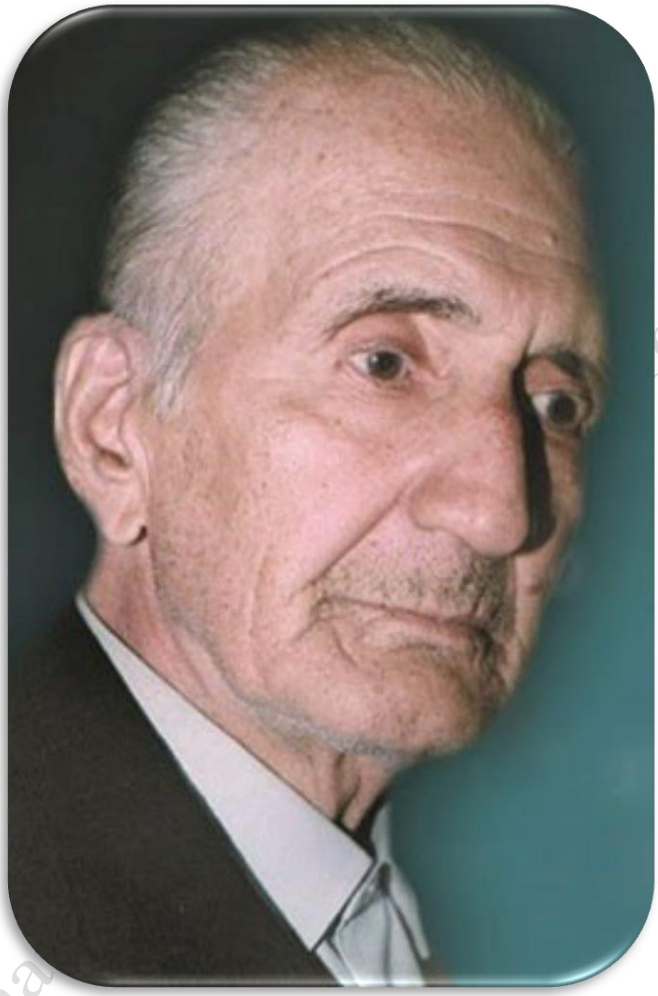
Iranian Society of Microbiology

Kerman University of Medical Sciences

Kerman University of Medical Sciences

20th International Congress of Microbiology

27- 29 August 2019 Kerman, Iran



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Founder of Kerman University

Kerman University of Medical Sciences

20th International Congress of Microbiology

27- 29 August 2019 Kerman, Iran

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Kerman University of Medical Sciences

20th International Congress of Microbiology

27- 29 August 2019 Kerman, Iran

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Razi vaccine and Serum Reaserch Institute

مرکز تحقیقات پاتولوژی و سلولهای بنیادی
دانشکده پزشکی افضلی پور

With Cooperation of:

HIV/STI Surveillance Research Center, and WHO Collaborating Center for HIV Surveillance

Shahid Bahonar University of Kerman

Dr. Hamidreza Rashidinejad

The president of congress and the chancellor of Kerman University of Medical Sciences, Kerman, Iran

Prof. Shahla Mansouri

Scientific Secretory of the Congress

Prof. Mohammad Mehdi Feizabadi

Head of Iranian Society of Microbiology

Dr. Davood Kalantar-Neyestanaki

Executive Secretary of Congress

Message of the President of the Congress

It is a great honour for me to invite you to the 20th International Congress of Microbiology to be held from the 27-29 of August 2019 in Kerman, Iran. The main goal of the congress is to provide an opportunity to exchange scientific ideas, update information and become familiar with the developments in the different fields of microbiology. In order to achieve this, the scientific committee has endeavoured to gather national and international experts from both clinical medicine and microbiology. I would like to thank all researchers who have shared with us their valuable viewpoints and actively engaged in the planning and execution of this congress. The presence of microbiologists, clinicians, and health authorities in the 20th International Congress of Microbiology will undoubtedly enhance the ability of participants and executives of the country to serve a healthier human life. I strongly hope that this congress through its scientific programs makes solid inputs into improving the quality of our knowledge. I also wish you all a pleasant stay in Kerman seasoned with unforgettable memories of its historical and monumental places.

Dr. Hamidreza Rashidinejad

Message of the Scientific Secretary of the Congress

On behalf of the scientific committee I would like to invite you all to join us and participate in the 20th Iranian International Congress of Microbiology that will be held from the 27-29 of August 2019 in Kerman, Iran. Our aim is to offer you high-quality research works along with exceptional presentations by competent internationally recognized microbiologist keynote lecturers.

We will do our greatest to provide you opportunities for collaborating and sharing knowledge with other scientists and to familiarize you with significant discoveries in the field of microbiology and the latest developments in various topics of clinical and other related subjects in Microbiology and Human Health areas.

During this congress many oral and poster communications will be presented and will cover many hot topics. Several practical workshops are also planned for fresher colleagues and beginners in the field.

Your participation in this event will be valuable for us and would improve the scientific quality of the congress.

We look forward to meeting you in Kerman with our warmest regards.

Prof. Shahla Mansouri

Message of the President of the Iranian Society of Microbiology

The Iranian Microbiology Society commemorates you at the 20th International Congress of Microbiology, Iran, Kerman and wish you a happy stay, along with academic achievements. Special thanks to the organizing authorities, especially the President and the Board of Kerman University of Medical Sciences. Without the university's scientific and administrative support, it would not have been possible to hold a conference in Kerman. We also welcome the presence of international speakers in Congress. New science and technology based on microbiology is emerging with new insights into new concepts and understandings of microbes' ability to use in different fields of biology, medicine and the functions of these organisms in their pathogenicity or health. The role of microbiologists in the evolution of these attitudes has been of primary importance and therefore more attention to the field of microbiological knowledge will bring valuable achievements. I seize the opportunity and thank you very much for the hard work of our colleagues, especially the Congress Secretary, Professor Shahla Mansouri as the Scientific Secretary and Dr. Davood Kalantar-Neyestanaki as the Executive Secretary of the Congress and their colleagues in the secretariat.

Prof. Mohammad Mehdi Feizabadi

International Invited Speakers

Prof. Kurt G. Naber

Technical University of Munich Medical School, Germany

Prof. Leonardo Antonio Sechi

University of Sassari, Italy

Prof. Helmut Hotzel

Institute of Bacterial Infections and Zoonoses

Friedrich-Loeffler-Institute, Germany

Scientific Committee

Members of Scientific Committee

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Dabiri	Shahriar	Kerman University of Medical Sciences
Dabirian	Shahriar	Ph.D. of food hygienic and quality assurance of Iran Dairy Industries
Dadashi	Masoud	Alborz University of Medical Sciences
Daneshvar	Hamid	Kerman University of Medical Sciences

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Iman Eini	Mohammd	Tehran University of Medical Sciences

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Kerman University of Medical Sciences

20th International Congress of Microbiology

27- 29 August 2019 Kerman, Iran

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Scientific Program

Kerman University of Medical Sciences

20th International Congress of Microbiology

27- 29 August 2019 Kerman, Iran

1st day, Tuesday, August 27

Opening Ceremony		
8:15 - 8:20	Quran Recitation	
8:20 - 8:30	Anthem of Islamic Republic of Iran	
8:30 - 8:40	Kerman at a glance	
8:40 - 8:50	**Welcome Speech**	**Dr. Hamidreza Rashidinejad:** The president of congress and the chancellor of Kerman University of Medical Sciences, Kerman, Iran
8:50 - 9:00	**Opening Speech**	**Prof. Mohammad Mehdi Feizabadi:** Head of Iranian Society of Microbiology
9:00 - 9:10	**Overview of Congress**	**Prof. Shahla Mansouri:** Scientific Secretory of the Congress
Session 1 **Oral Presentation**		
Board of Directors: Prof. Mohammad Reza Pourmand, Prof. Shahla Mansouri, Prof. Fereshteh Shahcheraghi, Prof. Rasoul Yousefimashouf, Prof Gholamreza Irajian.		
9:15 - 9:40	**Dr. Ali Eshaghi,** Head of Razi Vaccine and Serum Research Institute, Iran:	
9:40 - 10:05	**Prof. Leonardo Antonio Sechi**, University of Sassari, Italy: Infections and autoimmunity	
10:05 - 10:30	**Dr. Kurt G. Naber**, Technical University of Munich, Germany: Non-antibiotic treatment of uncomplicated UTI – quo Vadis?	
Break and Poster Visiting **10:30-11**		
Session 2 **Oral Presentation**		
Board of Directors: Prof. Mohammad Reza Pourmand, Dr. Abbas Abdollahi, Prof. Fereshteh Shahcheraghi, Prof. Rasoul Yousefimashouf, Prof. Gholamreza Irajian, Prof. Nour Amir-Mozafari.		
11:00 - 11:10	**Prof. Ali Zarei Mahmoudabadi:** The potency of Luliconazole, against Clinical and Environmental *Aspergillus Niger* Complex	
11:10 - 11:20	**Sahar Serajian:** Prevalence, antimicrobial susceptibility, serotyping and virulence determination of *Listeria monocytogenes* strains at a tertiary care hospital in Tehran, Iran	
11:20 - 11:30	**Samaneh Saedi:** Multilocus Sequence Analysis (MLSA) of *Mycobacterium tuberculosis* from Clinical Isolates in Northeast of Iran	
11:30 - 11:40	**Mortaza Haghighi Hassan Abad:** Vertical Transmission of *Chlamydia trachomatis* at Birth Time and Eye Colonization in New-borns	

1st day, Tuesday, August 27

Panel 1 **11:40-12:40** **Antimicrobial Resistance and Control of Nosocomial Infections** **Coordinator:** Prof. Mohammad Reza Pourmand **Panel Staff:** Dr. Abbas Abdollahi, Prof. Fereshteh Shahcheraghi, Prof. Rasoul Yousefimashouf, Prof. Gholamreza Irajian, Dr. Kurt G. Naber, Prof. Nour Amir-Mozafari.	
Prof. Mohammad Reza Pourmand: An overview on strategies for control of nosocomial infection	
Prof. Gholamreza Irajian: The organization and team of nosocomial infection control committee in Iran	
Prof. Rasoul Yousefimashouf: Barrier to success in control of nosocomial infection	
Dr. Abbas Abdollahi: Using Bioinformatics to Prediction Hospital Acquired Infections	
Prof. Fereshteh Shahcheraghi: Impact of rapid diagnosis of carrier patients on prevention and control of healthcare-associated infections (HAIs), A case report	
12:20 - 12:40	**Discussion, Question and Answer**
Praying, Lunch and Poster Visiting **12:40-14:00**	
Session 3 **Oral Presentation**	
Board of Directors: Prof. Mohammad Mehdi Feizabadi, Dr. Rostam Yazdani, Dr. Seyed Ali Mohammad Arabzadeh, Dr. Mehrdad Farrokhnia, Dr. Nader Mosavari, Prof. Azar Dokht Khosravi, Dr. Keyvan Tadayon, Dr. Saeed Zaker Bostanabad, Dr. Saeed Sharafi.	
14:00 - 14:20	**Dr. Seyed Alimohammad Arabzadeh:** Mycobacterial infection in HIV infected patients
14:20 - 14:30	**Masoud Keikha:** The PI3K-Akt/mTOR signalling pathway and tuberculosis pathogenesis; the first system biology report

1st day, Tuesday, August 27

Panel 2 **14:30-15:30** **Mycobacteria & Acid-Fast Bacteria** **Coordinator**: Dr. Nader Mosavari **Panel Staff:** Prof. Mohammad Mehdi Feizabadi, Prof. Leonardo Antonio Sechi, Dr. Rostam Yazdani, Dr. Seyed Ali Mohammad Arabzadeh, Dr. Mehrdad Farrokhnia, Prof. Azar Dokht Khosravi, Dr. Nahid Mojgani, Dr. Saeed Zaker Bostanabad, Dr. Saeed Sharafi.	
Dr. Nader Mosavari: Isolation and Identification of Mycobacterium from Captured Cats and Mice Belonging to Tuberculosis Infected Farms	
Dr. Rostam Yazdani: Case report: a 48 years old male with bilateral pulmonary consolidation and positive AFB of BA	
Dr. Mehrdad Farrokhnia: Latent Tuberculosis	
Dr. Nahid Mojgani: Isolation and purification of ESAT6/CFP10 complex antigen secreted by *Mycobacterium tuberculosis* strains C	
Dr. Saeed Zaker Bostanabad: Molecular Characterization of Epidemiology of MDR Mycobacterium tuberculosis isolated from tuberculosis patients resistant to Ofloxacin and Ciprofloxacin	
Dr. Saeed Sharafi: Tuberculosis in Iran: current situation and the role of medical laboratories as a unique national network in control of disease	
15:10 - 15:30	**Discussion, Question and Answer**
Break and Poster Visiting **15:30-15:50**	
Session 4 **Oral presentation**	
Board of Directors: Prof. Reza Ghotaslou, Dr. Amin Talebi Bezmin Abadi, Dr. Omid Tadjrobehkar, Dr. Fereshteh Saffari, Dr. Soheila Moradi Bidhendi, Prof. MohammadYousef Alikhani, Dr. Davood Darban-Sarokhalil, Dr. Seyed Sajad Khoramrooz.	
15:50 - 16:00	**Morteza Karami Zarandi:** Identification of Non-Tuberculosis *Mycobacterium* species in suspected patients to tuberculosis by Line Probe Assay method
16:00 - 16:10	**Farideh Kamarehei:** Designing a novel ELISA method based on CagA, NapA recombinant antigens to increase sensitivity and specificity of *Helicobacter pylori* whole cell antigen detection
16:10 - 16:20	**Mehrdad Hallaji:** Comparison of prevalence of Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) among *Staphylococcus aureus* isolates in a burn unit with non-burning units in Isfahan, Iran
16:20 - 16:30	**Sara Hamzehee:** Molecular identification of *Candida* species, assessment of the antifungal susceptibility and the genetic relationship of *Candida albicans* isolated from immunocompromised patients in Kerman, Iran
16:30 - 16:40	**Dr. Mehdi Fatahi Bafghi**: Isolation and Identification of the *Nocardia* spp from clinical specimens
16:40 - 16:50	**Hamid Reza Hagh Ranjbar:** Bioconversion of genistein to orobol by spore display tyrosinase
16:50 - 17:00	**Dr. Javid Sadeghi:** Frequency of *Staphylococcus aureus* isolates collected from men semen and women endocervix and detection of antibiotic resistance

1st day, Tuesday, August 27

Panel 3 **17:00-18:00** **New Laboratory Methods for Diagnosing Infectious Diseases** **Coordinator:** Prof. Reza Ghotaslou **Panel Staff:** Dr. Amin Talebi Bezmin Abadi, Dr. Omid Tadjrobehkar, Dr. Fereshteh Saffari, Dr. Soheila Moradi Bidhendi, Prof. MohammadYousef Alikhani, Dr. Davood Darban-Sarokhalil, Dr. Seyed Sajad Khoramrooz.	
Dr. Seyed Sajad Khoramrooz: Optical Biosensors for Detection of Pathogenic Microorganisms	
Dr. Davood Darban-Sarokhalil: Recent Updates on Laboratory Diagnosis of Tuberculosis	
Dr. Amin Talebi Bezmin Abadi: Recent Advance in Diagnosis of *Helicobacter pylori*: Invasive and Non-invasive Approaches	
Prof. MohammadYousef Alikhani: Loop-Mediated Isothermal Amplification (LAMP) Test for Detection of *Brucella* spp	
Dr. Soheila Moradi Bidhendi: Innovations in salmonella detection	
17:40 - 18:00	**Discussion, Question and Answer**

2nd Day, Wednesday, 28 August 2019

8:00-8:10	**Quran Recitation**
Session 5 **Oral Presentation**	
Board of Directors: Dr. Rainak Ghaderi, Prof. Iraj Sharifi, Dr. Gholamreza Hashemi Tabar, Dr. Ali Safar Maken Ali, Prof. Taghi Zahraei Salehi, Dr. Mohammed Zeinali, Dr. Peyman Keihani, Dr. Keivan Tadayon.	
8:10 - 8:35	**Dr. Helmut Holtzel,** Friedrich-Loeffler Institut, Germany: *Chlamydophila psittaci* and zoonotic infections, the German perspective
8:30 - 8:55	**Mr. Daniel Feakes,** Chief of Biological Weapons Convention Implementation Support Unit UN: The Biological Weapons Convention (BWC), a historical perspective
8:55 - 9:20	**Prof. Abbass Pardakhti:** Lipid vesicular drug delivery systems in treatment of intracellular infection: capabilities and challenges
9:20 - 9:30	**Dr. Samaneh Babaee:** Frequency of multi drug resistance and molecular characteristics of resistance to colistin among *Acinetobacter baumannii* isolated from hospitalized patients in ICU of Qazvin& Masih Daneshvari hospital, with ventilator associated pneumonia
9:30 - 9:40	**Dr. Razieh Tavakoli:** The role of resistance genes expression in meglumine antimoniate non-healing and healing isolates of anthroponotic cutaneous leishmaniasis due to *Leishmania tropica*
Panel 4 **9:40-10:40** **Zoonotic Diseases** **Coordinator:** Prof. Taghi Zahraei Salehi **Panel Staff:** Prof. Iraj Sharifi, Dr. Gholamreza Hashemi Tabar, Dr. Helmut Hotzel, Dr. Ali Safar Maken Ali, Dr. Nader Mosavari, Dr. Mohammed Zeinali, Dr. Peyman Keihani, Dr. Keivan Tadayon, Dr. Rainak Ghaderi.	
Dr. Nader Mosavari: Design and optimization of diagnostic glanders cassette using immunoblotting method based on immunoreactive proteins of *Burkholderia mallei*	
Dr. Rainak Ghaderi: Comparative Molecular Characterization of wild Isolates and Vaccine Strain of *Mycoplasma agalactiae* in Iran	
Dr. Gholamreza Hashemi Tabar: The role of Veterinary Medicine in controlling of Crimean-Congo Hemorrhagic Fever (CCHF)	
Dr. Mohammed Zeinali: Epidemiological situation of crimian congo haemorrhagic fever in 1998-2018 in Iran	
Prof. Iraj Sharifi: Leishmaniasis as a zoonotic disease in Iran	
Dr. Keivan Tadayon: Genetic structure of Mycobacterium bovis population a search for most globally frequent clonal complexes in the Iran	
Dr. Peyman Keihani: Molecular detection of *Borrelia burgdorferisensulato* in tick infested dogs in Isfahan province, Iran	
17:40 – 18:00	**Discussion, Question and Answer**
Break and Poster Visiting **10:40-11:00**	

2nd Day, Wednesday, 28 August 2019

Session 6 **Oral presentation**	
Board of Directors: Prof. Majid Fasihi Harandi, Dr. Pejvak Khaki, Dr. Daniel Feaks, Dr. Mohammad Mehdi Gouya, Dr. Mostafa Salehi-Vaziri, Dr. Mahdi Rohani, Dr. Saber Esmaeili, Dr. Mehdi Hassanshahian.	
11:00 - 11:10	**Dr. Jalil Mehrzad:** Investigation on the effects of *Salmonella enterica* Subsp enterica serovar Typhimurium on the apoptosis/necrosis of avian macrophage-like monocytes
11:10 - 11:20	***Mahtab Alsadat Madani Borugeni:*** The effect rates of Staphylococcus bacteriophages on resistance clinical isolates of *Staphylococcus aureus*
11:20 - 11:30	**Fatemeh Gholami:** Prevalence and characterization of toxin profiles in *Clostridium perfringens* isolates from infants and children in Iran
11:30 -11:40	**Sanaz Gharaei:** Optimization of biosurfactant production by an oil-degrading *Bacillus* isolated from petroleum-contaminated soil
Panel 5 **11:40-12:40** **Emerging & Re-emerging Infections** **Coordinator:** Prof. Majid Fasihi Harandi **Panel Staff:** Dr. Pejvak Khaki, Dr. Mohammad Mehdi Gouya, Dr. Mostafa Salehi-Vaziri, Dr. Mahdi Rohani, Dr. Saber Esmaeili, Dr. Mehdi Hassanshahian.	
Dr. Mohammad Mehdi Gouya: Preparedness against emerging and re-emerging infectious diseases: Are we ready to meet the challenges?	
Dr. Mostafa Salehi-Vaziri: Emerging Aedes-borne viruses	
Dr. Mahdi Rohani: Present status of Tularemia and Francisella infections in Iran	
Dr. Saber Esmaelli: Genotyping of Coxiella burnetti in Iran	
Dr. Pejvak Khaki: Leptospirosis in Iran	
12:20 - 12:40	**Discussion, Question and Answer**
Praying, Lunch and Poster Visiting **12:40-14:00**	
Session 7 **Oral presentation**	
Board of Directors: Dr. Mojtaba Nofeli, Prof. Saeid Bouzari, Dr. Seyed Mohsen Zahraei, Dr. Seyed Reza Banihashemi, Dr. Mohammad Mahdi Mohammadi, Dr. Hamid Daneshvar, Dr. Seyed Fazlollah Moosavi, Dr. Ali Afgar.	
14:00 - 14:10	**Mojtaba Ali Molaei:** Oral immunization with *Lactobacillus casei* vectored vaccines surface-expressed *Clostridium perfringens* toxoids
14:10 -14:20	**Ahmad Mehravaran:** Encapsulation of Imiquimod Adjuvant and Soluble *Leishmania* Antigen into Liposomes as a Vaccine in the Cutaneous Leishmaniasis Model
14:20 – 14:30	**Ali Mohammad Behroozikhah:** Determination *Brucella* vaccine strains of genetic typing with use VNTR-PCR HOOF PRINT
14:30 - 14:40	**Sajad Aslani:** Dissemination of incompatible plasmid groups among " *Klebsiella pneumoniae* superbug" strains harboring New Delhi metallo-lactamase-1 gene, Kerman, Iran

2nd Day, Wednesday, 28 August 2019

Panel 6 **14:40-15:40** **New Approach in Immunization & Vaccine Technologies** **Coordinator:** Dr. Mojtaba Nofeli **Panel Staff:** Dr. Sima Rafati, Dr. Seyed Mohsen Zahraei, Dr. Seyed Reza Banihashemi, Dr. Mohammad Mahdi Mohammadi, Dr. Hamid Daneshvar, Dr. Seyed Fazlollah Moosavi, Dr. Ali Afgar.	
Dr. Seyed Mohsen Zahraei: National vaccination program: promotions and new challenges	
Dr. Ali Afgar: Application of microorganisms in the treatment of cancer	
Dr. Seyed Reza Banihashemi: Factors that may influence vaccine immune responses	
Dr. Mojtaba Nofeli: Vaccine design & manufacturing approaches; challenges and solutions	
Dr. Sima Rafati: Immunotherapy and Leishmania Vaccine Research	
15:20 – 15:40	**Discussion, Question and Answer**
Break and Poster Visiting **15:40-16:00**	
Session 8 **Oral presentation**	
Board of Directors: Dr. Reza Pilehchian Langroudi, Dr. Mehrdad Shamsaddini Bafti, Dr. Hamid Hakimi, Dr. Mohammad Moradi, Dr. Mojtaba Alimolaei, Prof. Mohammad Motamedifar, Dr. Mohsen Fathi Najafi.	
16:00 - 16:20	**Prof. Mohammad Motamedifar:** Epidemiology, bacterial load, antibiotic susceptibility and risk factor of toxigenic *Clostridium difficile* in hospitalized patients in southwestern Iran
16:20 - 16:30	***Nasibeh Khodaverdi*:** The association between fecal enterotoxigenic *Bacteroides fragilis* with colorectal cancer
16:30 - 16:40	**Ali Razaei Kalavani:** Prevalence of *Clostridium novyi* in slaughterhouses of Alborz province using traditional methods and Polymerase Chain Reaction
16:40 - 16:50	**Nagar Mohammadi:** Frequency of *Gardnerella vaginalis* in patients with vaginosis in Isfahan by molecular method
16:50 - 17:00	**Salman Odooli:** Selective plate count and tuf gene-based qPCR methods for quantification of *Bifidobacterium animalis* subsp. lactis BB-12 in commercial probiotic yoghurts

2nd Day, Wednesday, 28 August 2019

Panel 7 **17:00-18:00** **Anaerobic Infections** **Coordinator:** Dr. Mohsen Fathi Najafi. **Panel Staff:** Dr. Mehrdad Shamsaddini Bafti, Dr. Hamid Hakimi, Dr. Mohammad Moradi, Dr. Mojtaba Alimolaei, Prof. Mohammad Motamedifar, Dr. Reza Pilehchian Langroudi, Dr. Alireza Pardis.	
Dr. Alireza Paradise: An investigation on Clostridial toxins effect on cancerous cell	
Dr. Mohsen Fathi Najafi: Use of Clostridial toxins for stopping and killing breast cancer cells	
Dr. Hamid Hakimi: The preventive and therapeutic effects of enterotoxin (CPE)	
Dr. Mehrdad Shamsaddini: Application of *Clostridium perfringens* enterotoxin (CPE) in the treatment of gastrointestinal tumor cells	
17:40 - 18:00	**Discussion, Question and Answer**

3rd, Thursday, 29August 2019

8:00-8:10	**Quran Recitation**
Session 9 **Oral Presentation**	
Board of Directors: Prof. Gholamreza Irajian, Dr. Ali Hossininasab, Prof. Shahla Mansouri, Dr. Bijan Nomanpour, Dr. Mohammad Javad Nasiri, Dr. Shabnam Razavi, Dr. Omid Pajand	
8:10 - 8:20	**Sedigheh Nakhaee:** Dissemination pattern of multidrug resistant Carbapenemase Producing Klebsiella pneumoniae Isolates in Nemazee and Faghihi Referral hospitals in Shiraz, Southwestern Iran
8:20 - 8:30	**Dr. Zahra Farshadzadeh:** Growth Rate and Biofilm Formation Ability of Clinical and Laboratory-Evolved Colistin-Resistant Strains of Acinetobacter baumannii
Panel 8 **8:30-9:30** **Qualitative Control in Microbiology Laboratories** **Coordinator:** Prof. Gholamreza Irajian **Panel Staff:** Dr. Ali Hossininasab, Prof. Shahla Mansouri, Dr. Bijan Nomanpour, Dr. Mohammad Javad Nasiri, Dr. Shabnam Razavi, Dr. Omid Pajand	
Dr. Hosseini Nasab: The Importance of Experimental Drug Testing in Clinical Practice	
Dr. Bijan Namanpour: Quality control of drug susceptibility testing in medical diagnostic laboratory	
Dr.Shabnam Razavi: MIC and MBC quality control	
Dr. Javad Nasiri: Quality control in drug susceptibility testing in Mycobacterium tuberculosis	
9:10 - 9:30	**Prof. Shahla Mansouri – Prof..Gholamreza Irajian** **Discussion, Question and Answer**
Board of Directors: Prof. Gholamreza Irajian, Dr. Ali Hossininasab, Prof. Shahla Mansouri, Dr. Bijan Nomanpour, Dr. Mohammad Javad Nasiri, Dr. Shabnam Razavi, Dr. Omid Pajand	
9:30 - 9:40	**Mozhgan Kheirandish:** The study of ferritin-binding proteins in *Streptococcus pneumoniae* proteome
9:40 - 9:50	**Soheila Ghaderi:** Comparing structure and conformation of diphtheria toxin with its non-toxic mutant (E3149K) at 300K using molecular dynamics simulations
9:50 -10:00	**Maryam Khaleghi:** Antibacterial curcumin-loaded hydrogel based on Hyaluronic Acid-Polydimethylsiloxane (HA-PDMS) for wound dressing perspectives
10:00 - 10:10	**Maryam Rahmani:** Determination and comparison of antibacterial activity of *Enterococcus* species isolated from breast-fed neonates and adults feces in Kerman
10:10 - 10:20	**Tayebeh Saberi Shahmarvandi:** Investigation of the quantity of crude oil degrading bacteria in contaminated areas in Masjed Soleyman
10:20 - 10:30	**Dr. Mahdi Askari Badouei:** Molecular serotyping of Shiga toxin-producing *Escherichia coli* (STEC) from animal sources in Iran: Emergence of a potentially virulent O26: H29 strain
Break and Poster Visiting **10:30-11:00**	

3rd, Thursday, 29August 2019

Session 10 Oral Presentation	
Board of Directors: Dr. Hossein Dabiri, Dr. Marzeih Hosseininezhad, Dr. Abbas Ali Imani Fooladi, Dr. Mojtaba Boniadian, Dr. Mehdi Mirzaei, Prof. Fariba Sharififar, Prof. Asghar Sharifi, Dr. Mojtaba Hedayati.	
11:00-11:10	**Dr. Roya Ahmadrajabi:** Comparative analysis of virulence genes, antibiotic resistance and capsule locus polymorphism of *Enterococcus faecalis* isolated from canals of root- filled teeth with different clinical infections and fecal flora
11:10-11:20	**Zahra Rajabi:** Study the effect of extracted bacteriocin of *Lactobacillus fermentum* with probiotic potential on biofilm formation of isolated *Streptococcus mutans* from dental caries
Panel 9 **11:20-12:30** **Food, Industrial and Applied Microbiology** **Coordinator:** Dr. Hossein Dabiri **Panel Staff:** Dr. Marzeih Hosseininezhad, Dr. Abbas Ali Imani Fooladi, Dr. Mojtaba Boniadian, Dr. Mehdi Mirzaei, Prof. Fariba Sharififar, Prof. Asghar Sharifi, Dr. Mojtaba Hedayati.	
Dr. Mojtaba Hedayati: Detection of enterotoxigenic staphylococcal food poisonings by traditional and new methods	
Dr.Hossein Dabiri: The role of microbiology laboratory in diagnosis and control of foodborne diseases following disasters	
Dr. Marzeih Hosseininezhad: An overview on the Industrial Challenges of Lactic Acid Bacteria as Probiotics and Natural Food Preservatives	
Dr. Mehdi Mirzaei: Chronobiology, the future of medicine	
Dr. Mojtaba Boniadian: The importance of verotoxigenic E. coli as emerging foodborne infections	
12:15 - 12:30	**Discussion, Question and Answer**
Praying, Lunch and Poster Visiting **12:30-14:00**	

3rd, Thursday, 29August 2019

Panel 10 **14:00-15:00** **Medical ethics: Challenges in Research and Publication** **Coordinator:** Prof. Hossein Safizadeh. Panel Staff: Prof. Mohammad Khaksari, Prof. Aliakbar Haghdoost, Prof. Abbass Pardakhti, Prof. Abbas Abbaszadeh, Dr. Mohammad Setayesh, Dr. Firoozeh Mirzaie.	
Prof. Abbass Pardakhti: Alternative in vitro and in silico methods in animal studies	
Prof. Aliakbar Haghdoost: Science for science, is it ethical	
Prof. Abbas Abbaszadeh: Ethical Considerations in Publishing Research Works (Intellectual Property Research Works)	
Dr. Firoozeh Mirzaie: Training of research ethics	
14:40 - 15:00	**Discussion, Question and Answer**
Closing Ceremony	

First section: One Best Oral/Poster Presentation will be selected from each day. The Certificate for Best Oral/Poster Presentation will be awarded.

Second section: Introducing and Appreciation for pioneering Microbiologists.

The Gifts are Provided, On the Behalf of *Dr. Seyed Abdolkarim Arabzadeh*, The Pioneer in Diagnostic Laboratories.

Notice: **The abstract published in this abstract book were not edited either scientifically or literary. The 20th International Congress of Microbiology repudiates any error or incorrect statements made by the authors.**

Oral Papers

NO.	LastName	FirstName	Name	Date	Title
1	Ahmadrajabi	Roya	11:00 - 11:10	Third Day 8/29/2019	Comparative analysis of virulence genes, antibiotic resistance and capsule locus polymorphism of Enterococcus faecalis isolated from canals of root- filled teeth with different clinical infections and fecal flora
2	Ali Molaei	Mojtaba	14:00-14:10	Second Day 8/28/2019	Oral immunization with *Lactobacillus casei* vectored vaccines surface-expressed *Clostridium perfringens* toxoids
3	Arabzadeh	Seyed Alimohammad	14:00 - 14:20	First Day 8/27/2019	Mycobacterial infection in HIV infected patients
4	Askari Badouei	Mahdi	10:20-10:30	Third Day 8/29/2019	Molecular serotyping of Shiga toxin-producing *Escherichia coli* (STEC) from animal sources in Iran: Emergence of a potentially virulent O26: H29 strain
5	Aslani	Sajjad	14:30-14:40	Second Day 8/28/2019	Dissemination of incompatible plasmid groups among " *Klebsiella pneumoniae* superbug" strains harboring New Delhi metallo-lactamase-1 gene, Kerman, Iran
6	Babaee	Samaneh	9:20-9:30	Second Day 8/28/2019	Frequency of multi drug resistance and molecular characteristics of resistance to colistin among *Acinetobacter baumannii* isolated from hospitalized patients in ICU of Qazvin& Masih Daneshvari hospital, with ventilator associated pneumonia
7	Behroozikhah	Ali Mohammad	14:20-14:30	Second Day 8/28/2019	Determination *Brucella* vaccine strains of genetic typing with use VNTR-PCR HOOF PRINT

NO.	LastName	FirstName	Name	Date	Title
8	Farshadzadeh	Zahra	8:20-8:30	Third Day 8/29/2019	Growth Rate and Biofilm Formation Ability of Clinical and Laboratory-Evolved Colistin-Resistant Strains of Acinetobacter baumannii
9	Fatahi Bafghi	Mehdi	16:30 - 16:40	First Day 8/27/2019	Isolation and Identification of the Nocardia spp from clinical specimens
10	Ghaderi	Soheila	9:40-9:50	Third Day 8/29/2019	Comparing structure and conformation of diphtheria toxin with its non-toxic mutant (E3149K) at 300K using molecular dynamics simulations
11	Gharaei	Sanaz	11:30-11:40	Second Day 8/28/2019	Optimization of biosurfactant production by an oil-degrading *Bacillus* isolated from petroleum-contaminated soil
12	Gholami	Fatemeh	11:20-11:30	Second Day 8/28/2019	Prevalence and characterization of toxin profiles in *Clostridium perfringens* isolates from infants and children in Iran
13	Hagh Ranjbar	Hamid Reza	16:40-16:50	First Day 8/27/2019	Bioconversion of genistein to orobol by spore display tyrosinase
14	Haghighi Hassan Abad	Mortaza	11:30-11:40	First Day 8/27/2019	Vertical Transmission of *Chlamydia trachomatis* at Birth Time and Eye Colonization in New-borns
15	Hallaji	Mehrdad	16:10-16:20	First Day 8/27/2019	Comparison of prevalence of Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) among *Staphylococcus aureus* isolates in a burn unit with non-burning units in Isfahan, Iran

NO.	LastName	FirstName	Name	Date	Title
16	Hamzehee	Sara	16:20-16:30	First Day 8/27/2019	Molecular identification of *Candida* species, assessment of the antifungal susceptibility and the genetic relationship of *Candida albicans* isolated from immunocompromised patients in Kerman, Iran
17	Kalavani	Ali Razaei	16:30-16:40	Second Day 8/28/2019	Prevalence of *Clostridium novyi* in slaughterhouses of Alborz province using traditional methods and Polymerase Chain Reaction
18	Kamarehei	Faride	16:00-16:10	First Day 8/27/2019	Designing a novel ELISA method based on CagA, NapA recombinant antigens to increase sensitivity and specificity of *Helicobacter pylori* whole cell antigen detection
19	Karami Zarandi	Morteza	15:50-16:00	First Day 8/27/2019	Identification of Non-Tuberculosis *Mycobacterium* species in suspected patients to tuberculosis by Line Probe Assay method
20	Keikha	Masoud	14:20-14:30	First Day 8/27/2019	The PI3K-Akt/mTOR signalling pathway and tuberculosis pathogenesis; the first system biology report
21	Khaleghi	Maryam	9:50-10:00	Third Day 8/29/2019	Antibacterial curcumin-loaded hydrogel based on Hyaluronic Acid-Polydimethylsiloxane (HA-PDMS) for wound dressing perspectives
22	Kheirandish	Mozhgan	9:30-9:40	Third Day 8/29/2019	The study of ferritin-binding proteins in *Streptococcus pneumoniae* proteome
23	Khodaverd	Nasibeh	16:20-16:30	Second Day 8/28/2019	The association between fecal enterotoxigenic *Bacteroides fragilis* with colorectal cancer

NO.	LastName	FirstName	Name	Date	Title
24	Madani Borugeni	Mahtab Alsadat	11:10-11:20	Second Day 8/28/2019	The effect rates of Staphylococcus bacteriophages on resistance clinical isolates of *Staphylococcus aureus*
25	Mehravaran	Ahmad	14:10-14:20	Second Day 8/28/2019	Encapsulation of Imiquimod Adjuvant and Soluble *Leishmania* Antigen into Liposomes as a Vaccine in the Cutaneous Leishmaniasis Model
26	Mehrzad	Jalil	11:00-11:10	Second Day 8/28/2019	Investigation on the effects of *Salmonella enterica* Subsp enterica serovar Typhimurium on the apoptosis/necrosis of avian macrophage-like monocytes
27	Mohammadi	Nagar	16:40-16:50	Second Day 8/28/2019	Frequency of *Gardnerella vaginalis* in patients with vaginosis in Isfahan by molecular method
28	Motamedifar	Mohammad	16:00 - 16:20	Second Day 8/28/2019	Epidemiology, bacterial load, antibiotic susceptibility and risk factor of toxigenic Clostridium difficile in hospitalized patients in southwestern Iran
29	Nakhaee	Sedigheh	8:10-8:20	Third Day 8/29/2019	Dissemination pattern of multidrug resistant Carbapenemase Producing Klebsiella pneumoniae Isolates in Nemazee and Faghihi Referral hospitals in Shiraz, Southwestern Iran
30	Odooli	Salman	16:50-17:00	Second Day 8/28/2019	Selective plate count and tuf gene-based qPCR methods for quantification of *Bifidobacterium animalis* subsp. lactis BB-12 in commercial probiotic yoghurts
31	Pardakhti	Abbas	8: 55 - 9:20	Second Day 8/28/2019	Lipid vesicular drug delivery systems in treatment of intracellular infection: capabilities and challenges

NO.	LastName	FirstName	Name	Date	Title
32	Rahmani	Maryam	10:00-10:10	Third Day 8/29/2019	Determination and comparison of antibacterial activity of *Enterococcus* species isolated from breast-fed neonates and adults feces in Kerman
33	Rajabi	Zahra	11:10-11:20	Third Day 8/29/2019	Study the effect of extracted bacteriocin of Lactobacillus fermentum with probiotic potential on biofilm formation of isolated Streptococcus mutans from dental caries
34	Saberi Shahmarvandi	Tayebeh	10:10-10:20	Third Day 8/29/2019	Investigation of the quantity of crude oil degrading bacteria in contaminated areas in Masjed Soleyman
35	Sadeghi	Javid	16:50-17:00	First Day 8/27/2019	Frequency of *Staphylococcus aureus* isolates collected from men semen and women endocervix and detection of antibiotic resistance
36	Saedi	Samaneh	11:20-11:30	First Day 8/27/2019	Multilocus Sequence Analysis (MLSA) of *Mycobacterium tuberculosis* from Clinical Isolates in Northeast of Iran
37	Serajian	Sahar	11:10-11:20	First Day 8/27/2019	Prevalence, antimicrobial susceptibility, serotyping and virulence determination of *Listeria monocytogenes* strains at a tertiary care hospital in Tehran, Iran
38	Tavakoli	Razieh	9:30-9:40	Second Day 8/28/2019	The role of resistance genes expression in meglumine antimoniate non-healing and healing isolates of anthroponotic cutaneous leishmaniasis due to *Leishmania tropica*
39	Zarei Mahmoudabadi	Ali	11:00-11:10	First Day 8/27/2019	The potency of Luliconazole, against Clinical and Environmental *Aspergillus Niger* Complex

Topic: Anaerobic Infections

Oral immunization with Lactobacillus casei vectored vaccines surface-expressed Clostridium perfringens toxoids

Mojtaba Alimolaei1*, Mehdi Golchin2
1Department of Research and Technology, Kerman branch, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Kerman, Iran
2Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

Introduction and Objectives: Clostridium perfringens is a bacterium commonly found in the intestines of humans and animals and causes food poisoning. In some conditions resident bacteria may grow rapidly and produce large amounts of toxins that damage the intestines, facilitating the absorption of toxins to the bloodstream and make toxemia. Vaccines and antitoxins can protect against these toxins. As a step toward developing recombinant oral vaccines, we have explored the feasibility of surface expression of alpha, beta, and epsilon toxoid genes from C. perfringens by Lactobacillus casei.
Materials and Methods: Genetically engineered toxoid genes of α, β and ε toxins were synthesized, cloned in pT1NX expression vector and electroporated into L. casei ATCC 393. Expression of recombinant toxoids on the surface of L. casei was evaluated by immunoblotting, ELISA, and confirmed by immunofluorescence microscopy. The safety and efficacy of these recombinant vaccines were evaluated in response to challenge with different Minimum Lethal Dose (MLD) of toxins.
Results: The results indicated toxoids were expressed well and general and mucosal immune responses elicited by these vector vaccines were higher than those control groups.
Conclusions: In conclusion, these constructed vaccines are good candidates for stimulation of both mucosal and humoral immunity against C. perfringens lethal toxins. Expression of C. perfringens antigens by L. casei makes it possible to study these recombinant strains as oral vaccines to prevent Clostridial infections.
Keywords: Clostridium perfringens, Lactobacillus casei, Toxoid, Oral Immunization

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Topic: Anaerobic Infections

Prevalence of Clostridium novyi in slaughterhouses of Alborz province using traditional methods and Polymerase Chain Reaction

Ali Rezaee Kalvani[1*], Lida Abdolmohammadi khiav[1], Alireza Paradise[1]
Department anaerobic bacterial vaccine Production and Research, Clostridia Research laboratory, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.

Introduction and Objectives: *Clostridium oedematiens* or *novyi* is classified into several types which are pathogenic for human and animals. *C. novyi* type B is the causative agent of black disease in ovine and occasionaily in bovine. Bacteria produce a large amount of toxin such as alpha, beta, gamma, delta, epsilon and zeta toxins. Therefore, the detection of toxin and isolation of bacteria from gastrointestinal contents are used to diagnose of disease. In order to effective control of disease, the frequency of *C. novyi* isolates from infected animals is essential in IRAN. The aim of this study was to determine the prevalence of *C. novyi in* slaughterhouses of Alborz province.

Materials & Methods: In this study, 386 liver samples were collected from the slaughterhouses, then the bacteriological test (including culture in Blood agar and special medium, motility and gram staining) and biochemical tests (including Fermentation of sugars, lecithinase, lipase, gelatinase, Indol, milk digestion and catalase) were used for characterisation and typing, the samples were confirmed using PCR. So, the Forward and Revers primers were designed using toxin-alpha sequences.

Result*:* The results of this study showed that prevalence of *C. novyi* in the slaughterhouses was 37 (9.5%) that 33 cases (89.18%) had concurrent contamination with *C. novyi* and *Fasciola*, and were identified only in 3 cases without *Fasciola* infection.

Conclusion*:* Due to the heavily economic losses, vaccination is very urgent for prevention of disease.

Keywords: *Clostridium novyi*, PCR, Diagnosis, toxin-alpha, Vaccination

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Topic: Antimicrobial Resistance

The potency of Luliconazole, against Clinical and Environmental Aspergillus Nigri Complex

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Introduction and Objectives: Black aspergilli are, the most causes of otomycosis and Aspergillus niger and A. tubingensis are two more frequently isolates. Although, amphotericin B was a Gold standard for the treatment of invasive fungal infection for several decades, it replaced by fluconazole and /or voriconazole. Luliconazole, appears to offer the potential for in vitro activity against black aspergilli. The aim of the present study was to compare the in vitro activity of a novel antifungal agent, luliconazole, with commonly used antifungals against clinical and environmental strains of black aspergilli.

Materials and Methods: Sixty-seven strains of black aspergilli were identified using morphological and molecular tests (β-Tubulin gene). Antifungal susceptibility test was applied according to CLSI M38 A2. The results were reported as minimum inhibitory concentration (MIC) range, MIC_{50}, MIC_{90} and MIC_{GM}.

Results: It was found that the lowest MIC range, MIC_{50}, MIC_{90}, and MIC_{GM} was attributed to luliconazole in clinical strains. Aspergillus niger was the common isolate followed by, A. tubingensis and 54.1% (clinical) and 30% (environmental) of isolates were resistant to caspofungin. The highest resistant rate was found in amphotericin B for both clinical (86.5%) and environmental (96.7%) strains. Clinical strains of Aspergillus were more sensitive to voriconazole (86.7%) than environmental strains (70.3%). On the other hand, 83.8% of clinical and 70% of environmental isolates were resistant to posaconazole, respectively.

Conclusion: In conclusion, luliconazole compare to routine antifungals is a potent antifungal for A. niger complex in vitro. The MIC range, MIC_{50}, MIC_{90} and MIC_{GM} of luliconazole against black aspergilli were the lowest among the representative tested antifungals.

Keywords: Black aspergilli, Luliconazole, Clinical and environmental isolates, Antifungal profile

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Topic: Antimicrobial Resistance

The effect rates of Staphylococcus bacteriophages on clinical isolates of Staphylococcus aureus

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Introduction and objective: *Staphylococcus aureus (S. aureus)* is considered one of the most important pathogens, responsible for nosocomial infections. Many clinical isolates are resistant to common anti-bacterial drugs. The widespread resistance to antibiotics push microbiologists to seek alternative therapeutic methods to replace with ordinary methods for preventing and therapy of bacterial infection. So, phage therapy and their potential in killing of bacteria at the end of infectious cycle are becoming more interesting. Phage therapy could be used as a chemotherapy alternative method. The aim of this study was to isolate and identify phages which had lytic effect on MRSA bacteria.

Materials and Methods: *S. aureus* were isolated from blood, sputum, urine, ear, eyes, nose, abscess, pleural fluid, peritoneal fluid, Broncho Alveolar Lavage, trachea, throat, wound secretions samples, and hospital environment of two education hospitals in Isfahan and Shiraz, Iran. The resistance to methicillin was measured and determined by disk diffusion method and *mecA* gene, was detected by PCR method. *Staphylophages* were isolated from Isfahan urban sewage samples. Double Layer Agar method, was used to detection of lytic phages and the developing of plaque, was considered the sign of destructive phage against S. aureus. The identification of phages was carried out based on morphology characteristics by TEM images.

Results: Out of 133 samples, 92.4% S. aureus isolates were Methicillin resistant which 88.7% of them carried *mecA* gene. Fifty-eight samples were lysed by isolated phage cocktails and produced plaques. They were belonged to families: *Siphoviridae*, *myoviridae*, *Tecticoviridae*, *Corticoviridae*, and *Microviridae*.

Conclusion: Different bacteriophages in four phage cocktails had good lytic effects on *S. aureus* clinical isolates. As using of phage cocktails may have potential to be an alternative chemotherapy specially against antibiotic resistant pathogenic isolates, it is necessary to study lytic effects in animal models.

Keywords: Bacteriophages, Phage therapy, *Staphylococcus aureus*, MRSA, *mecA*

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Topic: Antimicrobial Resistance

Dissemination of incompatible plasmid groups among " Klebsiella pneumoniae superbug" strains harboring New Delhi metallo-lactamase-1 gene, Kerman, Iran

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Introduction and objectives: New Delhi metallo-lactamase NDM– producing *K. pneumoniae* (CRKP) strains defined as superbugs. So far, twenty-one variants of bla_{NDM} gene have been described. The bla_{NDM-1} gene, is on plasmid incompatibility (Inc) groups including IncX, IncH, IncFII, IncL/M, IncN, IncR, and IncHIB -M/FIB-M.

Materials and Methods: this study was done on 37 clinical sample of non-duplicative NDM-producing *K. pneumoniae* strain. The bacterial isolates analyzed by PCR-sequencing and BioEdit 7.0.0, Lasergene 6 and MEGA 7.0 saftwares. After determination of of bla_{NDM} variants PCR-based replicon typing (PBRT) methods (5 multiplex PCR and 3 simplex PCRs) used for further evaluation of 18 distinct replicons including incompatible plasmid of FIA, FIB, FIC, HI1, HI2, I1-Ig, L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FIIA. Randomly Amplified Polymorphic DNA (RAPD) typing used for Strains clustering and molecular typing

Results: all 37 NDM-carrying strains had variant of bla_{NDM-1} gene which were on the conjugative plasmid. The frequency of plasmids were as ,FrepB (n=25[67.5%]), FIIA(s) (n=11[29.7%]), FIA (n=5[13.5%]) ,FIB(n=3[8.1%]) and for, I1-Ig, L/M, A/C , Y ,P and FIC plasmids reported as (n=2[5.4%]), (n=7[18.9%]), (n=7[18.9%]) ,(n=3[8.1%]),(n=1[2.7%]) and (n=1[2.7%]) respectively. In this study a strains was found that simultaneously carried I1, L/M, Y ,FIC, A/C and FIIS plasmids . Based on typing by RAPD method, our strains were divided into clusters 1 to 5. According to phylogenetic tree construction six strains belonged to cluster 1, five strains to cluster 2, eleven strain to clusters 3, six strains to cluster 4 and finally six strains to cluster 5

Conclusion: The success of the bla_{NDM-1} gene can not be attributed to a particular plasmid and a specific genus of bacteria. The results obtained from this is and other similar studies show that bla_{NDM-1} gene located on a wide range of conjugative incompatible plasmid group that leading to quick spread of bla_{NDM-1} gene among *Enterobacteriaceae* and other genus such as *Pseudomonas* and *Acinetobacter* hence considering the fast rise of superbugs and the danger they pose to human health worldwide, it is required to precisely monitor the molecular epidemiology of the bla_{NDM-1} gene as a powerful tool in superbagging, to make better counteractions and prevent their spread.

Keywords: bla_{NDM}, incompatible plasmid, superbugs, PBRT, RAPD-PCR

Topic: Antimicrobial Resistance

Frequency of multi drug resistance and molecular charachteristics of resistance to colistin among Acinetobacter baumannii isolated from hospitalized patients in ICU of Qazvin& masih daneshvari hospital, with ventilator associated pneumoniae

Masoumeh Aslanimehr

Introduction and Objectives: Antibiotic resistance is increasing among bacterial agents causing hospital infection. Acinetobacter Baumannii has been considered as a pathogenic organism with multiple antibiotic resistance (MDR) . Acinetobacter is the most common cause of pneumonia in patients who have been using the respiratory duct for more than 5 days. Today, Acinetobacter Baumannii is considered as one of the major pathogens in the ICU, which can be life-threatening due to the high drug resistance of this organism. Therefore, in this study, we evaluated the frequency of multiple antibiotic resistance and molecular properties of Clistin resistance in Acinetobacter Baumannii isolated from patients with pneumonia associated with ventilator admitted to special intensive care units of Masih Daneshvari Hospitals and hospital.

Materials and Methods: In this study, 200 isolates of Acinetobacter Baumanni I were collected from Bronchoalveolar lavage and aspiration tracheal of patients admitted to the ICU of Messiah Daneshvari and Qazvin hospital, with vap from 2011 to 2019. All isolated isolates were identified by conventional biochemical methods and then identified by the proliferation of the OXA-51 gene.

Determination of susceptibility of strains to different antibiotics by disk diffusion method for imipenem, ciprofloxacin, colistin, TG cyclin, Gentamycin, Amikacin, co trimoxazol, Piperacillin, Piperacillin-tazobactam,Cefotaxim, Ceftazidim, Cefepim disks. determination of antibacterial sensitivity to colistin by broth microdilution ,sequenced for mutation analysis in the Pmr CAB,mcr1 gens.

Result : Bacterial isolates were collected from clinical specimens of broncho alveolar lavage (32%) and tracheal (84%). Most of the samples in this study were isolated from Tehran (Mashhad Daneshvari Hospital), Among the 200 isolates of Acinetobacter baumannii 199 (99/5%) isolates were XDR, 1 (0/5%) isolates was PDR. According to our results the higher antimicrobial resistance rates were Imipenem, Ciprofloxacin, TG cyclin, Gentamycin, Trimetoprim – sulfometoxazol,Amikacin,Piperacillin, Piperacillin-Tazobactam, Cefotaxim, Ceftazidim, Cefepim (100%). In this study, MIC was reported at concentrations of 0.5-32.

80% of the isolates were sensitive to 0.5 µg / ml concentration and responded to colistin antibiotic. Consequently. The result of sequencing of PCR products by matching the NCBI site and valid molecular databases on the genes studied shows, as shown, the confirmation of mutation in the PMR CAB gene. The result of sequencing of PCR products by matching the NCBI site and valid molecular databases on the genes studied shows, as shown, the confirmation of mutation in the PMR CAB gene & The presence of the plasmid MCR1 was not confirmed.

Due to the presence of Colistin-resistant Acinetobacter isolates in the samples and its increased MIC level, it shows a change in the pattern of resistance of these strains to colistin. Therefore, more careful monitoring and administration of antibiotics along with improved infection control measures It is essential in health systems to prevent the spread of resistant strains. Therefore, greater monitoring and control over the administration and use of antibiotics along with the improvement of infection control measures in health systems is necessary to prevent the spread of resistant strains.

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Topic: Antimicrobial Resistance

Dissemination pattern of multidrug resistant Carbapenemase Producing Klebsiella pneumoniae Isolates in Nemazee and Faghihi Referral hospitals in Shiraz, Southwestern Iran

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Introduction and Objectives: *K. pneumonia* is one of the most important causes of health care associated infection. Carbapenemases have increasingly been reported in Enterobacteriaceae, especially in K. pneumonia. The objective of this study was to determine antibiotic resistance patterns, and molecular epidemiology of multi-drug resistant *K. pneumoniae* isolates obtained from hospitalized patients in Shiraz, Iran.

Materials and Methods: In this study, 60 K. pneumonia isolates were collected from Nemazee and Faghihi referral hospitals. Antibiotic susceptibility testing and MIC were performed by disk diffusion test and E-test, respectively. Carbapenemases genes were identified by polymerase chain reaction and sequencing. Then, clonal relationships were analyzed using PFGE.

Result: 33 out of 60 *K. pneumonia* isolates were resistant to carbapenems. Among the isolates, 86.6% were MDR (Multi Drug Resistant). Polymyxin B (18.3%) and tigecycline (23.3%) was shown to be the most active agent against *K. pneumonia* isolates. In our study, the high prevalence of bla_{NDM} (45%) and bla_{OXA48} (10%) was detected. PFGE analysis showed 11 clusters and 45 pulsotypes (PTs) based on an 80% similarity level.

Conclusion: The results of this study revealed that the carbapenemase genes are often located on the plasmids, so it may be the reason of the spread of them. Also, PFGE analysis showed that there we similar genetic patterns among *K. pneumonia* isolates and these patterns were responsible for dissemination of infection in hospitals.

Keywords: Klebsiella pneumonia, pulsed field gel electrophoresis (PFGE), Carbapenemases.

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Topic: Antimicrobial Resistance

Comparison of prevalence of Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) among Staphylococcus aureus isolates in a burn unit with non-burning units in Isfahan, Iran

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Introduction and Objectives: *Staphylococcus aureus* is one of the most important pathogens in burn infections that can be colonized in the nose and increase the risk of infections.

Materials and Methods: A total of 85 *S. aureus* isolates were isolated from clinical and nasal hospitalized patients and health care workers (HCWs) in a burn unit and non-burn units. Genes encoding penicillin binding protein 2a *(mec*A) and adhesive surface proteins, including fibronectin binding proteins (*fnbA, fnbB*), fibrinogen binding protein (*fib*), laminin binding protein(*eno*), collagen binding protein (*cna*), elastin binding protein *(ebps),* intracellular adhesion operon (*ica*A and *ica*D) were detected using PCR method.

Results: The rate of methicillin-resistant *S. aureus* (MRSA) among burn and non-burn isolates were 62% (18/29) and 25% (14/56), respectively. The most prevalent MSCRAMMs genes in burn units were *eno* (86%) and *fib* (66%). The most common gene pattern in burn center was *ica*A+*fib*+*eno.* The frequency of *ica*D, *fib* and *ebp*S was higher in clinical samples than nasal samples. No relation was found between the MSCRAMMs genes in the burn unit and non-burn units.

Conclusion: The high prevalence of MRSA in burn center can be a new challenge for clinicians. The higher frequency of *ica*D**,** *fib* and *ebp*S in clinical isolates than nasal isolates may reflect the important role of these genes in colonization and pathogenesis of *S. aureus*.

Keywords: Staphylococcus aureus, MRSA, Surface Proteins, MSCRAMM proteins

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Topic: Biological Products

Designing a novel ELISA method based on CagA, NapA recombinant antigens to increase sensitivity and specificity of Helicobacter pylori whole cell antigen detection

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Introduction and Objectives: *Helicobacter pylori* infection as the worldwide problem is related to many gastrointestinal disorders such as gastritis, gastric cancer, non-ulcer disease, peptic ulcer disease and duodenal ulcer.

Materials and Methods: We produced and purified recombinant *CagA* and *NapA* antigens in *Escherichia coli* and extracted their antibodies from a panel of positive sera specimens. We designed a novel enzyme linked immunoassay direct method in combination with the whole cell for the qualitative and quantitative detection of *Helicobacter pylori* antigens in human stool. Assay performance was evaluated by histopathology staining and urease activity.

Results: The sensitivity and specificity of assay was determined as 91.7 [95% confidence interval: 89.3-95.6%] and 93.1% [95% CI: 91.2-96.4%], respectively. Novel ELISA exhibits enhanced sensitivity and specificity of *Helicobacter pylori* detection in comparison with another commercially available kit.

Conclusion: Combination of the recombinant antigens and whole cell of *Helicobacter pylori* in immunoassay designing is a new approach about early diagnosis, treatment and fallowing up of the *Helicobacter pylori* infected patients, especially in peptic cancer cases.

Keywords: *CagA* protein, *Helicobacter pylori***,** Neutrophil activating protein A, Enzyme-linked immunosorbent assay.

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Topic: Biotechnology

Optimization of biosurfactant production by an oil-degrading Bacillus isolated from petroleum-contaminated soil

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Introduction and Objectives: Chemical surfactants are used in various industries. they are toxic and low degradability. biosurfactants are potentially used in many commercial applicationsins. because of their low toxicity, environmental compatibility can be a good alternative to surfactants, but due to the high cost of production and low production rates, they can not compete with chemical surfactants in commercial use. Therefore, finding new bacteria with a higher production rate in a shorter time, optimizing fermentation conditions to increase production and reducing final costs is very important. The main objective of this study is to evaluate the effect of some physical and chemical conditions on the duration and amount of biosurfactant production in the in-vitro condition.

Materials and Methods: Two-level fractional factorial design was employed to identify the most important chemical composition and physical factors affecting on biosurfactant production. The investigated factors were divided into two categories Physical factors included: temperature and chemical factors including: glucose (%), glycerol (v/v %), olive oil(v/v %) as a carbon source, yeast extract (g/l), peptone(g/l), NaNO3 (g/l) as a source of nitrogen and ILCO percentage (v/v %).According to the fractional factorial design, nineteen experiments were performed regarding one replicate and three center points. Then the results were analyzed with the Design Expert 7.0.0 package.

Results: The highest amount of biosurfactant production was observed at temperature 25 c° , glucose (1.25 %), glycerol (3.3% v/v), olive oil (1.6 %v/v) , yeast extract (1.2 g/l), peptone(2 g/l), NaNO3 (0.6 g/l) and ILCO percentage (0.5%v/v) And the production time decreased from 10 days to 32 hours.

Conclusion: The result of this study indicated the optimization of the conditions of cultivation on the production and duration of the production has a significant effect. Therefore, this study is very important in research on biosurfactants.

Keywords: Biosurfactant, Biodegradation bacteria, Optimization, Experimental design

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Topic: Biotechnology

Comparing structure and conformation of diphtheria toxin with its non-toxic mutant (E349K) at 300K using molecular dynamics simulations

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Introduction and Objectives: The molecular dynamic simulations have provided detailed information on the fluctuations and conformational changes of proteins. Mutation of GLu349 of diphtheria toxin (DT) to Lys inhibits the molecular cytotoxicity in mammalian cells. In this work, we aim to evaluate the effect of the mutation on the structure and the conformation of DT using the molecular dynamic simulation.

Materials and Methods: The protonation fixing process was done with the diphtheria toxin types, wild and mutant (E349K) to prepare in the natural pH (6.5) by using H++ server which was the input for molecular dynamics simulation the molecular dynamics simulations were done by GROMACS 4.5.4 package.

Result: The results of our analysis indicated that amino acid residue fluctuations in catalytic domain(C) of DT are more than those its mutant (E349K). Also, residue fluctuations in the region including Ile344-val347, Tyr514-Ser525 and Ile533-Lys534 of DT were more than those of the mutant (E349K). However, residue fluctuations in the region including Cys186-Cys201 and Glu349-Val351 of DT were less than those of E349K. In addition, the disulfide bridge (Cys186–Cys201) formed in DT, whereas it was not observed in the mutant. The secondary structure analysis showed that the beta sheet content of E349K decreased compared with DT. Also, the conformation of DT was different from that of E349K in the hinge loop regions (Ala379–Thr386 and Tyr514-Ser525). The radius of gyration (Rg) and the root mean square deviation (RMSD) of E349K were more than those of DT.

Conclusion: The conformational stability and compactness of DT are more than those of E349K. This method can be used to evaluation conformational change of toxins and proteins as well as vaccine candidates. Because it demonstrates structural details of the toxins that is an important aspect for predicting of activities of the receptor binding and the catalytic domains.

Keywords: Theoretical biology; Secondary structure; Targeted mutagens

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Topic: Food Microbiology

Selective plate count and tuf gene-based qPCR methods for quantification of Bifidobacterium animalis subsp. lactis BB-12 in commercial probiotic yoghurts

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Introduction and Objectives: Development of easy, accurate, and rapid procedures for qualitative and quantitative control of probiotic products is necessary since probiotic bacteria require a minimum concentration of 10^6 CFU/ml to exert their beneficial effects. Traditional culture-dependent methods have some limitations and disadvantages. The majority of quantitative real-time PCR (qPCR) procedures, as an alternative culture-independent method, are based on the quantification of the 16S rRNA gene. However, the quantitative data obtained by 16S rRNA-based qPCR are ambiguous because the 16S rRNA genes are present in the bacterial genome in multiple copies.

Materials and Methods: A new specific primer set targeting a highly conserved sequence of the single-copy *tuf* gene was designed for *Bifidobacterium animalis* subsp. *lactis* BB-12 and its specificity was evaluated through PCR reactions with DNAs extracted from prevalent probiotic Bifidobacterial and Lactobacilli strains. *Tuf* gene-based qPCR assay was developed for the quantification of BB-12. Finally, BB-12 was detected and enumerated through *tuf* gene-based PCR, tuf gene-based qPCR, and selective plate count during shelf life and after the expiry date of commercial probiotic yoghurts.

Results: Designed *tuf* gene-based primer set was specific for BB-12. The obtained standard curve of tuf gene-based qPCR reactions from 10^4-10^9CFU/ml was linear (R2=0.98) with the efficiency of 90.4%. Significantly decrease in the BB-12 counts was observed through both selective plate count and qPCR methods during shelf-life. However, these counts were according to the CODEX standard (10^6 CFU/ml) until the expiry date. The BB-12 count decreased rapidly and fell below the CODEX standard after the expiry date. Totally, significant differences were observed between the BB-12 counts derived from the qPCR and selective plate count, so that the bacterial counts obtained with the qPCR were higher than selective plate count.

Conclusion: Despite the fact that the new single-copy *tuf* gene-based qPCR assay developed here is a specific, rapid, and easy method for quantification of both cultivable and dormant BB-12 cells, it does not distinguish dead and viable cells. Moreover, selective plate count method doesn't quantify dormant bacterial populations. We deduce that the choice of enumeration method for probiotic bacteria may have a significant effect on the results of the analysis. So, qPCR assessments can serve as a complementary procedure for culture-based methods, and traditional cultural methods must still be used as a complementary golden standard for molecular approaches. For the sake of equitable judgment, we propose the use of Propidium monoazide (a DNA-intercalating dye that can selectively enter dead cells, covalently bind to DNA, and inhibit the PCR) in combination with single-copy based qPCR (PMA-qPCR), and subsequently comparing obtained results with the respective single-copy based qPCR and selective plate count.

Keywords: *Bifdobacterium* BB-12, Quantification, *tuf* gene, qPCR, Plate count, Probiotic yoghurt, Shelf-life.

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Topic: Fungal Infections

Molecular identification of Candida species, assessment of the antifungal susceptibility and the genetic relationship of Candida albicans isolated from immunocompromised patients in Kerman, Iran

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Introduction and Objectives: The goal of this study was to identify the *candida* isolates to the species level using conventional as well as molecular methods and to assess the in vitro susceptibility of *C. albicans* isolates.

Materials and Methods: A total of 80 clinical samples of immunocompromised patients were collected. Yeast isolates were identified to the species level using conventional as well as PCR-RFLP methods. Also, four primers were used for RAPD analysis of *C. albicans* strains. All *C. albicans* isolates were tested for their in vitro susceptibility to the Fluconazole, Itraconazole, Amphotericin B and Nystatin according to the CLSI M27-A3 standard.

Results: Of the sixty-one *Candida* isolates, the most common species was *C. albicans* (34.42%), followed by *C. glabrata* (24.59%), *C. parapsilosis* complex (18.03%), *C. krusei* (14.75%), *C. kefyr* (3.27%), *C. lusitaniae* (3.27%) and *C. dubliniensis* (1.63%). RAPD-PCR results indicated *C. albicans* isolates allocate into three clusters (A, B, C) with higher than 80% homology level. The antifungal susceptibility results suggest that *C.albicans* isolates are the most susceptible to Amphotericin B (100%) followed by Itraconazole (90.47%).

Conclusions: Our funding indicated that identification to species level is important for choosing proper antifungal treatment mainly in immunocompromised patients. We also could observe that RAPD assay was able to identify genetic variability among *C. albicans* isolates.

Keyword: *Candida* spp., RFLP-PCR, RAPD-PCR, Antifungal susceptibility, Iran.

Topic: Industrial and Applied Microbiology

Bioconversion of genistein to orobol by spore display tyrosinase

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Introduction and Objectives: Flavonoids are compounds with low molecular weight, which consist of 15 carbons with a structure of C6-C3-C6, and two benzene rings that are bonded together by carbon chain. Isoflavones are a group of flavonoids with a limited distribution in plants, especially in *Papilionoideae* subfamily. Soybeans are the main source of isoflavones. Genistein is the simplest isoflavone and Soybean is a rich source of this compound. Genistein (4´,5,7-trihydroxyisoflavone) comprises two phenolic rings linked to a three-carbon bridge, which in this case is an oxygenated heterocyclic ring with three hydroxyl groups at carbons 4´, 5, and 7. Genistein has a wide range of biological activities such as antioxidant, anti-inflammatory, anticancer and antimicrobial effects. Bioconversion is a process which transforms a molecule into another molecule with new features. In this paper, we transformed genistein to orobol by spore displayed tyrosinase. Tyrosinase is an oxidoreductase enzyme which catalyze hydroxylation of a monophenol to diphenol and oxidation of diphenol to the corresponding quinone.

Materials and Methods: Genetically modified *Bacillus subtilis* DB104 (pSDJH-cotE-tyr) was used for bioconversion of genistein to orobol. The production of orobol from genistein was confirmed by TLC and HPLC. The different concentrations effect of genistein and orobol were investigated on MCF-7 cancer cell line and the anticancer effect was determined by flow cytometry and MTT tests.

Results: MTT test demonstrated concentrations of 300 to 500 µM of orobol had more inhibitory effect on MCF-7 cells than genistein in the same concentrations. Flow cytometry analysis showed genistein and orobol in 500 µM had 80 and 87 percent inhibitory effect respectively.

Conclusion: In contrast to genistein, orobol is not a natural compound found in nature. Spore displayed tyrosinase converts genistein (4´,5,7-trihydroxyisoflavone) to orobol (3´,4´,5,7-tetrahydroxyisoflavone) with more anticancer effect by adding a hydroxyl group on 3´ carbon.

Keywords: Orobol, Genistein, Spore displayed tyrosinase, MCF-7 cells

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Topic: Industrial and Applied Microbiology

Investigation of the quantity of crude oil degrading bacteria in contaminated areas in Masjed Soleyman

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Introduction and Objectives: Today, petroleum products such as gasoline and petrol have produced high levels of environmental pollution in comparison with other chemicals and through accidents, oil industry activities in the sea and in the coast, exploration and storage of fuel, hydrocarbons It is released into the environment and causes oil pollution. Biodegradation of Crude oil contaminants by bacteria is an important economic approach to the revival of oil-polluted sites.

Materials and Methods: Sampling from 7 contaminated areas including Bibiyan and the foreign school in Masjed Soleyman. After determining the abundance of bacterial populations by CFU and MPN methods and enrichment in Bushnell-Hass medium, isolation and identification of bacteria were performed using biochemical and molecular methods. And using the 16S rRNA sequence, the top strain of the parser was approved.

Results: After two weeks of screening for strain E belonging to the foreign school district and the D-strain belonging to the Bibiyan district, they respectively belonged to the genus *Arthrobacter citreus* and *Rhodococcus jostii*, which were identified as the superior degrading bacteria. The superior degradation after the sequential treatment reduced the decomposition time from ten days to two days. Crude oil degradation was reported in a mixture of more than 90%.

Conclusion: The mixed cultures of these bacteria with the help of nutrients showed a high ability to remove Crude oil. The frequency of bacteria in the infected areas is high and by identifying the genes and enzymes involved in the analysis of the genetic manipulation potential of these strains and the transfer of active genes to other indigenous bacteria in the region to facilitate and accelerate degradation.

Keywords: Bacteria, Biodegradation, Oil pollution, Crude oil

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Topic: Microbiota and Probiotics

Determination and comparison of antibacterial activity of Enterococcus species isolated from breast-fed neonates and adults feces in Kerman

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Introduction and Objective: The genus *Enterococcus*, previously recognized as fecal *stereptococcus*, are microbiota of the intestine of human and other animals. They usually exerts anti-Listerial activities, in addition their presence in breast milk helps maintain the baby's health. This bacterium is one the first bacteria colonized in the neonate intestine and meconium, it could emphasize on its importance. In this study we aim to determine the antibacterial activity of *Enterococcus* spp. from fecal sample of neonates and healthy adults with comparison of their activities on 10 different standard bacterial strains.

Material and Method: Totally, 87 *Enterococcus* strains were collected from feces samples of breast-fed neonates aged 3 to 7 days and healthy adults that had not used any antibiotics or probiotic products. Conventional methods were used for bacterial identification. Antibacterial testing was performed through spot test bilayer against *staphylococcus aureus*, ATCC25923 *listeria monocytogenes* CCUG 15527, *Enterococcus Fecallis* PTCC 1237, *pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, Enteropathogenic E. coli (EPEC) ATCC1269 bfp, *E.coli* O157.H7 NCTC 1290, Enterotoxigenic E. coli (ETEC*)* H10407, Enteroinvasive E. coli (EIEC) 85b, *klebsiella pneumoniae* ATCC 70063. Data of antimicrobial effects against indicator pathogens were recorded according to the following scale: no inhibition zone (-), inhibition but no clear zone (1≥ , ±).weak inhibition zone (1 to 2, +).good inhibition zone (2 to 4, ++). high inhibition zone(4<, +++).

Result: *E.facium* was the most commonly isolated species followed by *E.fecallis*. Many isolated exhibited inhibitory activities against pathogenic strains. More than 50% of the *Enterococcus* isolate had good and high antibacterial properties against *L. monocytogenes*, EPEC, *E.coli, E.fecalis* and *P. aeruginosa*. ETEC was the least inhibited pathogen. *Enterococcus* isolated from neonates had significantly higher antibacterial properties than those isolated from adults (p-value≤ 0.05).

Conclusion: *Enterococcus* isolates have good and high inhibitory activity especially against *L. monocytogenes* and at a lower level against EPEC, *E.coli, E.fecalis* and *p. aeruginosa*. More *in vitro* and *in vivo* clinical data is needed before any conclusion on the probiotic properties of the isolates could be drawn.

Keyword: neonate, adult, enterococcus, antibacterial effects

Topic: Microbiota and Probiotics

Study the effect of extracted bacteriocin of Lactobacillus fermentum with probiotic potential on biofilm formation of isolated Streptococcus mutans from dental caries

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Introduction and Objectives: Dental caries is one the most common disease in each person especially in children and having a lot of economic burden for people. Streptococcus mutans is the major organism in dental caries. and despite all the efforts used in the production of oral hygiene products , tooth decay is still in force. So we study the inhibitory effect of extracted bacteriocin of probiotic Lactobacillus fermentum on biofilm formation of isolated Streptococcus mutans from dental caries.

Material and Method: Lactobacillus fermentum isolated from some traditional food and their probiotic potential was done by heat ,acid and bile tolerance tests and molecular confirmation test done by sequencing of PCR product of 16SrDNA gene . Bacteriocin of isolated Lactobacillus fermentum extracted by partial purification with ammonium sulfate . Molecular weight of bacteriocin was assayed by sds-page.

Streptococcus mutans from dental caries was isolated by biochemical methods and molecular confirmation done by PCR for specific gene gtfD.

Studying the biofilm formation of Streptococcus mutans and inhibitory effect of bacteriocin , assayed by TTC(Triphenyl Tetrazolium Chloride) in 96wells-diffiusion plate.

Results: 3(13%) strains of 23 isolated Lactobacillus fermentum from traditional food had probiotic potential . Partial purification with ammonium sulfate showed , 2 isolated of probiotic Lactobacillus fermentum had potential for producing bacteriocin. Extracted bacteriocin had 63 and 35 Da molecular weight respectively.

Out of 100 samples of decayed dental, 39(39%) Streptococcus mutans was isolated . All of them could able to form biofilm. Results shown that extracted bacteriocin can decrease the biofilm formation each seperately and had similar effect with each other. Cumulative of two bacteriocin had no significant effect compared to individual mode.

Conclusion: Due to reducing role of bacteriocin on biofilm formation, using of them as natural product in dental hygiene sanitation can be effective and useful and it can play a significant role in reducing the cost of treatment for dental caries.

Keywords: Probiotic, Bacteriocin, Biofilm, Streptococcus mutans, Dental caries

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Topic: Molecular Diagnosis

Identification of Non-Tuberculosis Mycobacterium species in suspected patients to tuberculosis by Line Probe Assay method

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Introduction and Objectives: The genus Mycobacterium consists of over 150 species and except than *M. tuberculosis* complex and *M. leper* the other species known as Non-Tuberculosis Mycobacterium (NTM). NTM infections are increasing due to increase in immune deficient individuals in population. Isolation and identification of NTMs by conventional methods is time consuming and low efficient. New Molecular methods, such as Line Probe Assay (LPA) are efficient tools for rapid and accurate detection of NTMs. By using this method identification of NTMs can be performed under 14 days and this would be a good progress in treatment of life threating NTMs infections in immune compromised patients.
Materials and Methods: Clinical samples including: sputum, BAL, blood, CSF, soft tissue and wound samples from suspected patients to mycobacterial infections were transferred to reference tuberculosis laboratory. Decontamination was performed by Petroff method. Culture was performed on LJ medium and after incubation in 37°c for 10 days. Conventional methods such as pigment production, growth rate, tween 80 hydrolysis, quantitative catalase, nitrate hydrolysis and niacin production were used for identification of NTMs. Bacterial colonies of culture positive samples were collected and suspended in 1x TE buffer. DNA extraction was performed by using DNA extraction kit. LPA was performed according to LPA kit manual.
Results: NTMs rate among total samples was 1.1% and totally 40 NTMs isolates were isolated by culture. By using LPA method all of the isolates identified to species level. *M. simiae*, *M. fortuitum*, *M. kansasii*, *M. abscessus* and *M. avium* respectively were the most common isolated species. While, Conventional methods results were inaccurate and misleading and for most of the isolates conventional tests were unable to identify the exact species.
Conclusion: NTMs infections rate are life threating in susceptible patients and according to similar clinical manifestation of NTMs infection by tuberculosis diagnosis of this infection is very challenging. Conventional laboratory methods are very inaccurate and time consuming for identification of NTM species. LPA method is a reliable and accurate method that can identify exact NTM species in acceptable time.
Keywords: (Non-Tuberculosis Mycobacterium) (Line Probe Assay)

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Topic: Molecular Diagnosis

Frequency of Gardnerella vaginalis in patients with vaginosis in Isfahan by molecular method

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Introduction and objectives: Bacterial vaginosis is a common vaginal disorder characterized by a change in bacterial vaginal microbial complexity from a normal to an inhomogeneous state containing a complex population of organism mainly anaerobic and micro aerobic. *Gardnerella vaginalis* is a gram variable coccobacilli and non-moving that can grow in micro aerobic. Selective treatment for *Gardnerella vaginosis* is metronidazole and clindamycin, both of which are available in the form of oral capsules and vaginal ointment, but antibiotic resistance has been reported in recent years.

Materials and Methods: In this cross-sectional study, 110 women referring to Isfahan clinics for bacterial vaginosis were studied. Patient satisfaction was obtained and a patient-specific questionnaire was prepared, diagnosis of the disease with 4 pH-based laboratory methods, whiff test, observation of clue cell in direct incubation and secretion culture in specialty chocolate agar containing starch and extracts of heart and brain containing starch. Confirmation of identification was carried out by biochemical tests. Also, all samples were evaluated for molecular detection of *Gardnerella vaginalis.*

Results: Based on molecular identification from a total of 110 patients with bacterial vaginosis symptoms, 32 (29.09%) had bactericidal vaginosis due to *Gardnerella vaginalis*. The characteristics of patients with bacterial vaginosis and non-infected individuals were compared. All women with this infection were married and most of them (43.8%) belonged to the age group of 25-30 years old. Among the patients (59.37%), infected were resident in the village and non-infected (54.54%) in the city. There was no significant difference between the two groups in terms of their place of residence. There was no significant difference in terms of education, occupation, number of previous pregnancies, abortion rates, use of antibiotics, vaginal ointment, and rate of secretion between the two groups. The results of chi-square test showed a significant difference between patients with positive clue cell in both groups of infected and non-infected to *Gardnerella vaginalis* ($P < 0.01$).

Conclusion: The relatively high frequency of *Gardnerella vaginalis* in women with bacterial vaginosis indicates the importance of the role of bacteria in the incidence of bacterial vaginosis. Also, in the studied region, bacterial vaginosis is prevalent and has many complications and it is necessary that people at risk be screened and if necessary be treated.

Keywords: *Gardnerella vaginalis*, clue cell, bacterial vaginosis

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Topic: Molecular Epidemiology

Multilocus Sequence Analysis (MLSA) of Mycobacterium tuberculosis from Clinical Isolates in Northeast of Iran

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Introduction and Objectives: *Mycobacterium tuberculosis* is still one of the most dangerous human pathogens. Identification of the relationships between different clinical strains has remained a high priority for epidemiology research. In this study, we used MLSA (Multilocus sequence analysis) to generate a highly robust phylogeny of *M. tuberculosis*. MLSA, based on single nucleotide polymorphism (SNP) was performed on five genes fragments from the *Rpsl* (302 bp), *MprA* (559 bp), *LipR* (322 bp), *KatG* (488 bp) and *Fgd1* (266 bp), in order to identify polymorphic nucleotide sites, and the discriminatory power of each locus for all genes was measured with Hunter-Gaston Index (HGI).

Materials and Methods: Twenty *M. tuberculosis* isolates were selected from pulmonary tuberculosis patients, during 2017 in the Regional Reference Tuberculosis Laboratory, in north-east of Iran. H37RV (ATCC27294) was also included as a reference strain in this study. These genes were amplified with the primers designed, using Primer3 software and DNA sequencing was performed on the ABI 3730XL DNA Analyzer. SNP data analysis including sSNPs (synonymous) and nsSNPs (non-synonymous) were analyzed, using the Vector NTI software.

Results: The sequencing results were multiple aligned, using MEGA Software, and compared with the reference sequence H37Rv. In final analysis of the sequencing data and phylogeny, two models have been compared: the first model was character-based and the second model was distance-based. There are 31 SNP among 20 isolates, these polymorphisms comprised of 6 nsSNPs, 25 sSNPs. In *Rpsl*, three nsSNP, in *KatG* three nsSNP and 1 sSNP, in *MprA* five sSNP, in *LipR* ten sSNP and in Fgd_1 nine sSNP were found. In our study, sSNPs were much more abundant than nsSNPs. The mutation in codon 128 of *rpsl* led to changes of lysine to arginine, and the mutation in codon 1280 of *katG* led to changes of alanine to glycine. Finally, sequence type (ST) number was assigned to each unique allelic profile, and 9 sequence types were identified from 20 strains, these imply that there is a high diversity of strains in this area of country.

Cconclusion: Our study implies that there is a high diversity of strains in this area of the country. SNPs can also be used to measure evolutionary distances between strains, to estimate the time of divergence of strains from their genetic distance if a mutation rate is known. Analysis of partial gene sequences of *rpsl*, *lipR*, *katG*, *fgd1*, *mprA*, revealed that *M. tuberculosis* strains showed that *rpsl* phylogeny power is weaker than others.

Keywords: *Mycobacterium tuberculosis*, MLSA, Molecular study

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Topic: Molecular Typing

Determination Brucella vaccine strains of genetic typing with use VNTR-PCR HOOF PRINT

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Introduction and Objectives: The assessment of the genetic stability is one of the essential elements to guarantee the biological quality of the live anti-bacteria vaccines. In this study, genetic patterns in *Brucella* vaccinal strains were evaluated using VNTR method.

Materials and Methods: 410 bacterial strains were investigated in this study included B. *melitensis Rev. 1, B. abortus S.19* (respectively, 92 & 100 commercially prepared vaccine production lots) and field strains of B. *melitensis, B. abortus* & B. *suis* (respectively, I II, 95 & II strains from RVSRIm culture collection). A reproducibility evaluation was integrated into the protocol by analyzing every sample twice in independent assays (16 parallel PCR reaction for individual sample). The genotypic fingerprints for each isolate was generated from the allelic profile and evaluated by the program ESGEM and all eight loci of that isolate were measured. HOOF Printslbased on multi-locus characterization of a variable number, eight base pair, tandem repeat in 8 microsatellite loci which has been named VNTRsII, introduce a survey method for assessment of genetic variation in *Brucella* strains.

Results: Vaccinal strains were genetically more homogenous, as Rev. I & S.19 vaccine strains, respectively, clustered 15 & 8 HOOF Prints genotypes. Field strains of B. *melitensis* and B. *abortus*, respectively, divided into 88 & 65 genotypes. But they showed less variation in fingerprinting patterns. it seems that HOOF Prints can significantly contribute to epidemiological trace-back analysis of Brucella infections and may advance surveillance and control of human brucellosis.

Conclusion: The outcome of the present study indicates that genetic patterns could be an essential assay to guarantee the quality and stability *Brucella* vaccinal strains. The validity of biovars established by classical microbiological methods could not be confirmed by microsatellites loci in assays. It seems that assay can significantly contribute to epidemiological trace-back analysis of Brucella infections and may advance surveillance and control of human brucellosis.

Keywords: VNTR-PCR, Brucella, Genetic pattern, vaccinal strains, IRAN

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Topic: Molecular Typing

Prevalence, antimicrobial susceptibility, serotyping and virulence determination of Listeria monocytogenes strains at a tertiary care hospital in Tehran, Iran

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Introduction and Objectives: *Listeria monocytogenes* is the etiological agent of listeriosis, a highly fatal infection which causes miscarriage or stillbirth in pregnant women. The objective of this study was to detect the prevalence, serotypes, antimicrobial susceptibility and virulence factors of *L. monocytogenes* isolated from the pregnant women with vaginitis, at a tertiary care hospital in Tehran, Iran.

Materials and Methods: During September 2015 to February 2017, a total of 400 clinical samples (vaginal swabs) were collected from the pregnant women with vaginitis at a tertiary care hospital in Tehran, and tested for the presence of *L. monocytogenes*. The presumptive isolates were characterized biochemically. All L. monocytogenes isolates were further analyzed by serotyping and antimicrobial susceptibility tests. All the positive samples for *L. monocytogenes* were analyzed for presence of virulence genes (*hly*A, *act*A, *inl*A, *inl*C, *inl*J and *prf*A).

Results: Twenty-two (5.5%) of the samples found positive for the presence of *L. monocytogenes*. Percentage of isolates resistant to antibiotics in this study was as following: penicillin G 45.45%, gentamicin 36.36%, ampicillin 45.45%, trimethoprim 81.82%, tetracycline 45.45%, ciprofloxacin 18.18%, sulfamethoxazole 81.82%, erythromycin 45.45%, streptomycin 45.45%, and chloramphenicol 54.55%. The majority of tested isolates (59.10%) belonged to serotype 4b, followed by 1/2a (22.73%), 1/2b (13.63%), and 3c (4.54%). The *hly*A, *act*A, and *inl*A were detected in all of the 22 *L. monocytogenes* isolates but, two, three and five isolates were found to lack *inl*C, *inl*J and *prf*A, respectively. Only one isolate lacked three *inl*C, *inl*J and *prf*A genes, also two isolates simultaneously lacked both *inl*J and *prf*A genes.

Conclusion: In conclusion, evaluation of the virulence factors and antimicrobial susceptibility can be highly helpful to develop effective treatment strategies against *L. monocytogenes* infections. This study is noteworthy in that it documents prevalence, virulence characteristics, and antimicrobial resistance of *L. monocytogenes*.

Keywords: *Listeria monocytogenes*, Pregnant women, Antimicrobial susceptibility, Serotyping, Virulence genes, Iran.

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Topic: Mycobacteria and Acid-Fast Bacteria

Isolation and identification the *Nocardia* species from clinical specimens

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The genus *Nocardia* is a Gram-positive aerobic bacterium and has mycolic acid, peptidoglycan, and phospholipid components in cell wall structure. The gold standard for nocardiosis identification is the isolation and culture of *Nocardia*. This bacterium causes pulmonary and extra pulmonary nocardiosis in human and isolated of Bronchoalveolar lavage (BAL), Sputum, Abscesses, Blood, Cerebrospinal fluid (CSF), and etc. Decontamination methods such as sodium hydroxide and N-acetyl-L-cysteine are toxic for the genus *Nocardia*. There are various methods and media for *Nocardia* isolation of clinical specimens such as paraffin baiting method, paraffin agar, media with antimicrobial agents such as Thayer-Martin agar and Buffered charcoal yeast extract (BCYE), Ogawa agar, conventional media such as blood agar, nutrient agar, brain-heart infusion agar (BHI). There are various phenotypic tests [colony morphology, pigment production, specific and conventional staining methods such as Gram, acid-fast and partial acid-fast, growth to lysozyme broth, aerial hyphae production, Growth at 45 °C, enzyme production, carbohydrate utilization, hydrolysis of amino acids, matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), and etc.] and molecular methods [technique polymerase chain reaction (PCR) base sequencing, PCR-restriction fragment length polymorphism (RFLP), Whole genome sequencing (WGS), and DNA-DNA hybridization] for Nocardia identification at the genus and species level. The most common species such as Nocardia asteroides complex, Nocardia nova complex, and Nocardia otitidiscaviarum have been isolated from clinical specimens from Iran.

Keyword: Nocardia, Isolation, Identification

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Topic: Parasitic Infections

Encapsulation of Imiquimod Adjuvant and Soluble Leishmania Antigen into Liposomes as a Vaccine in the Cutaneous Leishmaniasis Model

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Introduction and Objectives: The current therapy for the treatment of leishmaniasis is unsatisfactory because it has multiple side effects, and resistance has been reported among the parasites that cause these diseases. Attempts to produce vaccines for leishmaniasis need adjuvants to trigger the kind of immune reaction required for protection. In this study, we examined the properties of the TLR7 agonist imiquimod, a vaccine adjuvant, making use of a live model of infection where the immune reactions could be identified prior to and following the challenge of infection.

Materials and Methods: The liposomes of EPC containing the imiquimod adjuvant were provided and identified for protein concentration, surface charge, and particle size. Vaccination was done using the soluble *Leishmania* antigen (SLA) as a first-generation vaccine's model in the liposomal state to vaccinate BALB/c mice against the challenge of *leishmania major*. BALB/c mice were vaccinated subcutaneously, three times at a two-week interval. Parasite burden, footpad swelling, IgG isotype, as well as the level of IL-4 and IFN-γ were assessed as the protection criteria.

Results: The group of mice vaccinated by Lip+Imiquimod+SLA demonstrated a lower amount of footpad swelling and parasite burden than the buffer group. In addition, the greatest amount of IFN- γ and the smallest amount of IL-4 production was noticed in the splenocytes of the mice vaccinated by the formulation of Lip+Imiquimod+SLA.

Conclusion: These results imply that imiquimod added to the formulation of liposomes is able to modulate the immune reaction of the BALB/c mice vaccinated preferably to a Th1 reaction rather than a Th2 reaction; it can also lead to partial protection against the challenge of *Leishmania*.

Keywords: Leishmaniasis, vaccine, Imiquimod, Liposome, Immune response

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Topic: Parasitic Infections

The role of resistance genes expression in meglumine antimoniate non-healing and healing isolates of anthroponotic cutaneous leishmaniasis due to Leishmania tropica

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Introduction and Objectives: Anthroponotic cutaneous leishmaniasis (ACL) treatment is being challenged by the emergence of drug resistance against the first line of treatment, pentavalent antimonials (SbV) including meglumine antimoniate. Identification of Sb(V) resistance mechanisms through reliable molecular markers is critical for consolidating strategies to monitor the emergence and spreading of Sb(V) resistance in countries where CL is endemic. The aim of the present study was to assess the expression of AQP1, γ-GCS, MRPA, TDR1 and TR as resistance genes in clinical antimony non-healing and healing *L. tropica* isolates obtained from patients with ACL for exploring the potential of targeted expression profiling as a surveillance tool for monitoring the Sb(V) unresponsiveness in field isolates.

Materials and Methods: The expression of five major antimony resistance-associated genes, i.e., aquaglyceroporin1 (AQP1), γ-glutamylcysteine synthetase (γ-GCS), multidrug resistance protein A (MRPA), trypanothione reductase (TR) and thiol dependent reductase 1 (TDR1) in non-healing and healing *Leishmania tropica* field isolates was analyzed by quantitative real-time PCR (qPCR) in comparison with sensitive and resistant reference strains.

Results: Gene expression analysis showed the down-regulation of AQP1, γ-GCS and TDR1 by 1.9-fold, 1.7-fold and 3.55-fold in non-healing isolates compared to those of healing ones, respectively. Also, the average RNA expression level of MRPA showed an increase of 1.9-fold in the non-healing group. Additionally, a strong positive linear correlation between gene expression of AQP1 and γ-GCS was exhibited in isolates. Negative correlation between the AQP1 and γ-GCS expression level and lesion duration in healing patients, indicated the potential role in diagnosing drug-unresponsive parasites in endemic areas of ACL.

Conclusion: Due to the inconclusive outcomes of the resistance in clinical isolates, the expression analysis of a set of influential genes can be beneficial to identify distinctive biomarkers between the antimony non-healing and healing patients.

Keywords: Non-healing and healing patients, *Leishmania tropica*, Resistance genes, Real-time qPCR

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Topic: Pathogenesis and Virulence Factors

The PI3K-Akt/mTOR signaling pathway and tuberculosis pathogenesis; the first system biology report

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Introduction and Objectives: Tuberculosis (TB) is a major public health problem. It estimated that there are about 10.7 million TB patients throughout the world. The phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K-Akt-mTOR) signaling pathway has key role in cell growth, differentiation, apoptosis, autophagy, metabolism and infectious disease tuberculosis. Dysregulations of the PI3K-Akt/mTOR signaling pathway seem to have a role in tuberculosis. We performed analysis of the PI3K-Akt/mTOR expression changes in active-TB and latent-TB patients and healthy controls.
Materials and Methods: The gene expression profiles of CD4+ T-cells of tuberculosis and healthy individuals was obtained from Gene Expression Omnibus (GEO) database (Accession number: GDS4966; GPL570 platform). Subsequently, the GEO2R analysis was performed for detection of differentially expressed genes (DEGs) in active-TB, LTBI and healthy controls as per Benjamini-Hochberg FDR-adjusted p-values <0.05.
Results: Analysis performed revealed that inflammatory cytokines, JAK-STAT signaling, MAPK signaling pathway, autophagy gene expression profiles were distinctly different among TB, LTBI and healthy donors. Foxp3, CTLA-4, TIM1, JAK1, Cox11, BCL11A, TMX3, CXCL10 or PDL2 are overexpressed in active-TB compared to LTBI and healthy controls.
Conclusion: Our findings suggest that PI3K-Akt/mTOR signaling pathway is altered during active tuberculosis that may result in altered T regulatory cell function via FOXO1. Previous studies showed that T reg cells are overexpressed in active-TB patients which may result in suppression of Th1 response. Hence, expression of the PI3K-Akt/mTOR signaling pathway could be utilized to determine the activity of tuberculosis infection and monitor its progression and prognosis.
Keywords: *Mycobacterium tuberculosis*; the PI3K-Akt/mTOR signaling pathway; system biology; pathogenesis.

Topic: Pathogenesis and Virulence Factors

Investigation on the effects of Salmonella enterica Subsp enterica serovar Typhimurium on the apoptosis/necrosis of avian macrophage-like monocytes

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Introduction and Objectives*: Salmonella enterica* Subsp *enterica* serovar Typhimurium (SEST) is one of the most important serovars of the genus *Salmonella* in human and animals. Because of its intracellular tropism, monocytes/macrophages are pivotal in killing of *Salmonella* serovars; they are also responsible for transport of SEST to extra-intestinal organs. Study on the mechanistic. To see the mechanistic effects of the SEST on the apoptosis of avian innate immune cells, specially homogeneous macrophage-like monocytes (MLMs).

Materials and Methods: The MLMs were isolated from peripheral blood mononuclear cells of many healthy broilers. The MLMs were then divided in two groups: control (without SEST) and treatment (challenged with SEST clinical isolates) groups. Cellular-molecular damage caused by SEST in MLMs was assessed with bioluminescence [(BL), specific method measuring caspases activity and intracellular ATP contents] and flow cytometry (specific method for measuring apoptosis/necrosis).

Results: Results of BL-based apoptotic assays revealed higher caspases 3/7 and 9 activity (BL intensity). Furthermore, a significant diminished intracellular ATP content (BL intensity) was observed. The results of flow-cytometry based necrosis of the MLMs cell revealed stable the MLMs in both SEST-treated MLMs and non-treated/control, although slightly more necrotic MLMs was observed in SEST-treated MLMs compared to the non-treated/ctrl counterparts.

Conclusion: The results herein indicate that SEST weakens MLMs particularly through caspases activation/apoptosis. These findings can open a new window on the molecular aspect of *Salmonella*-macrophage interactions and immunopathology/pathogenicity of salmonellosis in animals especially chickens.

Keywords: Apoptosis, Avian macrophages, Bioluminescence, Flow cytometry, Salmonellosis, *Salmonella enterica* Subsp *enterica* serovar Typhimurium.

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Topic: Pathogenesis and Virulence Factors

Comparative analysis of virulence genes, antibiotic resistance and capsule locus polymorphism of *Enterococcus faecalis* isolated from canals of root-filled teeth with periapical lesions with those from different clinical infections and fecal flora

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Introduction and Objectives: *Enterococcus faecalis* (*E.faecalis*) is considered normal flora in human but has been considered a leading cause of hospital-acquired infections. In the oral cavity, it is commonly detected from canals of root-filled teeth with periapical lesions. The aim of this study was to compare antibiotic resistance patterns, the presence of virulence factors and capsule locus polymorphism among *E. faecalis* isolates recovered from different sources.

Materials and Methods: Eighty-eight *E. faecalis* isolates recovered from canals of root-filled teeth with apical periodontitis (n=22), human blood and CSF as invasive isolates (n=22), urine and wound as non-invasive isolates (n=22) and fecal flora of healthy individuals (n=22) were examined. Resistance to different antibiotic agents was determined by disk diffusion method. Phenotypic method was used to determination of gelatinase production and biofilm formation. Polymerase chain reaction technique was used to detection of *esp,ace*, *ebp*, *gelE*, *cyl*, *cps1*, *cps2*,*cps5* and *cbh* genes.

Result: The antibiotic susceptibility of the isolated dental, invasive, non-invasive and fecal *E.faecalis* showed the presence of multidrug resistant isolates. This property was significantly more common in non-invasive compared with fecal isolates (P= 0.022). Antimicrobial resistance rates were not significantly different between isolates from root canals and fecal flora (p>0.05). There were significantly different among isolates from root canals and fecal flora in gelatinase production (p= 0.009) and strong biofilm formation (p = 0.003). *E. faecalis* isolates from root canals carried *cyl* gene at a significantly higher frequency than invasive infections (p =0.002) and fecal flora (p =0.035). The presence of the *esp* gene was also significantly different between root canals isolates and the other isolates (p=0.000) .The most common type of capsule locus polymorphism among the root canals isolates was CPS 1 (63%) which suggests the lack of essential genes in *cps* operon for capsule production in these isolates .

Conclusion: This study demonstrated that there are major genetic differences between the *E. faecalis* isolates from root canal infections and those from clinical and normal flora. Knowledge about the characterizations of *E. faecalis* isolated from different sources may help to find appreciated procedures preventing enterococcal infections.

Keywords: *Enterococcus faecalis*; virulence factors; antibiotic resistance; apical periodontitis; infection; normal flora;

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Topic: Pathogenesis and Virulence Factors

The association between fecal enterotoxigenic Bacteroides fragilis with colorectal cancer

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Introduction and Objective: Enterotoxigenic *Bacteroides fragilis* (ETBF) is an enterotoxin-producing bacterium that possibily has a role in the occurrence and progression of colorectal cancer (CRC) by modulating the mucosal immune response and inducing epithelial cell changes. The aim of this study was to investigate the frequency of ETBF in stool samples of CRC patients and healthy volunteers.

Materials and Methods: A total of 60 stool samples from confirmed CRC patients and 60 stool samples from healthy volunteers with no personal or familial history or diagnosis of colorectal disease were collected. Stool samples were screened for direct detection of *B. fragilis* using PCR targeting the marker genes of neu and bft. Enterotoxin isotypes bft-1, bft-2 and bft-3 were also detected in *B. fragilis* positive samples.

Results: The frequency of *B. fragilis* among CRC and control cases was 58.3% and 26.6%, respectively ($P< 0.05$). Enterotoxin isotype bft-2 was detected with higher frequency among CRC patients than healthy control ($P<0.05$).

Conclusions: Our results show the association between fecal ETBF and CRC, and we suggest that detection of ETBF may be a potential marker for colorectal cancer diagnosis. However, additional investigations on tumor and paired normal tissue samples are required to substantiate this possible correlation.

Keywords: *Bacteroides fragilis*, Enterotoxin, bft gene, Colorectal cancer, Stool

Topic: Pathogenesis and Virulence Factors

Growth Rate and Biofilm Formation Ability of Clinical and Laboratory-Evolved Colistin-Resistant Strains of Acinetobacter baumannii

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Introduction and Objectives: Two different mechanisms of resistance to colistin in Acinetobacter baumannii have been described. The first involves the total loss of lipopolysaccharide (LPS) due to mutations in the lpxACD operon, which is involved in the lipid A biosynthesis pathway. The second entails the addition of ethanolamine to the lipid A of the LPS resulting from mutations in the PmrAB two-component system.

Materials and Methods: To evaluate the impact of colistin resistance-associated mutations on antimicrobial resistance and virulence properties, four pairs of clinical and laboratory-evolved colistin-susceptible/colistinresistant (ColS/ColR) A. baumannii isolates were used. Antimicrobial susceptibility, surface motility, in vitro and in vivo biofilm-forming capacity, in vitro and in vivo expression levels of biofilm-associated genes, and in vitro growth rate were analyzed in these strains.

Results: Growth rate, in vitro and in vivo biofilm formation ability, as well as expression levels of biofilm-associated gene were reduced in ColR LPS-deficient isolate (the lpxD mutant) when compared with its ColS partner, whereas there were not such differences between LPS-modified isolates (the pmrB mutants) and their parental isolates. Mutation in lpxD was accompanied by a greater reduction in minimum inhibitory concentrations of azithromycin, vancomycin, and rifampin than mutation in pmrB. Besides, loss of LPS was associated with a significant reduction in surface motility without any change in expression of type IV pili.

Conclusion: Collectively, colistin resistance through loss of LPS causes a more considerable cost in biological features such as growth rate, motility, and biofilm formation capacity relative to LPS modification. Therefore, ColR LPS-modified strains are more likely to spread and transmit from one patient to another in hospital settings, which results in more complex treatment and control.

Keywords: Acinetobacter baumannii, colistin resistance, biofilm formation, growth rate, antimicrobial resistance

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Topic: Pathogenesis and Virulence Factors

The study of ferritin-binding proteins in Streptococcus pneumoniae proteome

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Introduction and Objectives: Iron is the one of the vital elements for bacteria and they absorb it from limited Iron sources. Ferritin is an intracellular and extracellular protein that stores iron into cells and releases it in a controlled manner. The present study was aimed to identify the ferritin- binding proteins from *Streptococcus pneumoniae.*

Materials and methods: *S.pneumoniae* was cultured in BHI broth containing ferritin (1094 ng/ml) at 37°C for 4h and ferritin levels were measured using ELISA assay. Bacterium was separately cultured in BHI broth and its proteome were electrophoresed in SDS-PAGE and transferred in PVDF nitrocellulose membrane. In the next step the membrane incubated into ferritin solution and finally, the identification of ferritin-binding proteins carried out using anti-ferritin monoclonal antibody conjugated to HRP enzyme.

Result: The western blot analysis revealed that there were not any proteins with binding activity to ferritin. Ferritin levels were significantly ($p<0.05$) decreased following bacterium growth in medium containing ferritin.

Conclusion: The western blot analysis reveals that *S. pneumonia* doesn't have ability to produces ferritin-binding proteins and it is unable to use ferritin as Iron source, directly. However, the decreasing ferritin levels following bacterium growth in medium containing ferritin show that Iron may be provided by *S.pneumoniae* using a protease-mediated ferritin destroying mode.

Keywords: *Streptococcus pneumoniae*, ferritin-binding proteins, proteome

Topic: Prevention and Control of Infectious Diseases

Frequency of Staphylococcus aureus isolates collected from men semen and women endocervix and detection of antibiotic resistance

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Background and objects: Urogenital tract and asymptomatic cervical infections due to *Staphylococcus aureus* (*S. aureus*) have been recognized with infertility. This study was aimed to determine the frequency of *S. aureus* in semen and endocervix cultures obtained from infertile male and female patients in infertility centers in Tabriz.

Materials and Methods: In this study, 100 infertile couples were selected. Standard semen and vaginal speciemens analysis were performed according to WHO guidelines. Isolation and identification of *S. aureus* was carried out using phenotypic and genotypic methods. Antibiotic susceptibility testing was conducted by CLSI guidelines. Polymerase chain reaction (PCR) was used to detection of *mecA* and *tst* genes.

Results: *S. aureus* were isolated from seminal fluid of sixteen (16%) of infertile men and endocervix of twenty six (26%) women. Ten (62.5%) of the subjects had abnormal sperm motility and morphology and 3 (18.8%) of the subjects had abnormal seminal fluid density. The susceptibility of *S. aureus* isolates to co- trimoxazole, ciprofloxacin, gentamicin, and penicillin was 94.6, 78.5, 78.4, and 17.3%, respectively. Regarding PCR results, *mecA* gene was detected in 3 (18.7%) isolates of men and 7 (26.9%) isolates of women, whilst the *tst* gene was not detected in any of clinical isolates.

Conclusion: The results of this study revealed that the prevalence of *S. aureus* was very high in infertile women and it would appear that the *S. aureus* may be an exacerbating factor in the deterioration of male sperm quality and fertility. Therefore, it is required that all patients who are referred to infertility treatment centers are fully examined for infection with *S. aureus*.

Keywords: Infertility, *Staphylococcus aureus*, Seminal fluid, Endocervix, *mecA*

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Topic: Prevention and Control of Infectious Diseases

Vertical Transmission of Chlamydia trachomatis at Birth Time and Eye Colonization in Newborns

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Introduction and Objectives: Chlamydia *trachomatis (*C. *trachomatis)* is leading bacterial cause of sexually transmitted infections (STIs) in men and women, worldwide. Pregnant women can transmit this microorganism vertically to their newborns and cause conjunctivitis secondary to STIs. While there no program for screening of STIs among Iranian pregnant women, limited data are existed on the vertical transmission rate of *C. trachomatis* from our country. To address this gap, we aimed the current study to evaluate the frequency of *C. trachomatis* infection in a sample of pregnant women before vaginal delivery and then, to assess the rate of vertical transmission for this microorganism from genital tract of infected mothers to the eyes of their newborns.
Materials and Methods: Endocervical and conjunctival swabs were collected from pregnant women and their newborns before and after vaginal delivery, respectively. DNA was extracted from specimens and tested by an in-house PCR assay to amplify a 241bp fragment of C. *trachomatis.*
Result: Chlamydia *trachomatis* was detected in 9.6% (11 of 125) and 1.6% (2 of 125) of pregnant women and newborns, respectively. Both infected infants were born from asymptomatic infected women. The vertical transmission rate of C. *trachomatis* in this study was calculated as 18.1% (2 of 9). Additionally, our results revealed that presence of C. *trachomatis* in the eyes of newborns after birth is significantly in relation with genital C. *trachomatis* infection in their mothers (OR= 0.16, 95% CI= 0.03-0.7, P= 0.002).
Conclusion: Pregnant women with asymptomatic infection of C. *trachomatis* have a key role in the distribution of chlamydial conjunctivitis in newborns. Since ocular prophylaxis in neonates is not effective for chlamydial conjunctivitis, therefore, education and screening of pregnant women, as well as treatment of infected cases, remain as the best approach for controlling the disease.
Keywords: Chlamydia trachomatis, Pregnant Women, Vertical Transmission, Newborns

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Topic: Prevention and Control of Infectious Diseases

Antibacterial curcumin-loaded hydrogel based on Hyaluronic Acid-Polydimethylsiloxane (HA-PDMS) for wound dressing perspectives

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Introduction and Objective: Several studies indicate the antibacterial effect of curcumin. The aim of this study was to synthesis a novel curcumin-loaded composite hydrogel based on HA-PDMS and evaluation the antibacterial potential of it.

Materials and Methods: HA was cross-linked with polydimethylsiloxane-diglycidyl ether terminated (PDMS-DG) in alkaline condition, for 2 hours at 37°C. Cross-linking was between OH (from HA) and epoxy, leading to the ether bond formation. Immediately, curcumin solution was added to the resulting hydrogel (hydrogel A) or added after about 2 days to the semi dried hydrogel (hydrogel B) and then the hydrogel kept in dark. Room temperature dried curcumin-loaded hydrogels were characterized using NMR and swelling analysis. Antibacterial activity of the curcumin-loaded hydrogels was investigated against *Psudomonas aeruginosa* PAO1 using the disk diffusion and bactericidal efficiency (10 mg of each hydrogel in a 2 ml bacterial suspension at 10^6 CFU/mL) methods.

Results: HA-PDMS hydrogel was a transparent hydrogel with vial inversion property. Loading with curcumin, changed the color of the hydrogel to red and orange for hydrogel A or B, respectively. NMR analysis showed that the HA-PDMS hydrogel and curcumin-loaded hydrogels were synthesized successfully. Swelling assay showed the high water uptake capacity for the hydrogels. According to disk diffusion test on Mueller Hinton agar, 10 mm inhibition zone around the curcumin-loaded hydrogels was observed against *P. aeruginosa*. Also, bactericidal efficiency test revealed 39.28%, 57.14% and 14% inhibition in *P. aeruginosa* growth in the presence of curcumin-loaded hydrogel A, curcumin-loaded hydrogel B, and HA-PDMS pure hydrogel, respectively.

Conclusion: Based on these data, loading with curcumin increased the antimicrobial effect of the HA-PDMS hydrogel. Moreover, curcumin-loaded hydrogel B has the most antimicrobial effect and can be proposed as a wound dressing material.

Keywords: Hyaluronic acid, PDMS-DG, Curcumin, Antibacterial hydrogel

Topic: Treatment and New Drugs

Lipid vesicular drug delivery systems in treatment of intracellular infections: capabilities and challenges

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Lipid vesicles composed of amphiphilic compounds and cholesterol are classified as new drug delivery systems for transfer of vast range of chemical and biological ingredients through biological membranes. Liposomes, niosomes, ufasomes and many other "somes" are used nowadays for treatment of intracellular infections such as tuberculosis, leishmaniasis, malaria and AIDS. For example, in order to better penetration of paromomycin, a cationic aminoglycoside compound through biological membranes including macrophage cytoplasmic membrane, stratum corneum of skin and amastigote/promastigote cell membranes, we designed niosomal formulation and assessed its in vitro and in vivo antileishmanial effect. Furthermore, niosomal zinc sulfate, amphotericin B, glucantime and dapsone were also formulated, pharmaceutically characterized and practically evaluated and utilized, in some cases, in clinical trials to treat *Leishmania major* or *L. tropica* cutaneous infections.
Hereby, some commercialized antimicrobials formulated as lipid vesicles will be presented and their clinical application(s) will be shown. Then, some new aspects of these micro/nano-particulate dosage forms, including new formulations prepared in Kerman University of Medical Sciences (KMU), will be introduced. Some challenges such as sterile formulation and packaging, active pharmaceutical ingredients (APIs) stability and probable cytotoxicity or side effects will also be discussed.

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Topic: Zoonotic Diseases

Molecular serotyping of Shiga toxin-producing Escherichia coli (STEC) from animal sources in Iran: Emergence of a potentially virulent O26: H29 strain

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Introduction and Objectives: Shiga toxin- producing *Escherichia coli* (STEC) have been considered as one of the most important food-borne pathogens worldwide. Although a wide range of *E. coli* serogroups has been implicated in human infections, most severe cases has been related to certain serogroups. Lack of the availability of serotyping data in most developing countries has been a public health challenge to track outbreaks and to monitor the possible sources. We aimed to investigate the distribution of major STEC serotypes in a collection of STEC strains isolated from different provinces and variety of sources for the first time in Iran.

Materials and Methods: Total of 75 non-duplicate STEC strains isolated in previous studies (2008 to 2016) was selected. The isolates were obtained from cattle (35), sheep and goats (22), pigeons (14), and other sources (4). All isolates were subjected to two multiplex - PCR assay detecting the major virulence genes (*stx1, stx2, stx2f, eae, Ehly*) and phylotypes. Then, they were tested for the 15 important O-groups including O26, O45, O103, O111, O113,O121, O145, O157, O5, O55, O75, O91, O104, O118, and O128 by PCR. Finally, 20 strains were selected for identification of H-genotypes by PCR and sequencing.

Results: The predominant serogroup was O113 as it was detected in 9 isolates from different sources (5 cattle, 2 goats and 2 deer). O26 and O111 were found only in cattle isolates. O5 was only detected in ovine, but O128 was found in goats and pigeons. Some serogroups were not present in any sources (O157, O45, O121, O145, O55, O91, O104 and O118). The important recognized serotypes were O113: H21 (cattle, goats), O113:H4 (deer), O111: H8 (calves), O26: H11 (cattle), O128:H2 (goats, pigeons), and O5:H19 (sheep). Importantly, one strain from cattle that carried *stx1*, *stx2* and *eae* belonged to O26: H29 serotype.

Conclusion: This study provides the first serotyping (O: H typing) data documented in STEC strains of animal origin in Iran. Most strains with determined O-groups were from the bovine source that highlights the importance of cattle as reservoir of potentially pathogenic serovars. The recognized O26:H29 strain carried the essential facility for development of severe infections in human needs further investigations as a possible emerging strain.

Keywords: VTEC, animals, serotypes, Iran, virulence

Poster Presentation

Poster Code	Time and Date of Presentation
P95 - P118 P184 - P201 P269 - P287 P306 - P315	Morning of First Day- 27th Ague
P29 - P94	Afternoon of First Day- 27th Ague
P2 - P12 P202 - P238 P316 - P333 P334 - P340	Morning of Second Day- 28th Ague
P119 P120 - P165 P239 - P257	Afternoon of Second Day – 28th Ague
P13 - P28 P166 - P188 P258 - P268 P288 - P305 P341 - P342	Morning of Third Day – 29th Ague

Topic: Anaerobic Infections

P9: New Classification of *Clostridium perfringens* based on the production of major and minor toxin

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Introduction and Objectives: *Clostridium perfringens* (*C. perfringens*) causes many enterotoxic diseases in humans and animals as a result of its ability to produce potent protein toxins. *C. perfringens* produces at least twenty extracellular toxins but the classification of isolates was done on their ability to produce a combination of only major alpha, beta, epsilon, and iota toxins.

Materials and Methods: In this review key words cheese minor toxin of *C. perfringens* in search engines and databanks, including Elsevier Sciences, PubMed, and Google Scholar were searched.

Results: Therefore, *C. perfringens* has been classified into five types (A–E). The researchers characterized a novel toxinotyping scheme of *C. perfringens* by typing of minor toxins. Many toxins are encoded on plasmid and need to consider in classification. Recently based on these criteria new toxinotypes have been established.

Conclusion: .In this new classification the ability of producing minor toxins such as enterotoxin, binary enterotoxin, netB, netE, and netF considered. C. *perfringens* types F and G consists of isolates that can related to type A but discharge the miner toxins that imported then separated names. This strains responsible for *C. perfringens* human food poisoning and chickens necrotic enteritis. There are new *C. perfringens* toxinotypes can formally be proposed and accepted but further experimental work is required before.

Keywords: *Clostridium perfringens*, Typing, Toxin, Molecular diagnosis.

Topic: Anaerobic Infections

P8: Survey major toxins of Clostridium perfringens isolated of ruminants by molecular method in the southeast of Iran

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Introduction and Objectives: *Clostridium* is a common bacterium that is responsible for involving live stocks; poultries even humans and may lead to large economic losses. *Clostridium perfringens (C. perfringens)* produces a lot of toxins. This is divided into five types, from A to E, based on their ability to produce any of the four major lethal toxins (alpha, beta, epsilon and iota). The aim of this research is identifying of various genotypes of *C. perfringens* in the southeast of Iran.

Materials and Methods: Sampling strategies was according to the livestock population. Firstly samples were cultured on enriched medias under anaerobic conditions after that some differential medias used for culture and identification of *C. perfringens* speies . DNA extraction of all isolated carried out and multiplex PCR reaction was performed by using specific primers to indicate types of *C. perfringens*.

Results: The results showed that upon of half of isolated was type A in three groups (sheep, goat and cow), furthermore The type B was less than other types. Phylogenic tree, nucleic acids affinity, comparison of genetic similarity percent of C. perfringens isolates with standard strains and submitted gene in Gen Bank amplified that the same reaction fragments by PCR related to *C. perfringens*.

Conclusion: C. perfringens type A was dominant than other types. These isolated strains can be used in research and manufacturing projects.

Keywords: *Clostridium perfringens*, Diagnosis, Toxin, Multiplex PCR.

Topic: Anaerobic Infections

P7: Comparison of Spore Germination Rate between Toxigenic and Nontoxigenic *Clostridium difficile* Isolates in the ½ × MIC of Ceftazidime alone and in Combination with Clindamycin and Vancomycin

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Introduction and Objectives: Germination of spore in *Clostridium difficile* (*C. difficile*) can be occurred in the presence of some antibiotics. The aim of this study was to compare the spore germination rate in toxigenic and nontoxigenic isolates in the sub-MIC of ceftazidime alone and in combination with clindamycin and vancomycin.

Materials and Methods: The MIC and FIC of *C. difficile* isolates were performed by microdilution and checkerboard microdilution method, respectively. About 10^6 CFU/mL spores were inoculated to pre-reduced medium with ½ × MIC of each antibiotic alone and ½ × FIC in combination. The number of remaining spores were counted after 24 hours by viable spore count/mL.

Results: The ungerminated spores' rate were different between toxigenic and nontoxigenic isolates. The toxigenic isolates of *C. difficile* germinated more than nontoxigenic isolates at ½ × MIC and ½ × FIC of antibiotics. All isolates (toxigenic and non-toxigenic) of *C. difficile* germinated at ½ × MIC of ceftazidime.

Conclusion: The rate of toxigenic isolates are increasing at the presence of some particular antibiotics. So that the nontoxigenic isolates are replaced by toxigenic ones, and as a result, the rate of *C. difficile* associated diarrhea is increased.

Keywords: *Clostridium difficile*, Spore germination, sub-MIC, Antibiotics, Combination

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Topic: Anaerobic Infections

P251: Expression of Virulence Factors of *Clostridium difficile* at sub-Minimum Inhibitory Concentration of Antibiotics; a Review

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Introduction and Objectives: *Clostridium difficile* (*C. difficile*) generally colonizes in the intestinal tract of hospitalized patients who receive antibiotics for a long time. *C. difficile* expresses its virulence factors which are associated with pathogenesis. Naturally, the expression of these virulence factors may be influenced by antibiotics.

Materials and Methods: The databases (PubMed, ScienceDirect, google scholar and so on) were searched by appropriate criteria.

Results: The effect of antibiotics has investigated at a sub-minimum inhibitory concentration (MIC) on virulence factors. The expression of virulence factors of *C. difficile* was varied and depended on the type of antibiotic and *C. difficile* isolate. Some of the antibiotics at sub-MIC upregulated virulence factors and, while others downregulated theirs. Meanwhile, some antibiotics had no detectable effects on the regulation of virulence factors. Most of the antibiotics at sub-MICs regulate genes expression of virulence factors, toxin production, spore formation, and germination by several mechanisms especially SOS response system.

Conclusion: In order to achieve a clear understanding of the effect of antibiotics at sub-MIC on the expression of genes of virulence factors, which are related to the pathogenesis of *C. difficile*, further and wider investigations are needed, especially on the issue of the numbers of isolates.

Keywords: Virulence factors, sub-MIC, *Clostridium difficile*, Antibiotics

Topic: Anaerobic Infections

P194: Photomodulation potential of antimicrobial photodynamic therapy with nano-chitosan doped with curcumin versus *Porphyromonas gingivalis*

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Introduction and Objectives: *Porphyromonas gingivalis* is an etiological agent frequently found in both chronic and aggressive periodontitis as well as peri-implantitis. This study assessed the effect of antimicrobial photodynamic therapy (aPDT), as an alternative treatment modality, by nano-chitosan doped with curcumin (CNPs-Cur), as a photosensitizer, on the virulence features of cell-surviving aPDT against *P. gingivalis*.
Materials and Methods: *P. gingivalis* photosensitized with CNPs-Cur was irradiated with diode laser at a wavelength of 435 nm for 3 min, and then bacterial viability measurements were done. The biofilm formation ability, metabolic activity, and antimicrobial susceptibility profiles were assessed for cell-surviving aPDT.
Results: CNPs-Cur-aPDT resulted in a significant reduction of cell viability (89.2%), biofilm formation capacity (57.4%), and metabolic activity (44.6%) of *P. gingivalis* when compared to the control group ($P < 0.05$).
Conclusion: The virulence of *P. gingivalis* strain reduced in cells surviving aPDT with CNPs-Cur, indicating the potential implications of aPDT for the treatment of *P. gingivalis* infections in periodontitis and peri-implantitis *in vivo*.
Keywords: Antimicrobial photodynamic therapy, *Porphyromonas gingivalis*, Nano-chitosan,Curcumin

Topic: Anaerobic Infections

P313: Induction of alpha toxin production and reduction of time of cultivation of *Clostridium novyi* for vaccine production

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Introduction and Objectives: *Clostridium novyi* is an anaerobic bacterium and has a longer growth period than other *Clostridum* strains, which itself produces long-time toxin production and inactivates and hydrolyzes toxin over a long period of time. The aim of this work was reduction of time of growth and high toxin production with medium optimization and growth conditions.

Materials and Methods: in this study, based on the characteristics of the bacterium and its metabolism, different media with various source of carbons and nitrogen and their percentage in the media were analyzed. In this step, growth of *C. novyi*, time of growth and toxin production were controlled. The optimized medium and the old one was used for alpha toxin production in class bottle (16 litters) for final vaccine production. Also, the optimized medium was used for growth of *C. novyi* in fermenter condition. Final results were analyzed for the best medium and growth condition for vaccine production.

Results: The bacterial growth conditions decreased from 72 hours to 24 hours, with quantitative and qualitative changes in the culture medium and the use of appropriate amounts as well as material displacement. Alpha toxin increased significantly due to the high growth of the bacterium as well as the reduction of growth time. In these changes, carbon source displacement has created favorable conditions for the growth and production of toxin. Also, removal of liver extract as an unnecessary capacity in the culture medium reduced the cost of consumable raw materials. Adding small quantities of vitamin and mineral compounds stimulated more and better growth and was effective in the rate of toxin production. Comparison of production conditions in class bottle (16 litters) culture with two old and new culture medium formulations showed that the new formulation culture medium has higher growth rates and more toxin production than the old formulation culture medium. The growth rate has dropped to about one-third and the amount of toxin has increased significantly. The evaluation of the presence of toxin by SDS-PAGE and ELISA control methods indicated that the presence of toxin levels was approximately 3 times higher in new culture. The results of this study showed that growth of the bacterium in the fermenter with new medium formulation was effective in producing 40-50% more of the toxin compare with other methods and the production process is much easier and more voluminous.

Conclusion: These changes in media and growth conditions reduce production costs by at least 30 percent in the consumption of materials and also more than 100 percent in the cost of the production process. This achievement is currently underway at Razi vaccine and serum research Institute of Mashhad as a production process for the production of black disease vaccine.

Keywords: *Clostridium novyi*, alpha toxin, fermenter production, medium optimization, growth conditions.

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Topic: Anaerobic Infections

P2: Effect of incubation time on epsilon Toxin Production by Clostridium perfringens

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Introduction and Objectives: The epsilon toxin is one of 20 protein toxins produced by Clostridium perfringens, a Gram-positive, spore-forming, anaerobic bacterium that is an important pathogen of humans and livestock.C. perfringens virulence is largely dependent upon toxin production, and epsilon toxin (ETX) is the most potent of all C. perfringens toxins that produced by type B and D and cause enterotoxemia and enteritis in livestock after proliferating in the intestines and producing epsilon-toxin (ETX).

Materials and Methods: Growth and epsilon toxin production by Clostridium perfringens strains was determined in Thioglycollate medium..The five C. perfringens type D isolates were cultivated in Thioglycollate medium and incubated at 37 °C for 6, 12, 18 and 24 hours .Toxinogenesis and presence of the toxin was demonstrated with a C. perfringens epsilon toxin enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instruction and Positive or negative readings were obtained according to the instructions, furthermore a Minimum lethal Dose test (MLD50/ml) did on samples too.

Results: Results of ELISA test show that epsilon toxin production was positive in all four time and in MLD test results of isolates that was incubated 24 hours show higher result, so the overnight incubation is the best time for producing epsilon toxin.

Conclusion: The amount of alpha toxin that produced may be influenced by the time of being held in incubator.

Keywords: C. perfringens, Toxin, ELISA, MLD.

Topic: Anaerobic Infections

P5: Isolation and identification of the most common anaerobic bacteria isolated from different clinical samples

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Introduction and Objectives: Anaerobic bacteria cause a diverse range of serious and fatal infections. Increasing evidences have reported approximately 20% mortality rate due to anaerobic infections. As identification of anaerobic bacteria are difficult, and time- and cost-consuming task, therefore anaerobic infections are usually treated by empirical therapy. It seems that screening and detection of these bacteria in clinical samples are must for treatment of patients. This study were, therefore isolation and identification of the most common anaerobic bacteria isolated from different clinical samples.

Materials and Methods: clinical samples were collected from 100 hospitalized patients in different part of Imam Khomeini hospital in Tehran, Iran between May 2018 and May 2019. The samples were

Processed for Gram staining and anaerobic cultures were done following standard techniques. Species identification was performed by VITEK 2 system.

Results: Among 100 studied patients, anaerobic bacteria were isolated from 22 patients (22%).

Anaerobes were predominantly isolated from abdominal collection (9%), blood (5%) and wound infection (5%). The most frequent anaerobes were *Bacteroides* (n=12) and *Clostridioides* (n=5).

Conclusion: Anaerobic bacteria were isolated from different clinical samples. Improving and setting up of anaerobic bacteriology in laboratories as well as information about most common anaerobic bacteria in clinical samples may assist to clinicians for empirical therapy.

Keyword: Anaerobic bacteria, clinical samples, diagnosis

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Topic: Anaerobic Infections

P3: Cloning and expression of the gene encoding beta toxin of *Clostridium perfringens* type B into *E. coli* strains BL21 (DE3) and RosettaTM (DE3)

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Introduction and Objectives: So far, 231 specious of Clostridia were classified which at least 15 specious are cause of disease in human and animals. *Clostridium perfringens* is an important specious that produces four major toxin. Beta toxin is produced by *C. perfringens* types B and C, which is cause of fatal in animals. The aim of this research was cloning and expression of recombinant beta toxin of *C. perfringens* type B in *Escherichia coli* strains.
Materials and Methods: *C. perfringens* type B were cultured into selective medium and genomic DNA was extracted using Phenol-Chloroform method. Then beta toxin was amplified using specific primers. In next stage, it was ligated in pET22b (+) and cloned into *E. coli* strains BL21 (DE3) and RosettaTM (DE3). Then Recombinant protein was expressed after IPTG induction. Expression recombinant gene was optimized using induction of different concentrations IPTG. Then its expression was evaluated using SDS-PAGE technique. Finally, the recombinant protein was purified via Ni-NTA and was analyzed using western blot.
Results: Recombinant protein was expressed after IPTG induction in strain RosettaTM (DE3) and can improve the expression of the recombinant beta toxin. However it was not expressed in *E. coli* strain BL21 (DE3).
Conclusion: The result of this study showed that *E. coli* strain RosettaTM (DE3) can improve the expression of the gene encoding beta toxin.
Keywords: Cloning, expression, beta toxin, *Clostridium perfringens*, *E. coli*

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Topic: Anaerobic Infections

P4: Comparison of Indirect ELISA and MNT method for detection and evaluation of *Clostridium perfringens* epsilon antitoxin in vaccinated rabbits

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Introduction and Objectives: *Clostridium perfringens* is a spore forming anaerobic bacterium which divided into five types based on the production of major toxins. Epsilon toxin is one of the major toxins which are produced by *C. perfringens* B and D types. Epsilon toxin is the responsible for enteric diseases, generically called enterotoxaemia, in sheep, goats, and other animals. Therefore, evaluation of epsilon antitoxin is urgent for diagnosis and evaluating of the vaccine. MNT has some problem include large number of used animals and Long-duration testing. The purpose of this study was to evaluate the ELISA as an alternative method for measuring of epsilon antitoxin.

Materials and Methods: First, epsilon toxin was purified and amount of protein was measured using Lowry method. Then, positive and negative control antisera and samples antisera were prepared. Finally, we were measured epsilon antitoxin by ELISA and MNT simultaneously and the results were analyzed by SPSS software.

Results: The negative control cut-off was estimated 0.485 and cross-examination test was shown that epsilon toxin had not cross reaction with other toxins of *Clostridium* spp.

The results of this study showed that there is a significant agreement between In vivo and In vitro tests. Linear regression analysis gave significant correlation coefficients of 0.697 for the indirect ELISA ($P<0.01$).

Conclusion: Finally, ELISA assay could be used as an alternative MNT test. However, further research for evaluation of epsilon antitoxin of *Clostridium perfringens* is needed for target animals.

Keywords: ELISA, enterotoxaemia, *Clostridium perfringens*, epsilon antitoxin, MNT

Topic: Anaerobic Infections

P310: Conventional and molecular identification of vaccine strain of *Clostridium septicum*

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Introduction and Objectives: Genus *Clostridium* are ubiquitous which are cause disease in human and animal. Disease caused by *Clostridium* species have been existed from years ago in Iran widespread. For prevention require effective vaccines. So, toxigenic strains are needed. The purpose of this study is characterization of vaccine strain of using microbiological and biochemical properties, molecular approach and toxicity titration.
Materials and Methods: Vaccine strain (CN913) was cultured anaerobically in liver broth for 24 h. After growth, this strain was characterized using microbiological (including Gram staining, culture on Blood agar and LB agar and motility) and biochemical tests (including Fermentation of sugars, lecithinase, gelatinase, Indol, and catalase). Then genomic DNA was extracted using phenol and chloroform extraction method. The target gene was amplified through PCR by specific primer which was taken from gene bank and designed using oligo software. Finally, Toxicity titration was performed using injection intravenously into white mice per dilution. The inoculated mice were observed and titer was calculated.
Result: *C. septicum* colonies were smooth and slightly translucent colonies surrounded by beta haemolysis. In Gram staining was observed gram positive straight or curved rod shape. The other microbiological and biochemical properties of vaccine strain are also found similar to those of *C. Septicum*. On the other hand, Vaccine strain was confirmed using PCR approach. The titer of lethal toxin produced was 1:50 per ml.
Conclusion: The result showed that vaccine strain is confirmed with reliable references and could be used as vaccine strains.
Keywords: *Clostridium septicum*, PCR, Diagnosis, toxin-alpha

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Topic: Anaerobic Infections

P10: *Clostridium perfringens* typing in ruminants in south of Kerman province by PCR

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Introduction and Objectives: *Clostridium perfringens* (*C. perfringens*) is a gram positive, sporulating bacterium that is extremely pathogenic and responsible for a wide spectrum of anaerobic diseases in animals and humans. This bacterium is classified into five toxinotypes (A, B, C, D, and E). The aim of this study was *Clostridium perfringens* typing in ruminants in south of Kerman province by PCR.

Materials and Methods: A total of 495 fecal samples were obtained from different ruminants and analyzed for typing *C. perfringens* by multiplex PCR. Specific primers for of α, β, ε, and ι toxins genes were used

Results: Out of 495 investigated samples, 906 bacterial isolates were morphologically selected for microbiological examination. Only 53 *C. perfringens* strains were confirmed by multiplex PCR. Interestingly, the predominant *C. perfringens* toxovar was type A (50 isolates), but also types D (3 isolates) could be identified as pathogens ruminants in south of Kerman province. Other types of *C. perfringens* (B, C and E) were not detected.

Conclusions: The detection of toxigenic *C. perfringens* isolates with PCR was performed for the first time in this area. Results showed that multiplex PCR is a useful and reliable tool for *C. perfringens* genotyping in routine veterinary diagnostics and epidemiological studies.

Keywords: *Clostridium perfringens*, PCR, Ruminants, South of Kerman.

Topic: Anaerobic Infections

P242: Impact of culture media kind on alpha toxin production of Clostridium perfringens

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Introduction and Objectives: Clostridium perfringens phospholipase C (PLC), commonly known as alpha toxin, is the lethal, dermonecrotic toxin produced by all strains and is considered a major virulence factor in clostridial myonecrosis. ELISAs have relatively high sensitivity and can detect small amounts of toxin in samples
Materials and Methods: Three culture media, that are selected was liquid Thioglycolate medium, the base media of *c. perfringes* and also c. novyi vaccine that contain pepton and the other nutrients. 10 Strains of C. perfringens was cultured in these three culture medias and incubated at 37 C for 6 hours under anaerobic conditions.an enzyme-linked immunosorbent assay (ELISA) with antibodies specific to alpha toxins of C. perfringens was done on all Culture supernatants by using a Bio-X *Alpha toxin ELISA* kit. The results determined optical densities by means of a microplate spectrophotometer with a 450 nm filter
Results: Interpreting the results show that the presence of toxin in all samples was positive but there were differences between ODs.
Conclusion: the amount of alpha toxin produced by *c. perfringens* may be influenced by the kind of the media.
Keywords: c. perfringes, culture medias, alpha toxin, ELISA

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Topic: Anaerobic Infections

P339: Characterization of virulence determinants and antimicrobial resistance patterns in toxigenic clinical isolates of *Clostridioides (Clostridium) difficile*

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Introduction and Objectives: *Clostridioides* (*Clostridium*) *difficile* infection as a serious healthcare-associated infection can cause life-threatening infectious diarrhea in hospitalized patients. The aim of the study was to evaluate the virulence determinants and antimicrobial resistance patterns of *C. difficile* isolates obtained from hospitalized patients in Shiraz, Iran.

Materials and Methods: This study was performed on 45 toxigenic *C. difficile* isolates. Determination of toxin profiles was done using polymerase chain reaction method. Antimicrobial susceptibility to vancomycin, metronidazole, clindamycin, tetracycline, moxifloxacin, and chloramphenicol were determined by the agar dilution method. The genes encoding antibiotic resistance were detected by the standard procedures.

Results: The *tcdA* and *tcdB* genes were detected in 95.6% of the isolates ($tcdA^+$, $tcdB^+$), and 4.4% of strains harboured only one toxin associated gene ($tcdA^-$, $tcdB^+$). The genes encoding CDT were also found in six (13.3%) isolates. Predominant toxin profile (82.2%) was A^+ B^+ CDT^-. Resistance to tetracycline, clindamycin and moxifloxacin were observed in 66.7%, 60% and 42.2% isolates, respectively. None of the strains showed resistance to vancomycin, metronidazole, and chloramphenicol. The distribution of the *ermB* gene was 57.8% and the *tetM* and *tetW* genes were found in 62.2% and 13.3% of the strains, respectively. The substitutions Thr82 to Ile in GyrA and Asp426 to Asn in GyrB were seen in moxifloxacin resistant strains.

Conclusion: Our data contributes to the present understanding of virulence and resistance traits amongst the isolates. Infection control strategies should be implemented carefully in order to curb the dissemination of *C. difficile* strains in hospital.

Keywords: *Clostridioides* (*Clostridium*) *difficile*, CDI, toxins, antibiotic resistance, Iran

Topic: Anaerobic Infections

P45: An investigation of some Clostridium genus on cancer therapy

Alireza paradise

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Currently, cancer has one of the highest mortality rates worldwide. Common therapeutic approaches (chemotherapy, radiotherapy and surgery) in cancer therapy are somewhat limited. Bacteria were known as enemies in the past, but at present are known as friends. They have shown great potential for cancer therapy. Bacteria of many species demonstrate the amazing ability to attack and colonize solid tumors. Successful cancer treatment remains a major challenge. As a result, alternative therapies for tumor therapy are being sought. One of these is the use of live species of Clostridium, Clostridia is probably the most widespread of all pathogenic bacteria that produce the highest number of toxins of any type of bacteria.
Bacterial-based tumor-targeted therapy is an area of growing interest and holds promise for the treatment of solid tumors. Upon systemic administration, various types of non-pathogenic obligate anaerobes and facultative anaerobes have been shown to infiltrate and selectively replicate within solid tumors. The tumor specificity is based upon the unique physiology of solid tumors, which is often characterized by regions of hypoxia and necrosis. Prokaryotic vectors can be safely administered and their potential to deliver therapeutic proteins has been demonstrated in a variety of preclinical models. There are several issues however that are still unknown and remain major challenges. Although past results have fuelled skepticism about its clinical use, recent promising findings emphasize the potential of Clostridium-directed tumor therapy. In this lecture, using Clostridium as prototypical agents, I will discuss the major advantages, challenges and shortcomings of bacterial systems for tumor-specific therapy.
Keyword: tumor-targeted therapy, non-pathogenic anaerobes, Clostridium

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Topic: Antimicrobial Resistance

P341: The first report of the *bla*$_{NDM}$ carbapenemase genes in *Salmonella* spp. isolates in Kerman, Iran

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Introduction and Objectives: spreading of multidrug resistant (MDR) strains of *Salmonella* spp. is an important global health issue. In fact, these MDR strains of *Salmonella* spp. act as a reservoir and subsequent horizontal spreading of antibiotic resistance genes to non-resistant ones. In this study, we aimed to identify the antimicrobial resistance genes (*bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{NDM}$) in *Salmonella* spp., isolated from chicken feces by PCR method.
Materials and Methods: In this study, a total of 45 *Salmonella* spp. isolates were collected from chicken feces samples. The isolates confirmed as *Salmonella* spp. by standard biochemical tests and presence of antimicrobial resistance genes including *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{NDM}$ determined by PCR methods.
Results: The results revealed that 16 (35.5%), 6 (13.4%) and 1 (2.3%) of *Salmonella* spp., carried *bla*$_{TEM}$, *bla*$_{NDM}$ and *bla*$_{CTX-M}$ genes, respectively, while *bla*$_{SHV}$ gene was not detected.
Conclusion: The *bla*$_{TEM}$, *bla*$_{NDM}$ and *bla*$_{CTX-M}$ genes are common among the *Acinetobacter* spp., *Escherichia coli* and *Klebsiella* spp and numerous isolates from human patients, and some environmental niches such as water and sewage and also recently have been isolated from farm (livestock) animals in China. Based on our results presence of *bla*$_{TEM}$, *bla*$_{NDM}$ and *bla*$_{CTX-M}$ genes in investigated isolates indicates a serious alarm for the prevalence of these genes and subsequent spread from animals to people. We recommended that the use of antibiotics in chicken food must be tightly regulated to avoid such outcomes.
Keywords: *Salmonella*, Antimicrobial resistance genes, PCR

Topic: Antimicrobial Resistance

P118: Patient with MDR Chronic Suppurative Otitis Media: A Case Report

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Introduction and Objectives: Chronic inflammation of middle ear is defined as chronic suppurative otitis media (CSOM). Infection can cause complications such as mastoid abscess, deafness, meningitis, and intracranial abscess. The most common bacteria isolated from CSOM are *P. aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumonia*, and *Escherichia coli* [7, 8]. CSOM caused by *p. aeruginosa* usually is treated with topical ciprofloxacin. One of the mechanisms behind fluoroquinolone resistances (FRs) in bacteria such as *P. aeruginosa*, is mutations in *gyrA* and *parC* genes.

Materials and Methods: A 24-year-old male patient with CSOM was reported. CSOM was prolonged for ten years and physician prescribed topical ciprofloxacin drops, pus suctioning and ear pH alteration. The treatment wasn't effective and the patient came back to the clinic with relapse of suppurative otitis media. Antibiotics resistance investigated with disc diffusion method for ten antibiotics. We investigated three fluoroquinolone resistance genes including *gyrA*, *parC*, and *nfxB* by polymerase chain reaction (PCR) (Table 1).

Results: *P. aeruginosa* was isolated as the cause of CSOM and the isolate was resistant to ciprofloxacin, aztreonam, imipenem, gentamicin, doripenem, cefepime, levofloxacin, amikacin and susceptible to colistin and ceftazidime. We observed two mutations in *gyrA* and eight mutations in *nfxB* genes. parC gene had no mutation.

Conclusion: CSOM is a major public health concern associated with hearing loss. Mutations in *gyrA*, *parC*, *nfxB* genes are the main causes of fluoroquinolone resistance (FRs) including ciprofloxacin resistance [8]. Any change in the structure of the middle ear can cause CSOM. In our study, the first mutation in the *gyrA* gene (threonine 83 isoleucine) was similar to the study of Xiaoyan Yang *et al.* in 2015 [14]. The routine and primary treatment for CSOM did not seem sufficient and tympanomastoidectomy is suggested to be the best treatment approach for these patients.

Keywords: *Pseudomonas aeruginosa*, fluoroquinolones resistance, suppurative otitis media, tympanomastoidectomy.

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Topic: Antimicrobial Resistance

P337: High prevalence of phylogroup B2 in Extended-Spectrum-β-Lactamase- producing Escherichia coli isolates in patients with *urinary tract* infections *in Tehran*

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Introduction and Objectivess: Uropathogenic Escherichia coli (UPEC) are the major causing of urinary tract infections (UTIs). Incidence of UTI due to Multi-drug Resistant and Extended- Spectrum Beta-Lactamases (ESBLs) producing Escherichia coli (E.coli) are increasing worldwide. Most *E. coli* strains are classified under four main phylogeny groups B2, B1, A, and D. The present study was carried out to determination common phylogroups in ESBLs producing E.coli in children with UTIs.

Materials and Methods: Current study was carried out between April 2017 to January 2018 in 100 isolated *E.coli* from urine of children with symptomatic UTI in Tehran; Iran. Screening for ESBL-producing *E. coli* isolates was done by standard methods according to CLSI 2017 guidelines. Phylogenetic grouping of all positive ESBL isolates were done by using of the triplex PCR method using by based on combination of three genetic markers *chuA*, *yjaA* and *TspE4.C2*.

Results: The ESBLs were detected in 100 of 220 *E.coli* isolates. Among these, the predominant phylogenetic group was B2 (*n*=84; 76.3%), followed by A/C (*n*=15; 13.7%) and D (*n*=9; 10 %).

There was a statistically significant difference between phylogeny groups and antibiotic resistance to ciprofloxacin (69% in group B2), trimethoprim + Sulfamethoxazole (90% in group B2) and nitrofurantoin (18.2% in group A vs. 7% in groupB2). All the isolates of *E. coli* group D were sensitive to nitrofurantoin and amikacin. Due to high prevalence to resistance to ciprofloxacin (62% in ESBL positive isolates), it is is suggested that ciprofloxacin as empirical therapy for UTI should be reconsidered.

Conclusion: Our study showed that phylogroup B2 and D was significantly associated to highly antibiotic resistance than group A.

Keywords: urinary tract infection, extended spectrum β-lactamases, phylogenetic group

Topic: Antimicrobial Resistance

P70: Epidemiology of urinary tract infection and antibiotic resistance pattern of *E. coli* in patients referred to Shahid Beheshti hospital in Zanjan, Iran

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Introduction and Objectives: Urinary tract infection (UTI) is one of the most common bacterial infections. Antibiotic resistance patterns vary in different regions. According to the increasing antibiotic resistance among strains of *E. coli*, aim of this study was to determine the current pattern and also to investigate changes in the antibiotic resistance patterns of *E. coli* in outpatients referred to the Shahid Beheshti hospital in Zanjan.

Materials and Methods: In this study, 3225 urine samples were examined during a twelve months period from December 2016 to April 2017. They were all cultured and then examined for *E. coli*. Morphological study and identification of isolated bacteria by differential biochemical tests and using hot dyeing were performed. Antibiogram profile of the bacteria was determined by disk-diffusion test (Kirby-Bauer) according to CLSI standards.

Results: The urine test result was positive in 552 (17.11 %) patients of 3225 eligible ones. The most common isolated bacteria were *E. coli*; 464 (84.05%). Based on the results of antimicrobial resistance test, the highest resistance was with co-trimoxazol (50.64%), nalidixic acid (47.41%), ceftriaxone (24.35%) and ciprofloxacin (20.47%). Furthermore, the lowest resistance belonged to nitrofurantoin (5.81%), amikacin (6.68%), ceftizoxim (8.40%) and gentamicin (12.28%), respectively.

Conclusion: The highest sensitivity was to nitrofurantoin and the highest resistance was to Co-trimoxazol. According to the data, the treatment of UTIs should be done according to the susceptibility and resistance pattern of the area in order to prevent the occurrence of a drug resistance and treatment failure that leads to the complication of the infection.

Keywords: Antibiotic resistance pattern, *E. coli*, Urinary tract infections.

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Topic: Antimicrobial Resistance

P32: Prevalence of plasmid-mediated AmpC in clinical isolates of *Escherichia coli* in Gazvin

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Introduction and Objectives: One of the causes of resistance to third generation cephalosporins is production of AmpC beta-lactamases which can be encoded by chromosome or plasmids. The purpose of this study was to determine the prevalence of six plasmid-mediated AmpC (pAmpC) among *Escherichia coli* isolates in Qazvin.
Materials and Methods: The 196 isolates of *E. coli* were collected from patients with different infections during 2018 to 2019 from four hospitals in Qazvin. For all isolates, antimicrobial susceptibility testing for cefoxitin (30 µg) was performed using the Kirby–Bauer disk diffusion method and the results were interpreted according to the Clinical Laboratory Standards Institute guidelines. PCR was performed on non-susceptible cefoxitine isolates for detection of six plasmid-mediated AmpC-specific families (MOX, FOX, CIT, EBC, DHA, and ACC).
Results: With respect to phenotypic resistance to cefoxitin, 32 isolates were non-susceptible to cefoxitin (28 resistant and 4 intermediate susceptible). The prevalence of pAmpC families among non-susceptible cefoxitin *E. coli* isolates was 34.3%. In other words, the plasmid-mediated *ampC* genes were detected in 11 out of 32 isolates which 7 isolates from three different hospitals were found to harbor bla_{CIT} gene and 4 isolates from a hospital carried bla_{DHA} gene. However, the remaining pAmpC families including MOX, FOX, EBC, and ACC were not were not detected in our isolates.
Conclusion: In this study, we showed that more than one-third of non-susceptible cefoxitin isolates harbored at least one pAmpC families. Further studies are needed to find the cause of cefoxitin resistance among remaining non-susceptible isolates which is probably mediated by chromosomal AmpC β-lactamase.
Keywords: *Escherichia coli*, plasmid-mediated AmpC β-lactamases (pAmpC), Prevalence

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P62: Genotypic and phenotypic assessment of AmpC beta-lactamase in *Klebsiella pneumoniae* isolates from educational hospitals in Qazvin

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Introduction and Objectives: Advent of resistance to beta lactam antibiotics in Gram negative pathogens, especially *Escherichia coli* and *Klebsiella pneumonia*, frequently results from the production of β-lactamase enzymes. In the Enterobacteriaceae, AmpC enzymes are encoded by either chromosomal or plasmid-mediated genes. Majority of the plasmid-mediated AmpC β-lactamases can be found in *klebsiella* and *E. coli*. *K. pneumonia* is mostly plasmid mediated AmpC β-lactamase (PABls) producer. Since PABls represent a new threat of spread to other organisms within a hospital or geographic region, the aim of the study was to evaluate the occurrence of PMABLs in clinical isolates of *K. pneumonia*.

Materials and Methods: A total of 183 *Klebsiella pneumonia* isolates were recovered from urine culture of patients admitted in four major educational hospitals of Qazvin city, between 2017-2019. Screening for AmpC β-lactamase production was done using cefoxitin disks. Confirmatory phenotypic identifications were done for the Cefoxitin-resistant isolates using Boronic Acid for combined and AmpC induction tests using azteronam, amoxicillin- clavulanic acid and ceftazidime disks. PCR was used as the genotypic confirmatory test using DHA, CIT, MOX, FOX, EBC and ACC primers.

Results: The AmpC-producing isolates among all identified K. pneumonia were 18% (32/183) as detected by cefoxitin screening method. Among AmpC-producing isolates, 9% (3/33) were positive for AmpC by combined disc method (Cefoxitin and Boronic Acid) and induction test. Eighteen percent (6/33) and 12% (4/33) of AmpC-producing isolates were positive for presence *CIT* and *DHA* Plasmid-mediated AmpC genes, respectively. Other PABL genes including *ACC*, *EBC*, *MOX* and *FOX* were not found among AmpC-producing isolates.

Conclusion: Accurate and fast identification of AmpC beta-lactamases using combined disc method (Cefoxitin and Boronic Acid) and induction test in the routine diagnostic microbiology laboratories can help reduce the burden of these pathogens.

Keywords: AmpC beta-lactamases, *Klebsiella pneumoniae*, Qazvin

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P159: Effect of lactobacillus plantarum consumption on hematologic parameters in rats with bone marrow inflammation

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Introduction and Objectives: Bone marrow inflammation is a disease that affects the entire bone. The most important pathogen responsible for this disease is Staphylococcus aureus which penetrates the osteoblasts from the immune system and therefore does not respond to common antibiotic treatments. It has been recently shown that Probiotics enriched with selenium have a high inhibitory effect on pathogens. in the present study Effect of lactobacillus plantarum consumption on hematologic parameters in rats with bone marrow inflammation Was investigated After induction Bone inflammation with Staphylococcus aureus.

Materials and Methods: in this study the number of 26 male wistar rats was prepared and divided into 6 separate groups including: control group(infected with staphylococcus areus), group infected with staphylococcus areus that treated with lactobacillus plantarum, group inoculated with staphylococcus areus that treated with selenium enriched lactobacillus plantarum, Piercing the foot bone and injecting 100 µl of Staphylococcus aureus suspension has done in group that was inoculated with staphylococcus areus. after treatment, blood sampling was directly performed from the animal heart. The number of blood cells and their indexes were analyzed using MINDRAY CBC device. After the completion of blood sampling, the bone marrow of the right leg of the rats was carefully separated from the middle of the bone and then homogenized in Serum Physiology buffer, and then the supernatant isolated after centrifugation. 100 µl of supernatant was cultured in Muller hinton Agar medium and incubated at 37°C for 24hrs. the number of bacteria was counted after incubation.

Results: Results showed that the level of White blood cells in group inoculated with staphylococcus areus($P \leq 0/001$) and group inoculated with staphylococcus areus that treated with lactobacillus plantarum($P \leq 0/05$) increased. Although the level of them were increased in group infected with staphylococcus areus that treated with selenium enriched lactobacillus plantarum, but there was no significant difference with control group. The variations in other factors in the experimental groups were not significant in comparison to the control group. Number of single Staphylococcus aureus colonies in group inoculated with staphylococcus areus and in group that treated with selenium enriched lactobacillus plantarum, was significantly decreased in compared to group inoculated with staphylococcus areus .while the number of this colonies in group treated with lactobacillus plantarum showed no variation in compared to group infected with staphylococcus areus.

Conclusion: It seams that selenium enriched lactobacillus plantarum reduced infections caused by staphylococcus areus through it's antioxidant and antibacterial activity and thos leads to reduction of white blood cells and number of staphylococcus areus colonies. While other blood parameters are not affected by this condition.

Keywords: Acute bone marrow inflammation, Staphylococcus areus, lactobacillus plantarum, Selenium

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P258: Inhibition of biofilm formation and virulence factors production in *Pseudomonas aeruginosa* PAO1 by vitamin E

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Introduction and Objectives: *Pseudomonas aeruginosa*, a common Gram-negative bacterium in hospital-acquired infections, is an opportunistic human pathogen that exhibits intrinsic multi-drug resistance and causes death in many burn victims, cystic-fibrosis and neutropenic cancer patients. It is known that *P. aeruginosa* biofilm maturation and production of cell-associated and extracellular virulence factors are under the control of a quorum-sensing (QS) system. Among several proteins involved in the *P.aeruginosa* QS network, LasR, PqsR and RhlR play an important role in its cascade signaling system. In this research, vitamin E used as an antibacterial agent for the first time, based on the structure similarity with PqsR ligand, called PQS.

Materials and Methods: Effect of Vitamin E was investigated in both biofilm formation and virulence factors production including pyoverdine, pyocyanin and protease. The effect of vitamin on other major QS pathways was also evaluated. Moreover, the compounds used in combination with tobramycin to estimate whether its activity enhanced against *P. aeruginosa*. Finally, *in silico* docking analysis was performed to assess possible interactions.

Results: Vitamin E not only suppress biofilm formation but inhibit virulence factors and homoserine lactones (HSLs) production as the main components in other QS pathways of *P. aeruginosa*. Vitamin E also showed synergistic effect with tobramycin against the pathogen.

Conclusion: This is the first report on anti-QS and anti-biofilm activity of vitamin E. These findings suggest that vitamin E can be used as potential inhibitors to control *P. aeruginosa* pathogenicity.

Keywords: Vitamin E; Biofilm; *Pseudomonas aeruginosa*; Quorum sensing; PqsR

Topic: Antimicrobial Resistance

P42: Antibiotics resistance in isolated *Salmonella* from Urmia's poultry farms

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Introduction and Objectives: *Salmonella* is a genus bacillus gram-negative bacteria of the Enterobacteriaceae family. *Salmonella* is the causative agent of bacillary white diarrhea and fowl typhoid in poultry.

Materials and Methods: At this study sampling was done from 150 cases of 500 broilers was *referred to Urmia university veterinary clinic; and nine of them was infected with* Salmonella.

After necropsy, *sampling was done from broilers' liver, bile sac, and* colon components. after 18h incubation in Selenite F Broth medium, we have grown bacterias in Brilliant Green Agar (BGA) medium and separate negative lactose colonies; we did IMViC and TSI testes on negative lactose colonies for definitive detection.

Antibiotics were used as a plate and it was included common antibiotics used.

For antibiotic sensitivity test (antibiogram) we grown separated Salmonella's colonies in Muller-Hinton Agar (M-H Agar) medium; after 24h incubation, we put antibiotic plates in medium and then incubate it for 24h.

Results: We recognize that enrofloxacin and ampicillin are the most powerful and effective antibiotics against salmonellosis and all of the samples had resistance against tylosin and tiamulin.

Conclusion: overuse and unnecessary use of antibiotics are one of the main reason for antibiotic resistance and according to this article the best antibiotics for against salmonellosis are enrofloxacin, ampicillin, flumequine, and neomycin.

Keywords: *Salmonellosis*, antibiotic, poultry

Topic: Antimicrobial Resistance

P50: Antibiotic resistance plasmids gene profile in *Staphylococcus aureus* isolates from burn patients

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Introduction: Bacterial resistant to antibiotics, especially those that are multidrug resistant, are increasingly major health care problem around the world. The antibiotic resistance of bacteria is achieved through the concerted activities of mobile genetic elements. These elements are able to move within or between DNA molecules, which include insertion sequences, transposons, and gene cassettes/integrons, and those that are able to transfer between bacterial cells, such as plasmids and integrative conjugative elements. This study aims to outline the presence of the antibiotic resistance plasmid gene in *Staphylococcus aureus*, as one of the hospital pathogens.

Materials & Methods: Total of 295 isolates were obtained from burn patients during one year (January 2018 to January 2019). Isolates were evaluated for detection of *S. aureus* by standard biochemical and bacteriological tests. Antibacterial Resistance pattern was determination by disk diffusion method (CLSI 2018). Confirmed samples were extracted for plasmid by MAXI preparation alkaline lysis with SDS. By using specific primers, resistant genes (*blaZ*, *dfrA*, *ermB*, *ermC*, *ileS*, *mphBM* and *msrA*) were investigated by PCR method on extracted plasmids.

Results: Out of 295 isolates, 93(31.52%) *S. aureus* were detected. The frequency of studied genes were as follows: *blaZ* 91(97.8%), *dfrA 85(91.4%)*, *ermB 26(28%)*, *ermC 87(93.5%)*, *ileS* 20(21.5%), *mphBM* 85(91.4%) and *msrA* 74(79.6%). The prevalence of antibiotic resistance genes showed, that a significant correlation was found between the behavior of these antibiotic resistance and antibiotic resistance plasmid genes.

Conclusion: The plasmids plays a central role in facilitating genetic exchange and promote the acquisition and spread of antibiotic resistance genes among prevalent staphylococcus isolates. This exchange is a perilous step in formation and increasing the risk of missing antibiotic treatment of Staphylococcus bacteria.

Keywords: *Staphylococcus aureus*, Burn patients, Antibiotic resistance, plasmid

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P191: Lytic effect of specific bacteriophage cocktail against *Salmonella (enteritidis, typhimurium, and infantis)* in HEP2 cell line

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Introduction and Objectives: Salmonellosis is one of the most important infectious diseases among humans and animals, and it has many problems in response to common antibiotic treatments. Today, due to the emergence of drug resistance to antibiotics, there should be a good alternative to drugs. Bacteriophages or phages for short, are viruses that attack bacteria and destroy them. Due to their specific properties, the lytic bacteriophages can be considered as an appropriate choice for the treatment of infectious diseases.

Materials and Methods: Standard strains of *Salmonella* (*enteritidis*, *typhimurium* and *infantis*) were prepared from the Faculty of Veterinary Medicine, University of Tehran, and their specific bacteriophages were isolated using soft agar method. In the next step, the direct effect of each strain on the target cell line was investigated, and the effect of the inoculated bacteriophage was studied individually and finally as a cocktail on the strains mentioned.

Results: Particular plaques of bacteriophages isolated from *salmonella* were specific to each *salmonella*, and had no lytic performance against other *salmonella* and other intestinal pathogenic bacteria. The binding and pathogenicity of *Salmonella* strains and cocktails prepared, as was expected, was positive after inoculation, as well as their lytic function, were observed.

Conclusion: Overall, due to the increasing prevalence of antibiotic resistance among *salmonella* and concerns about their treatment, it is anticipated that in the future, these phages can well be used as a proposed for disease control and prevention, both *in vivo* and *in vitro* conditions.

Keywords: *Salmonella*, Bacteriophage, Salmonellosis, HEP2 cell line

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P86: Antibiotic resistance and prevalence of Extended Spectrum ß- Lactamase (ESBL) in non-infectious children under three years old in Ahvaz city

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Introduction and Objectives: *Escherichia coli* is one of the first bacterial species that is colonized in the gastrointestinal tract of newborns and could be an important source of antibiotic resistance genes. Extended-Spectrum ß-lactamase (ESBL)- producing bacteria are worldwide significant threat. The aim of the present study was investigation of the antimicrobial resistance and prevalence of Extended Spectrum ß- Lactamase (ESBL) among *E. coli* strains isolated from feces of non- infectious children under three years old in Ahvaz city.

Materials and Methods: Two hundred one stool samples were collected from non-infectious children and cultured to isolate *E. coli* strains. Antimicrobial resistance pattern of isolates was detected using Kirby-Bauer disk diffusion method. The investigated antibiotics were included nalidixic acid, ampicillin, tetracycline, cefotaxime, ceftazidime, trimethoprim-sulfamethoxazole, ciprofloxacin, and gentamycin. ESBL-producing isolates were detected by combined disk (CD) test. The CD method was performed using cefotaxime, cefotaxime-clavulanic acid and ceftazidime, ceftazidime- clavulanic acid.

Results: The highest resistance was observed against ampicillin (64.7%). The percent of resistance related to nalidixic acid, tetracycline, cefotaxime, ceftazidime, trimethoprim-sulfametoxazole, ciprofloxacin, and gentamycin was as follow: 43.8, 42.3, 49.8, 40.8, 50.2, 25.4 and 6.5 respectively. Of these isolates 31 (15.42%) strains were ESBL carries.

Conclusion: Our study showed high resistance to antimicrobial agents among commensal isolates; therefore, the control of antibiotics use is necessary. The prevalence of ß- lactamases leads to a high resistance to antibiotics, especially ß- lactams and ultimately leads to an increase in infectious diseases.

Keywords: Commensal *Escherichia coli*, antibiotic resistance, Disc diffusion method, ESBL

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P94: Detection of *bla-IMP, bla-VIM* and *bla-spm* genes among *Pseudomonas aeruginosa isolated* from clinical isolates in Tehran, Iran

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Introduction and Objectives: Carbapenems are considered first-line drugs for the treatment of multi-drug resistant bacteria, including *Pseudomonas aeruginosa*. Unfortunately, carbapenem-resistant *P. aeruginosa* (CRPA) has been increasing in recent years. The aim of this study was to determine antibiotic susceptibility patterns and metallo-beta-lactamase (MBL)-mediated resistance in clinical *P. aeruginosa* isolates.

Materials and methods: In this cross-sectional study, 270 *Pseudomonas aeruginosa* isolates were collected from the various clinical specimens in Tehran hospitals from 2014 to 2016. After identification of isolates by biochemical tests then antibiotic susceptibility and detection of MBLs using the Kirby-Bauer and combined double-disk synergy test methods, respectively. The frequency of *bla-IMP* and *bla-VIM* and *bla –SPM* among MBL producing *P. aeruginosa* was investigated using Polymerase Chain Reaction (PCR).

Results: In this study, cephpodoxim and polimixin with 98.6% and 1.7% resistance showed the highest and lowest resistance against isolates. Moreover, 165 (61.1%) of isolates were detected as multi drug resistance strains. A total of 270 isolates, 112 (41.4%) isolates were carbapenem- resistant. From 112 isolates, 51 (45.5%) isolates were metallo-beta-lactamase producer which 48 isolates (42.8%) and 33 isolates (29.4%) carried *bla*IMP-1 and *bla*VIM-1 genes, respectively. Also 12 (10.7%) isolates carried both of the *bla*IMP and *bla*VIM genes simultaneously.

Conclusion: The prevalence of MBLs-producing *Pseudomonas aeruginosa* strains detected in this study is a main concern and highlights the need for infection control measures are needed to prevent further dissemination of these organisms.

Keywords: Carbapenem, Metallo-β-lactamase genes, *Pseudomonas aeruginosa*, PCR, MDR

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P47: Diagnosis and Identification of Macrolic Resistance (Clarithromycin) *Mycoplasma Pneumonia* in Patients with Respiratory Tract Infections in Tehran, Iran

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Introduction and Objectives: *Mycoplasma pneumoniae* is one of the major causes of community acquired pneumonia (CAP). Macrolics are used as the primary treatment for pneumonia caused by *Mycoplasma pneumoniae* that in recent years, the widespread use of macrolics has led to the rapid and global emergence of macrolic resistance to *Mycoplasma Pneumonia* (MRMP) as a result of the nucleotide displacement at specific positions in the domain V of the 23SrRNA gene.

Materials and Methods: In this study, 100 samples of throat swab were collected from patients with respiratory tract infections. After the extraction of the DNA of the samples using Roch kit, PCR technique was performed with specific primers for P1 gene for *Mycoplasma pneumoniae* species and 23SrRNA gene and then was performed for *Mycoplasma pneumoniae* MIC samples with microdilution broth with Claritromicine antibiotic and finally, the PRC product of 23SrRNA gene was sequenced to detect mutations associated with macrolic resistance in the V domain of the 23SrRNA gene.

Results: In this study, using specific primers, 6 cases (6%) were reported positive for *Mycoplasma pneumoniae* species. Also, after analyzing the PCR sequence of the 23SrRNA gene, one it was specified that one of the samples indicated a mutation in the A2431G and G2491A positions that by measuring MIC amount, all of the positive samples for *Mycoplasma pneumoniae* species and 23SrRNA genes were susceptible to the Clarithromycin antibiotic and no macrolic resistance was reported for clarithromycin antibiotics.

Conclusion: In this study, after analyzing the PCR product of 23SrRNA gene, no macrolic resistance in *Mycoplasma pneumonia* was reported against clarithromycin antibiotic. Therefore, in order to prevent the emergence of macrolic resistance in *Mycoplasma pneumonia* MRMP in Iran, the use of macrolic antibiotics should be limited.

Keywords: *Mycoplasma Pneumonia*, Macrolic Resistance, Clarithromycin, 23SrRNA gene

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P81: Identification of Metallo-beta-lactamase (MBL) producing in *Escherichia coli* by phenotypic methods and PCR

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Introduction and Objectives: Among urine pathogens, *Escherichia coli* causes 80% of urinary tract infections. Due to destruct nature of penicillins, cephalosporins and carbapenems (with the exception of manobactam, such as aztreonam), carbapenemase enzymes have created many problems for treating infectious diseases. The study aim was to investigate the phenotypic and molecular characterization of MBL genes produced by *E. coli* isolates in an educational hospital 2016-17.

Materials and Methods: In this cross-sectional study, 80 UTI samples affected by *E. coli* were investigated. To identify MBL enzyme producing strains, phenotypic tests containing Modified Hodg Test, EDS Test and AmpC Disk were performed. The frequency of VIM and IMP genes were determined by PCR.

Results: Between 80 *E.coli* samples, phenotypic tests including, Modified Hodg Test, EDS Test and AmpC Disk Test showed the positivity of 15(18.75%), 15 (18.75%) and 8(10%) isolates, respectively($P<0.001$). PCR test result for VIM gene was 19 (23.75%) positive isolated from *E. coli*, but IMP gene was not observed in any of the isolates($P<0.001$).

Conclusion: The emergence of E. *coli* producing MBLs enzymes is a serious threat amongst clinical infections. The findings of this study indicated the presence of E.*coli* producing MBL. These enzymes can degrade carbapenems antibiotics, the last class current treatment of MDR infections.

Keywords: *E.coli*, drug resistance, VIM, IMP

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P190: Effect of phage on *Acinetobacter baumannii* biofilm

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Introduction and Objectives: Biofilm-associated infections caused by *Acinetobacter baumannii are* extremely tenacious to antibiotic treatment. According to this fact, phage therapy is a good choice for alternative treatment. The aim of this study is to investigate the phage effect on *A. baumannii* biofilm formation.

Materials and Methods: The clinical bacteria that isolated were confirmed by phenotypic and PCR method with bla oxa gene, then among of them MDR isolates with disk diffusion method, chose for further investigation. The MIC method was performed accroding to CLSI 2018. Biofilm formation was measured by microtitre plate. Phage isolation from the environment was performed. Finally, the effect of phage was tested on the biofilm formation of *A. baumannii* by co–incubation.

Results: Fifteen MDR isolates detect and in the MIC test, demonstrate the high resistance value, also in the biofilm formation assay 60%, 26.6% and 13.3% of isolates show strong, moderate and weak biofilm formation respectively. among of those isolates one isolate, that has strongest biofilm and the most resistance, choose for the effect of phage in MoI 10, 1, 0.1, 0.01, 0.001. our study demonstrates that the phage in MoI 0.01 has the best effect on the biofilm formation of that bacteria.

Conclusion: Our study demostrate that phage in MoI 0.01 could be effective in eradication of biofilm formation in *Acinetobacter baumannii*. Efficient bacteriolytic activity and significant reduction of the bacterial biofilm suggests its therapeutic potential to be used to treat infection caused by *A. baumannii.*

Keywords: multi-drug resistant Acinetobacter baumannii, biofilm, phage, phage therapy

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P66: Comparative Evaluation of the Prevalence of Extended Spectrum Beta-Lactamases (ESBLs) Producing *E. coli* in Fecal and Urine Samples of Women with Community Acquired UTIs

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Introduction and Objectives: Uropathogenic *E. coli* (UPEC) which are the principal agent of urinary tract infections (UTIs) can originate from intestinal microbiota or from vaginal microbiota, contaminated foods and environments. The ability of commensal or uropathogenic *E. coli* to produce extended spectrum beta-lactamases (ESBLs) result in resistance to the beta-lactam antibiotics that are important drugs for treatment of UTIs. So, the aim of this study was to evaluate and compare the prevalence of ESBL-producing *E. coli* in fecal and urine samples of women with community acquired UTIs.

Materials and Methods: During seven months, fecal and urine samples were collected from 30 non-hospitalized women with UTIs who referred to different hospitals of Kerman, Iran. The 60 samples were directly cultured on MacConkey agar and lactose-fermenting colonies were confirmed as *E. coli* by conventional biochemical tests. Susceptibility of the 60 isolates to cefotaxime, ceftazidime, and ceftriaxone were analyzed by Kirby-Bauer method. To determine the ESBL activity, the isolates which were resistant to at least one of the tested antibiotics were submitted to phenotypic confirmatory test using cefotaxime disks with and without clavulanic acid according to CLSI guidelines.

Results: Prevalence of resistance to cefotaxime was 10.0% vs. 28.5% in fecal and urinary *E. coli* isolates. These prevalence for ceftazidime and ceftriaxone were 13.3% vs. 21.4% and 10.0% vs. 14.2%, respectively. Totally, six (10%) *E. coli* isolates were confirmed as ESBL- producer. Rates of ESBLs-positive *E. coli* in fecal and urinary isolates were 3.3% and 17.8%, respectively.

Conclusion: Comparative evaluation of the prevalence of antibiotic resistance and rate of ESBLs activity in fecal and urinary *E. coli* isolates revealed no significant difference ($P > 0.05$). The data warns increasing of resistance to beta-lactam antibiotics in *E. coli* which could limit the therapeutic options and increase the mortality rate of UTIs patients.

Keywords: Antibiotic resistance, ESBLs, UTIs, Commensal and uropathogenic *E. coli*

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P227: In vitro Synergistic Effect of Vancomycin and some Antibacterial Agents against Clinical Methicillin-Resistant and Sensitive *Staphylococcus aureus* Isolates

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Introduction and Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) can be responsible for serious long-term infections. Sometimes monotherapy can be ineffective for the treatment of these infections; hence, it is hypothesized that combined drug treatment can be more potent in these cases. The aim of this study was to investigate the synergistic effect of vancomycin and eight other antibacterial agents, in order to identify the best combination pattern in the management of MRSA.

Materials and Methods: AZDAST (Ameri-Ziaee Double Synergism Test), double-disc, checkerboard, and time-kill methods were used to assess the synergistic effect in 24 isolates of *Staphylococcus aureus* (*S. aureus*), including 22 MRSA and two Methicillin-sensitive *Staphylococcus aureus* (MSSA). Furthermore, based on the results, handmade combined antibiotic discs were prepared to evaluate the results of the checkerboard, and time-kill methods at the plate level.

Results: All the isolates were sensitive to vancomycin, linezolid, and daptomycin. Furthermore, penicillin had the highest resistance (100%) in all isolates. The synergistic activities were observed, when the vancomycin was combined with the imipenem, using three double-disc, checkerboard, and time-kill methods. The sub-minimum inhibitory concentration (MIC) amount of the combined discs could increase the diameter of the inhibition zone, confirming the results.

Conclusion: The data obtained from this study suggested that vancomycin and imipenem together, even at sub-MIC, could be effective against MRSA and MSSA infections. Based on the results, the double-disc and combined discs tests can be valuable for an in-house screening in the hospitals' laboratories to faster diagnose the best-combined drugs for therapy of MRSA infections.

Keywords: *Staphylococcus aureus*; MRSA; MSSA; Vancomycin; Synergy; Antimicrobial drug.

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Topic: Antimicrobial Resistance

254: Prevalence of Hemolysins in methicillin resistant *Staphylococcus aureus* isolates of healthy community

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Introduction and Objectives: Methicillin resistant Staphylococcus aureus (MRSA) is the main cause of infections ranging from minor skin infections to serious infections in hospitals and community settings. Alpha hemolysin (hla) and beta hemolysin(hlb) are the most common survival factors in *S. aureus*. The current study is aimed to detect hla and hlb genes in nasal methicillin resistant *S. aureus* isolates in the student population.

Materials and Methods: A total of 400 samples were obtained from the nasal of students' schools in areas 1 and 3 at Tabriz city. After confirmation of *S. aureus* strains by standard biochemical tests, the antibiotic resistance pattern was determined by disk diffusion method. Phenotypic test of MRSA strain was performed using oxacillin 30 µg /disc, and methicillin sensitivity test was performed by cefoxitin 30 µg/disc. The presence of mecA, hla and hlb genes was examined by PCR reaction.

Results: Of 400 samples, 15% (60 cases) were positive *S. aureus*. Among them, 18.34% (11 samples) were MRSA based on disk diffusion. The highest antibiotic resistance was observed to ampicillin (100%) and the highest sensitivity was against vancomycin (100%). Based on PCR results, 60% cases were positive mecA. These results were indicating the emergence of Oxacillin susceptible mecA positive strains (OS-MRSA) for the first time in health community in IRAN.In this study, 88.33% of cases were positive for hla gene and 63.36% were positive for hlb gene.

Conclusion: The prevalence of carriage of hla/hlb positive *S. aureus* indicates an essential need for monitoring of nasal carriers in healthy community to prevent subsequent infections.

Keywords: *Staphylococcus areus*, MRSA, MEC-A, hla, hlb.

Topic: Antimicrobial Resistance

P77: Molecular study of CTX-M-1, CTX-M2, CTX-M9, mcr-1, NDM-1 genes among *Klebsiella pneumonia* isolates from hospitalized patients in Taleghani hospital; Tehran, Iran

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Introduction and Objectives: *Klebsiella pneumonia*, is a major cause of hospital infections and the prevalence of drug resistance among them is increasing globaly. The aim of this study was molecular evaluation of CTX-M-1, CTX-M-2, CTX-M-9, mcr-1, NDM-1 genes among *Klebsiella pneumonia* isolates from hospitalized patients in Taleghani hospital.

Materials and methods: *Klebsiella pneumonia* isolates from patients who referred to Taleghani hospital were collected during2016-2017.All were confirmed by standard bacteriologic tests. Antimicrobial susceptibility test (AST) was done by Kirby-Bauer method based on CLSI protocol. After DNA extraction, frequency of selected resistance genes was determined by PCR method and further sequencing. Statistical analysis was done by SPSS software.

Results: among 70 isolates of ***Klebsiella*** *pneumonia*, the highest resistance were against: Ampicillin74%, Cefpodoxime65%, Ceftriaxone 57%, and 55.6% to Aztreonam. The most sensitivity was to Fosfomycin/Trometamol 86.5%, Amikacin, Imipenem, Doripenem, Meropenem, Ertapenem 71.6%.

PCR results showed that 71% of the isolates had CTX-M-1 and 31% CTX-M-2, 23% CTX-M-9, 1.4% NDM-1. All were negative for *mcr-1gene*.

Conclusion: with the release of new information on drug resistance and the detection of CTX-M 1,2, 9, mcr-1 and NDM-1 genes in *Klebsiella pneumoniae* isolates, doing molecular tests are recommended which may help physicians to prescribe the best and most appropriate antibiotic.

Keywords: *Klebsiella pneumoniae*, mcr-1, antibiotic resistance, CTX-M gene, NDM-1

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P231: The Effects of Berberine and Palmatine on Efflux Pumps Inhibition with Different Gene Patterns in *Pseudomonas aeruginosa* Isolated from Burn Infections

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Introduction and Objectives: Related Multidrug Resistance (MDR) to efflux pumps is a significant problem in treating infections caused by Pseudomonas aeruginosa (P. aeruginosa). Plant compounds have been identified as Pump Inhibitors (EPIs). In the current study, the potential effect of Berberine and Palmatine as EPIs were investigated on efflux pump inhibition through focusing on different gene patterns in P. aeruginosa isolated from burn infections.

Materials and methods: All isolates were collected and identified using the standard biochemical tests. Antimicrobial sensitivity was performed based on disk agar diffusion method for 12 antibiotics. MIC-MBC tests were also performed based on the broth microdilution method to detect synergistic relationship between ciprofloxacin, Berberine and Palmatine. Detection of mexA, mexB, mexC, mexD, mexE, mexF and mexX was conducted by PCR assay. Fisher's Exact test and Logistic Regression were used as statistical tools.

Results: A total of 60 P. aeruginosa isolates were collected. The highest and lowest levels of resistance were found to be respectively against clindamycin and tigecycline. Comparing the MIC with MBC distribution, it was found that Berberine and Palmatine lower the MIC-MBC level of ciprofloxacin. The PCR results indicated that the highest frequency is about MexAB-OprM operon. The statistical analysis among different gene patterns of efflux pumps showed that there were no significant relationships between the effectiveness of Berberine and Palmatine ($p>0.05$).

Conclusion: It can be speculated that Berberine and Palmatine both act as EPIs and can be used as auxiliary treatments with the purpose of increasing the effect of available antibiotics as well as decreasing the emergence of MDR bacteria. The efficiency of these combinations should be studied further under in vivo conditions to have a more comprehensive conclusion regarding this issue.

Keywords: Berberine, Palmatine, Pseudomonas aeruginosa

Topic: Antimicrobial Resistance

P90: Antimicrobial susceptibility profiles in *Streptococcus agalactiae* isolated from pregnant women in Yasuj city

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Introduction and Objectives: Group B Streptococcus (GBS) causes severe infections in very young infants and invasive disease in pregnant women and adults with underlying medical conditions. The aim of this study was to evaluate PCR assay compared with conventional culture method and confirmed by PCR and evaluation of antimicrobial susceptibility for isolated bacteria.

Materials and methods: Total of 400 paired vaginal and anul swabs were collected from women at 35-37 weeks of pregnancy from May 2017 through May 2018 for detection of *Streptococcus agalactiae* using PCR assay targeting *cfb* and *atr* genes and culture method following broth enrichment. Antibacterial susceptibility testing was performed on isolated bacteria

Results: Prevalence of *Streptococcus agalactiae* was determined as 13.4% (n=107) using culture method of which 34.6% were confirmed by PCR on *atr* and *cfb* genes. Microbial susceptibility to Chloramphenicol (77%), Ampicillin (77%), Penicillin (23.1%), Erythromycin (7.7%), Azithromycin (3.8%), Clindamycin (3.7%), Cefotaxime (84.6%), Ciprofloxacin (92.3%), Linezolid (100%) and Vancomycin (100%) were observed.

Conclusion: The results showed that the suspected bacteria isolated were necessary with the PCR test, and antibiotics were prescribed for treatment according to the antibiogram test result.

Keywords: *Streptococcus agalactiae*, Pregnancy, PCR

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P217: New antibacterial wound dressing designed with DOE

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Introduction and Objectives: As antibiotic resistance is rising, use of intrinsic antimicrobial polymer like chitosan is increasing. chitosan is biodegradable, wound healing material for delivery drugs as wound dressing. Hydroxyl group in structure of *polyvinyl alcohol* (PVA) interacts with rigid molecule of chitosan and make hydrogen bonding on molecular level. Zinc oxide nanoparticles (ZnO NPs), individually or in combination with antibiotics loaded in chitosan, can be considered as good candidates for struggling against drug resistant bacteria. Major Nosocomial infections by multi-drug resistant (MDR) *Pseudomonas aeruginosa* is difficult to treat with usual antibiotics, these pathogens cause tardiness in wound healing.

Materials and Methods: Design Of Experiments (DOE), central composite designs were used to optimization of drugs in formulation of films and three parameters, the amount of ceftazidime(x_1), ZnO (x_2), sucralfate as antimicrobial agents. Antimicrobial effect (Y_2) as response were checked. Formulations were prepared by solvent casting method. 1.5% chitosan and 5% PVA were dissolved in acetic acid 1% and distilled water (1:1) under magnetic stirring at room temperature for overnight, drugs and ZnO NPs were loaded on polymer. Antibacterial activity of formulations against 3 MDR *P. aeruginosa* strains, isolates from burn wounds and 3 standard strains of *P. aeruginosa* were tested by viable cell counting method. Determination of folding endurance of each formulation was carried out by repeatedly folding the film at 180-angle at the same place until it broke.

Results: Antimicrobial activity of 17 formulations were tested against standards and isolates *P. aeruginosa.* All of studied strains were inhibited by formulation number 12 with 20µg/ml of ceftazidime and 6% ZnO NPs and 14% sucralfate more than 90%. The folding endurance of formulation number 12 was found to range of standard.

Conclusion: formulation number 12 with 20µg/ml of ceftazidime and 6% ZnO NPs and 14% sucralfate has the highest antibacterial effect and the best folding endurance needs further in vivo study for wound dressing application.

Keywords: antimicrobial resistance, chitosan, wound dressing, ZnO NPs, ceftazidime

Topic: Antimicrobial Resistance

P48: Isolation and antibiotic resistance pattern determination of bacteria causing urinary tract infections in patients referred to the central laboratory of Poldokhtar city in Lorestan, Iran

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Background and Objectives: Urinary tract infections are one of the most common bacterial infections in humans. Increasing resistance to antimicrobial agents in bacteria is a major problem around the world in treatment of urinary infections.

Materials and Methods: In this descriptive study, 2150 urine samples were collected from admitted patients. Urine specimens were cultured in Blood agar and Eosin-methylene blue medium (EMB), then were identified the specimens that were positive in term of culture. Resistance and sensitivity tests of urinary pathogens to conventional antibiotics were evaluated using a standard diffusion method according to CLSI standard.

Results: The urine test result was positive in 107 (4.9%) patients of 2150 eligible ones. They were 10.3% male and 79.7% female. The most common pathogens isolated were *Escherichia coli* (83.2%), *Staphylococcus epidermidis* (7.5 %), *Citrobacter* species (2.8%), *Proteus* species (2.8%), and *Klebsiella* (2.8%). The most antibiotic resistance was related to co-trimoxazole (58.1%) and the Highest sensitivity was related to nitrofurantoin antibiotics (90.1 %) and gentamicin (88%).

Conclusion: The present study showed that *Escherichia coli* is the most common pathogen causing the urinary tract infection. Due to the high resistance of co-trimoxazole to antibiotics, it is recommended to avoid overuse, as well as is antibiogram tests are essential for treatment of urinary tract infections.

Keywords: urinary tract infection, pattern of antibiotic resistance, *Escherichia coli*

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Topic: Antimicrobial Resistance

P335: Evaluation of antibiotic resistance in *Aeromonas hydrophila, Streptococcus iniae, Yersinia ruckeri*

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Introduction and Objectives: The aquaculture industry is very important to produce nearly one-third of the world's seafood supplies. One of the biggest problems in this industry is bacterial infectious diseases. The aim of this study was to measure the susceptibility or resistance of 3 bacterial pathogens to antibiotics commonly used in treatment.

Materials and Methods: After preparing bacterial culture from each specimen in Muller Hinton Broth medium, the samples were compared for opacity with 0.5 McFarland (10^8 bacterial). For antibiotic test bacterial was cultured on a Merck Germany medium and the effects of antibiotic disks were evaluated using the CLSI (Clinical and Laboratory Standard Institute) method. According to the standard, the diameter of the zone was determined to prevent the growth of bacteria. The absence of zone formation or diameter less than the sensitivity standard of bacteria was reported as resistance to the antibiotic. Antibiotic disks used in this study were obtained from the Iranian Antibacterial Medicine Company, which included: tetracycline 30 µg, amoxicillin 25 µg, ampicillin 10µg, erythromycin was 15 micro grams. So, after the bacterial culture of the bacteria on the agar culture medium, the antibiotic discs were incubated on the agar medium and incubated after 48-72 hours and after 5 days the diameter of the Growth bacterial was measured.

Results: This research was based on three control treatments (Injection *Streptococcus iniae*, *Yersinia ruckeri* and *Aeromonas hydrophila*) and 15 treatments (3 bacteria and 5 antibiotics) were evaluated in 4 replications. Data analysis was performed using SPSS software version 18. For The results showed that there was a significant difference between the two aerobic bacteria of *Aeromonas hydrophila* during the 24 hours and the *Yersinia ruckeri* against antibiotic amoxicillin. In the case of *Aeromonas hydrophila* and *Yersinia ruckeri*, there was no significant difference between 24, 48, and 72 hours in the 3rd time, but the *Streptococcus iniae* had a significant difference with 48 hours and 72 hours with the first of 24 hours. Also, the data showed that 3 bacteria tested at different times showed no significant difference with the antibiotics of oxytracycline, but there was a significant difference in comparison with each other. In contrast to erythromycin, there was a significant difference between different times for *Aeromonas hydrophila* bacteria but there was no significant difference for *Streptococcus iniae* infection in different times. In the case of *Yersinia ruckeri* bacteria, there was a significant difference between 48 hours and 24 hours and 72 hours. Against tetracycline, there was a significant difference in the *Yersinia ruckeri* time of 24 hours with two other times.

Conclusion: The bacteria of these 3 bacterial pathogens are still susceptible to 5 antibiotics and can be introduced for the treatment of aquatic diseases**.**

Keywords: Aquatic Disease Bacteria, Antibiotic Disc, Zone of Growth

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Topic: Antimicrobial Resistance

P193: Investigating the antimicrobial and antibiofilm effects of *Euphorbia hebecarpa* extract on some pathogen bacteria resistant to antibiotics

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Introduction and Objectives: Spurge, also known as *Euphorbia hebecarpa*, belongs to Euphorbiaceae family. *Euphorbia hebecarpa* is mostly located in humid areas, along streams and in wheat or barley farms and cause disturbance like a weed. This plant is distributed all around the world while some species are exclusively seen in Iran. Alkaloids, saponin, tannin, flavonoids and cardiac glycosides are among the known compounds in this plant.
Materials and methods: This study aimed to assess the effects of *Euphorbia hebecarpa* on Gram-positive bacteria of *Staphylococcus aureus*, *Bacillus cereus* and *Streptococcus pneumonia* and Gram-negative bacteria of *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*. The anti-biofilm effect of this plant was determined using microtiter plate. In addition, the effects of this plant on the dehydrogenase activity of bacteria was also investigated.
Results: *Euphorbia hebecarpa* was mostly effective on the planktonic forms of *Bacillus* and *E. coli*. Assessment of plant's capacity to form biofilm using BATH and microtiter plate tests showed that *Streptococcus pneumonia* has the strongest biofilm while the weakest biofilm is formed by *Bacillus cereus*. Considering their inhibitor effects on biofilm formation, the most inhibiting effect was seen in ethanol extract of *Euphorbia hebecarpa* on *Staphylococcus aureus* (99.5%) while the ethanol extract on *Bacillus cereus* was effective in elimination of biofilm structures. Moreover, the lowest level of dehydrogenase activity was observed in *Bacillus cereus* treatment with ethanol extract of *Euphorbia hebecarpa* (98.58%).
Conclusion: In this study, the role of herbal extracts in the elimination of biofilm structures was shown and these extracts were presented as an appropriate alternative to address the various properties of biofilms.
Keywords: Biofilm, Resistance, Inhibition, Plant extract, Antibiotics

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Topic: Antimicrobial Resistance

P93: Prevalence of *bla* $_{CTX-M}$ Genes among ESBL-Producing *Klebsiella pneumoniae* in Bushehr Province, Iran

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Introduction and Objectives: CTX-M-type extended-spectrum β-lactamases (ESBLs) are the most prevalent ESBLs in bacterial members of *Enterobacteriaceae* family including *Klebsiella pneumoniae*. The global spread of CTX-M-producing *K. pneumoniae* is a major concern in most countries as well as Iran. The aim of this study was to determine the prevalence of CTX-M types of β-lactamases in ESBL-producing *K. pneumoniae*.

Materials and Methods: A total of 212 non-duplicate *K. pneumoniae* were collected from different clinical specimens and confirmed using amplification of malate dehydrogenase (*mdh*) gene. ESBL production was confirmed using combine disk test (CDT). In addition, PCR technique was used for genotypic detection of CTX-M types in ESBL-Producing isolates.

Results: Fifty-six (26.41%) out of 212 isolates were confirmed as ESBL producer. Based on the results of PCR, *bla* $_{CTX-M}$ was found in 83.92% of ESBL-producing *K. pneumoniae*. The prevalence of bla $_{CTX-M-1}$ and *bla* $_{CTX-M-9}$ genes among CTX-M harboring isolates was 97.8% and 2.1%, respectively. Neither $_{CTX-M-2}$ nor $_{CTX-M-8}$ was found in our isolates.

Conclusion: Our data revealed the low prevalence of ESBL-producing *K. pneumoniae* in Bushehr province although the prevalence of *bla* $_{CTX-M-1}$ genes was high among ESBL-producing *K. pneumoniae* isolates. Screening of ESBL production in clinical isolates could help in controlling infection in healthcare and community settings.

Keywords: *Klebsiella pneumoniae*, ESBLs, CTX-M genes, PCR.

Topic: Antimicrobial Resistance

P78: Detection of Carbapenem-resistant *Enterobacteriaceae* by the phenotypic methods in Tabriz during 2018

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Introduction and Objectives: Carbapenem-resistant *Enterobacteriaceae* (CRE) are a major concern associated with morbidity and mortality in the world. CRE often is becoming a cause of therapeutic failure in both hospital and community acquired infections. The aims of this study were to determine carbapenem resistance *Enterobacteriaceae* by the phenotypic methods.

Materials and Methods: Fifty non-duplicated CRE were recovered from Tabriz, Iran. Antimicrobial susceptibility testing were performed by the phenotypic methods. The carbapenem resistance mechanisms such as carbapenemase genes and AmpC mechanism were determined by the phenotypic methods.

Results: Fifty CRE (41 *Klebsiella pneumoniae*, 6 *Escherichia coli* and 3 Enterobacter spp.) from urine (52%), wounds (22%), blood (20%) and other body fluids (6%) isolated from Jan 2018 to Dec 2018. According to Carba NP Test, all isolates were positive for carbapenemase, and Modified-Hodge test detected 49 isolates for carbapenemase activity. AmpC mechanism found in six isolates (three Enterobacter spp. and three *K. pneumoniae*). All CRE isolates were susceptible to Colistin.

Conclusion: Our findings show that CRE isolates have high-level resistance to antimicrobial agents. Carba NP Test and Modified-Hodge test are cheaper than molecular assays, can detect carbapenemase activity.

Keywords: Antibiotic resistance, Carbapenem-resistant Enterobacteriaceae, Phenotypic method

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Topic: Antimicrobial Resistance

P84: Phenotypic and Genotypic Identification of Carbapenemase-producing *Klebsiella pneumoniae* in Bushehr Province, Iran

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Introduction and Objectives: The emergence of carbapenem-resistant pathogens particularly, the increasing prevalence of carbapenemase-producing Klebsiella pneumoniae poses a serious threat to public health worldwide. The aim of this study was to evaluate the prevalence of carbapenemase-producing Klebsiella pneumoniae *using phenotypic and genotypic methods.*

Materials and Methods: A total of 151 non duplicate *K. pneumoniae* isolates were collected from six hospitals in Bushehr province and confirmed using PCR of malate dehydrogenase (*mdh*) gene. Antimicrobial susceptibilities were determined by disk diffusion and E-test methods. The modified carbapenem inactivation method (mCIM), EDTA-modified carbapenem inactivation method (eCIM) and OXA-48 Disk Test were used for phenotypic confirmation of carbapenemase production. The resistance genes including bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{IMP}, bla_{VIM}, and blaGES were detected by PCR method.

Results: Totally 12 (7.9%) carbapenemase-producing *K. pneumoniae* isolates were identified. Antibiogram results via disk diffusion revealed colistin (99.4%) as the most effective of all 18 tested antimicrobial agents. Carbapenemase-producing isolates exhibited a range of imipenem MIC values from 4 µg/ml to ≥32µg/ml. The results of molecular detection were in accordance with phenotypic confirmatory tests. PCR results indicated that bla_{NDM-1} and bla_{OXA-48} genes were found in 11(91.6%) and 4 (33.3%) carbapenemase-producing isolates respectively. It is notable that the coexistence of bla_{NDM} and bla_{OXA-48} was seen in 3 (25%) isolates. In addition, bla_{KPC}, bla_{IMP}, bla_{VIM}, and bla_{GES} were not found in our isolates.

Conclusion: Our findings highlight the emergence and dissemination of NDM-producing *K. pneumoniae* and emphasize the need for intensive surveillance and precautions.

Keywords: Klebsiella pneumoniae, KPC, NDM-1, OXA-48, Carbapenemases

Topic: Antimicrobial Resistance

P206: Synthesis and characterization of Ag nanoparticles by ultrasonic method and their antibacterial effects investigation on methicilin resistant *Staphylococcus aureus* isolates

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Introduction and Objectives: Serious infections are associated with methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria and this can lead to many deaths in the world. This mortality rate is due to an increase in antibiotic resistance among bacteria. Nano-silver particles have found many applications as alternative antimicrobials in recent years. The aim of the present study was synthesis of silver nanoparticles to evaluate the antibacterial effect against Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates from clinical samples.

Materials and Methods: Silver nanoparticles was synthesized by micellelation assisted ultrasonic method. The as-prepared silver nanoparticles were characterized with X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM). The XRD studies showed that pure Ag nanoparticles have been produced after calcination.The antibacterial effect of silver nanoparticles was performed using Agar well diffusion assay and minimum inhibitory concentration (MIC) was determined.

Results: Based on obtained findings, synthesized silver nanoparticles showed favorable effects on the bacterial isolates. The mean MIC and MBC value were determined as 0.015 mg/ml and 0.07 mg/ml, respectively. All MRSA isolates were susceptible to more than MIC of nanosilver in contrast showed high resistance to multiple classes of antibiotics.

Conclusion: silver Nanoparticles have high inhibitory activity against MRSA, thus can be proposed as an alternative or adjuvant with antibiotics for MRSA treatment. Further investigations are required to assess the safety and efficacy of nano- silver particles in the body.

Keywords: Silver nanoparticles, Methicillin-Resistant *Staphylococcus aureus*, Antibacterial effects.

Topic: Antimicrobial Resistance

P275: Flow cytometry as a tool for study of antibiotic resistance pattern in Multi drug resistant Acinetobacter baumannii isolates

Nahid Rahimi

Introduction and Objectives: Acinetobacter baumannii is one of the most cause of nosocomial infections and MDR bacteria in worldwide. The rapid identification of MDR isolates is particular important. Flow cytometry is a rapid method that can analysis thousands of cells per second and can be used for identification, determination of cell viability in microbial populations and determination of bacterial antimicrobial susceptibility. The aim of this study was to investigate antibiotic resistance patterns of Acinetobacter baumannii isolates by flow cytometr.

Materials and Methods: 55 isolates of Acinetobacter baumannii were isolated from clinical specimen of patients and were identified by biochemical tests. Antibiotic resistance patterns were studied by disc diffusion method for 7 antibiotics based on CLSI 2017 and MDR strains were selected. MIC of Meropenem and Piperacillin were determined by microdilution broth method. Also antibiotic resistance pattern of isolates was determined by coloring with Rhodamine-123 and flow cytometry.

Results: The highest and lowest resistance were for pepracilin (100%) and tetracycline (65.7%), respectively. 98% of isolates were MDR. The MIC ranges for maropenem were 8 - 256 µg / ml and for piperacillin were 128-1024 µg / ml. By flow cytometry demonstrated that at concentrations of 8, 4 and 2 µg / ml of meropenem, only 1.96%, 1.44% and 0.59%, of cells were killed. At concentrations of 64,128 and 16 µg / ml of piperacillin, 13.8%, 11.3% and 5.9%of cells were killed. Reducing the number of living bacteria was observed with increasing concentrations of both antibiotics. Up to 95% of isolates were resistant to both antibiotics by flow cytometry.

Conclusion: The similarity between the results of flow cytometry and both agar and broth antibacterial susceptibility methods showed that flow cytometry as reliable and rapid test can be used for this purpose.

Keywords: Antibiotic susceptibility, Acinetobacter baumannii, MDR, flow cytometer

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Topic: Antimicrobial Resistance

P56: Emergence of *bla*OXA-Carrying Carbapenem Resistance *Acinetobacter baumannii* in the Intensive Care Unit

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Introduction and Objectives: *Acinetobacter baumannii* as a major human pathogen is associated with nosocomial infections especially in intensive care units (ICUs). Nowadays, increasing in the frequency of multiple drug-resistant (MDR) and (XDR) *A. baumannii* infections is an excessive dilemma in all part of the world. Carbapenems were highly efficacious antibiotics to eradicate *A.baumannii* strains. Unfortunately, a globally rise in carbapenem-resistant *A. baumannii* (CRAB) strains has been reported during the last 10 years. The production of oxacillinases is the main resistance mechanism among CRAB. *A.baumannii* strains are predominantly express Class D OXA-type enzymes such as the intrinsic OXA-51 enzyme, and the acquired OXA- 23, OXA-24, OXA-58. This study was aimed to detect the OXA-type genes among clinical strains of *A. baumannii* isolated from hospitalized patients in the north of Iran.

Materials and methods: In a cross – sectional study during 6-month period in 2018, 700 samples were collected from ventilator, burn wound and bloodstream of hospitalized patients in ICUs of three hospitals in the north of Iran. All isolates were identified by conventional biochemical and microbiological tests and were confirmed by amplification of *blaoxa- 51*-like gene. Antibiotic susceptibility test was performed by Kirby-Bauer method and for Colistin and Imipenem, minimum inhibitory concentration (MIC) was determined by E-test. Plamid DNA of all *A.baumannii* isolates were extracted using plasmid extraction kit. Multiplex PCR was used for detecting of *blaOXA-23*-like, *blaOXA-24*-like and *blaOXA-58*-like genes.

Results: Totally, 59 (8.4%) non duplicate *A. baumannii* isolates were obtained from collected samples. The majority of *A. baumannii* strains (42 strains) were isolated from ventilator (71.2%) followed by burn wounds (20.3%) and bloodstream (8.5%). The results of antibiotic susceptibility test revealed that all isolates were resistant to meropenem, cefepim, imipenem and ceftazidime. The least resistance rate was observed against Doxycyclin (42.4%). Also, MIC results showed that all clinical isolates of *A. baumannii* were susceptible to colistin but were resistant to imipenem. Among 59 clinical isolates of *A. baumannii*, *bla*-OXA-23 was the most prevalent *bla*-OXA gene (86.4%) followed by *bla*-OXA-24 (69.5%). None of clinical isolates harbored *bla OXA*-58 gene.

Conclusion: In the present study, a high frequency of MDR *A.baumannii* isolates (76.3%) and XDR (23.7%) were detected which demonstrate a high distribution of carbapenemase-encoding genes and high resistance to cephalosporins, aminoglycosides, floroquinolones and ampicillin-sulbactam combination in this region. Fortunately, the full susceptibility of all CRAB to colistin was seen in this study. Results of this study indicate that colistin is still an option of drug for the treatment of infections caused by *A. baumannii* in Iran hospitals.

Keywords: *Acinetobacter baumannii*, nosocomial infections, Antibiotic susceptibility, Oxacillinases

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Topic: Antimicrobial Resistance

P131: Antibiotic Susceptibility of *Ornithobacterium rhinotracheale* (ORT) Isolated in broiler chickens of Arak, Markazi province 2017

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Introduction and Objectives: Respiratory diseases cause considerable damage to the poultry industry in worldwide and various bacterial and viral agents involved in its creation. In recent years, *Ornithobacterium rhinotracheale* (ORT) bacterial disease has been reported from different parts of the world frequently including of Iran as poultry respiratory infections.

Materials and Methods: In this study, 231 samples from each bird with respiratory signs were collected by taking of lungs, trachea, ocular sinus swaps and corps from Arak poultry farms during August 2011 to March 2013. The samples were cultured on blood agar medium with 5% sheep blood containing Gentamicin and Polymyxin B and then incubated with 7.5% CO_2 at 37 °C for 48 to 72 hours. The bacteria identification was carried out using biochemical tests.

Results: The antimicrobial susceptibility of the bacteria to 17 common antibiotics which used in poultry farms was determined by Kirby & Bauer method as well. In this study, the ORT bacteria were isolated from the 15 out of 231 (6.5%) samples that means 20% of Arak poultry farms are infected by ORT .All of the isolates were resistant to Gentamicin, Enrofloxacin, Erythromycin, Penicillin, Amoxicillin, Colistin, Lincomycin and Polymyxin B completely. These isolates also were mostly sensitive to Nitrofurantoin.

Conclusion: The results of this study indicate that 20% of the examined poultry farms of Arak city are infected with the *Ornithobacterium rhinotracheale* (ORT) and have highly resistant to current antibiotics.

Keywords: *Ornithobacterium rhinotracheale*, poultry, respiratory infections, Kirby & Bauer.

Topic: Antimicrobial Resistance

P72: Antibiotic resistance patterns in *Acinetobacter baumannii* isolates obtained from Emam-Ali hospital of Zahedan

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Introduction and Objectives: *Acinetobacter baumannii* is a gram negative, aerobic and non-motile bacillus that generally is part of the normal body flora but also have described as a leading nosocomial pathogen. *A. baumannii* is known as the causative agent of a wide variety of local and systemic infections like pneumonia, septicemia and wound infections.

Materials and Methods: In this study, 180 clinical isolates of *A. baumannii* were obtained from clinical samples. Identification was made by colony morphology and biochemical tests. Susceptibility to antimicrobial agents was determined by using the disk diffusion method. The following antimicrobial agents were used: Amikacin (AN), Imipenem (IMP), Colistin (Col), Trimethoprim-sulfamethoxazole (SXT), Cefepime (CPM), Ciprofloxacin (CP)

Results: Antimicrobial sensitivity pattern showed that was Ciprofloxacin(CP) the most effective drug since it inhibited 70.1% of the isolates, antimicrobial sensitivity to Other antibiotics were as follow: AN(48.4%) , SXT(66.2%) ,CPM(16.1%) ,IPM(27.2%),COL (57.7%).

Conclusion: The results showed that, Ciprofloxacin is a promising drug in the treatment of *A. baumannii* infections Due to the high frequency of multi-drug resistance strains of this bacterium, Antibiotic resistance screening is a very important process and could be employed as a preventive strategy in prohibition of antibiotic resistance development.

Keywords: *A. baumannii*, Antibiotic resistance, Infection

Topic: Antimicrobial Resistance

P174: Identification of drug-resistant *Candida* species causing recurrent vulvovaginal candidiasis, a new report

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Introduction and Objectives: To investigate the drug resistance in women with vulvovaginal candidiasis which has long been treated with azoles, susceptibility of *Candida* species isolated from VVC cases to clotrimazole and fluconazole and molecular screening of *ERG3* gene in the azole resistant *Candida* species was performed.

Materials and Methods: For the identification at the species level of isolated *Candida* species differential media, CHROM agar Candida, GT test and Corn meal agar were used and confirmed by PCR-RFLP. A disc diffusion method was performed based on the standard guidelines of the National Committee for Clinical Laboratory Standards to determine level of susceptibility against fluconazole and clotrimazole. An azole resistance factor, *ERG3* mutant gene was screened.

Results: Among all Candida isolates, 76.3% (74 cases) were *Candida albicans* followed by *C. glabrata* and *C. krusei* 9 (9.3% each) and other non albicans *Candida* species 4 (4.1%). From the *C. albicans* isolates resistant to Clotrimazole, 8(53.3%) had *ERG3* gene and 7(46.7%) did not. Among all isolates resistant to Clotrimazole, 40% carried *ERG3* mutant gene against 60% of others that did not show the gene. Also, in 50% of the isolated *C. glabrata ERG3* mutant gene was detected.

Conclusion: As a conclusion, the mutant *ERG3* gene can be detected in a considerable number of azole resistant *Candida* species.

KeyWords: Candida, vulvovaginal candidiasis, drug resistant, ERG3

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Topic: Antimicrobial Resistance

P267: Serotyping and Antimicrobial Susceptibility Pattern of *Escherichia coli* Isolates from Urinary Tract Infections in kidney transplant patients

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Introduction and Objectives: Urinary tract infections (UTIs) are the most common infections in renal transplant recipients and are considered as a potential cause of bacteremia, sepsis, and affects graft outcomes(1). The aim of this study was to isolate and serotype and the *E. coli* isolates in kidney transplant patients with urinary tract infections (UTIs) and to assess their antibiotic susceptibility patterns.

Materials and Methods: In this study, 53 *E. coli* isolates were collected from Kidney patients of Labbafinejad hospital and Yekta Labs during Apr to May 2019. All isolates were reconfirmed by standard bacteriologic methods and kept in 10% glycerol +TSB at -70°C. Antimicrobial susceptibility test (AST) was done according to CLSI 2018 protocol. Serotyping of E. *coli* isolates was identified by slide agglutionation method and using BaharAfshan antiserum kit.

Results: Only 50 isolates reconfirmed as *E. coli* after biochemichal tests and 3 samples were excluded of this study. By slide agglutination, the frequency of serotypes were as: 25 isolates serotyping I (O126,O55,O111) and 6 samples serotyping II (O86,O127), 12 samples serotyping III (O44,O125,O128) and 4 samples serotyping VI (O120,O114).. Based on AST,
highest antibiotic susceptibility were to: doripenem, fosfomycine and ertapenem (100%), Imipenem 95%, also, the highest antibiotic resistance were to: ampicillin (86%), cefotaxime (80%), cefazolin and cefpodoxime (77%).

Conclusion: Because of high resistant rate to common antibiotics based on AST results and critical effect of accurate antibiotic prescription on the graft's destiny among Kidney transplant patients, smart local screening and doing AST before any prescription should be mandatory.

Keywords: *E. coli*, drug resistance, kidney transplantation

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Topic: Antimicrobial Resistance

P36: Three Years Cross Sectional Study on Resistant Nosocomial Infections at Shiraz burn center

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Introduction and objectives: Nosocomial infection and drug resistance against them are a global concern in the worldwide. Recently invasive infection is associated strongly with mortality and morbidity rate, especially in immunosuppressive patients like burned victims. So knowledge about burn infection and antibiotic susceptibility pattern can contribute to the effective control of infection. The current study investigates the prevalence of infectious agent and antibiotic resistant pattern in order to improve treatment policy in burn patients.

Materials and methods: This cross sectional study was conducted during April 2016 to March 2019 in Amir-Al momenin burn center affiliated with Shiraz University of Medical Sciences. A total number of 1180 samples were taken from blood, urine, stool, wound and evaluated to detect the most prevalent infections during 3 years. Patients with sustained deep burns (degree 2 and more) included in the study.

Results: Out of 1180 patients, 60.8% were males while 39.2% were females. Patient's age range from 1 to 89 years with mean 32.07 ±20.88 .According to infectious agent; *Pseudomonas aeruginosa* was the most frequent isolates (46.9%) followed by *Klebsiella (11.3%) and Acinetobacter Baumannii* (6.8%). Based on the sensitivity pattern; highest resistance against *P.aeruginosa* and *Klebsiella* was Ciprofloxacin which was estimated 40.93 and 7.28% respectively. The antibiotics to which *A. Baumannii* was most resistance was Imipenem and Ceftazidime (5.93%)

Conclusion: A major barrier to the treatment of nosocomial infections is the increase in antibiotic-resistant organisms especially for burn victims. Hand hygiene, considering stewardship program and especial attention to infection control programs will help to improve the patient's surveillance.

Keywords: Nosocomial infection, antibiotic-resistance, Burn Patients, Shiraz.

Topic: Antimicrobial Resistance

P87: Antibiotic resistance and biofilm formation of *Pseudomonas aeroginosa* strains isolated from clinical samples in Kerman, Iran

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Introduction and objectives: *P. aeruginosa* strains are common pathogens in hospitals as they have ubiquitous nature, ability to survive in moist environments and innate resistance to many antibiotics. The aim of this study was the survey of biofilm formation and drug resistance of *Pseudomonas aeruginosa* strains.
Materials and methods: A total 15 isolates of *Pseudomonas aeroginosa* were isolated during April to June 2018 from different clinical samples obtained from hospitals in Kerman. All isolates were identified on the basis of their cultural, morphological and biochemical characters and antibiogram was evaluated by Kirby-Bauer's disk diffusion method as well as MIC against common antibiotics by CLSI2016 guide line. Cell surface hydrophobicity (CSH) test and biofilm formation on glass and polyprolene surfaces in shaking and static states were also performed.
Results: 20 strains of *P.aeruginosa* were identified by characteristics as oxidase-positive, motile bacteria with production of a blue, red or brown pigment on King's medium. They were resistant to tetracycline (95%), Chloramphenicol (80%), Imipenem (75%), ceftizoxime (65%), norfloxacin (30%), and Gentamycin (15%). MICs were observed in different values. Maximum cell surface hydrophobicity was 81% about *P.aeroginosa* IAUK8717 was reported with maximum biofilm formation in shack and static states on glass and polypropylene.
Conclusion: Antibacterial surveillance should be performed periodically to monitor the present resistance patterns of *P. aeroginosa* in different parts of local hospitals such as ICU. Finding accurate information about multidrug resistant strains of *P. aeruginosa* will allow us for better programming in resistance interruption in the future.
Keywords: *Pseudomonas aeruginosa*, biofilm, Gentamycin.

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Topic: Antimicrobial Resistance

P57: Investigation of the frequency of MDR *Staphylococcus aureus* strains in of hospital food and stool samples in patients with diarrhea in three hospitals of Tehran

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Introduction and objectives: Increased frequency of Methicillin-resistant *S. aureus* (MRSA) infections imposes a high and increasing burden on healthcare resources. The epidemiology of MRSA is constantly changing, which results in variation in its drug-resistance patterns throughout regions and countries. We aimed to investigate the frequency of multidrug-resistant *S. aureus* (MDR-SA) in the hospital food and stool samples in patients with diarrhea.

Materials and Methods: A total of 258 faecal samples from patients with diarrhea and 35 food samples were used to investigate infection with *S. aureus*. Methicillin-resistant *S. aureus* (MRSA) was characterized by the cefoxitin disk diffusion method in Mueller Hinton agar medium supplemented with 1% NaCl. PCR amplification of enterotoxin genes (*sea*, *sec*, and *see*) was carried out on all *S. aureus*. Susceptibility to 11 antimicrobial agents were analyzed by the standard disk diffusion method according to CLSI guidelines.

Results: *S. aureus* was detected in 22.09% (57/258) of the stool samples and 14.28% (5/35) of food samples. Nearly, 10.5% (6/57) and 8.7% (5/57) of the strains from stool samples and 20% (1/5) and 20% (1/5) of the strains from food samples were characterized as MRSA and MDR, respectively. Resistance to most of the antibiotics was <20%, while highest one detected against tetracycline (24.5%). Low frequency of MDR patterns (3DR, 4DR, 5DR, and 6DR) were detected in the fecal and food *S. aureus* isolates. Among them, panta-drug resistant *S. aureus* was detected in 3.5% of the patients' isolates and triple-drug resistant phenotype was the only MDR pattern was detected in the food samples (2.8%). Nearly, 43.8% (25/57) of the strains carried the enterotoxin genes; the most common was sea^+ (17.5%), sea^+/see^+ (5.2%), sec^+(15.7%), sea^+/sec^+(3.5%), and $sea^+/sec^+/see^+$(1.7%). These genes were significantly higher among MDR compared to non-MDR *S. aureus* strains isolated from the fecal or food samples (100% *vs* 39.2%).

Conclusion: Involvement of MDR and enterotoxigenic *S. aureus* strains in the occurrence of gastroenteritis and their carriage in medical food samples highlighted the importance of food controls in prevention of gastrointestinal diseases, both in the community and clinical settings.

Keywords: MDR-SA, MRSA, Enterotoxigenic *S. aureus*, Diarrhea.

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P37: An Investigation into Antibacterial Activity of Fluoroquinolone-Derived Compounds on Two Gram-Negative Bacteria: *Escherichia coli* and *Pseudomonas aeruginosa*

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Introduction and objectives: Quinolones are known as a bunch of antibiotics inhibiting two central enzymes involved in DNA replication and transcription; i.e. DNA gyrase and topoisomerase IV. Among them, fluoroquinolones can be developed via substituting fluorine atoms at the sixth position of core quinolone structure, thereby enhancing antibacterial activity. As a result, growth and proliferation of bacteria may be prevented through new compounds derived from fluoroquinolones and somehow strengthen their antibacterial effects.

Materials and Methods: The present study was designed and conducted to measure inhibitory concentrations of N-4-methyl (phenyl)-2,2,2-trifluoroacetimidoylciprofloxacin (**5a**) and N-4-methyl (phenyl)-2,2,2-trifluoroacetimidoylnorfloxacin (**5b**) as two synthetic derivatives of fluoroquinolones. In addition, real-time PCR (RT-PCR) technique was used to assay the performance of these two derivatives on DNA gyrase gene expression levels in *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) as gram-negative bacteria. Broth microdilution method and disc diffusion test were also employed to determine minimum inhibitory concentration (MIC) of these synthetic compounds in comparison with conventional antibiotics of gentamicin and ciprofloxacin (a fluoroquinolone).

Results: According to the findings; **5a** compared with the two antibiotics in gram-negative bacteria showed inadequate efficacy at phenotypic and molecular levels, and it could also have antibacterial effects assumed less than the given antibiotics. In contrast, **5b** generated a larger diameter of the zone of inhibition (ZOI) in *E. coli* and *P. aeruginosa* compared with the two antibiotics that were reported statistically significant (p=0.00). The results of the broth microdilution method also confirmed findings from disc diffusion test. On the other hand, **5b** brought about a significant reduction of DNA gyrase expression levels in both bacteria, while **5a** had did not show such a significant effect in this domain.

Conclusion: The results of this study suggested that **5b** could be used as a new and alternative antibiotic candidate for gentamicin or ciprofloxacin against infections caused by *E. coli* and *P. aeruginosa*. However, further research focused on various dimensions, including corresponding complications, as well as clinical trials were required to draw a definite conclusion on these synthetic compounds.

Keywords: Fluoroquinolone, N-4-Methyl (Phenyl)-2,2,2-Trifluoroacetimidoyl Ciprofloxacin (**5a**), N-4-Methyl (Phenyl)-2,2,2-Trifluoroacetimidoyl Norfloxacin (**5b**), *Escherichia coli*, *Pseudomonas aeruginosa*

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P54: Plasmid mediated quinolone resistance determinant qnr B and qnr S in *Escherichia coli* isolated from poultry colibacillosis

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Introduction and Objectives: Colibacillosis is one of the most important bacterial infections in poultry industry that causes high economic losses annualy in the world wild. Quinolones are the potent antibiotic agents used in the prophylaxis and treatment of infections caused by *E. coli*. Due to the widespread use of these drugs, frequency of resistant to these antibiotics is steadily increasing. Given the fact that food chain is an important route of transferring antibiotic resistance to human, the presence of resistance strains in poultry flocks is a serious threat for public health.

Materias and Methods: The aim of this study was to investigate the quinolone resistance of 100 *Escherichia coli* recovered from broilers referred to the Faculty of Veterinary Medicine hospital in Semnan University. Antimicrobial susceptibility of E. coli isolates were determined by disk diffusion method and the presence of plasmid mediated quinolone resistance determinant *qnr B* and *qnr S* were detected using PCR-specific primers.

Results: The highest phenotypic resistance was observed for Nalidixic acid (46%), Ciprofloxacin (30%), Levofloxacin (25%) and Ofloxacin (20%) respectively. Also13% and 7% of the isolates harbored *qnr B*, and *qnr S* genes.

Conclusion: The results of this study indicate that plasmid mediated *qnr* genes has observed in poultry farm in Semnan, and the use of appropriate therapeutic approaches and the rational administration of antibiotics to control the resistance to these drugs should be considered.

KeyWords: *Escherichia coli*, Colibacillosis, quinolone resistance

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P63: Prevalence assay of quinolone resistance among *Enterobacteriaceae* isolated from urban and hospital wastewater in Karaj

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Introduction and Objectives: Hospitals are hotspots for antimicrobial-resistant bacteria (ARB) and will be ejected from hospitals via wastewater systems. In this study, we investigated the prevalence of quinolone resistance in Entrobacteriaceae strains isolated from hospitals and urban wastewater in Karaj.

Materials and Methods: A total of 60 *Entrobacteriaceae* strains isolated from hospitals and urban wastewater in Alborz province during the spring of 2018. Bacterial strains were identified by standard microbiological and biochemical tests. The antimicrobial susceptibility test to ciprofloxain, levofloxacin, ofloxacin, norfloxacine and nalidxic acid was determined according to Kirby Baur assay.

Results: Among the organisms cultured, *Escherichia coli* (70%) was the most common organism followed by *Citrobacter freundii* (11.66%) and *Citrobacter diversus* (5%). Antibiotic resistance pattern were observed as follows: nalidxic acid 71.66%, norfloxacine 28.33%, ciprofloxain 26.66%, ofloxacin 23.33% and levofloxacin 20%.

Conclusion: The wide geographic spread of antibiotic-resistant strains may be connected with their transmission between hospitals, urban wastewater and environment. Antimicrobial resistance should now be seen as an environmental pollutant and new wastewater treatment processes must be assessed for their capability in eliminating antimicrobial-resistant bacteria, especially from hospital effluents.

Keywords: *Entrobacteriaceae*, quinolone resistance, wastewater

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P35: Amino acid substitution mutations of *gyrA* and *parC* in clinical *Enterococcus faecalis* isolates conferring high level fluoroquinolone resistance

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Introduction and Objectives: *Enterococcus faecalis (E. faecalis)* is known as one of the most important nosocomial pathogens causing various infections such as the urinary tract infection (UTI). Fluoroquinolones have been frequently used to treat *E. faecalis* UTIs, and the emergence of fluoroquinolone-resistant *E. faecalis* isolates has recently been considered as global concern. The objective of this study was to determine the amino acid substitutions of GyrA and ParC proteins in high level quinolone resistant isolates of *Enterococcus faecalis* from Kerman, Iran.

Materials and Methods: Minimum inhibitory concentrations (MICs) of ciprofloxacin against 20 isolates of multidrug-resistant *E. faecalis* were determined using agar dilution method. Amino acid mutation profiles of GyrA and ParC amplicons of 20 high quinolone-resistant isolates were determined by DNA sequencing. Sequences were compared with the *gyrA* and *parC* genes reference sequence of *E. faecalis* V583 using Vector NTI AdvanceTM.

Results: Ciprofloxacin -resistant isolates exhibited MICs that ranged from 64 to ≥256µg/ml. The amino acid substitutions in GyrA and ParC were found in 65% and 75% of isolates respectively. Sequencing of *gyrA* gene showed 1 amino acid substitution, serine 83 to isoleucine, in 92.3% (n=12) isolates and serine 83 to tyrosine in 7.7% (n=1) isolate. Sequencing of *parC* gene showed 1 amino acid substitution, serine 80 to isoleucine in 86.6% (n=13) isolates and serine 80 to leucine in 13.3% (n=2) isolates. In one isolate (6.6%) tyrosine 84 was changed to Phenylalanine. In two isolates with MIC 64 and 128 µg/ml, only amino acid changes in ParC were observed.

Conclusion: The results suggest that acquisition of mutations in certain positions of *gyrA* and *parC* genes confers high-level resistance to quinolones. However, since in some of resistant isolates of this study, there were no amino acid substitutions, other mechanisms such as efflux pumps may be involved in quinolone resistance.

Keywords: *Enterococcus faecalis*, Quinolone resistance gene, Sequencing, Mutations

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P280: *In vitro* and *in silico* assessment of ketoprofen as quorum quenching compound against *Pseudomonas aeruginosa* PAO1

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Introduction and Objectives: The *Pseudomonas aeruginosa* quorum-sensing (QS) is a complicated network of genome-wide regulation, activated in response to bacterial population density. One of the main components is the auto-regulating *Pseudomonas* quinolone signal (PQS) system that controls the construction of several virulence factors and biofilm-related factors. Therefore, inhibiting the QS circuitry is a promising approach for anti-virulence agents with much lowered resistance development possibility.

Materials and Methods: Here, we tested a member of NSAID (Non-Steroidal Anti-Inflammatory Drugs) family, ketoprofen, *in silico* and *in vitro* as an anti-quorum sensing compound due to its chemical structure similarity to the natural *P. aeruginosa* signaling molecules (PQS). Its effect was assessed with colorimetric method by spectrophotometer against biofilm formation and several virulence factors production, besides evaluation of the synergistically effect with tobramycin. Moreover, its influence on the other major QS pathways (*las* and *rhl*) was also depicted.

Results: Our study revealed that ketoprofen decreased the expression of virulence factors including pyocyanin, protease, pyoverdine and also lactonic compounds, inhibited the biofilm formation and reduced the effective dose of tobramycin against *P. aeruginosa* PAO1.

Conclusion: As it was anticipated, ketoprofen is a quorum quenching compound and could be utilize in order to attenuate *Pseudomonas aeruginosa* pathogenicity.

Keywords: *Pseudomonas aeruginosa*, quorum-sensing, ketoprofen, biofilm, virulance factor

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P83: Frequency of vancomycin resistance genes (VanA, VanB, VanC) in *enterococci* isolated from clinical specimens of patients admitted to Payambare azam Bandar Abbas Hospital in 2017

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Introduction and Objectives: The genus *Enterococcus* as enteric gram-positive cocci, is one of the most important nosocomial multidrug-resistant organisms. Isolation of vancomycin-resistant enterococci (VRE) is increasingly reported from around the world.
Materials and Methods: A descriptive study was performed on clinical samples of hospitalized patients in different wards at Payambare-azam hospital in 2017.
Results: Ninety-one clinical isolates of enterococci including *E. faecalis* (n=14) and E. *faecium* (n= 9) were examined for the presence of vanA, vanB, vanC and ddl genes by a PCR. sixty-three (69%) of isolates recovered from urine sample. The highest number of isolates (26%) were recovered from intensive care unit. Seventeen percent of isolates were vancomycin-resistant. The ddl primers yielded a product of 941 bp for *E.faecalis* and 550 bp for *E. faecium*. VanA and vanC were detected in 7(8%) and vanB in 9 (10%) of isolates. The distribution of resistance genes based on different species was as follows. *VanA* and *vanB* gens in 1 isolate of *E.faecalis* (1.1%) and *E.faecium*. VanC in 2 isolate of *E.faecalis* (2.2%). None of the *E.faecium* was carriers of the vanc.
Conclusion: These findings suggest that multidrug-resistant *Enterococcus* with transferable vancomycin resistance genes will emerge as important nosocomial pathogens.
Keywords: *Enterococcus*, Vancomycin resistance, VanA ligase protein.

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Topic: Antimicrobial Resistance

P82: The study of the prevalence of shv, ctxm and per betalactamase genes in clinical isolates of *Acinetobacter baumannii* from the shahid mohammadi hospital in Bandar abbas

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Introduction and Objectives: *Acinetobacter baumannii* is one of the most important agents of nosocomial infections and has a crucial role as opportunistic pathogens that generally are resistant to many classes of antibiotics such as cephalosporines. Beta- lactamases enzymes are the main causes of resistance to cephalosporins. This study aimed to determine the prevalence of antibiotic resistance and β- lactamase genes including bla_{PER}, bla_{CTXM}, bla_{TEM}, bla_{SHV} in clinical isolates of *A. baumannii* in Shahid Mohammadi hospital during the period March - October2016.

Materials and Methods: In this study, clinical isolates were identified by biochemical methods to genus level and PCR for bla $_{OXA-51}$ and beta-lactamase encoding genes was carried out. Antibiogram by disk diffusion method was carried out for 18 antibiotics.

Resuls: The prevalence of *A.baumannii* among 61 *Acinetobacter* was 93.4%. The most resistance to antibiotics including, piperacillin, cefepime, ceftriaxone, meropenem, ciprofloxasin, ticarcillin, ertapenem, cefotaxime, ceftazidime, doripenem(100%), followed by imipenem (96.4%) and gentamicin (87.7%), doxicycline (82.4%),amikacin (77.1%), tobramicin (73.6%), ampicillin-sulbactam(45.4%) were observed. The prevalence of β lactamses genes was as follow: bla_{TEM} 23(40.3%), bla_{SHV} 5(8.7%), bla_{PER} 2(3.5%) and $bla_{CTXM,}$ not found in any of isolates.

Conclusions: The results of this study indicated increasing resistance of *A.baumannii* to these antibiotics. Despite of low prevalence of resistance genes against cephalosporins, high resistance to these antibiotics indicated over use of antibiotics in hospital which lead to activation of efflux pumps in bacterial nosocomial agents.

Keywords: *Acinetobacter baumannii*, Beta- lactamases, cephalosporin's

Topic: Antimicrobial Resistance

P91: *Terbinafine Resistance of dermatophyte* Detection of OXA Beta Lactamases among Clinical Isolates *Acinetobacter baumannii* in educational hospitals of Sari

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Introduction and Objectives: Acinetobacter baumannii is a non-motile Gram-negative bacterial pathogen with the history of vast resistant to antibiotics. The spread of carbapenem-resistant *Acinetobacter baumannii* is a global concern. The aim of this study was to determine the possibility of existence of OXAs genes among clinical isolates of *Acinetobacter baumannii* in educational hospitals of Sari.

Materials and Methods: A total of 100 isolates were identified as A. baumannii by common biochemical and molecular tests. The susceptibility to different antibiotics was assessed with Kirby-Bauer disk diffusion method. Phenotypic Detection of MBLs was performed with CDT test and PCR assay was also performed for detection of *blaOXA-23*-like, *blaOXA-51*-like genes.

Results: All isolates of *A. baumannii* showed high-level of resistance to all antibiotics except for colistin. The *blaOXA-51*-like and *blaOXA-23*-like *genes* were detected in (100%) and (86.6%) of. *Acinetobacter baumannii* isolates, respectively.

Conclusion: Due to the results, treatment of *A. baumannii* isolates by carbapenems is ineffective and Tigecycline or colistin could be used for treatment. Other studies for detection of other mechanisms for Carbapenem resistance are recommended.

Keywords: *Acinetobacter baumannii*, Carbapenemase, PCR

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Topic: Antimicrobial Resistance

P179: Terbinafine Resistance of dermatophyte species Caused by Point Mutations in the Squalene Epoxidase Gene

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Introduction and Objectives: With regard to increasing number of antifungal-resistant dermatophytes, antifungal susceptibility testing of dermatophytes serves as a useful tool in managing clinical dermatophytosis. Terbinafine is one of the allylamine antifungal agents whose target is squalene epoxidase (SQLE). Terbinafine resistance has been predominately attributed to point mutations in *SQLE* target gene a key enzyme in the ergosterol biosynthetic pathway leading to single amino acid substitutions. Inhibition of this enzyme leads to accumulation of squalene inside the fungal cells, depletion of ergosterol, and finally causes cell death. This study aimed to determine point mutations in terbinafine-resistant isolates.

Materials and Methods: Dermatophyte species (n= 99) was confirmed by sequencing of ITS region. Antifungal susceptibility testing of all isolates was assessed to terbinafine agent using CLSI M38-A2 guidelines.

Results: Based on our results, among 99 tested isolates, 5 (5%) showed reduced terbinafine susceptibility (MIC>32 µg/ml), of which for two species *T. rubrum* and *T. tonsurans* were found to be related to amino acid substitution Leu393 by Phe in the squalene epoxidase protein. We reported terbinafine resistance for dermatophytes isolated from tinea pedis and tinea corporis. This is the first case of terbinafine-resistant *T. tonsurans* strain isolated from patient. Previous studies showed *T. rubrum* isolates resistant to TER were fully cross-resistant to naftifine, butenafine, and tolnaftate. Therefore, it is suggested antifungal susceptibility testing of isolates resistant to TER against the mentioned antifungals.

Conclusion: This increase of terbinafine resistance of dermatophyte isolates is worrisome warranting antifungal susceptibility testing and mutation analysis for monitoring this emerging resistance.

Keywords Squalene epoxidase, Point mutation, Terbinafine resistance

Topic: Antimicrobial Resistance

P64: Molecular characterization of class 1, 2 and 3 integrons in multi-drug resistant *Escherichia coli* clinical isolates that collected from Intensive Care Units (ICUs)

Introduction and Objectives: *Escherichia coli* (*E.coli*) are important pathogen in hospital-acquired infections, and very commonly isolated organism from the ICUs. On the other side recently, the frequency of MDR isolates of *E. coli* is increasing and can make serious concerns in public health. Integrons are one of the most important reasons to produce Multidrug resistance (MDR) strains. The aim of this study was to determine the Frequency of classes I, II, III integrons, gene cassettes and multiple drug resistance among clinical isolates of *Escherichia coli isolated* from Intensive Care Units (ICU) of Qazvin, Karaj and Tehran hospitals.

Materials and Methods: In this study, 215 *E. coli* isolates collected from ICUs of 16 hospitals of Qazvin, Karaj and Tehran during 2014-2017 from deferent sources. Antimicrobial susceptibility pattern of strains was determined by disk diffusion method, according to the (CLSI) criteria. Multidrug resistance (MDR) strains were determined according to standard definitions of the CLSI, EAUCAST and FDA PCR was used to detect classes I, II, III integrons and gene cassettes.

Results: Out of the 215 isolates 201 (93.5%) isolates carried integrons and 204 (94.9%) isolates were MDR. Among MDR isolates, 190 (93.1%) isolates carried integrons [165 (80.9%) isolates carried only classes I integrons, 25 (12.3%)isolates carried class I and class II integrons and 1 (0.5%) isolate carried only class II integrons). Class 3 integrons were not found in any isolates. Most commonly found gene cassettes in the integron positive isolates were *dfrA17-aadA5* casset.

C**onclusion:** The results of this study show that the prevalence of MDR *E. coli* isolates in ICUs of Qazvin, Karaj and Tehran hospitals is high and can be considered as an important problem in hospitals. On the other side, in this study the high rate of integrons presence in MDR strains detected and because of the efficacy of integrons in distribution of antimicrobial resistance in clinical isolates of *E. coli*, this result can be very important.

Keywords: Class I integrons, class II integrons, class III integrons, *Escherichia coli*, ICUs, MDR, gene cassettes

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Topic: Antimicrobial Resistance

P71: Antimicrobial resistance, biofilm formation and alginate production in *Pseudomonas aeruginosa* isolates obtained from respiratory tract specimens

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Introduction and Objectives: *P. aeruginosa* is one of the most common opportunistic bacteria in nosocomial infections, which has a significant resistance to antimicrobial agents. *P. aeruginosa* is a biofilm-forming bacterium which can result in serious health problems. The purpose of this study was to investigate the biofilm formation, alginate production and antimicrobial susceptibility patterns among *P. aeruginosa* isolated from respiratory tract specimens.

Materials and Methods: In this study, 36 isolates of *P. aeruginosa* were recovered from respiratory tract specimens. *P. aeruginosa* isolates were identified and confirmed by phenotypic methods and PCR test. Antimicrobial susceptibility of the isolates has been specified by the disk diffusion method. Biofilm formation and alginate production of these isolates were measured by microtiter plate and carbazole assay, respectively.

Results: Antimicrobial susceptibility test showed that 12 (33.3%) isolates were sensitive to all antibiotics; on the other hand, 4 (11.1%) isolates were resistant to all antibiotics. 11 (30.5%) of *P. aeruginosa* isolates were multidrug-resistant (MDR). The most effective antibiotic was piperacillin-tazobactam as 83.3% of isolates were sensitive, and the most resistant was observed for ofloxacin (36%). All isolates were biofilm and alginate producers, in which, 36.1% were strongly biofilm producers, and the rates of moderate and weak biofilm producers were 52.8% and 11.1%, respectively. The production of alginate in 93% of strong-biofilm forming isolates was more than 250 µg/ml, while 75% of low-biofilm forming isolates produced less than 250 µg/ml alginates.

Conclusion: In this study, a high prevalence of biofilm and alginate production was observed in *P. aeruginosa* isolates from respiratory tract specimens. Also, the results of this study indicated a relationship between the rate of alginate production and level of biofilm formation in *P. aeruginosa* isolates.

Keywords: Alginate, Biofilm, *Pseudomonas aeruginosa*

opic: Antimicrobial Resistance

P76: Antibiotic resistance in clinical isolates of *Acinetobacter Bumanni* in Tabriz hospitals

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Introduction and Objectives: *Acinetobacter baumannii* due to high propensity of biofilms formation and high frequently of multi-drugs resistant (MDR) is an important microorganism causes nosocomial infections. The aim of this study was investigation of antibiotic resistance patterns in clinical isolates of *A. baumannii*

Materials and Methods: One hundred *A. baumannii* isolates were collected from three University affiliated hospitals in Tabriz, Iran during 2015-2016. The antibiotic resistance patterns were evaluated by the disk diffusion method.

Results: Eighty-eight *A. baumannii* isolates were MDR and have high resistant to cefepime, third generation cephalosporins, imipenem (92%), ciprofloxacin(92%), cotrimoxazole(75%), amikacin(60%), meropenem(52%), and gentamicin(51%). Resistance to colistin (4%) was observed in 4 isolates.

Conclusion: The results of this study indicate that high levels of drug resistance of clinical isolates of *A. baumannii*. These results highlight the need to draw up detailed plans for the control of infectious diseases caused by *A. baumannii* in our hospitals.

Keywords: *Acinetobacter baumannii*, antibiotic resistance

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Topic: Antimicrobial Resistance

P43: Identification of Methicillin-resistant *Staphylococcus aureus* from personnel and equipment of Vali-e-Asr hospital-Bafgh-Yazd in 2018

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Introduction and Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common pathogens in hospitals that has been increasing worldwide over the past three decades. The purpose of this study was to isolate and identify MRSA from hospital personnel and equipment.
Materials and Methods: This is a cross-sectional study. The samples collected of personnel and equipment of different parts of the hospital, including lab, Internal, Pediatric, Surgical, CCU, ICU, Operating room, and Dialysis sections. One hundred samples were cultured on blood agar and eosin methylene blue agar. To continue, phenotypic tests, including hemolysis production, pigment production, Gram stain, catalase, coagulase, mannitol salt agar, and DNase were used for *Staphylococcus aureus* identification. Cefoxitin was used for MRSA identification by disc diffusion method.
Results: The results of 100 samples from personnel and equipment in different parts of the hospital showed that the highest rate was for Gram-positive bacteria. Twenty five isolates were positive for *Staphylococcus aureus* and six isolates were positive for MRSA.
Conclusion: Considering the increasing MRSA infections, disinfection of hospital equipment and eradicating it from carriers is essential.
Keyword: *Staphylococcus aureus*, Medical equipment, MRSA

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P209: Inhibitory effects of *Mentha longifolia* and *Menthol* on efflux pumps in imipenem and ciprofloxacine resistant *Acinetobacter baumannii* isolates

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Introduction and objectives: *Acinetobacter baumannii* is an opportunistic pathogens that cause nosocomial infections especially in patients in intensive care units (ICU). Accordingly, the aim of our study was investigation on inhibitory effect of the essential oil of *Mentha Longifolia* and menthol on *Ade ABC* efflux pumps in ciprofloxacin and imipenem resistance clinical isolates of *A. baumannii*.
Materials and Methods: A total of 75 clinical isolates of *A. baumannii* were collected. The presences of efflux pump genes were detected by Polymerase Chain Reaction (PCR). Minimum Inhibitory Concentration (MIC) of the essential oil of *Mentha Longifolia* and Menthol and their combined effect with antibiotics were measured by microbroth dilution and Fractional Inhibitory Concentration (FIC) index.
Results: The frequency of *ade A*, *ade B* and *ade C* genes in clinical isolates of *A. baumannii* were 93.3%, 96%, and 97.3%, respectively. When the essential oil of *Mentha longifolia* with ciprofloxacin and imipenem were combined, MICs decreased 4 and 8 fold, respectively. In menthol combination with imipenem, in 90 %(63/70) of the isolates the resistance to imipenem reduced from 0 to 16 folds.
Conclusion: The presence of efflux pump genes, in more than 90% of *A.baumannii* isolates, indicates the potential role of efflux pump in inducing imipenem and ciprofloxacin resistance in this bacterium. Menthol has an antimicrobial effect as an effective ingredient in *Mentha Longifolia*. In the future, the combination of medicinal plants with antibiotics can be used as a complement to treat diseases caused by drug-resistant bacteria such as *A.baumannii* infections.
Keywords: *Acinetobacter baumannii*, Efflux Pump, Menthol, *Mentha Longifolia*

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Topic: Antimicrobial Resistance

P79: Study of antibiotic resistance pattern and ESBls in *Pseudomonas aeruginosa* isolates obtained from hospitalized patients from Amiralmonenin hospital, Zabol, southeast of Iran

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Introduction and objectives: *Pseudomonas aeruginosa* is an opportunistic pathogen of human and animals that causes different kind of infections. The aim of this study is detection of the antibiotic resistance pattern and the ESBLs in *P. aeruginosa* local isolates which were obtained from hospitalized patients in Amiralmonenin hospital, Zabol, Iran.

Materials and methods: Study population were included, 80 local isolates of *P .aeruginosa* that had been cultivated from patients bedridden in Amiralmonenin hospital during 2017-2018. This research is a cross-sectional descriptive-analytic study. *P. aeruginosa* isolates were identified by biochemical tests and sensitivity to antibiotics was measured by disk diffusion method. Combined Disk Diffusion method (CDD) was used to detect ESBLs (Extended Spectrum- beta Lactamases) in these isolates. ESBL genes were screened by PCR method.

Results: The results obtained from CDD test showed that among the 80 isolate of *P .aeruginosa*, 32 samples (40%) were ESBL positive. Resistance to Azithromycin was 72.5%, Amoxicillin 97.9%, Amoxiclav 90%, Imipenem 0%, Gentamicin 50%, Cotrimoxazole 75%, Ciprofloxacin 28.8%, Cefotaxime 52.5%, Cefalexin 81.3% Ceftriaxone 60%, Ceftazidime 75% and Nitrofurantoin 75%. ESBL genes including, *bla*(shv), *bla*(ctxm1)، *bla*(ctxm2), *bla*(ctxm3) and *bla*(oxa) were existed in 15.6% of isolates. *bla*(ctxm3) gene were the most prevalent ESBL gene (84.4%) in compare to the others and *bla*(ctxm2) gene was not detected in any isolate.

Conclusion: The results of the study indicated high rate of the resistance to various classes of antibiotics among the *P. aeruginosa* isolates. Therefore, it could be concluded that detection of antibiotic resistance pattern of the *P. aeruginosa* isolates have to be done before any antibiotic prescription in any infection attributed to this pathogen.

Keywords: *Pseudomonas aeruginosa*, antibiotic resistance, Extended spectrum β-lactamases (ESBL)

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Topic: Antimicrobial Resistance

P256: Identification of capsular vaiants of *Klebsiella pneumoniae* clinical isolates and antimicrobial resistance profile in educational hospitals of Tehran

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Introduction and Objectives: The capsular polysaccharide is a main virulence factor in *Klebsiella pneumoniae* and serotypes K1and K2 are the most virulent types in human infections. The aim of this study were to assess the antimicrobial susceptibility and to determine the distribution of serotypes K1, K2 by detecting of *wzc* and *orf10* genes in clinical isolates of *K. pneumoniae*.

Materials and methods: A prospective study was carried with a total of 120 consecutive, non-duplicate isolates of *K. pneumoniae* recovered from various clinical specimens from patients admitted to educational hospitals of Tehran. Antibiotic susceptibility of isolates was determined using a disk diffusion method. The presence of *wzc* and *orf10* genes was detected by polymerase chain reaction. The data were analyzed by using descriptive statistics and chi-square.

Results: Totally, 75 (62.5%) of isolates showed multidrug resistance (MDR) pattern. The highest and lowest rates of resistance were related to amoxicillin 68.2% and ciprofloxacin 91.8%, respectively. Results of the PCR assay showed that 57 (47.5%) isolates related to K1serotype, 12 (10%) to K2 serotype and 51 (42.5%) of isolates were belonged to non K1/ K2 serotypes. no significant association was seen between serotypes K1, K2 and MDR pattern ($P< 0.05$).

Conclusion: The present study indicated considerable rate of serotypes K1, K2 with MDR pattern among clinical isolates of *K. pneumoniae* collected from Tehran hospitals. Considering the major role of these serotypes with high rates of drug resistance in different clinical infections, using of appropriate infection control measures and treatment strategies are essential to prevent further dissemination of these virulent and resistant isolates in clinical settings.

Keywords: *Klebsiella pneumonia*, capsular polysaccharide K1, capsular polysaccharide K2, multiple drug resistance

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Topic: Antimicrobial Resistance

P41: Study the expression of *rpos* gene in a ciprofloxacin medium resistant *E.coli* mutant

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Introduction and Objectives: *E. coli* is a gram negative bacterium belongs to *Enterobacteriacea* family. Some of its strains cause urine infection. Ciprofloxacin is a selective treatment against this infection. This antibiotic belongs to the second-generation of fluoroquinolone and its target is A and B subunits of DNA gyrase. Recent studies have shown that ciprofloxacin enhances the expression of *recA* and *recF* genes in ciprofloxacin-resistant mutants. *recF* locates in a four-gene operon and can be expressed by either operon promoter or its own promoter. Special promoter is activated by RpoS sigma factor in stationary phase. Therefore, the aim of this study was to study the expression of *rpoS* gene in *E.coli* mutant with intermediate resistance to ciprofloxacin after treatment with this antibiotic.

Materials and methods: For determination of relative expression of *rpoS*, firstly, RNA was extracted and then cDNA was synthesized. Real-time PCR method then was used to determine the gene expression.

Results: The result of present study showed that the *rpoS* gene expression level did not increase at logarithmic phase following treatment with ciprofloxacin.

Conclusion: The increase in *recF* expression after treatment with ciprofloxacin is not related to its own promoter and is relevant to the operon promoter.

Keywords: *E.coli*, *rpos* gene, Real time PCR, ciprofloxacin

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P141: Surveillance study for epidemiology of multidrug resistant *Campylobacter* strains in symptomatic patients with diarrhea and poultry meat samples distributed in 22 regains of Tehran, Iran

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Introduction and Objectives: *Campylobacter* is among main enteropathogens that are responsible for inflammatory diarrhea in human populations. Failure of antibiotic regimens against invasive infections of this bacterium in human could be caused through transmission of resistance genes from the strains exists in food animal reservoirs. To show this relationship, a prospective surveillance study was done in 22 regions of Tehran.

Materials and Methods: Infection rate of *Campylobacter* spp. in 400 symptomatic patients with diarrhea and contamination of 100 chicken meat samples distributed across 22 regions of Tehran was analyzed. The presence of *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis* among the isolates were characterized by PCR methods. Resistance patterns to 7 antibiotics and multidrug resistance patterns were detected by E-test and disc diffusion methods, as described by EUCAST and CLSI guidelines published 2018. MIC50 and MIC90 were reported based on the determined values. Statistical analyses were done to show correlation of the resistance phenotypes among common resistance phenotypes between the human and chicken meat isolates. MAMA PCR for detection of quinolones resistance determining region (QRDR) of gyrA was done on C. jejuni isolates with defined MIC values.

Results: The poultry meat samples were related to 41 different brands, which their weight ranged from 0.76 Kg to 2.71 Kg**.** *Campylobacter* was isolated from 35% of chicken meet samples (*C. jejuni*, 23%; *C. coli*, 1%; *C. lari*, 2%; other species, 9%), while isolated from 6.7% of the patients with community acquired diarrhea (*C. jejuni*, 5.7%; *C. coli*, 0.5%; *C. lari*, 0.14%; other species, 0.25%). Higher rates of isolation were detected among children with diarrhea and meat samples of ≥1.5 Kg weight. Resistance to tetracycline (62.8% and 55.5%), ciprofloxacin (51.4% and 29.6%), nalidixic acid (42.8% and 29.6%), erythromycin (37.1% and 40.7%), gentamicin (31.4% and 33.3%), ampicillin (17.1% and 51.8%), and clindamycin (17.1% and 40.7%) was common among the meat and feces isolates, respectively. MDR phenotype was detected in 42.8% of the *Campylobacter* isolates from the chicken meat and 51.8% of the stool samples, respectively. The resistance patterns were not linked to specific brands of the chicken products, the production date, and city regions.

Conclusion: Frequency of *Campylobacter* strains with higher rates of resistance to most of the antibiotics among the chicken meat isolates and similarity of their characteristics at species level between the human stool and chicken meat isolates proposed their risks for transmission to human population through food chain in Tehran.

Keywords: *Campylobacter*; Chicken meat; Human stool; Diarrhea; Antimicrobial resistance, MAMA PCR; MDR; Tehran.

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Topic: Antimicrobial Resistance

P233: Antimicrobial action of mesoporous silica nanoparticles loaded with cefepime and meropenem separately against multidrug-resistant (MDR) *Acinetobacter baumannii*

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Introduction and Objectives: The overuse of antibiotics is causing a continuous emergence of drug resistance in pathogenic bacteria. The multidrug resistance (MDR) by reason of extensive use of antibiotics is being a major challenge in world public health. *A. baumannii* is becoming an increasingly important human pathogen due to the emergence of MDR strains. The aim of this study was to prepare positive charge mesoporous silica nanoparticles (MSN) which are loaded by cefepime (CFP) and meropenem (MEM) to improve efficacy and antibacterial activity to combact MDR strains.

Materials and Methods: An amine functionalized MSN (MSN-NH_2) was synthesized and loaded by CFP and MEM. The characterization of prepared nanoparticles was done by some methods such as Scanning Electron Microscopy (SEM), nitrogen adsorption/desorption isotherms, Fourier Transform Infrared (FT-IR) spectroscopy and X-ray Diffraction (XRD) spectroscopy. Broth microdilution and well diffusion methods were used to determine antibacterial activity against MDR *A. baumannii.*

Results: The results showed the CFP and MEP loaded MSNs-NH_2 (CFP@MSNs-NH_2 and MEM@MSNs-NH_2) were prepared correctly having high pay load and drug release kinetics is pH-sensitive. The antimicrobial tests results against multi drug resistant *A. baumannii* isolate were showed that drug loaded MSNs-NH_2 more effective than free drug.

Conclusions: The results of present study demonstrated that CFP@MSNs-NH_2 and MEM@MSNs-NH_2 potentiate antimicrobial activity than free drug and enhanced the possibility of combat against *A. baumannii* isolate.

Keywords: Antibiotic resistance, *Acinetobacter baumannii*, mesoporous silica nanoparticles, Cefepime, Meropenem

Topic: Antimicrobial resistance

P31: Relative frequency of mutation in QRDR (quinolone resistance determining region) among ciprofloxacin-resistant and susceptible pseudomonas aeruginosa strain isolated from burns of hospitalized patients in Rasht Valayet Hospital in 2018

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Introduction and Objective: *Pseudomonas aeruginosa* is one of the most common opportunistic pathogens which cause death in the patients with burn wounds infection. Fluoroquinolones such as ciprofloxacin could prevent DNA replication of bacterial agents via inhibiting DNA gyrase and topoisomerase IV. The main mechanism of resistance to floroqinolones in *p. aeruginosa* is alterations in quinolone resistance determining regions (QRDR) of gyrA and parC genes. The aim of present study was to find out the role of these mutations in ciprofloxacin resistant strain.

Materials and Methods: 118 isolates of *P. aeruginosa* were collected from Rasht Valayet Hospital. Afterward isolates were analyzed for antimicrobial susceptibility, polymerase chain reaction amplification of quinolone resistance determining regions (QRDR), finally mutation detection with sequencing.

Results: 54 isolates were resistant to ciprofloxacin **Thr**-83→Ile substitution in the (QRDR) of gyrA was found in ciprofloxacin resistant isolates. Ser-87→Leu substitution in parC was observed. All parC mutations were observed in isolates which carried a gyrA mutation.

Conclusion: It was found that mutation in gyrA and parC genes might involve in resistance to ciprofloxacin of *P. aeruginosa* isolates.

Keywords*: Pseudomonas aeruginosa*, Antibiotic Resistance, QRDR

Topic: Antimicrobial resistance

P74: Prevalence and antibiogram pattern of bacteria isolated from blood cultures in Emam hospital Jiroft, 2019

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Introduction and objectives: Septicemia is one of the main causes of mortality and morbidity especially in neonates and children. Blood culture is the most common and important method for diagnosis of bacterial systemic infections. The mortality rate is reported from 20% to 50% in cases of bacteremia. Emergence of multidrug resistant bacterial strains is a main problem in the management of the disease. The current research was undertaken to study the common bacterial pathogens isolated from blood cultures and to determine their antibiotic susceptibility pattern

Materials and Methods: In this cross-sectional study, blood specimens were collected from patients referring to emam hospital in Jiroft, south of Iran. The isolated bacteria were identified using conventional methods. Antibiotic susceptibility testing was performed by modified Kirby-Bauer disk diffusion method.

Results: In this study, gram-positive bacteria were isolated from 38.1 % of blood cultures and gram-negative bacteria were recovered from 61.9 % of blood cultures. Streptococcus spp were the most common bacteria isolated from blood cultures. Streptococcus spp were the most common gram-positive bacteria and Escherichia coli were the most common gram-negative bacteria isolated from blood cultures. It was found to be 87.5%-gram positive bacteria were sensitive to Nitrofurantoin. About 61.5% of gram-negative bacteria were sensitive to Gentamicin.

Conclusion: Present study showed that both gram positive and gram-negative bacteria were responsible for blood stream infections. Streptococcus spp and Escherichia coli were the most common bacteria isolated from blood cultures.

Keywords: Antibiogram, bacteria, blood culture

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Topic: Antimicrobial resistance

P30: Investigating antimicrobial resistance pattern and detection of the *sul* genes in *Stenotrophomonas maltophilia* isolates in Bushehr, Iran

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Introduction and Objectives: *Stenotrophomonas maltophilia* can be resistant to various antimicrobial drugs. Emerging resistance to trimethoprim-sulfamethoxazole (SXT) poses a serious threat to the therapy of infections caused by this organism. The *sul* and *dfrA* genes could contribute to the resistance to SXT. We aimed to determine antimicrobial resistance pattern and to investigate the presence of *sul* and *dfrA* genes in *S. maltophilia* isolates in Bushehr, Iran.

Materials and Methods: A total of 87 *S. maltophilia* isolates (67 clinical and 20 environmental isolates) were collected in Bushehr, Iran. The clinical isolates were collected from three hospitals and the environmental isolates were collected from various sources in two hospitals and one dental clinic. The isolates were identified by biochemical methods and PCR. Antimicrobial susceptibility testing was performed by disk diffusion and determination of MIC using MIC Test Strip. To assess the presence of *sul1*, *sul2*, *sul3*, *dfrA1*, *dfrA5*, *dfrA12*, *dfrA17*, class 1 integron, and insertion sequence common region (ISCR) elements, PCR was performed.

Results: All isolates were susceptible to minocycline and levofloxacin. Eighty-four isolates (96.6%) were susceptible to SXT and three isolates (3.4%) which were environmental, were SXT-resistant. The MIC values of SXT for three SXT-resistant isolates were ≥32µg/ml. Resistance rate to ceftazidime, ticarcillin-clavulanate, and chloramphenicol were 75.9%, 69%, and 65.5%, respectively. All three (100%) SXT-resistant isolates carried *sul1* gene and class 1 integron. Among 84 SXT-susceptible isolates, 14 (16.7%) and 7 (8.3%) were *sul1*-positive and *sul2*-positive, respectively. Of the 17 *sul1*-positive isolates, 15 harbored class 1 integron. The *sul3* and *dfrA* genes as well as ISCR were not detected.

Conclusions: Our results support that SXT, levofloxacin, and minocycline are good therapeutic options for *S. maltophilia* infections in Bushehr. The presence of SXT-resistant *S. maltophilia* in hospital environment, suggests that it may act as a reservoir for this bacterium.

Keywords: *Stenotrophomonas maltophilia*, sul genes, trimethoprim-sulfamethoxazole

Topic: Antimicrobial resistance

P59: Evaluation of Antibiotic Resistance of Urinary Tract Pathogens among hospitalized patients in Ghaem hospital, Mashhad, Iran

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Introduction and objectives: Nosocomial urinary tract infections (UTIs) are among the most common bacterial infection diseases. In this study we established the microbial etiologies of UTI and anti-microbial resistance pattern of bacterial pathogens causing UTIs in hospitalized patients at the Ghaem hospital, Mashhad, Iran.

Materials and Methods: Cross-sectional study was conducted from April 2017 to March 2019 in Ghaem hospital. Urine samples were collected and processed following standard operating procedures. Antimicrobial susceptibility testing was done following Clinical Laboratory Standards Institute 2014 guidelines.

Results: We identified 3634 microorganisms (2731 bacteria and 903 yeasts). Escherichia coli was the most frequently microorganism isolated (44%) followed by Klebsiella pneumonia (16%) and Eenterococcus faecalis (16%). E.coli was highly resistant to ampicillin (83.6%), piperacillin (81.9%), cefazolin (67.9%), ceftriaxone (63.15%) and cefixime (63%) while less resistance rate was found for nitrofurantoin (7.6%), amikacin (7.3%) and meropenem (4.1%). The most antibiotic resistance in Klebsiella pneumonia was to ampicillin (95.7%), cefazolin (78.9%), ceftriaxone (70.3%), ceftazidime (65.8%) and cefepime (61.4%) and the least antibiotic resistance was to amikacin (31.%), nitrofurantoin (39.7%) and imipenem (40.1%). For Eenterococcus faecalis strains, the most and the least antibiotic resistance was observed to gentamicin (61.2%) and linezolid (4.6%) respectively.

Conclusions: In this study, family of enterobacteriaceae were the most common causes of UTIs. and their antimicrobial resistance patterns is advantageous and necessary in order to design a guideline for empirical therapy.

Keywords: Antibiotic Resistance, Urinary Tract, infections

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Topic: Antimicrobial resistance

P80: Prevalence and Antimicrobial Susceptibility Pattern of Bacterial Isolates from Respiratory Tract Infections in Ghaem Hospital, Mashhad, Iran

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Introduction and objectives: Respiratory tract infection is a serious concern for public health in the worldwide, and imposes a lot of pressure on health facilities, specifically in developing countries along with economic restrictions. This study evaluated the prevalence of bacterial pathogens and their antibiotic sensitivity pattern among patients with Respiratory Tract Infection in Ghaem hospital, Mashhad, east of Iran.

Materials and Methods: The etiological and antimicrobial susceptibility profile of respiratory tract infection over a period of six month at Ghaem educational hospital was studied. The specimens were collected and processed according to the standard microbiological methods. Antibacterial susceptibility testing was performed by disc diffusion method following clinical and laboratory standards institute (CLSI) guidelines.

Results: There were 868 clinical samples of respiratory tract, which 679 (77.8%) samples were recognized to be culture positive. Out of 679 culture positive, 44% were related to female and 56% to male. The most frequency of respiratory tract infections were occurred in the intensive care unit (ICU) about 60.5% (411/679). Most isolates were obtained from the age group of 60-80 years old (211/679; 31%). The most common isolated were *Acinetobacter spp.* (35%; 239/679) and *Klebsiella pneumonia* (20%; 138/679). Antimicrobial profile of *Acinetobacter spp* and *Klebsiella pneumonia* showed maximum sensitivity to Amikacin respectively 20.3% and 50.5%; and maximum resistant to ceftazidime respectively 98.1% and 91.2%.

Conclusions: According to this study, reducing respiratory infections are effect on reducing mortality and health-care costs. Also, the duration of hospitalization in the ICU especially surgery ICU, is associated with an increased risk of respiratory infection. Therefore, infection control plays an important role in the ICUs.

Keywords: Respiratory Tract Infections, Antibacterial susceptibility test, intensive care unit

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Topic: Antimicrobial resistance

P264: Prevalence of virulence gene encoding type 1 fimbriae (*fimH*) among UPEC and relationship with antimicrobial resistance

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Introduction and Objectives*: Escherichia coli* is the most causative pathogen of causing urinary tract infection (UTI), the most common microbial infectious disease found in the clinical setting, leading to high medical costs and significant morbidity. Better understanding of the properties of virulence and its antibiotic resistance pattern helps clinicians anticipate the improvement of infection in the patients.

Materials and Methods: *E. coli* strains were recovered from patients with urinary tract infections (UTI) who admitted in several major hospitals in Hamedan. Antibiotic susceptibility testing was done, according to CLSI guidelines. PCR screened the uropathogenic *E. coli* strains for the prevalence of virulence gene encoding type 1 fimbriae (*fimH*).

Results: In total, 90 E. coli strains were subjected to the study. *E. coli* isolates were resistant to cefepime (78%), Cefotaxime (38.6%), Nalidixic acid (50%), Cotrimoxazole (64.28%), Amikacin (4.2%), cefixime (41.21%), ceftriaxone (35.86%), Gentamicin (9.8%), Nitrofurantoin (1.4%), and Cephalothin (74%) and susceptible to imipenem (100%) and meropenem (100%). The prevalence of gene coding for fimbrial adhesive systems was 63% for *fimH*. The strains isolated from hospitalized patients displayed a great diversity of gene associations compared to those isolated from ambulatory patients.

Conclusion: The result showed that antibiotic resistance is escalating rapidly. UPEC strains causing infections are more likely to harbor specific virulence genes. In the current study, high resistance was observed against antibiotics widely used for the treatment of urinary tract infection; therefore, to reach better therapeutic outcomes, empiric treatment regimens have to be modified.

Keywords: *E. coli*, *fimH*, antibiotic resistance

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Topic: Antimicrobial resistance

P53: Frequency of surgical infection, bacteria causing and antibiotic resistance pattern in Ghaem educational hospital

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Introduction and Objectives: Surgical site infections (SSIs) determine to increase post-operative complications and medical expense. The current study is assumed to evaluate the frequency of SSI with pointing to factors contributing to it and the antimicrobial susceptibility pattern of the organisms.

Materials and Methods: This cross-sectional study was performed during six months (Jun 2018-December 2018). 1696 samples were collected from surgical site infections. The pus samples were cultured and antibiotic susceptibility determined by Kirby Bauer's disc diffusion method following clinical and Laboratory Standards Institute (CLSI) 2018 recommendation.

Results: Of 1696 patients we reported 312 (18.3%) with Surgical site infections. The high frequency was among patients operated on neurosurgery basis in surgical unit. Among bacterial isolates, the highest prevalent bacterium belonged to the Escherichia coli with prevalence 64(21%) followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter sp*. with prevalence of 60(19%),34 (11 %) and 32(10%), respectively. Antimicrobial profile of Escherichia coli revealed maximum sensitivity to novobiocin, colistin and linezolid and maximum resistant to oxacilin and cefotaxime. The highest and lowest resistance of *Klebsiella pneumoniae* Isolates were related to ampicillin (84.4%) and amikacin (11.9%), respectively. Pseudomonas aeruginosa displayed the highest and lowest resistance to ciprofloxacin (86.5%) and linezolid (5.4%).

Conclusions: This study suggest that bacterial resistance is a prevalent and current problem in neurosurgeries. Escherichia coli is still the most frequently involved pathogen, showing high resistance rates. novobiocin and colistin are still the best therapeutic options to treat these infections

Keywords: Surgical site infection, Antibiotic susceptibility, *Escherichia coli*

Topic: Antimicrobial resistance

P228: In Vitro Antimicrobial Activity of Dermcidin-1L against Extensively-Drug-Resistant and Pan drug-Resistant Acinetobacter baumannii

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Introduction and Objectives: *Acinetobacter baumannii* are currently considered one of the greatest causes of nosocomial infection. The rapid emergence and spread of resistance to most conventional antibiotics highlight the need to identify novel antimicrobial agents. Antimicrobial peptides (AMPs) are introduced as potential therapeutic alternatives. Human anionic antimicrobial peptide, dermcidin-1L (DCD-1L), has shown antimicrobial activity against a wide range of nosocomial pathogens; however, it is still unknown whether it exhibits such properties against *A. baumannii*. For the first time, the present study was conducted to examine the antimicrobial activity of DCD-1L against biofilm forming extensively-drug-resistant (XDR) and pan drug-resistant (PDR) isolates of *A. baumannii*, belonging to different clonal lineages.

Materials and Methods: Dermcidin-1L was examined in terms of antimicrobial properties against 1 biofilm-forming representative XDR isolate from each clonal lineage and 1 PDR isolate via minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) analyses and time-kill assay. Dermcidin-1L resistance mutation frequency in *A. baumannii* was also determined.

Results: Minimum inhibitory concentration and MBC of DCD-1L against all 8 representative XDR and standard (ATCC 19606) isolates were 16 and 32 μg/mL, respectively, while the corresponding value for 1 PDR isolate was 8 μg/mL. The time-kill assay results revealed that the bactericidal effects were more rapid against PDR than XDR strains. In addition, the tested AMPs showed a low tendency to develop resistance.

Conclusions: The present results showed that DCD-1L exhibits interesting antibacterial properties against PDR *A. baumannii* strains, making it a promising candidate for the development of new anti-infective therapies.

Keywords: Antimicrobial Peptide DCD-1L, Drug Resistant, *Acinetobacter baumannii*

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Topic: Antimicrobial resistance

P260: Assessment of the relationship between *Klebsiella pneumoniae*, urinary tract infections and virulence factors

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Introduction and objectives: Bacteria belonging to the genus Klebsiella frequently cause human infections, including life threatening nosocomial infections. In particular, *Klebsiella pneumoniae*, accounts for a significant proportion of hospital-acquired urinary tract infections (UTI), pneumonia, septicemias, and soft tissue infections. Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks. As opportunistic pathogens, the organism primarily attack immunocompromised individuals who are hospitalized and suffer from severe underlying diseases such as diabetes mellitus or chronic pulmonary obstruction. This study was undertaken to assess the frequency of *K. pneumoniae* in UTI in in-patients, demographic data and the virulence factors involved.

Materials and Methods: The clinical study was conducted at the Microbiology unit of Sina hospital's Laboratory for the period 2018- 2019. Bacterial isolation and identification were carried out following standard cultural and biochemical techniques. The pertinent information on any underlying disease and other demographic data were collected and analyzed. Susceptibility profiles of pathogens gathered were determined according to CLSI standards. Capsule associated virulence factors were studied by performing multiplex PCR.

Results: Among the total isolates collected (n=188), the highest resistance was found for cefazolin (95.8%), followed by ceftazidime (91.4%). None of the isolates showed resistance to colistin. Around 43% of the isolates were found to produce extended spectrum beta-lactamase. Serotype K54 was the most prevalent. K5 and K20 serotypes had a higher prevalence in clinical specimens collected from the females than males ($p< 0.05$). In contrast, the distribution of K54 serotype with five capsule associated virulence factors did not differ in both genders.

Conclusion: Information about the distribution of capsular serotypes in UTI, demographic details and pattern of antibiotic resistance could help physicians for prescribing the appropriate treatment.

Keywords: *Klebsiella pneumoniae*, Serotype, Capsule, Virulence, Antibiotic resistance

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Topic: Antimicrobial resistance

P51: Prevalence of Resistant plasmids and integron Classes in new emerged *Staphylococcus lugdunensis* infection in burn patients

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Introduction and objectives: *Staphylococcus lugdunensis* (SL) is coagulase-negative staphylococci may cause various infections with unusual severity. In resentment of various antibiotics treatment, infections caused by these bacteria are still significant and the frequency of antibiotic resistance genes, especially by Integron structures and plasmid gene increases this issue. The object of this study was to investigate the antibiotic susceptibility pattern, the presence of integron classes and presence of antibiotic resistance plasmids gene in SL isolates.

Materials and Methods: Sampling for this study was carried out over a period of one year from burn patients. Twenty-eight isolates of SL were confirmed by phenotypic and Antibacterial Resistance pattern was determined by disk diffusion test (CLSI 2018). The prevalence of antibiotic resistance genes (*bla*Z, *dfr*A, *erm*B, *erm*C, *ile*S, *mph*BM and *msr*A) on plasmids and Integron classes' detection were investigated by PCR method.

Results: The frequency of SL was about 21.8% in burn patients of recent study. The presence of resistance genes was as follows: *dfr*A 28(100%), *mph*BM 27(96.4%), *msr*A 25(89.3%), *bla*Z 24(85.7%), *erm*C 24(85.7%), *erm*B 15 (53.6%), *ile*S 8(28.6%). The prevalence of integron classes I and II was 7 (25.00%) and 2 (7.14%) respectively while class III integron was detected. The association of antibiotic resistance and the presence of integron classes was not significant. A significant relation between resistance genes and antibiotic resistant was seen.

Conclusion: The results indicated the probability of association between antibiotic resistance pattern and presence of resistance plasmid genes. Presence of genes on plasmids increase the probable of resistant transferring in nosocomial infections. Therefore, checking of resistant transfer mechanisms and high risks is helpful in controlling the hospital infections caused by this bacterium.

Keywords: *Staphylococcus lugdunensis*, Integrons, Shiraz, Antibiotic resistance, plasmid

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Topic: Antimicrobial resistance

P210: Evaluation of the antimicrobial activities of leaf extracts from three *Onosma* species

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Introduction and Objectives: *Onosma* L. species belonging to the *Boraginaceae* family are widely used as a folk medicine to treat skin burns and wound healing. In this context, the n-hexane-dichloromethane, ethyl acetate and methanol extracts from aerial parts of three *Onosma* L. species (*O. dichroantum* boiss., *O. sericea* Willd. and *O. elwendicum* Wettst.) were screened for potential antimicrobial activity against medically important bacterial strains including Escherichia coli and *Staphylococcus. aureus*

Material and Methods: For antimicrobial activity assay, well-diffusion method was used according to standard protocols. After 24 hours of incubation, antibacterial activity was expressed as inhibition zone diameters (mm) produced by the plant extracts. An inhibition zone of 10 mm was arbitrarily chosen as representative of bacterial susceptibility to the Extracts.

Results: The results showed that ethyl acetate extracts of the three *Onosma* species; and methanol extracts of *O. dichroantum* and *O. sericea* exhibited significant activity against *E. coli* and *S. aureus* with inhibition zone diameter > 20 mm. The highest antibacterial activity was observed at concentration of 100mg/ml for the ethyl acetate extract of *O. sericea*. The n-hexane-dichloromethane extracts of the three *Onosma* species did not show any significant antimicrobial activity.

Conclusion: These primary results confirm the possible antibacterial potential of the aerial parts extracts of the three *Onosma* L. species. Further studies are needed to evaluate their therapeutic potential during infectious diseases.

Keywords: Antimicrobial, leaf extracts, *Onosma*

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Topic: Antimicrobial resistance

P67: Phenotypic and Genotypic Characterization of Antimicrobial Resistance in *Escherichia coli* Isolates from Dogs and Their Owners

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Introduction and Objectives: Today there are growing concerns about the increased prevalence of antibiotic resistance. Excessive and inappropriate use of antibiotics has an influence on the rate of antimicrobial resistance of not just the pathogenic bacteria but also has effect on the normal flora of the exposed individuals (animals and humans) or populations. The objective of this study was the phenotypic and genotypic characterization of antimicrobial resistance in *Escherichia coli* (*E. coli*) isolates obtained from feces of the dogs and their owners.

Materials and Methods: A total of 112 *E. coli* strains isolated from feces of 28 healthy dog–owner pairs (two isolates from each individual) and confirmed by standard biochemical tests. Phenotypic characterization of antimicrobial resistance was done by the Kirby-Bauer method using tetracycline and streptomycin disks. Genotypic analysis was done on two streptomycin resistance genes (*strA* and *strB)* and four tetracycline resistance genes (*tetA*, *tetB*, *tetC* and *tetD)* using PCR method.

Results: According to phenotypic analysis, the rate of resistance to tetracycline were 46.7% vs. 25%, and for streptomycin were 67.7% vs. 58.9% in dogs' and owners' isolates, respectively. Prevalence of 14.3% vs. 8.9% for *strA*, 12.5% vs. 1.8% for *strB*, and 58.9% vs. 48.2% for *tetA* genes were detected in dogs' and owners' isolates, respectively. Prevalence of both *tetB* and *tetC* genes in dogs' and owners' isolates were the same (8.9%). *tetD* gene was not found in none of the isolates. *tetA* was the most prevalent gene in dogs' and owners' isolates.

Conclusion: There were no significant differences between prevalence of tetracycline and streptomycin resistance genes in *E. coli* isolates from dogs and their owners. This could show the possibility of sharing of *E. coli* or their resistance genes between dogs and their owners which was due to common environment and close relationship between them.

Keywords: Antibiotic resistance, Streptomycin, Tetracycline, *E. coli*, Dogs and their owners

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Topic: Antimicrobial resistance

P61: Frequency of PMQR genes of isolated *Pseudomonas aeruginosa* strains from patients in Kerman hospitals, Iran

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Introduction and Objectives: Emerging antibiotic resistance is a major worldwide threat to human's health. Understanding and fighting in this direction requires a full assessment of the current status on the prevalence of antibiotic resistance genes and understanding their correlation with each other. Plasmid mediated quinolone resistance (PMQR) genes of *Pseudomonas aeruginosa* strains have arisen as a significant concern in recent years. Accordingly, the aim of the present research was to determine the frequency of PMQR genes related to the isolates of *P. aeruginosa* obtained from clinical samples of Kerman hospitals located in south east of Iran.

Material and Methods: In this sectional study, clinical samples were used to isolate different strains of *P. aeruginosa*. Accordingly, in the first step, antibiotic resistance was evaluated using Kirby-Bauer and CLSI criteria. In the second step, following DNA extraction, *qnr* and *aac (6)-Ib-cr* genes were amplified using PCR method and results were analyzed**.**

Results: Cefexim (80%), Nalidixic acid (75%) showed maximum antibiotic resistance and imipenem (25%), ciprofloxacin (35%) had the minimum antibiotic resistance. 10 samples of *qnr A* (16.66%) and 8 samples of *qnrB* (13.33%) as well as 7 samples of *qnrS* (11.66%) were positive and 5 samples of *aac(6)-Ib-cr* (8/33%) were positive.

Conclusion: Using molecular methods for diagnosis of pathogenic bacteria such as *P. aeruginosa* and rapid diagnosis of their resistant genes has proved to be very important and critical. On the other hand, it is necessary to monitor for the spread of PMQR genes of clinical isolates and to ensure careful antibiotic use in a hospital setting.

Keywords: *Pseudomonas aeruginosa*, antibiotic resistance, PMQR genes frequency, *qnr, aac(6)-cr*, PCR.

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Topic: Antimicrobial resistance

P52: Role of antigen-43 on biofilm formation and horizontal antibiotic resistance gene transfer in non-O157 Shiga toxin producing *Escherichia coli* strains

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Introduction and Objectives: The objectives of this study were to evaluate the antibiotic resistance profiles, biofilm formation, presence of antigen 43 (*Ag*43) gene, and transfer of antibiotic resistance phenotype among non-O157 Shiga toxin producing *Escherichia coli* (STEC).

Material and Methods: From October 2014 to November 2015 a total of 276 stool samples were collected from healthycalves, goats and 395 patients with the sign of nonbloody diarrhea and screened for presence of *stx* and serotype O157 genes by polymerase chain reaction (PCR) technique. Susceptibility to 14 antibiotics was determined as per CLSI guideline. Presence of Ag43 and intimin (*eaeA*) genes were detected by PCR. Biofilm formation was measured by microtiter plate method. Conjugation was carried out by membrane filter technique.

Results: We isolated 74 (93.6%) non-O157 STEC strains from 41 calves, 33 goats and 5 (6.3%) patients' stools, however, no O157 serotype was detected in our study. Resistance was observed most commonly to tobramycin (66.2%), kanamycin (48.6%), and amikacin (29.7%) and less frequently to ciprofloxacin (4.1%), amoxicillin-clavulanic acid (5.4%), and ceftriaxone (9.5%) in isolates recovered from calves and goats fecal samples, whereas, all human isolates were sensitive to ceftazidime, ciprofloxacin, tobramycin and imipenem, respectively. Furthermore, Ag43 was detected in 60 STEC isolated from animals and 5 human origins (no *eaeA* gene was found in this study). Biofilm formation from Ag43+ and Ag43- colonies
showed 20 isolates with strong biofilm activities. Cefotaxime resistance phenotype was transferred to *E. coli* ATCC 25922.1 (Nalr) by conjugation at a frequency of 1.6×10-4.

Conclusion: From the above results we concluded that, human infections with non-O157 STEC were significantly low in Kerman. Ag43 was insignificant with biofilm quantity in most cases.

Keywords: Shiga toxin producing *E. coli*, Antibiotic resistance, Biofilm, PCR, Conjugation

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Topic: Antimicrobial resistance

P222: Antimicrobial and anti-biofilm activities of biosurfactant produced by *Shewanella* on antibiotic-resistant bacteria

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Introduction and objectives: There are a lot of concerns about the use of antibiotics to treat various diseases of humans and other living organisms. Because of high levels of antibiotics, it can cause disorders and adverse effects on human health. Despite the demonstration of the antibiofilm and anti-bacterial action of biosurfactants, they can also be used to solve this problem. Biosurfactants are biodegradable, non-toxic, harmless and environmentally friendly compounds. Therefore, these compounds can be a good alternative to antibiotics. In this study, our goal was to assess the in vitro antimicrobial and anti-biofilm abilities of biosurfactant produced by *Shewanella*.

Materials and Methods: We determined biosurfactant minimum inhibitory concentration (MIC) against both Gram-positive and negative antibiotic-resistant bacteria by agar well diffusion method. Also, the anti-biofilm activity of biosurfactant against the biofilm produced by clinically isolated bacterial strains was investigated by microtiter plate.

Results: The biosurfactant produced by Shewanella non-selectively showed activity against both Gram-positive and negative bacterial strains. The highest zone of inhibition (30 mm) was observed at concentration of 1 mg/ml against *Acinetobacter*. Obtained results of the biofilm formation revealed that biosurfactant disrupted the biofilm of *Pseudomonas aeruginosa* (90%) at 100 mg/ml concentrations.

Conclusion: The result of this study indicated that antibacterial and antibiofilm agents on the bacteria studied but the use of biosurfactant in biomedicine and the replacement of antibiotics needs further investigation.

Keywords*:* Biosurfactant, Antibiotic resistant, Biodegradation bacteria, Antimicrobial activity, Antibiofilm activity

Topic: Antimicrobial resistance

P69: Prevalence of *Staphylococcus aureus* and MRSA among Medical and Medical Laboratory students of Shahid Sadoughi of Medical Sciences, Yazd, Iran

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Introduction and Objectives: *Staphylococcus aureus* is a major pathogen within hospital and in the community. This bacterium colonizes the skin and in the anterior nose of about 25-30%of healthy people. During the past four decades, methicillin- resistant *staphylococcus aureus* (MRSA) has spread in hospital and community worldwide. The spread of MRSA indicates that preventive strategies in world societies are inadequate implemented. Determination of nasal carriage frequency and antibiotic resistance of *S. aureus* isolated from medical and paramedical students of Shahid Sadoughi University of Medical Sciences was the major aim of the present study.

Materials and Methods: Following completing a questioner sheet for 177 students, a nasal swab was collected and inoculated on MSA and after 24 hours incubation; suspected colonies were further applied for gram stain, catalase and coagulase tests. The isolated *S. aureus* was then proceeding for antibiogram with Disk Diffusion Method using: penicillin, gentamycin, erythromycin, rifampin, ciprofloxacin, tetracycline, clindamycin, trimethoprim- sulfamethoxazole, cefoxitin, chloramphenicol, nitrofurantoin. Inducible clindamycin resistance was identified using D-zone test.

Results: Among 177 students, 100 were Medical and the remaining 77 were Medical Lab students. Overall, 38 cases (21.5%) were nasal colonized of *S, aureus*; in which 28 (73.7%) were medical and 10 (26.3%) Medical Lab students. The most sensitive antibiotic were found to be nitrofurantoin and rifampin (100%) and the resistance antibiotic was penicillin (100%). note that (2.64%) of isolated *S. aureus* was resistance (MRSA) and the remaining were sensitive to cefoxitin (97.36%). D test was positive in 7.9% of cases.

Conclusion: Although isolation of MRSA in this survey was limited, high frequency of *S. aureus* carriage among medical students is highly considerable.

Keywords: MRSA, antibiotic resistance, Medical students, Medical Lab students, *Staphylococcus. aureus*, carrier

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Topic: Antimicrobial resistance

P34: Isolation of *Streptomyces* with antimicrobial effect on Gentamicin resistance *Escherichia coli*, from bakhtegan lake

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Introduction and objectives: Urinary tract infections are one of the most clinical bacterial infections. *Escherichia coli* is the organism that causes UTIs in most patients. Many drug resistances have been reported, unfortunately, drug resistance is increasing. "Researchers are looking for alternative to replace common drug. One of alternatives are the metabolites produced from *Streptomyces*. *Streptomyces* are filamentous bacteria that isolation from soil and water. Some species of them can secretion antibiotics or other metabolites that kill or inhibit the growth of other microorganism. In this study we were screening for *Streptomyces* that had antimicrobial effect on Gentamycin resistance *E. coli*, from Bakhtaran Lake.

Materials and Methods: For isolation of Streptomyces samples were tested by serial dilution method and culture in starch casein agar then incubated in 28^0c for 7 day. Gypsum and powder colonies were detected. For metabolite produced isolated bacteria were cultured in ISP2 broth in 28 ^{0}c and 150 rpm for 7 day. Then centrifuged and the same volume, ethyl acetate was added and incubated in 28^0c for 5h. Then liquid transferred to Separator funnel . The supernatant phase was transferred to distiller. This liquid was used for antimicrobial tests. Resistance to Gentamicin and other common antibiotics was studied from 50 selected patients. Then, the isolated metabolites from *Streptomyces* were examined by disc diffusion method on these bacteria. Finally, streptomyces were identified by 16srRNA.

Results: 17 *Streptomyces* were isolated from Bakhtegan Lake. One of them has shown antimicrobial effect. The diameter of the non-growth zone was 19±1.24, Results from 16srRNA indicate that these bacteria is 99.9% similar to *Streptomyces Streptomyces sp. strain 11K402.*

Conclusion: *Streptomyces* have high ability to produce antimicrobial metabolites. By purification and formulation these metabolites, they can be used of them as an alternative to common antibiotics or antiagents.

Keywords: *Streptomyces*, *Actinomycete*, Water, Antibiotic, *Escherichia coli* , Gentamicin resistance

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Topic: Antimicrobial resistance

P136: Assessment of colistin-resistant *Escherichia coli* isolates from Chicken in Qazvin

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Introduction and Objectives: Colistin is considered as the last-resort of treatment for multi-drug-resistant gram-negative bacteria infections (MDR-GNB) in both human and animals. In recent studies, the presence of plasmid-mediated colistin resistance genes has been detected in various countries. In this study, we examined the presence of these genes in *Escherichia coli* isolates from chiken.

Materials and Methods: The 60 isolates of *Escherichia coli* were collected from chicken fecal samples in Qazvin, between 2017 and 2019. Colistin resistance was determined using the broth microdilution method by colistin Minimum inhibitory concentration (MICs) to characterize their antimicrobial susceptibility based on Clinical Laboratory Standards Institute guidelines 2019, and PCR was used to detect the plasmid-mediated *mcr-1*,and *mcr-2* genes.

Results: Among 60 isolates, 7 (11.6 %) were resistant to colistin with MIC $\geq 4\ \mu g/ml$. No isolates were detected to harbor *mcr-1*, *mcr-2* genes.

Conclusion: There was no relationship between resistance to colistin and presence of *mcr* genes. It seems that other mechanisms are involved in colistin-resistant isolates and further studies are need to find out the colistin resistance mechanisms in our isolates.

Keywords: *Escherichia coli*, Colistin, plasmid-mediated *mcr*

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Topic: Antimicrobial resistance

P197: Isolation and identification of Aeromonas species from leeches' gut and its antagonistic effects on human pathogens

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Introduction and objectives: The continued increase in infectious diseases has led to the extensive use of commercially available antibiotics that has been proving to be a challenging phenomenon of antibiotic resistance, This phenomenon is described as the future bomb of human society. Several studies have been reported on the assessment of the antimicrobial effects of living organisms on human pathogenic bacteria, the purpose of this study was to isolate and identify the biochemical and molecular characteristics of the *Aeromonas* species isolated from the Caspian leech intestine, and to investigate the antimicrobial effects on human pathogens by disk diffusion and well methods.

Materials and Methods: In this study, intestinal secretions from indigenous Caspian leech were cultured on Ryan's artificial environment at 30 ° C for 24 hours. The dark green colonies with the black center were identified by biochemical methods. For molecular identification DNA from the desire colonies were extracted by GeneAll kit and the product was performed by 16sr DNA pcr sequencing method and the resulting sequence was aligned by chromas software. The latex from identified colonies were obtained by centrifugation with 14000 rpm at 4 ° C for 20 minutes. Antibacterial effects from latex were evaluated against human pathogens including clinical *E. coli* , *Pseudomonas aeruginosa* with PTCC 1811, *Klebsiella pneumonia* with PTCC 9310 and *Staphylococcus aureus* with PTCC 1764 by disc diffusion and well methods.

Results: Biochemical and molecular identification confirmed the presence of *Aeromonas hydrophila* from leeches gut extract. in the disc diffusion method the inhibition zones respectively against *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli , Klebsiella pneumonia* were 15mm , 13mm , 9mm , 0mm .In the well-being method , the inhibition zones respectively against *Staphylococcus aureus , Pseudomonas aeruginosa, E.coli , Klebsiella pneumonia* were *15mm , 12mm ,7mm 0mm*

Conclusion: According to the results, the bacterial latex from isolated bacteria was effective against a wide range of gram-negative and positive bacteria. As well as is seemed disc diffiusion method in contrast to well method to be effective for avaluation of antibacterial activity. Accordingly, in the future production of natural antibacterial from animal sourses can be replaced synthetic antibiotics.

Keywords: disk diffusion, leech, 16sr DNA sequencing, latex, aeromonas *hydrophilla*

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Topic: Antimicrobial resistance

P255: Detection of toxic shock syndrome in MRSA nasal carriages in Tabriz, North-West of Iran

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Introduction and Objectives: Methicillin resistant Staphylococcus aureus (MRSA) is often the cause of wide range of infections ranging from minor skin infections to serious infections such toxic shock syndrome (TSS) in hospital and community settings. Toxic shock syndrome toxin 1 (TSST-1) super antigen is the main cause of TSS. The aim of current study is detection of methicillin resistance (*mecA*) and TSST-1 genes in healthy community.

Materials and Methods: A total of 400 samples were obtained from the nasal of students' high school schools in Tabriz. After confirmation of *S. aureus* strains by standard biochemical tests, the antibiotic resistance patterns were determined by the disk diffusion method. The presence of *mecA* and tsst-1 genes was examined by PCR reaction.

Results: from 400 students, 15% (60 samples) were positive for *S. aureus*. Antimicrobial susceptibility testing was performed against 12 antibiotic disks. The highest resistance rate was observed against ampicillin (100%) and the highest sensitivity was observed against vancomycin antibiotic disk. Also, 18.34% of *S. aureus* isolates were resistant to methicillin (MRSA) while the presence of the *mecA* gene was confirmed in 54.54% of cases. These results were indicating the emergence of Oxacillin susceptible *mecA* positive strains (OS-MRSA) for the first time in health community in IRAN. Based on PCR results, only one of isolates was positive for TSST-1 gene.

Conclusion: The results of this study indicate prevalence of MRSA nasal carriages in healthy population so there is an essential need for continuous monitoring of nasal carriages in health community in order to prevent subsequent infections.

Keywords: *Staphylococcus aureus*, MRSA, mecA, Tsst-1, high school students

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Topic: Antimicrobial resistance

P253: Prevalence of virulence factors in methicillin resistant *Staphylococcus aureus* isolates in healthy students

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Introduction and Objectives: *Staphylococcus (S.) aureus* is the most common cause of skin and soft tissue infections. Arginine catabolic mobile element (ACME) and Panton–Valentine leukocidin (PVL) are known as survival factors of *S. aureus* on the skin, mucous membrane and soft tissue. The current study is aimed to detect ACME-*arcA* and PVL genes among nasal methicillin resistant *S. aureus* isolates in the student population.

Materials and Methodes: A total of 400 nasal samples were obtained from high school students of Tabriz, Iran. The antibiotic resistance profile of *S. aureus* isolates were determined by the disk diffusion method. The presence of *mecA* and ACME-*arcA* and PVL genes was examined by PCR reaction.

Results: Of the 65 positive *S. aureus* isolates, 2.75 % (11/400) cases were MRSA nasal carriage and 27.69% of isolates were multidrug resistance (MDR). Based on PCR results, 20 (30.76%) of isolates were positive for *mecA* and 28 (43.07%) isolates for ACME-*arcA* gene and 10 (15.38%) isolates were positive for PVL. There is a significant relationship between the presence of *ACME-arcA* gene and the frequency of mecA positive strains (P <0.05).

Conclusion: The prevalence of carriage of ACME-*arcA*/ PVL-positive *S. aureus* indicate an essential need for monitoring of nasal carriers in healthy community to prevent subsequent infections.

Keywords: *S. aureus, nasal carriage*, MRSA, PVL, ACME-*arcA*

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Topic: Antimicrobial resistance

P205: *In vitro* antibacterial activity of curcumin-meropenem combination against extensively drug-resistant (XDR) bacteria isolated from burn wound infections

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Introduction and Objectives: Burn wound infection is a severe complication of thermal injury. Patients with severe burn injuries need urgent care to diminish complications after severe burns. Wound infections are commonly considered one of the most serious burn complications, particularly those that are caused by extensively drug-resistant (XDR) bacteria with few therapeutic choices. The objective of this study was to determine *in vitro* activity of meropenem and curcumin, alone and in combination, against antibiotic-susceptible Gram-positive, and antibiotic-resistant and antibiotic susceptible gram-negative bacteria isolated from burn wound infections.

Materials and Methods: The antimicrobial activity of meropenem and curcumin was investigated alone and in combination, against antibiotic-susceptible and antibiotic-resistant bacterial (XDR) strains isolated from burn patients. In addition, the cytotoxic effect of curcumin on human's epithelial cell lines, was determined.

Results: In this study, minimum inhibitory concentrations of meropenem decreased considerably in the presence of curcumin (2- to 16-fold reductions), with synergy observed. Curcumin exerted no cytotoxic effect at concentrations 256-512 µg/ml on human epithelial cell lines.

Conclusion: We suggest that curcumin-antibiotic combinations may provide an alternative approach for treating infections with MDR and XDR bacteria.

Keywords: Curcumin, Meropenem, Extensively drug-resistant (XDR), Antibacterial, Wound Infections

Topic: Antimicrobial resistance

P65: Survey of *ermA, ermB, ermC* and *mecA* genes among *Staphylococcus aureus* isolates isolated from patients admitted to hospitals in Tehran, Iran by PCR and sequencing.

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Introduction and Objectives Staphylococcus aureus is one of the gram-positive bacteria that has created many problems in treatment. The antibiotic resistance is an important problem and debatable topic in the world. In recent years, because of overuse of antibiotics and transition of resistance genes, frequency of resistant staphylococcal infections, are increasing. Clindamycin inductive resistance causes failure in treatment. The aim of current study is detection of clindamycin inductive resistance S. aureus isolates among patients admitted to Tehran hospitals by multiplex PCR.

Materials and Methods: A total of 80 isolates of S. aureus were collected from hospitalized patients in Tehran. The antibiotic susceptibility tests were applied by MIC and disk diffusion methods. The identification of clindamycin inductive resistance isolates was performed by D-zone test. To detection of *ermA, ermB, ermC and mecA* genes, multiplex PCR was administrated.

Results: In current experiment, among 80 isolates, resistance rate to erythromycin and clindamycin were 70% and 45% respectively. By D-zone test, 15 samples were positive. The frequency of *ermA, ermB and ermC* genes in S. aureus isolates were 5%, 7.5% and 10% respectively. The results of this study demonstrated that the antibiotic resistance is a main problem in patient's treatment.

Conclusion: By identification of resistant isolates and apply appropriate treatment, can be somewhat prevent from outspread of resistant isolates.

Keywords: Methicillin resistance *Staphylococcus aureus* (MRSA), Clindamycin inductive resistance, D-zone test

Topic: Antimicrobial resistance

P73: The study of antibiotic resistance of isolated bacteria from upper respiratory tract in Adults in Shahid Bahonar Hospital. Kerman. Iran

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Introduction and Objectives: Upper respiratory tract infection is one of the most common bacterial infections in humans and the second reason for the high administration of antibiotics. The upward trend of antibiotic resistance in bacterial pathogens is a worldwide challenge.

Materials and Methods: In this study, the upper respiratory tract samples of patients were evaluated during a three-month period from February 2019 to April 2019. Respiratory specimens were cultured on blood agar and nutrient agar media. The bacteria were grown on the plates then identified by conventional biochemical tests. The disc diffusion antibiotic susceptibility test was performed according to CLSI standards to 5 antibiotics. In addition, the minimum inhibitory concentration was done by broth macro dilution method for antibiotic resistant isolates about gentamicin antibiotic Next, the percentage of cell surface hydrophobicity for 13 strains was determined.5 isolated with the highest and lowest hydrophobicity were selected and used to study biofilm formation on glass, polypropylene surface with stable and shake condition.

Results: 59 bacterial isolates with positive respiratory culture, were respectively including: 37.28% *Staphylococcus* spp.,20.33% *Klebsiella* spp.,16.94% *Pseudomonas* spp.,6.77% *Escherichia Coli* ,5.08%*Streptococcus* spp., *and Citrobacter* spp. *Serratia* spp. *Proteus* Spp. *Corynebacterium* Spp.3. 38%. *Antibiotic* susceptibility test for isolated showed maximum resistance to tetracycline and most of isolates sensitive to gentamicin. The MIC test showed that mostly of isolated were sensitive to gentamicin. The results of biofilm production on the selected surface, was indicate increasing the biofilm formation on propylene surfaces and in shaker condition.

Conclusion: According to the results, there is a direct relationship between antibiotic resistance and biofilm formation potential in isolates. The incorrect prescription of antibiotics for treatment of this infection has increased the resistance of pathogens to antibiotics and also the production of biofilm. An effective program can be developed by evaluating antibiotics susceptibility patterns and new therapeutic methods.

Keywords: Upper Respiratory Tract Infection, Bacteria, Antibiotic Resistance, Biofilm

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Topic: Antimicrobial resistance

P257: Serotyping, and Molecular Characterization of Antibiotic Resistance Genes in *Listeria monocytogenes* Isolated from Pregnant Women with a History of Abortion

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Introduction and objectives: *Listeria monocytogenes* show a high mortality among pregnant women and newborns. The objective of our study was to detect *L. monocytogenes* in pregnant women with a history of abortion and assess the serotypes, antibiotic susceptibility patterns, and its resistance genes.

Materials and Methods: A total of 400 vaginal swabs were taken from pregnant women with a history of abortion in the past few years in a tertiary care hospital in Tehran, Iran, during 2015- 2018. Antibiotics susceptibility to a panel of 10 antibiotics was determined using the standard disk diffusion method and the isolates serotyped by the agglutination method. The antimicrobial-resistant isolates were also screened for the presence of *tet*M, *erm*B and *dfr*D genes by PCR.

Results: From 400 samples, a total of 22 *L. monocytogenes* isolates were identified. High rates of resistance were observed for trimethoprim (50%; n=11), sulphamethoxazole (50%; n=11), tetracycline (45.45%; n=10) and gentamicin (36.36%; n=8). From 22 *L. monocytogenes* isolates, 13 (59.10 %), 5 (22.73 %), 3 (13.63 %) and 1 (4.54%) belonged to serotypes 4b, 1/2a, 1/2b, and 3c, respectively. The genetic determinant *tet*M was detected in 70% of the tetracycline-resistant isolates. Out of 11 trimethoprim-resistant isolates, 27.27% isolates contained *dfr*D. Moreover, the *erm*B gene was found in 83.33% of the erythromycin-resistant isolates.

Conclusion: The resistance to antibiotics most commonly used in human listeriosis treatment is an important public health concern. Therefore, it is necessary to continue monitoring and management antimicrobial resistance and to diminish its further emergence and spread.

Keywords: *Listeria monocytogenes*, Serotyping, Antibiotic resistance genes.

Topic: Antimicrobial resistance

P237: A Study of antimicrobial effects of essential oil of Thyme on *Escherichia coli* isolated from broiler chicken flocks with colibacillosis

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Introduction and Objectives: Avian pathogenic *Escherichia coli* (APEC) is the major cause of colibacillosis in poultry. This disease has an important economic impact on poultry production worldwide. Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis. However, resistance to existing antimicrobials is widespread and of concern to poultry veterinarians. Nowadays, antibacterial effects of some medicinal plant oils are considered as an appropriate alternative of antibiotics.
Materials and Methods: The purpose of the this study was to determination of antimicrobial effects of essential oil of Thyme against 178 E.coli isolated from broiler chicken flocks with colibacillosis infection during 2014 and 2015. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of this medicinal plant oil were determined for each isolate using micro-dilution technique. The antibiotic resistance of isolates for common antibiotics on Iranian poultry industry were evaluated using Kirby-Bauer disc diffusion method test.
Results: The highest resistance was to Tetrayclin (99.43%), Erythromycin (97.75%), Trimethoprime-sulphadiazine (80.34%), Enrofloxacin (77.53%), Neomycin (75.84%), Colistin (68.54%) and Florfenicol (58.99%). Furthermore, essential oil of Thyme showed significant inhibitory effect on E.coli isolates.
Conclusion: The results of this study show the high frequency of resistance to antimicrobial agents commonly used in Iranian poultry industry and also Thyme essential oil have antimicrobial activity against E.coli isolates. So, there is using essential oil of Thyme as a substitute of common antibiotics.
Keywords: *E coli*, antibiotic resistance, thyme, broiler flocks

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Topic: Antimicrobial resistance

P245: High Rate of Fecal Carriage of Extended-Spectrum-β-Lactamase Producing Escherichia coli in healthy People in the south of Tehran, IRAN

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Introduction and objectives: The incidence of Multi-Drug-Resistant (MDR) Escherichia coli is alarming in the worldwide. E. coli is a main inhabitant of human gut flora. Fecal colonization by Extended-Spectrum-B-Lactamase (ESBL) producing E. coli (EPE) in healthy individuals contributes in their dissemination. The aim of this study was find out the prevalence, phenotypic resistance pattern and molecular detection of blaCTX-M genes of ESBL producing E. coli in feces of healthy people in Tehran.

Materials and methods: A total of 200 stool samples were taken from healthy people at ages of 18-35 years from January to July 2018. All red colonies on Chromagar ESBL and Chromagar carba media were selected as EPE and conventional biochemical tests were used to identification of the E. coli. Antimicrobial susceptibility tests in EPE were performed using disc diffusion method for, quinolones, carbapenems and aminoglycosides antibiotics. Presence of ESBL enzymes were confirmed by phenotypic standard methods. Every EPE were screened for blaCTX-M genes (blaCTXM-15 and blaCTX-M14).

Results: In present study, 155/200 (77/5%) E. coli isolates were grown on media which ESBL confirmed in 137/200 (88/5%). The blaCTX-M genes were detected in all isolates that high prevalence was related to blaCTX-M15 (119l137; 86/8%). The blaCTX-M14 gene was detected in the 6/137 (4/3%) only. 86/137 (62/7%) isolates were resistance to quinolones also 11/137(8/02) and 12/ 137(8/7%) isolates resistance to carbapenems and aminoglycosides respectively.

Conclusions: The current study demonstrates the high rate of fecal carriage of ESBL producing E. coli harboring bla CTX-M-15 in low income region of Tehran. it should aware us to possibility of increase transmission of these MDR isolates in the part of the city with limited access to well medical care, high population density and poor hygiene. To reduce the risk of accession and subsequent transmission of these isolates in healthy people, fecal carriage of high-risk people in community should be the center of attention.

Keywords: Antibiotic resistant, Intestinal microbiota, Community carriage

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Topic: Biological Products

P289: T cell-mediated immune responses of chicken inoculated with fowl pox vaccine

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Introduction and Objectives: Fowl pox is a viral disease that is widely distributed throughout the world. The disease has an economic impact on the poultry industry, and even the outbreak has been reported in vaccinated flocks. This study was designed to evaluate the T cell-mediated immune responses of chickens induced by fowl pox (FP) vaccine.

Materials and Methods: Three groups of 40 non-pathogenic 21-day-old chicks were used. They were vaccinated with either the local or commercial FP vaccines. Peripheral blood mononuclear cells were isolated and the percentage of $CD3^+$, $CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$ T lymphocytes were analyzed by flow cytometric method. The concentration of inflammatory cytokines (IFN-γ) and anti-inflammatory (IL-4) in peripheral blood mononuclear cells were also measured using the ELISA method.

Results: The results showed that, after 7 days post immunization, a maximum (90-100%) swelling formation ("take") on the site of vaccinated chickens were observed. The ratios of $CD4^+$ to $CD8^+$ T-lymphocytes in both groups of commercial and local FP vaccinated groups were significantly higher ($P < 0.05$) than the control group. The percentages of $CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$ T-lymphocytes were increased ($P < 0.05$) in vaccinated chickens with commercial and local FP vaccines. The results of cytokine analysis showed that the ratio concentration of IFN-γ to IL-4 in supernatant cell culture of vaccinated chickens in both groups were higher than the control group. The results show that the flow cytometric method and the cytokine assay are appropriate for measuring IFN-γ and IL-4 by T-cells ($CD4^+$ and $CD8^+$).

Conclusion: This study revealed that the protective immunity may be associated with increased cellular immunity (predominantly Th1 type-reaction), enhancing the proliferation of T cells and increasing $CD4^+$ to $CD8^+$ ratios due to vaccination of the FP vaccine.

Keywords: FP vaccine, IFN-γ, flow cytometry, CD4 and Cd8 T-cells

Topic: Biological Products

P215: Identification of novel antimicrobial peptide from Asian sea bass (*Lates calcarifer*) by *in silico* and activity characterization

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Introduction and Objectives: The global crisis of antibiotic resistance increases the demand for the new promising alternative drugs such as antimicrobial peptides (AMPs). Accordingly, we have described a new, previously unrecognized effective AMP, named dicentracin-like, from Asian sea bass and characterized its antimicrobial activity by comparison with moronecidin.

Materials and Methods: Gene expression analysis demonstrated the expression of dicentracin-like peptide in tissues of the immune system such as the skin and the head kidney, which is an important endocrine and lymphoid organ. Moronecidin and dicentracin-like exhibited a higher antibacterial activity against gram-positive bacteria relative to gram-negative ones, while both peptides showed a greater binding ability to gram-negative bacteria compared to gram-positive ones. This contradiction between antibacterial activity and binding affinity may be related to the outer membrane from gram-negative bacteria. Compared with moronecidin, dicentracin-like peptide showed more potent binding ability to all gram-positive and gram-negative bacteria. In addition, dicentracin-like peptide exhibited a high antibacterial activity against the investigated microorganisms, except against Staphylococcus aureus. A direct relationship was found between the binding affinity/cationicity and the antibiofilm activity of the peptides wherein, an elevation in pH corresponded to a decrease in their antibiofilm property. Time kill kinetics analysis against clinical Acinetobacter baumannii isolate indicated that bactericidal effect of dicentracin-like and moronecidin at inhibitory concentration (1XMIC) was observed after 4 and 6 hours, respectively, while bactericidal effect of both AMPs at concentration of 2XMIC was observed after 2 hours. Dicentracin-like peptide showed higher inhibitory activity at subinhibitory concentration (1/2XMIC), relative to moronecidin. Compared with moronecidin, dicentracin-like peptide possessed greater binding affinity to bacteria at high salt concentration, as well as at alkaline pH; In addition, dicentracin-like exhibited a higher antibiofilm activity in comparison to moronecidin even at alkaline pH. Hemolytic analysis against human RBC revealed that hemolytic activity of moronecidin was more potent than that of dicentracin-like, which is consistent with its greater non-polar face hydrophobicity.

Conclusions: In the present study, In Silico comparative sequence analysis and antimicrobial characterization led to identify a new, previously unrecognized antimicrobial function for named dicentracin-like peptide by comparison with moronecidin, representing a possible template for designing new effective AMPs and improving known ones.

Keywords: Antimicrobilal peptide, Acinetobacter baumannii, Staphylococcus aureus, in silico analysis

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Topic: Biotechnology

P150: Isolation and screening of novel local yeast strains for L-asparaginase production

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Introduction and Objectives: L-asparaginase is an enzyme with great potential for biotechnological applications including the pharmaceutical and food industries. Investigation of novel L-asparaginase producers may advance the commercial development of the enzyme. In this study, some local yeast strains were isolated and screened for L-asparaginase production.

Materials and Methods: Ten soil samples were collected and serially diluted on Rose Bengal Chloramphenicol Agar medium. The isolated yeasts were identified by sequencing of the D1/D2 domain of the LSU rRNA gene. The isolates and ten identified yeast strains obtained from Iranian Biological Resource Center (IBRC) were spot-inoculated on modified Czapek agar media containing 0.009% phenol red or 0.008% bromothymol blue. After incubation at 25 °C for 72 h, the diameter of the zones was measured. Positive strains were cultured on the modified Czapek broth for quantitative estimation of L-asparaginase production. After incubation at 25 °C for 4 days, enzyme activity was determined by measuring the amount of ammonia formed by nesslerization and expressed as International Unit of L-asparaginase activity per volume of culture (IU/ml).

Results: Three yeast strains were isolated from three soil samples and identified and designated as *Rhodotorula* sp. F1, *Rhodotorula* sp. F2 and *Sarocladium* sp. F3. These strains and five strains obtained from IBRC including *Aureobasidium mangrovei* IBRC-M 30265, *Fereydounia khargensis* IBRC-M 30116, *Coniochaeta iranica* IBRC-M 30187, *Graphiola fimbriata* IBRC-M 30158 and *Starmerella orientalis* IBRC-M 30204 showed positive reaction in the plate assay. In liquid culture, L-asparaginase production by the strains were estimated at the range of 0.08–1.68 IU/ml. The strains *Sarocladium* sp. F3 and *Fereydounia khargensis* IBRC-M 30116 showed the highest production level of 1.68 and 1.11 IU/ml, respectively.

Conclusion: In the present study, seven local L-asparaginase producing yeast strains were reported. Among them, five yeast species were introduced as L-asparaginase producers for the first time.

Keywords: Isolation, L-asparaginase, screening, yeast

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Topic: Biotechnology

P161: Comparison of CXCL10 expression in *E. coli* and *Pichia pastoris*

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Introduction and Objectives: Bacterial protein expression system is one of the most widely used system to produce recombinant proteins due to two important features, fast and high production ability and low cost of production. In contrast, yeasts are often used to produce recombinant proteins as a eukaryotic host. In addition to grow fast and cost less, this host is used to recombinant proteins that cannot be produced in the prokaryotic system because of the need for proper folding and glycosylation. The aim of this study was Comparison of Expression of inflammatory chemokine CXCL10 in *E. coli* and *Pichia pastoris*.

Materials and Methods: The encoding sequence of the CXCL10 was codoned in *E. coli BL21 (DE3)* or *Pichia pastoris*. Then, the coding sequences were cloned in frame with 6 His-tagged sequence into pET28a plasmid for bacterial expression, and pPICZA plasmid for yeast expression and the expression was analyzed by SDS-PAGE, dot- and Western blotting.

Results: Protein expression was investigated in *E. coli* by incubating at 180 rpm, 37 ° C for 4 hours after induction by IPTG 1 mM. Also, protein expression in the *Pichia* was evaluated at 200 rpm, 28 ˚C for 96 hours, with induction by methanol every 24 hours.

Conclusion: By analyzing the SDS-PAGE results, it was found that the recombinant protein was well expressed in both hosts. The expression level in the bacteria was significantly higher than the yeast, as well the results of this study indicate that the expression of the recombinant protein in the *Pichia* started from 48 hours after induction and the highest expression of this protein in the yeast was observed in 96 hours after induction, while this level is considerably lower than protein expression in bacterial host. Of course due to PTM, it is suggested that expression in the yeast should be optimized.

Keywords: Expression, *E. coli*, *Pichia pastoris*, *Expression assessment*, *Comparison of protein expression*

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Topic: Biotechnology

P240: Phylogenetic analysis of *Brucella* field isolates according to *rpo B* gene modifications

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Introduction and Objective: Brucellosis is a zoonotic disease in humans and animals.Nowadays,several serological tests for brucellosis diagnosis are available but all of these tests have their own limits.The aim of the present study was to use Amos PCR and Multiplex PCR and Bruce ladder techniques according to *rpo* B gene for the molecular analysis of the individuals with brucellosis.

Materials and Methods: 50 clinical isolates were studied.11 patients were diagnosed with positive immunological tests such as Wright,Coombs Wright,Rosbangal.Samples of blood were cultured (BACTEC) and incubated at 37 degrees Celsius for 5 days and then they were cultured for 3 days on Brucella agar.The nucleotide sequence of *rpo* B gene in the studied strains was obtained from the NCBI,BLAST.Draw phylogenetic tree of *rpo* B gene for the species examined using MEGA6 software Was performed.Analysis of the structure of *rpo* B gene was carried out in terms of nucleotide differences and phylogenetic tree mapping by neighborjoining method.

Results: In this study,all the samples selected were seropositive from which the colonies were obtained.The results of the amplification reaction and the amplified bands of 1432 bp for *rpo* B gene indicate the validity of the primers.According to this study,protected areas form a small part of the gene sequence,which indicates the high polymorphism of this gene as well as its susceptibility to nucleotide changes and mutations,especially in area 81 bp.

Conclusion: We showed that PCR and SNPs retrieved from the B. melitensis draft full genomes were sufficient to resolve the interspecies relationships between B. melitensis strains and to discriminate between the vaccine and endemic strains.

Keywords: Brucella, Phylogenetic analysis, Molecular typing, Brucella field isolates, *rpo B*

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Topic: Biotechnology

P138: In vitro and in vivo effects of *Agrobacterium tumefacience* strain IAUK2307 on growth of *Helianthus annuus*

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Introduction and Objectives: Biofertilizers constructed by obtained bacteria from rhizosphere of plants are good alternative for chemical fertilizers. *Helianthus annuus* is an important strategic oilfield crop. The aim of this research was to isolate beneficial plat growth promoting rhizobacteria and evaluation their effects on vegetative stage of *Helianthus annuus* in pot experiment.

Materials and Methods: Sampling was performed from rhizospheric soils of different fields in Kerman district. Different bacterial strains were isolated from rhizospheric samples. Direct mechanisms were carried out as zinc and phosphate solubilization, nitrogen fixation, ammonia production as well as evaluation of their resistance to environmental stress. Molecular characterization using 16R rRNA sequences was used to identify the selected isolate. A pot experiment was also conducted to investigate the effects of selected PGPR isolate on the growth of *Helianthus annuus*.

Results: Strain numbers of isolates were considered as IAUK2301-2330. All of selected strains were resistant to the different temperatures between 25-42ºC as well as having normal growth in salt concentrations between 5-7 and PH=4-8. Maximum phosphate solubilization efficiency was 350 and related strain was selected for further experiments. Molecular characterization using 16R rRNA sequences suggested the identity this isolate as *Agrobacterium tumefacience* strain IAUK2307. Pot experiment by this strain revealed meaningful plant growth promoting traits in seedling stage on shoot height, root length and dry weight of *Helianthus annuus*.

Conclusion: According to the In vitro and in vivo results, *Agrobacterium tumefacience* IAUK2307 may have a good potential for field experiments before usage as the new biofertilizer.

Keywords: *Agrobacterium tumefacience* IAUK2307, PGPR, Phosphate solubilization, Pot experiment, *Helianthus annuus* L

Topic: Biotechnology

P293: Optimization of induction conditions and purification of glycoprotein B cytomegalovirus

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Introduction and Objectives: In recent years, cytomegalovirus has been identified as an important cause of neonatal congenital abnormalities, and important factors transmission infection including blood transition, blood products and organ transplants, are known. Some viral glycoproteins play an important role in the life cycle of the virus. Among them, glycoprotein B (gB) is a surface glycoprotein virus. gB is site for binding anti-virus antibodies that can be used to target subunit vaccines.

Materials and Methods: In this study, to produce gB in Full Length, after bioinformatics and optimization studies, the sequence of this gene was synthesized in pET15b vector and then transformed into E. coli BL21. In order to obtain the gB protein, Ni-NTA chromatography resin was purified using microtubes method. Optimization of protein expression in prokaryotic system was performed by evaluating effective parameters and SDS PAGE and Western Blot were used to confirm the gene expression vector. An expressive protein was prepared for final purification and immunological evaluations.

Results: The induction of transfected BL21 bacteria with IPTG resulted in expression of gB, The highest yield of recombinant gB occurred in the presence of 1 mM of IPTG in LB Broth medium after 8 h at 37°C. Thereby purifying the chromatography and purification of this protein and confirming the western blot.

Conclusion: considering the ability to express the glycoprotein expressed in the BL21 bacteria, this protein can be a suitable candidate for immunological evaluation and vaccine production against cytomegalovirus.

Keywords: cytomegalovirus, purification, induction

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Topic: Biotechnology

P148: Identification of a bacterial isolate from Sarcheshmeh copper mine (in south east of Iran) used for the biosynthesis of Bismuth nanoparticles

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Introduction and Objectives: There is an increasing attention on usage of Bismuth nanoparticles (BiNPs). The main applications are electrochemical applications, such as in the electrolyte or cathode of solid oxide fuel cells, in bio-medical and cancer imaging and for other photoconductive characteristics in thin films. Biosynthesis of BiNPs is a good alternative for chemical synthesis of this element. Accordingly, the aim of this research was to isolate and identify bacteria with capability to synthesize BiNPs.

Materials and Methods: Soil samples were collected from Sarcheshmeh copper mine in Kerman district (South east of Iran). Screening of bismuth producing bacteria was followed by inoculation of serially diluted samples on two sets of nutrient agar with and without bismuth salts. All positive isolates were identified using molecular tests using 16S rRNA gene. In order to biosynthesize of bismuth nanoparticles, each bacterial strain was subcultured on nutrient agar supplemented with bismuth subnitrate and this followed by biological purification of nanoparticles by an organic-aqueous system.

Results: From soil samples of Sarcheshmeh copper mine, one bacterium with BiNPs synthesis ability was isolated and identified as *Pseudomonas* sp. strain 1061. The ability of this isolate to reduce bismuth subnitrate was used for biosynthesis of bismuth nanoparticles and this synthesis was approved by conformational tests.

Conclusion: Although the chemical synthesis of BiNPs has been reported in the several research articles, but in comparison there are a few reports about its biological synthesis. This research focuses on isolation of a bacterium as well as its biological method for BiNPs synthesis.

Keywords: Bismuth, nanoparticle, Biosynthesis, *Pseudomonas* sp. strain1061

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Topic: Biotechnology

P55: Frequency of *higBA and relBE* Toxin-Antitoxin System in multiple drug resistant *Acinetobacter baumannii*, isolated from burn wound infections

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Introduction and Objectives: *Acinetobacter baumannii* is an opportunistic pathogen which due to the development of multi-drug resistant infections and the ability to form biofilms, especially in patients with burn wound infections. TA systems are combined of a protein toxin and its cognate antitoxin, which may be an RNA or a protein. type II TA systems were first discovered on plasmids in the mid-1980s.One of the most effective genes in the production of biofilms and antibiotic resistance is *higBA* and *relBE*. These genes are located on the plasmids in the *A. baumannii*, where antibiotic-resistant genes are also present. The study aims to determine the frequency of the *higBA* and *relBE* genes in the bacteria that isolated from burn wound infections which are multi-drug resistant.

Materials and Methods: Biochemical and molecular tests were used for identification of the *A. baumannii* and antibacterial susceptibility test was performed using disk diffusion methods. The *higBA* and *relBE* toxin-anti toxin gene was detected in the isolates by PCR molecular method.

Result: The results of PCR on higBA gene showed that 7.91%of the isolates possess the gene. The results of PCR on relBE gene showed that 94.96% of the isolates possess the gene. Of the 11 isolates with higBA gene, only two cases did not have the relBE gene.

Conclusion: Our results reflect the high frequency of *higBA* and *relBE* genes in antibiotic resistance in bacteria.

Keywords: toxin-antitoxin system, *higBA, relBE, multi* drug resistant

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Topic: Biotechnology

P121: Optimizing the Immobilization of Biosurfactant-Producing Pseudomonas aeruginosa in Alginate Beads

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Introduction and Objectives: optimizing the immobilization of *pseudomonas aeruginosa* as a biosurfactant producer was studied.

Materials and Methods: Biosurfactant production by *p. aeruginosa* was confirmed through the hemolysis test, emulsification index (EI), and surface activity measurement. Calcium alginate encapsulation technique was used in order to entrap the *P. aeruginosa* cells. Full factorial design was employed to optimize bead preparation. Furthermore, the morphology and the stability of beads were evaluated.

Results: It was proved that immobilized cells can be preserved the viability and biosurfactant production. Application of full factorial design indicated that the values of three parameters sodium alginate 3 %, $CaCl_2$ 1 % (w/v), and hardening time of 35 min, was found to be too stable for minimum surface tension and stable alginate gel beads.

Conclusion: Finally, it could be concluded that the alginate gel beads showed good stability during the growing process and the immobilized cells efficiently were viable. Alginate source, hardening time and the interaction between $CaCl_2$ concentration and hardening time influenced on the bead preparation.

Keywords: Immobilization; Alginate Beads; Optimizing; *Pseudomonas aeruginosa*

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Topic: Biotechnology

P151: Cloning of *Yarrowia lipolytica* extracellular lipase 2 gene in *Saccharomyces cerevisiae*

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Introduction and Objectives: Lipases have attracted a high domain of biocatalytic processes in many industries such as biodiesel production, food industry and synthetic pharmaceuticals. Microbial production of lipases, specially yeast lipases plays an important role in industry due to the capability of large-scale production with lower costs of production. *Yarrowia lipolytica* yeast is one of the most important species that produces several intracellular, cell-bound, and extracellular lipases. Among several lipases of this yeast, *Y. lipolytica* extracellular lipase (YlLIP2) has significant importance since it has high biocatalytic activity. Recently, systems and synthetic biology tools have taken for developing microorganisms such as *Escherichia coli* and *Saccharomyces cerevisiae*. In this study, we developed a shuttle vector containing *Y. lipolytica* extracellular lipase gene for cloning in *S. cerevisiae* as a cell factory, by synthetic biology method.
Materials and Methods: The *LIP2* lipase genes were isolated from *Yarrowia lipolytica* strain DSM3286 by PCR method and inserted in *Saccharomyces cerevisiae* CEN. PK113-7D strain. The purified *LIP2* gene and USER cloning vectors, PCFB3035 were prepared by with FastDigest AsiSI. Digested lipase genes and recombinants vectors were ligated. Qualitative analyses of lipases expression were tested on MSM with 10 mL/L tributyrin plats by the halo assay. After incubation at 29 °C for 72 h, the clear halos are observed where extracellular tributyrin was degraded.
Results: After incubation, the halos around of the engineered strain colonies had been extended, and it was about 17 mm diameter. However, no halo was observed for wild strain which due to the absence of any extracellular lipase activity.
Conclusion: The recombinant strain efficiently degrades hydrophobic substrates such as fatty acids, fats and oils by these lipases.

Keywords: *Saccharomyces cerevisiae*, Lipase, *Yarrowia lipolytica*, Synthetic biology.

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Topic: Biotechnology

P6: Purification and characterization of clostridium perfringense type A alpha toxin

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Introduction and Objectives: *Clostridium perfringens* is a gram positive, rod shape anaerobic, spore forming pathogenic bacterium with the ability to produce different bacterial exotoxins. The alpha toxin from clostridium perfringens type A is a metallophospholipase that causes food born disease marked by diarrhea and abdominal discomfort or even death.
Materials and Methods: The bacterium source was taken from Mashhad Razi vaccine and serum researches institute. For obtaining maximum bacterial growth and toxin production, different media were formulated and applied as growth media. The sources of nitrogen, carbon, minerals, and vitamins were subjected in the process. in addition, time and temperature conditions were optimized. During growth optimization, the amount of toxin was monitored with hemolysis and lecithinase assay and protein quantification. After optimization, the purification was performed with salting out, gel filtration and ion exchange chromatography and in each step the yield of toxin and its purity was controlled with SDS-PAGE and hemolysin activity. The zymography method was used for activity and structure of the protein. The selected medium with the highest toxin production yield consists of 0.5% carbon source, 4% nitrogen source, 0.2% trace elements and 0.25%phosphate buffer. The maximum activity of enzyme was in the presence of zinc and magnesium as metal sources. The SDS-PAGE results were shown that enzyme was successfully purified as a single band with estimated molecular weight 43 KD.
Results: According to the results, the optimized culture medium and growth conditions for alpha toxin production were applicable for vaccine production. Also, the purified toxin can be used in diagnostic application and vaccine researches.
Keywords: *Clostridium perfringens*, alpha toxin, enzyme, optimized, purification, characterization chromatography.

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Topic: Emerging Infectious Diseases

P117: Incidence of *Vibrio cholerae* infection (cholera) in Najaf Abad, Iran Between 2014 and 2018

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Introduction and Objectives: cholera is an enteric bacterial disease which could have different consequences from asymptomatic case to massive disrupting diarrhea. The causative organism is *Vibrio cholerae* which is divided into several serogroups based on the somatic O antigen. Only O1 and O139 serogroups are well known to cause epidemic and pandemic cholera. The cholera is still an endemic disease in Iran, which there are several reports in that respect, annually. At the present study, the incidence of cholera was investigated in Najaf Abad city, Isfahan.

Material and Method: A total of 8034 patients with watery diarrhea symptom were referred to Najaf Abad hospitals and health care centers over a period of 5 years from 2014 to 2018. For laboratory diagnosis, the rectal swab samples were collected and cultured according to the standard procedures. Serologic and biochemical tests were used for serotyping and identification of isolates.

Results: The prevalence of cholera over 2014 to 2018 was 6.3, 6.2, 0, 2.1, and 0.6 per 1000 patient, respectively. In total, the mean prevalence of cholera was 3 per 1000 patient with watery diarrhea. The results showed that 65% of the isolates were belongs to serogroup O1 and serotype Inaba.

Conclusion: It seems that the prevalence of cholera has decreased from 2014 to 2018. It is highly recommended that the patient with massive diarrhea have to screened for *Vibrio cholerae* infection and subsequently the costs attributed to cholera prevention and treatment will be decrease incredibly.

Keywords*:* *Vibrio cholerae*, Inaba serotype, Diarrhea.

Topic: Emerging Infectious Diseases

P292: Detection of Saffold virus as a New Emerging Picornavirus in Environmental Waters

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Introduction and Objectives: Saffold virus as a newly discovered virus, which seems to be related to acute gastroenteritis as with other enteric viruses and to human airway diseases in children belongs to *Cardiovirus* genus in *picornaviridae* family with 11 genotypes. Saffold virus initially was detected in America from infant stool sample. Saffold virus has also been detected in environmental water samples.

Objectives: Until now, two reports have demonstrated that sewage water sources are contaminated with Saffold viruses. Molecular detection of Saffold virus mostly depended on reverse transcription-PCR methods and RT-qPCR, which had targeted 5´UTR region of the viral genome. The present study aims to evaluate the molecular detection of Saffold virus in sewage water and river water specimens by RT-qPCR assay in Karaj, Iran.

Materials and Methods: 50 samples collected from environmental waters containing treated and untreated sewage water and river water samples were included in this study. After viral RNA extraction, the Real-time PCR was developed to amplify the 5´UTR sequence of Saffold virus genome and viral load was assessed.

Results: Out of 50 samples tested (consisting 28 river water samples and 22 sewage water samples), the Saffold virus genomic RNA was identified in 10/28 (35.7%) of river water samples and in 4/12 (33.3%) of treated and 4/10 (40%) of untreated sewage samples.

Discussion: Our results for the first time indicate that Saffold virus has apparently been circulating among Iranian peoples. Also, the viral load of Saffold virus in each of tested samples was within moderate to high in range.

Keywords: Saffold virus, Sewage Water, River Water, Real-time PCR

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Topic: Emerging Infectious Diseases

P152: Fish tank granuloma, a new neglected emerging disease in Mashhad

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Introduction and Objectives: *Mycobacterium marinum* is a free-living acid-fast bacterium which classified as atypical mycobacterium and causesa cutaneous lymphatic disease called Fish tank or swimming pool granuloma. This agent is the inhabitant of swimming pools, home aquaria and animals living inside salt or fresh water. In this case series study seven patients with fish tank granuloma are presented.

Materials and Methods: During 3 years (2016-2018), more than 300 people who had cutaneous lymphatic lesions on their extremities were referred to parasitology laboratory of Emamreza hospital and special clinics. All of the individuals have been clinically examined by dermatologists and introduced to the laboratory with primary diagnosis of cutaneous Leishmaniasis. For those patients whose direct Giemsa smears were negative for Leishman body and had history of direct contact with fish or swimming pool, Ziehl-Neelsen stained smear and culture on Lowenstein- Jensen medium was performed.

Results: Ziehl-Neelsen stained smears showed Acid fast bacilli: *Mycobacterium spp*. on 37 smears. The egg yolk like colonies grown on Lowenstein- Jensen media confirmed *Mycobacterium marinum* infection. Clinical manifestation of all the patients and result of treatment of some of the lesions were followed up.

Conclusion: Fish tank granuloma is an emerging sporadic skin disease in Mashhad and probably other regions in Iran. Skin lesions are similar to cutaneous Leishmaniasis especially sporotrichotic form. Patients with skin lesions, who have previous history of contact with aquarium water, manipulating fish or swimming inside fresh water pools, should be considered for fish tank granuloma.

Keywords: *Mycobacterium marinum*, Fish tank granuloma, Cutaneous Leishmaniasis, Mashhad

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Topic: Emerging Infectious Diseases

P332: Report of lesion of septicemic Salmonellosis in a sheep

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Introduction and Objectives: Salmonellosis is a common bacterial enteric infection with significant economic losses for the intensive production of cattle, sheep, and poultry. It is a food born disease. *Salmonella* species are zoonotic and human may be infected by contaminated animal products or direct contact. Clinical disease is characterized by two major forms including septicemia and enteritis. *Salmonella enterica* is a food pathogen causes infections associated with systemic infections including diarrhea and septicemia and may even lead to death in severe cases.

Materials and methods: A 5-year old sheep was referred to the Veterinary Clinic of Shahid Bahonar University of Kerman. In the clinical examination, body temperature was 38.5℃ and the heart rate was 84/minute with Muffled sounds. Dyspnea, reduced appetite, depression, seizure, neck stiffness and dehydration were other signs. This case died after primary treatment. Samples of affected tissues were referred to microbiological laboratory for detection of pathogen and are cultured directly onto Nutrient agar, Blood agar, MacConkey agar were used.

Results: In the necropsy examination, fibrinopurulent pericarditis and pleuritis, purulent peritoneum with adhesion and abscess. Also, thickening of the meninges and necrotic area of cerebrum were observed. In the microbiological examination pathogen wad founded in Blood agar and MacConkey agar. The suspicious *Salmonella* strain was tested with biochemical methods including urea test, TSI agar, citrate, indole and motility. After examination and serological confirmation *Salmonella enterica* was found. *Salmonella enterica* is a gram-negative bacterium, non-hemolytic, lactose negative and urease negative.

Conclusion: Prevention and control of salmonellosis is a major problem because of carrier animals and contaminated feedstuffs. The principles of control include preventing the introduction of carrier animals by maintaining a closed herd or by purchasing from a herd of a known health status

Keywords: *Salmonella enterica*, Sheep, Zoonotic, Peritonitis, Pericarditis

Topic: Emerging Infectious Diseases

P106: Contagious infectious diseases in hajj

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Introduction and Objectives: One of the biggest gatherings in the world is pilgrimage to Hajj pilgrimage to Saudi Arabia. During this great religious ceremony, the country of Saudi Arabia receives a large number of pilgrims throughout the year to perform the Hajj ritual. To this end, an average of around 3 million people come from around 140 countries each year with different cultures and cultures in a small geographic collection. This causes many diseases and exacerbations of chronic diseases. Therefore, it is clear that it is impossible to deny the necessity of observing health, safety and conduct of examinations before being sent to Hajj. The purpose of this study was to determine the distribution of common diseases among pilgrims, mortality rates and how to control these diseases.

Materials and methods: This research have been conducted in the field of scient metric studies in a survey (analytical) method. The community under study consists of all health-related articles on Hajj and pilgrimage on websites. All articles were extracted based on the number of scientific publications, authors, organizations, countries and countries' share in science production.

Results: 391 articles have been published on the health of pilgrims of Hajj. The number of review articles and clinical practice is very limited. The share of Iran is only 8 original articles. Eventually, 84 articles and publications were finalized during the period 2000-2019 And 4 cases of common and contagious diseases in Hajj were investigated and analyzed.

Conclusion: In the Ridicule of Hajj, pilgrims from all over the world travel to the land of revelation for the pilgrimage of Hajj. There may be a series of infectious and contagious diseases that can lead to a transfer from one person to another and the emergence of an epidemic in another society. Although they are vaccinated against diseases before the departure of the Hajjis, nonetheless, the likelihood of illness is high if they do not comply.

Keywords: Hajj, Hajj health, Visitor health

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Topic: Food Microbiology

P226: The potentiality of crude proteins from a newly domesticated Iranian native medicinal mushroom *Lentinus tigrinus* to suppress the growth of pathogenic fungi

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Introduction and Objectives: *Lentinus tigrinus* is a culinary-medicinal mushroom belonging to the Polyporales order, which possesses nutritious and biological activities. This study for the first time reports the antifungal potentiality of crude proteins extracted from an Iranian well-authenticated strain of this mushroom, which has been recently adapted for cultivation in artificial substrate.

Materials and Methods: The pure mycelia of *L. tigrinus* was cultured on the malt agar medium followed by being inoculated into wheat grains supplemented with 0.18% w/v CaCO3 and 1% w/v CaSO4. A lignocellulosic substrate composed of sawdust enriched with 1% wheat bran and limes 1% w/w was formulated to support vegetative and reproductive growth of this mushroom. The soluble protein was extracted from fresh mushrooms using PBS buffer precipitated by polyethylene glycol 6000. Inhibitory effects of the precipitated protein at various concentrations were quantifiably evaluated against *Verticillum dahliae* and *Phytophthora drechsleri*. The inhibition potency towards each fungal strain was calculated using the difference in the radial growth of fungal mycelia challenged with the protein-impregnated media in comparison with the not treated pathogens.

Results: The findings showed that the total amount of soluble protein was 5.06±0.9% (w/w in dry matter) based on the Bradford, while the figure was 10.7±0.8% (w/w in dry matter) using Lowry method. Among various concentrations of the *L. tigrinus* protein (400-2000 µg/mL), 800 µg/mL of the protein prevented the growth of *V. dahliae* by 47.33±2.5 % which was significantly greater than those obtained with the other concentrations ($p \leq 0.05$). By contrast, the activity of the protein was apparently weaker in *P. drechsleri* in comparison with *V. dahlia*.

Conclusion: The data presented in this study shows that this indigenous Iranian mushroom strain may be considered as a potential to be investigated for its biological activities, including antimicrobial effects against plant and human pathogens.

Keywords: Iranian wild-growing *Lentinus tigrinus*, medicinal mushroom, antifungal activity, soluble protein

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Topic: Food Microbiology

P127: Frequency of *Salmonella* spp. from raw milk and some traditional dairy products supplied in Yazd in 2018

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Introduction and Objectives: Milk and its products are highly nutritious and easily exposed to contamination, which is the source of some of the most important nutritional problems. One of the common diseases between human and livestock through milk and dairy products which can cause food poisoning in humans is salmonellosis. *Salmonella* bacteria is very important in terms of nutritional health. The main purpose of this study was to Salmonella frequency from raw milk and traditional dairy products in Yazd province and comparing it with the National Iranian Standard (#4413) in 2018.

Materials and methods: In this research, 125 samples of raw milk, 75 samples of traditional cheese, 75 samples of traditional Creamy and 75 traditional ice cream samples were randomly collected from 5 regions (north, south, center, east and west). All samples were tested for *salmonella* by standard method. Then, the results were compared with the national Iranian standard and were analyzed with using SPSS version 18 and chi-square and Fisher test.

Results: Based on microbiological tests, out of 350 samples, 23 samples (6%) were positive for *Salmonella* isolates. The prevalence of *Salmonella* was 1.9%, 1.1%, 1.6% and 1.6% respectively in, raw milk, cheese, creamy and traditional ice cream. In this study, the most Frequently was detected of *salmonella* in raw milk.

Conclusion: for the first time in the city of Yazd, the present study examined the prevalence of *Salmonella* isolates in raw milk and traditional dairy products**.** Due to 6% contamination of milk and dairy products with *Salmonella* bacteria in the city, health measures are needed to reduce the contamination of these products.

Keywords: *Salmonella.*, Raw milk, Traditional dairy products**.**

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Topic: Food Microbiology

P142: Isolation and identification of a potent histamine oxidase producing bacterium from nettle soils

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Introduction and Objectives: Histamine poisoning, an allergy like food poisoning, caused by ingesting scombroid fishes which have high amount of histidine in their muscle. Histamine is formed by decarboxylation of histidine through the activity of microorganisms. Though it is not present in fresh fish, Histamine in fish is a good indicator of hygienic food quality. Current methods used for histamine determination (HPLC) are complicated, time-consuming and expensive. Therefore, it is essential to implement rapid methods for analysis of fishery products. The main purpose of this study was the isolation of bacteria producing histamine oxidizing enzyme for development an enzymatic method for histamine determination.

Materials and Methods: For isolation bacteria with potent histamine oxidase, several soil samples collected at different places in Iran. We used histamine as main nitrogen and carbon source in culture media and isolation several histamine degrading bacteria. Then we designed primer sets for histamine oxidase gene and amplified by PCR. The histamine oxidase-producing bacteria were chosen from histamine-utilizing strains.

Results: From the samples of soil, about 100 histamine utilizing bacteria were isolated that 4-strain had histamine oxidase gene revealed by PCR amplification and gene sequencing. One strain, N1A3101 was selected and used for further experiments. Strain N1A3101 was found to be similar to *Glutamicibacter endophyticus* (homology: 98.5%, based on 16SrDNA). Crude enzyme showed potent activity toward histamine (208 unit/ml), whereas it was inactive toward other diamines and polyamines.

Conclusion: We have found a potent histamine oxidase from an indigenous bacterium that acts more specifically on histamine. By optimization of the cultivation condition and purification, the N1A3101 enzyme can be used for development of a test kit for histamine determination in fishery products.

Keywords: Histamine poisoning, Enzymatic methods, *Glutamicibacter endophyticus.*

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Topic: Food Microbiology

P23: The study of Prevalence of Parasitic Contamination of fresh vegetables in Tehran, Iran

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Introduction and Objectives: Parasitic diseases have created lots of health-economic problems in developing and developed countries. Consuming raw vegetable contaminated with parasites is regarded as one of the most prevalent ways of transmitting diseases. Regarding the importance of healthy vegetable consumption, awareness of vegetable status prevents from the infection. Therefore, the present study was investigated to determine the level of parasitic contamination of vegetables consumed in Tehran.

Materials and Methods: The present descriptive and cross-sectional study was conducted on the vegetable samples spread in Tehran from October 2017 to September 2018. The samples included 240 vegetables selected from10 types of vegetable including leek, basil, mint, spring onion, radish, parsley, lettuce, cress, tarragon, and coriander. Each sample was examined after passing the washing and centrifuging. Parasitic agents such as unicellular, egg and larva of the worms were studied. SPSS software was used to analyze the data.

Results: Based on the findings, parasitic infection was observed in 62 of the samples (25.8%). Coriander and lettuce had the highest and lowest rate of contamination, respectively. The results indicated the Rhabditoid larva 12.5% (15 cases) and Physaloptera egg 1.6% (2 cases) as the most and the least observed parasites. Other parasites such as Entamoeba, Giardia, Blastocystis, Hymenolepis, Ascaris, and the egg and larva of the Hookworms were observed, as well.

Conclusion: Despite the relative improvement of social, agricultural, economic and health conditions in Tehran, the prevalence of the parasitic infections is still observed. Factors such casusing modern waste collection methods, improving the urban sewage systems, preventing domestic animal traffic in the pasture, and promoting the relative knowledge of different classes of people could reduce the prevalence of such diseases. Conflict of interest: The authors declare no conflict of interest.

Keywords: Parasitic infection, Consumed vegetables, Tehran

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Topic: Food Microbiology

P67: Antibiotic Resistance Among *Staphylococcus aureus* and *Escherichia coli* Isolated from Traditional and Industrial Food Samples

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Introduction and Objectives: Foodborne diseases are one of the serious problems in the world. Every year, more than 100 million people are affected by foodborne and waterborne diseases particularly immunocompromised diseases. The aim of the present study was to evaluate bacterial load and antibiotic resistance pattern in bacterial isolates from food samples of meat, dairy, and pastry products from west of Tehran, Iran, during April 2007 to March 2008.

Materials and Methods: A total of 1625 different food samples including dairy products, meat and pastries were collected randomly from different parts of the west of Tehran. All samples were kept at 4°C. The samples were first cultured according to the standard bacteriological methods and then *Staphylococcus aureus* and *Escherichia coli* isolates were identified using standard bacteriological tests. Antimicrobial susceptibility test was performed by disk diffusion method according to Clinical & Laboratory Standards Institute (CLSI) guidelines.

Results: During 2007 and 2008, 2.8% and 3% of the food samples were contaminated with *S. aureus.* Similarly, 3.5% and 6.4% of the food samples were contaminated with *E. coli. E. coli* isolates were highly resistant to amikacin and cephotaxime and this resistance was increased in 2008. Similarly, *S. aureus* isolates were resistant to ciprofloxacin, cephotaxime, gentamicin, and tetracyclin. There was no significant difference during 2007-2008.

Conclusion: The rate of contamination during 2007 was 2.8% and during 2008 was 3% for *S. aureus.* This strain was isolated from the food samples. Further studies should be done to determine the changes of bacterial resistance pattern for various food samples. Thus, the baseline for comparison with future prospective studies should be established, enabling the determination of trends over time.

Keywords: Antibiotic Resistance, *Staphylococcus aureus*, *Escherichia coli*, Food

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Topic: Food Microbiology

P88: Antibiotic resistance pattern of methicillin-resistant *Staphylococcus aureus* strains isolated from poultry meat

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Introduction and Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are considered a major cause of foodborne diseases. Poultry meat play an important role in transmission of MRSA strains to human population. The present research was done to study the antibiotic resistance pattern of MRSA strains isolated from raw poultry meat samples.

Materials and Methods: Two-hundred and sixty poultry meat samples were cultured and MRSA strains were identified using cefoxitin (30 µg) and oxacillin (1 µg) susceptibility test. Antibiotic resistance pattern of MRSA strains were studied using simple disk diffusion method.

Results: Twenty-two out of 240 (9.16%) raw poultry meat samples were positive for *S. aureus* strains. Twelve out of 22 *S. aureus* strains (54.54%) were determined as MRSA strains. MRSA strains harbored the highest prevalence of resistance against penicillin (100%), ceftaroline (100%), tetracycline (100%), gentamicin (83.33%) and trimethoprim-sulfamethoxazole (75%) antibiotic agents. MRSA strains harbored the lowest prevalence of resistance against chloramphenicol (25%), rifampin (25%), levofloxacin (33.33%), ciprofloxacin (33.33%) and clindamycin (41.66%) antibiotic agents.

Conclusion: High prevalence of antibiotic resistance in the MRSA strains pose an important public health threat regarding the role of poultry meat in transmission of antibiotic-resistant MRSA strains to human. Chicken, turkey, quail and ostrich meat may be reservoir of MRSA strains.

Keywords: Methicillin-resistant *Staphylococcus aureus*, Antibiotic resistance pattern, Raw poultry meat.

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Topic: Food Microbiology

P192: Anti listeria effects of *Echinophora platyloba* extracts in broth medium and milk

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Introduction and Objectives: *Listeria monocytogenes* is one of the main foodborne bacteria that most of the time transmitted via dairy products. Recently, consumers demand to replace chemical preservatives with natural ones. The aim of this study was to investigate the antibacterial effects of *Echinophora platyloba* extracts on *Listeria monocytogenes* in broth medium and milk.

Materials and Methods: The standard method of microdilution was used to determine the minimum inhibitory concentration and the minimum bactericidal concentration of aqueous and ethanol extracts of *Echinophora platyloba* on tested bacteria.

Results: The results showed that the Minimum inhibitory concentrations of aqueous and ethanol extracts, were 50, 70 mg/ml respectively and the minimum bactericidal concentrations for these extracts, were 70, 100 mg/ml respectively.

Conclusions: Based on the results, both aqueous and ethanol extracts showed acceptable anti listeria effects at 4ºC and 25ºC in milk compare to the control group ($P< 0.05$). At the same concentrations, the aqueous extract showed a stronger effect on the *Listeria monocytogenes* than ethanol extract. Also, the results revealed that the antimicrobial effect of the aqueous extract was greater at 4ºC than 25ºC in the same concentration ($P< 0.05$).

Keywords: *Listeria monocytogenes*, extract, *Echinophora platyloba*, milk.

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Topic: Food Microbiology

P284: Study of the survival of Bacillus cereus bacteria in sheep using electron beam

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Introduction and Objectives: Mutton is one of the main sources of meat in the basket of Iranian households. Bacillus cereus is one of the carcinogenic microorganisms transmitted through meat and its products and is one of the food poisoning agents. The present study was conducted to study the survival of *Bacillus cereus* bacteria in sheep meat by electron beam strain.

Materials and Methods: In this study, mutton samples were sterilized to prevent the bacterial contamination of 20 kg, and then Bacillus cereus ATCC11778 was inoculated into specimens and infected meat samples.

Infected specimens were irradiated with rays of 1, 3 and 5 kg. The control and treatment groups were evaluated on days 1, 3, 5, 7 according to microbial standard number 2324.

Results: The results of this study showed that the average number of Bacillus cereus bacteria in mutton samples between different doses at 3 and 5 days was statistically significant. There was also a significant difference between Bacillus cereus numbers between 3 and 5 with control group ($p < 0.05$). The results showed that the highest inhibitory effect was related to a dose of 5 Kg, so that a dose of 5 Kg can reduce the visible bacteria on days 3 and 5.

Conclusion: Using electron beam technology, an effective step can be taken to remove Bacillus cereus bacteria from lamb samples and ensure microbial safety and consumer hygiene to this Bacterial contamination.

Keywords: Mutton, Bacillus cereus, electron beam, foodborne diseases

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Topic: Food Microbiology

P137: An investigation of the quality of ovine raw milk in East of Iran

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Introduction and Objectives: Dairy product quality assurance begins at the farm and ends in the hands of the consumer. In this regard, raw milk quality is essential. Sheep milk has a higher nutritional value and higher concentrations of proteins, fats, minerals, and vitamins, as compared to the milks of other domestic species. This study aimed to determine the hygienic status of ovine raw milk by evaluation of standard plate count, and detection of *Escherichia coli* and *Pseudomonas aeruginosa*.

Materials and Methods: A total number of 90 raw ovine milk were obtained from individual sheep after cleaning of the teat in East of Iran. 1 ml of milk was pour plated in Plate Count agar medium and incubated at 37 °C for 48 h. Detection of *E. coli* and *P. aeruginosa* were performed by culture of 0.1 ml of milk on the EMB and Cetrimide agar, respectively, the plates were incubated at 37 °C and 42 °C for 48 h, respectively.

Results: The mean of SPC was about 2×10^6. 43 out of 90 samples (47.78%) had a SPC of lower than 10000 and 29 out of 90 samples had a SPC of lower than 100000 and higher than 10000. 3 samples were contaminated with *E. coli* and none of the samples were contained *P. aeruginosa*.

Conclusion: The result of this study showed that ovine milk has a good hygienic status as about half of the samples had a good quality regarding SPC. Moreover, all of the samples were free of *P. aeruginosa*.

Keywords: raw milk, Ovine, Standard Plate Count, *Escherichia coli, Pseudomonas aeruginosa*.

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Topic: Food Microbiology

P144: Incidence of *Listeria* species in bulk tank milk and meat from sheep's in Kerman, Iran

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Introduction and objective: *Listeria* Spp, especially *Listeria.monocytogenes* are opportunistic pathogens affecting all individuals, although immunocompromised, elderly and pregnant women are especially at risk. These bacteria are among important food born infections around the world. This study was performed to determine the prevalence of *Listeria* Spp from milk and meat samples of sheep in Kerman.

Materials and Methods: Totally 300 sample of milk from sheep flocks (n=150) and meat (150 sample) from butcher shops were collected. The *Listeria* Spp were identified by standard biochemical tests and Microgen™ *Listeria*-ID System. All the culture positive isolates were confirmed by PCR methods using primers for identification of *hly*, *prf* and *iap* genes.

Results: A total of 18 isolates were identified as *Listeria* Spp, comprising 13 isolates from meat and 5 isolate from milk. The most frequent species were *L.ivanovii* (n=9), followed by *L.gravi* (n=7) and *L.welshimer*i (n=2). *L.monocytogenes* was not isolated in this study.

Conclusion: the prevalence of *Listeria* Spp in sheep samples in this study was low, with no incidence of the common pathogen of the group, *L.monocytogenes.* Since the foods can be contaminated during processing and the importance of this food born pathogen, study on other type of food products from animal origins such as cows and vegetables is necessary in order to control this opportunistic pathogen in the food chain.

Keywords: *Listeria.monocytogenes, Listeria.ivanovii,* sheep milk, sheep meat

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Topic: Food Microbiology

P147: Proteolytic and Antimicrobial Activity of Indigenous Probiotic Lactic Acid Bacteria Isolated from Traditional Cheese Samples

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Introduction and Objectives: Lactic acid bacteria (LAB) are extensively used in dairy products where they bestow desirable effects on the taste, odor, texture and flavor of the product. The proteolytic activity of the LAB is known to be responsible for the release and generation of biological peptides, including hypotensive peptides which inhibit angiotensin I-converting enzyme (ACE), opioid agonist and antagonist peptides, and mineral binding, immunomodulatory, antibacterial, and antithrombotic peptides The aim of the present study was to screen the LAB isolated from cheese sample for their probiotic properties. The isolates were also screened for their proteolytic and antimicrobial activity.

Materials and Methods: Twenty-one LAB isolated from 9 cheese samples were identified to genus and species level by biochemical and molecular methods. The isolates were tested for their pH, and bile tolerance and antibiotic sensitivity profile. The antimicrobial potential of the isolates against a number of gram positive and gram-negative pathogens was determined by agar well diffusion and Agar spot assay. The isolates were screened for their ability to produce protease on skim milk agar plate.

Results: *L.casei, L.acidophilus and L.plantarum* isolated from the cheese samples potential probiotic properties by demonstrating acid and bile tolerance. The selected isolates showed significant antibacterial activity. *S.aureus* was the most sensitive indicator strain while none of the isolates inhibited the growth of *Shigella dysenteriae*. Significant proteolytic activity was demonstrated by 12 of the isolates, while L.plantarum showed the strongest proteolytic activity on the tested media.

Conclusion: The results of this study concludes that dairy LAB isolates have probiotic potential and are significant protease producers with strong antimicrobial actions. Therefore, these isolates might have the potential to be used in therapeutics and as food bio-preservatives.

Keywords: lactic acid bacteria, probiotic, Proteolytic activity, Antimicrobial Activity, cheese.

Topic: Food Microbiology

P128: Lactic Fermentation of camel milk by Exopolysaccharide producing bacteria and Study antioxidant properties

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Introduction and Objective: Due to the problems concerned in urban life and the consequences of noncommunicable diseases such as diabetes, cardiovascular disease and cancer, it is necessary to change the eating habits as a way to help and slow down the diseases. Camel milk is a healthy valuable source that can be presented on the consumers table and can be fermented to extend its shelf life. This research has studied the thermal process of camel milk with producing Exololysaccharide bacteria which in addition exhibits antioxidant activity and is effective in improvement of the sensory characteristics of fermented process.

Materials and Methods: Camel milk has been heated at 85°C for 15 minutes and then is impregnated with Exopolysaccharide bacteria including lactobacillus casei TD4, lactobacillus casei T20, and lactobacillus plantarum. After incubation periods of first, seventh, fourteenth and twenty first days the physico-chemical characteristics particularly the antioxidant and sensory properties were evaluated.

Results: The findings determined that the thermal process apart from the safety increased the shelf life of the product. Strains producing Exopolysaccharide beside the products as the result of fermentation like lactic acid give the product more acceptability from consumer viewpoint and also have significant role in increasing the antioxidant activity. These products at refrigerator temperature after 14 days have the highest antioxidant property.

Conclusion: This research work indicated that the thermal process did not exhibit significant effect on the chemical composition and antioxidant activity of the product. In fact, fermentation by suitable lactic bacteria makes it valuable due to the increase in the shelf life.

Keyword: antioxidant ،Camel Milk،Exopolysaccharide،Fermentation ،Lactic Acid Bacteria

Topic: Fungal Infections

P169: Assessment of the *In vitro* antifungal efficacy of soil Streptomycetes isolates against *Fusarium oxysporum* and *Fusarium solani*

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Introduction and Objectives: Members of the genus *Fusarium* are common soil saprophytes and significant plant pathogens, which also cause a wide spectrum of diseases ranging from superficial to the life-threatening systemic infections in human as well as animals. Since the Streptomycetes are the largest taxon of antibiotic producers we aimed to assess the inhibitory effects of *Streptomyces* isolated from soil samples against *Fusarium* species.

Materials and Methods: Soil samples were collected from different regions of Kerman city in order to isolation of *Streptomyces*. Morphological as well as physiological characterization of the isolates was investigated according to the standard protocols. All isolates were evaluated in order to their antifungal activities against *Fusarium oxysporum* (PTCC 5115), and *Fusarium solani* (PTCC 5284). Afterwards, molecular identification of active *Streptomyces* isolates was conducted using 16Sr DNA gene.

Result: Out of 250 soil collected samples, fifty *Streptomyces* isolates were obtained. Among these isolates two strains showed the most antagonistic *in vitro* effect on *F. oxysporum* and *F. solani*. Besides, these two *Streptomyces* isolates were showed valuable lipase, amylase, protease, and Chitinase activities. According to the analysis of 16S rRNA gene sequences these isolates were identified as *Streptomyces rochei* (99% similarity).

Conclusion: The obtained results of this study indicated that *S. rochei* has obvious inhibitory effect against *Fusarium* species which could use as a potent source of bioactive compounds with antifungal activity.

Keywords: *Fusarium oxysporum*, *Fusarium solani*, Streptomyces, Antifungal, 16S rDNA.

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Topic: Fungal Infections

P177: Determination of Prevalence and Drug Resistance Profile of Candida Species in patients with vulvovaginal candidiasis from Zanjan, Iran

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Introduction and Objectives: Candida infection is one of the most common causes of vulvovaginitis in women. In recent decades, due to the increased use of Broad-spectrum azole agents, resistance to *Candida* species has been reported. The aim of this study was to determine species distribution and antifungal susceptibility pattern of *Candida* species causing vulvovaginal candidiasis.

Materials and Methods: In this study, 140 patients with suspected vulvovaginal candidiasis were examined. Samples were inoculated onto to Sabouraud Dextrose Agar (SDA) and CHROMagar. After identification, isolates were tested for in-vitro susceptibilities fluconazole, itraconazole and ketoconazole as described by the Clinical Laboratory Standards Institute (CLSI) M27-A3 and M27-S4 document guidelines.

Results: In total, 41 (29.3%) colonies of Candida spp. were isolated from 140 patients with Vulvovaginal Candidiasis. The most common identified species of Candida were C. glabrata (56.1%) and C. albicans (39%). The results indicate high resistance to azole drugs, as resistance to ketoconazole, itraconazole and fluconazole was found to be 65.9%, 5 and 58.5% and 26.8%, respectively.

Conclusions: The results showed that non-albicans species of Candida are more frequent than C. albicans in patients with vulvovaginal candidiasis. This result is consistent with some recent studies indicating that non-albicans species of Candida has increased and must be considered in gynecology clinics due to the reported azole resistance in these species. Also, it is important to conduct continuous epidemiological surveys to measure drug resistance profile and epidemiological changes.

Keywords: Candida, Candidiasis, Vulvovaginitis, Antifungal, Drug resistance

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P39: Investigation of UV lights on drug resistance pattern and possible gene regulation of *M.sympodialis* as a human skin microflora

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Background: Opportunistic lipophilic *Malassezia* yeasts are among Basidiomycota family of fungi and are among micro flora of human skin. In some situation, these commensal yeasts could be converted to opportunistic pathogens and can cause Pityriasis versicolor, saeboric dermatitis, folliculitis, atopic dermatitis. In this study, we investigated the possible effects of UV lights on *Malassezia sympodialis* (*M. sympodialis*) yeast. Furthermore, we study the possible changes in drug resistance pattern of this species before and after exposure as well as possible changes in gene regulations related to resistance and pathogenicity.

Methods: The *M. sympodialis* yeasts have been isolated from the body surface of healthy individuals. The obtained yeasts were grown on Dixon Agar plates and were exposed under UVA (365 nm) and UVB (302 nm) sources separately, for 5, 20 and 30 minutes, then incubated at 32°C for 5 days. *In vitro* activity of KTC against *M. sympodialis* and a quantitative Real Time PCR was performed for evaluation of the antifungal susceptibility testing and expression level of *MSY001_1493* and *Malas13* genes after UV exposure.

Results: *M. sympodialis* yeasts showed greater resistance (MIC_{90}) when were exposed to UVA and UVB for 20 and 5 min, respectively. There were no differences between the MICs of samples which were exposed to the UV for 30 min in compares to the normal sample, without UV exposure. The expression levels of *MSY001_1493* and *Malas13* genes distinctly increased after 5 and 20 min ($P<0.05$). While the expression levels of mentioned genes haven't changed in *M. sympodialis* which were exposed to UVA and UVB for 30 min ($P<0.05$).

Conclusions: The obtained results of the present study show the importance of proper duration of UV radiation on reduce drug susceptibility and progress towards pathogenicity of *M. sympodialis*. In addition, UV radiation could affect on related gene expression which could affect on the intensity of pathogenicity of this *Malassezia* species. **Keywords:** Ultraviolet Radiation; Antifungal Susceptibility Test*; Malassezia sympodialis.*

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P184: Erythrasma in Diabetic Patients

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I**ntroduction and objectives:** Erythrasma is a chronic superficial infection of *Corynebacterium minutissimum* which occurs as normal skin flora in the general population. Diabetes mellitus (DM) is the most common endocrine metabolic disorder. High levels of salivary glucose, low secretion of saliva, impaired chemotaxis, and defect of phagocytosis are reasons for making diabetic patients more susceptible to cutaneous infections. Our aim is to study the prevalence of erythrasma in diabetic patients which admitted to educational hospitals.

Materials & Methods: The study group consisted of 100 adults diabetic (non-insulin dependent) randomly with signs and symptoms of superficial infection in patients admitted to educational hospitals, Tehran, Iran.The control group consisted of 100 non-diabetic, non-immunocompromised patients. All patients had a thorough skin examination like wood's light, skin scrapings for potassium hydroxide (KOH) for microscopic examination and cultures of fungi. Demographic and clinical data were collected including age, sex, duration of diabetes mellitus, fasting blood sugar and percent of HbAIc, areas of involvement, clinical manifestation and body mass index (BMI).

Results: 100 adult diabetic and 100 non-diabetic patients were chosen. The overall prevalence of erythrasma was higher in diabetic than non-diabetic patients. The mean age of the patients was 53 ±12.6 years. 68% of diabetic patients were presented erythrasma (16% male, 52% female), especially among obese diabetics (BMI≥30). In nondiabetic patients, prevalence was 12% (2%male, 10 % female). The toes, below of breast and crural folds were the most commonly affected sites. Mean of duration of diabetes was 10 ± 3.4 (years). Mean of fasting blood sugar and HbAIc were 212 ± 63 (mg/dI), 10 ± 2(%), respectively.

Conclusion: In diabetics, erythrasma was the most commen infection. It appears that diabetic require more diagnostic, therapeutic and preventive care in terms of mycotic infection than has been previously thought.

Keywords: Erythrasma, Mycotic infection, Diabetes mellitus

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Topic: Fungal Infections

P183: Catheterized patients: Profile of *Candida* isolated from urinary tracts and pattern of antifungal susceptibility

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Introduction and objectives: *Candida* Urinary Tract Infections (UTIs) are an increasingly prevalent nosocomial problem. Candiduria, the presence of *Candida* species in the urine, is in most part associated with the use of urinary catheters. Our aim is to evaluate candiduria associated with in dwelling urinary catheters, to characterize different *Candida* speices and their susceptibility pattern to antifungal agents in patients admitted to educational hospitals.

Materials & Methods: Urine specimens from 230 catheterized patients were inoculated on Sabauraud Dextrose Agar, the species identification of *Candida* isolates was performed by CHROM agar *candida* medium and PCR-RFLP, then antifungal susceptibility testing was done according to CLSI (M27-S3) protocol against Amphotericin B, Fluconazole and, Itraconazole.

Results: *Candida* species was isolated in 32 (73.6%) specimens. *Candida albicans* was commonly isolated in 59.3% (n=19) followed by *C. glabrat*a, *C. tropicalis*, *C. guillermondi*, *C. krusei* and *C. parapsilosis*. All *C. krusei* and *C. glabrata* were consistently resistant to fluconazole. 12 of 19 *C. albicans* were also resistant to fluconazole. Emergence of resistance was also seen to Itraconazole in 14 of 32 isolates. Resistance to Amphotericin B was seen in 28 isolates. That was exhibited by 14 *C. albicans*, 8 *C. glabrata*, 4 *C. parapsilosis* and 2 *C. krusei* species.

Conclusion: *C. albicans* is an important nosocomial pathogen causing UTI in catheterized patients, nevertheless role of other species of *Candida* as emergent pathogens and resistance to antifungal drugs needs to be emphasized. Surveillance Urine specimens for fungi should be carried out in patients with urinary catheters. Results showed as about half of them are other than the *C. albicans* species and are likely to be more resistant, so species identification is important. In view of emergence of drug resistance amongst the *candida* species, antifungal testing can be useful to choose the antifungal agents for better therapy.

Keywords: Urinary catheters, *Candida* species, Antifungal susceptibility, PCR-RFLP

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Topic: Fungal Infections

P186: Identification of Candida species isolated from patients with onychomycosis by PCR-RFLP method and determination of their susceptibility to antifungal drugs in Mashhad

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Introduction and objective: Onychomycosis are the most common nail disorder and affects almost 5% of the world population. Yeasts especially candida species (Candida SP) are very important as a responsible pathogen for nail infections. Due to the resistance of some candida SP to antifungal drugs and because of the fact that the outbreak of this disease and its causes are different in various regions, the present study was designed to identify candida species in patients referred to the diagnostic laboratories of Mashhad University Hospitals and to determine their susceptibility to antifungal drugs.

Materials & Methods: In this study, a total of 210 patients clinically suspected to have onychomycosis were examined by both direct examination and culture. Clinical materials were collected from the abnormal nails. After direct examination using KOH 20%, the nail samples were inoculated on sabouraud dextrose agar containing chloramphenicol with and without cycloheximide. The cultures incubated in 25^0c and 37^0c aerobically for 4 weeks and checked twice weekly for any growth. Initial identification was done based on conventional methods and by using Candida chrome agar. After DNA extraction, PCR-RFLP method was done for identification of candida species.

Results: Of the 210 patients examined, 51(24.2%) were mycologically proven cases of candidal onychomycosis. Female with 37 (72.5%) affected more frequently than male 14 (27.5%) and in both sexes, those who were 30-39 years old, more infected. The age range of the patients studied was2-78 years old. Fingernails with 38 (74.5%) affected more frequently than toenails 11 (21.6%); 2 patients had both infections on finger and toe nails. The most frequent detected candida species was *Candida parapsilosis* (n=23), followed by *C.albicans* (n=21), *C.tropicalis* (n=2), *C.glabrata* (n=2), *C.guillermondii* (n=2) and *C.famata* (n=1). MIC90 clotrimazole, nystatin, itraconazole and fluconazole was 2 µg / ml, 1 µg / ml, 0.016 µg / ml and 0. 5 µg / ml for *Candida parapsilosis* respectively. The highest resistance was observed in non-albicans species to fluconazole and clotrimazole, so that resistance to these drugs was 50% in *C.parapsilosis*. The resistance to clotrimazole was also 50% in *C.glabrata*.

Conclusion: *C. parapsilosis* and *C. albicans* were the most common species involved Onychomychosis patients in Mashhad. The most resistant species to the antifungal drugs were *C.tropicalis* and *C.glabrata*.

Keywords: Candida, PCR-RFLP, Onychomycosis, antifungal drug

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Topic: Fungal Infections

P176: Genotype analysis of Candida albicans isolates using ALT repeat sequences obtained from patients with vulvovaginal candidiasis from Zanjan, Iran

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Introduction and Objectives: Candida infection is one of the most common causes of vulvovaginitis in women. molecular characterization of the isolates is essential to understand the epidemiology of the infections and for tailoring prevention strategies.
Materials and Methods: In this study, 140 patients with suspected vulvovaginal candidiasis were examined. Samples were inoculated onto to Sabouraud Dextrose Agar (SDA) and CHROMagar. After identification, we reported a PCR system targeting 25S rDNA and ALT repeat sequences in the repetitive sequence (RPS) for genotyping of C. albicans. And data has been analyzed with SPSS.
Results: In total, 41 (29.3%) colonies of Candida spp. were isolated from 140 patients with Vulvovaginal Candidiasis. The most common identified species of Candida were C. glabrata (56.1%) and C. albicans (39%).Genotype A3 C. albicans with 5 isolates (31.25%) constituted the majority of the isolates, followed by B2/3 with 4 isolates (25%) and A3/4 ، C3/4 ,و B3/4 with 2 isolates (12.5%) and C2/3 C3 with 1 isolate (6.25%), respectively
Conclusions: The results showed that non-albicans species of Candida are more frequent than C. albicans in patients with vulvovaginal candidiasis. The present results indicate that PCR amplifications targeting 25S rDNA and ALT repeats are useful for rapid genotyping and distinction of C. albicans in small scale and epidemiological surveys. In this study, like other studies Genotype A3 C. albicans have been the major type. but reports about genes A, B ,C are different .The reason for this variation can be because of the genomic variability within the Candida species
Keywords: Genotyping, *Candida albicans*, 25S rDNA, ALT repeat, RPS

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Topic: Fungal Infections

P185: Study the Fungal Infections in papsmear samples of women of Ahvaz

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Introduction and Objectives: Pap smear is a screening test for diagnosing cervical cancer in females and also many microbial infections can be detectet by this test. The aim of this study was to the investigation of the Fungal Infections in pap smear samples of women of Ahvaz, Iran.

Materials and Methods: In this research study, totally 200 Pap smear samples were collected from pathology laboratories in Ahvaz, Iran. The samples were stained using the conventional Papanicolaou technique, and hematoxylin staining, then evaluated by microscopy. For positive samples, Corn Meal Agar culture was used for chlamydospore production by *Candida albicans*, then samples were cultured on CHROM agar Candida medium. After incubation, growth coloration of Candida isolates compared with those of standard patterns.

Result: The results indicated that 4.5% samples had *Candida* vulvovaginitis, 88% cases were *Candida albicans* and 11% case was *C. tropicalis*. 9 strains were investigated for the presence of the *Candida* colonies, According to the *Corn Meal Agar culture* and CHROM agar *Candida* medium results, the formation of green or blue-green in 8 strains indicated the species *Candida albicans*, and the *FMC9* strain was blue with a dark grey with blue metallic violet margins was indicated *Candida tropicalis*.

Conclusion: Pap smear is an easy and economical screening method to detect Fungal Infections of the cervix. Corn Meal Agar and Chrom Agar can be combined for identification and accurate Separation between species of *Candida*. According to microscopic evaluations, the fungal infection causes changes in normality and the adhesion of cells.

Keywords: *Candida albicans*, papsmear**,** Fungal Infections, *Corn Meal Agar*

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Topic: Fungal Infections

P196: Antifungal effects of different fractions of standardized extract of *Myrtus communis L.* against nystatin-resistant and nystatin-susceptible *Candida albicans*

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Introduction and Objectives: The importance of *Candida albicans* as a model system for studying pathogenic fungi has only multiplied. The phenomenon of multidrug resistance in *C. albicans* and the related pathogenic species has taken toll on the clinicians because the management of fungal diseases has become extremely difficult. In order to explore alternative drug targets and develop modern age drugs, thorough understanding of the pathogen's biology has become vital. *Myrtus communis L.* (Family _ *Myrtaceae*) is traditionally used as an antiseptic, antifungal drug.

Materials and Methods: *Myrtus communis L.* was collected from Kerman province in June 2018. Total extract of the plant leaves was prepared by sonication method and petroleum ether, chloroform, ethyl acetate and methanol were used for fractionation. The standard strain of *candida albicans* (ATCC 10231) was purchased and nystatin-resistant *Candida albicans* samples also collected from patients referred to educational hospitals in Kerman. The minimum inhibitory concentration (MIC) of myrtle oil was determined using the M27-A3 method. In addition, nystatin was applied as positive control.

Results: From the five fractions of *Myryus communis L*, choloroform fraction had most effective antifungal against nystatine-susceptible and nystatine-resistant *Candida albicans*.MIC of chloroform fraction was 62.5. MIC of nystatin drug for the resistant and susceptible *Candida albicans* were 80,40 respectively.

Conclusion: It can be concluded that the active compounds of the plant belong to a specific group of metabolites,which according to the type of solvent, probably have non-polar nature.

Keywords: *C. albicans, Nystatin, Myrtus communis L.*

Topic: Fungal Infections

P168: Molecular identification of *Candida* isolates obtained from HIV/AIDS patients by Real time PCR- High Resolution Melting Analysis

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Introduction and Objectives: *Candida* species are the major important cause of opportunistic mycoses. Due to different antimycotic resistance patterns in various *Candida* spp, reliable and fast techniques for their identification and quantification are needed. High-resolution melting real-time PCR (PCR-HRM) is a new strategy for rapid diagnosis and characterization of Fungal Infections. In this study, we assayed Real-time PCR and HRM for the rapid identification of 46 Candida isolates obtained from oral cavity of HIV/AIDS patients.
Materials and Methods: In this study, 46 *Candida* isolates obtained from oral cavity of HIV/AIDS patients were identified by Real-Time PCR and high-resolution melting curve analysis.
Result: Considering reference and clinical isolates, 46 *Candida* species distinguished by Real-time PCR and HRM. Identified Eight species of *Candida* spp included 14 *C. albicans* (30.43%), 12 *C. kefyr* (26.09%), 11 *C. glabrata* (23.91%), 2 *C. krusei* (4.35%), 2 *C. tropicalis* (4.35%), 2 *C. lusitaniae* (4.35%), 2 *C. guilliermondii* (4.35%) and 1 *C. parapsilosis* (2.17%).
Conclusion: HRM is a reliable and simple approach for identifying important *Candida* species. It should be considered as a diagnostic method.
Keywords: *Candida* spp, High resolution melting, HIV/ADIS

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Topic: Fungal Infections

P167: Detection of *Candida* species isolated from oral cavity of healthy persons by Polymerase Chain Reaction

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Introduction and Objectives: *Candida* species are a part of normal human microbial flora. They can cause dangerous infections in immunocompromised persons such as recipients of organ or tissue transplants. The most commonly *Candida* species are C. *albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, *C. lusitaniae*, and C. *krusei.* Rapid detection of *Candida* species for prescribing appropriate antifungal drugs are necessary. The aim of this study was to detect of *Candida* species isolated from oral cavity of healthy persons by Polymerase Chain Reaction and ITS86-ITS4 primers.

Materials and Methods: In this study, 72 different isolates of *Candida* obtained from oral cavity of healthy persons detected with PCR and by ITS86-ITS4 primers.

Result: The primers used successfully amplified DNA from all tested *Candida* isolates. After amplification, products of 198 to 372 bp were obtained. Tested *Candida* included 22 *C. albicans* (30.56%) (282 bp), 7 *C. parapsilosis* (9.72%) (250 bp), 9 *C. glabrata* (12.5%) (360 bp), 17 *C. krusei* (23.61%) (294 bp), 9 *C. tropicalis* (12.5%) (270 bp), 6 *C. lusitaniae* (8.33%) (198 bp), 1 *C. guilliermondii* (1.39%) (320bp), 1 *C. Kefyr* (1.39%) (372bp).

Conclusion: PCR by ITS86-ITS4 primers is a sensitive technique for the rapid detection of *Candida* species isolated from oral cavity of healthy persons.

Keywords: *Candida* spp, PCR, Molecular diagnosis, ITS86-ITS4

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Topic: Fungal Infections

P181: Investigation about antimicrobial pattern that isolated from clients of health center and lab.in Kashmar-Khorasane Razavi, Iran. Spring 1398

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Introduction &objectives: One of the most infection is urinary tract infection (UTI) that caused by bacteria which is the most important reason is Entrobacteriacea special *E. coli* and *Kelebsila*. Antimicrobial pattern is one of the most important challenge for WHO & specially in developed countries.The purpose of this study is surviving from UTI and antimicrobial pattern that caused by bacteria, in health center and lab, in Kashmar, during 2months of spring.

Material &Method: This investigation was descriptive and cross-sectional study about clients of health center in city, village and lab, in Kashmar, during 2 months of spring. Urine sample (midstream) was cultured on selective medium and bacteria that grew up, separated with differential culture medium. Antibiogram test done by disk diffusion, Macfarland standard and CLSI 2018 and result were analyzed by spss16.

Result: Clients were 460person which 252 number were female (76.31%) and 109 number were male(23.69%) . Of the total of urine sample 126 were positive. *E. coli* by prevalence 63 was the most common and sensitive to *Amikacin, Piperacillin- Tazobaktam, Ciprofloxacin* & most resistant to *cefalotin* and *Ceftriaxone*.

Conclusion: As a result, most sufferers were Pediatrics (53case) and 20-35 years old women (50case). For prevention of UTI, detecting hypertension and nephritis is necessary.

Keywords: urinary tract infection, pediatrics, Entrobateriacea, disk diffusion, Antimicrobial test, Kashmar, Khorasane Razavi.

Topic: Fungal Infections

P187: Evaluation and monitoring of the amount of *malassezia Spp*. isolated from the students of an Institute for Medical Science in Mashhad, 1398

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Introduction &objectives: The opportunistic yeasts of the *Malassezia genus* belong to the *Basidimicheta* branch and the *Cryptococcus* family, which naturally inhabit human beings and warm-blooded animals. The proliferation and increase of their populations on the scalp, resulting in dandruff and crust, and causing itching, leads to folliculitis and gradual loss of head hair resulting in baldness. This study was conducted to evaluate the frequency of *Malassezia* in students of an Institute in Mashhad, 1398.

Materials and Methods: A cross-sectional study was conducted during the 2nd semester. The population studied was 206 students from a medical education center. The scalpel was sampled using scrubbing (scraping) from dandruff and fat and skin scrubs. After fixation on lam and staining, the samples were examined by optical microscopy at the center of mycology laboratory.

Results: The population of the study consisted of 206 students, 48 male students (23.31%) and 158 female students (76.69%), ages 20 to 26 years old, mean age 22. In this study, 81 cases (39.32%) of the shells were samples that had a low Malassezia yeast (*Pityrosporum oval*) (+1). Pea samples of 40 (19.41%) were samples that contained a relatively small amount of this yeast (2+). The shell skin samples of 32 (15.53%) were samples that had an average of this yeast (3+). Head skin samples 53 (25.72%) were samples that had high levels of this yeast (4+).

Conclusion: Regarding the high relative contamination rate, using simple, inexpensive and available methods, during practical training, students determine the amount of fungi and students who need to be treated and trained to be referred to a specialist physician and treated, cared and advised. And will prevent hair loss and side effects.

Keywords: *Pityrosporum Ovale*, *Malassezia ovalis*, Dandruff, Institute for Medical Science, Mashhad

Topic: Fungal Infections

P188: The Potency of Luliconazole against Clinical and Environmental Isolates of *Aspergillus flavus*

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Introduction and Objectives: Luliconazole, a new imidazole antifungal, is currently confirmed for the topical treatment of some superficial and cutaneous mycosis. The *in vitro* activities of luliconazole has also shown against some molds and yeasts. *Aspergillus flavus* is an important opportunistic fungus cause of *Aspergillosis*. Amphotericin B was as Gold standard antifungal agent for invasive fungal infection for more than 5 decades. However, it replaced by some azole antifungals. The aim of the present study was to evaluate the effects of luliconazole in comparison with amphotericin B, voriconazole, and caspofungin on clinical and environmental isolates of *A. flavus.*

Materials and Methods: Thirty-eight isolates of *A. flavus* (20 clinical and 18 environmental) were collected from Ahvaz, Iran. Based on PCR-sequencing of β-tubulin ribosomal DNA gene all isolates were detected as *A. flavus*. All isolates tested against luliconazole, voriconazole, amphotericin B and caspofungin using by CLSI M38-A2 guidelines. Minimum inhibitory concentration (MIC), MIC_{50}, MIC_{90} and MIC_{GM} were calculated for both environmental and clinical isolates.

Results: Luliconazole with MIC_{GM} 0.0024 showed potent activity against *A. flavus* isolates and followed by voriconazole inhibited 95% of isolates at ≤1µg/ml. 25% and 11.1% of the clinical and environmental isolates of *A. flavus*, respectively, were displayed resistant to caspofungin. All isolates were found to be resistant to amphotericin B. Also, we did not see any statistically significant difference between clinical and environmental strain and resistant to both voriconazole ($p > 0.11$) and caspofungin ($p > 0.13$).

Conclusions: Our results confirm that *in vitro* activities luliconazole is useful on A. flavus vs another antifungal studied.

Keywords: Luliconazole, Amphotericin B, Voriconazole, Caspofungin, *Aspergillus flavus*

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Topic: Fungal Infections

P172: Isolation, Identification and antifungal profiles of *Geotrichum*, and *Galactomyces* from Dairy Products

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Introduction and Objectives: *Geotrichum* species are yeast - like microorganisms that have both free living in environment and commensalism on mucous membranes of warm-blooded intestinal tract as normal mycobiom. Several reports have shown that *Geotrichum* normally isolated from urine, sputum, feces and vaginal discharge samples. On the other hand, dairy products are the important sources of *Geotrichum* species as well as fruits and vegetables, soil, and plants materials. The most common species is *Geotrichum candidum*, followed by *G. britannicum*, *G. carabidarum*, *G. eriense*, *G. fermentans*, *G. fragrans*, etc. The aim of this study was to identify different species of *Geotrichum* from dairy products.

Materials and Methods: 153 dairy products including, cheese (74), Yogurt (43), Dough (28) and milk (8) were collected. The 10 mL of sterile distilled water was added to each tube and vortexed. Finally, 5 µL of supernatants were cultured on Sabouraud dextrose agar, supplemented with chloramphenicole. Plates were incubated at ambient temperature for 2-3 days and then suspected colonies to *Geotrichum* species were examined microscopically. DNA was extracted from pure colonies using boiling method and subjected for PCR and sequencing with ITS primers. Antifungal assay was performed according to CLSI protocol, M27 A3.

Results: Out of 153 dairy samples, 47 cases (29.7%) were positive for different species of *Geotrichum* including, 23% from cheeses, 14.3% from Dough, and 60.5% from Yogurt. However, we could not isolate any fungal organisms for milk samples. Molecular analysis revealed that 25 (53.2%) of isolates were *Geotrichum candidum* and the rest of them (22, 46.8%) were *Galactomyces candidum*. Voriconazole was the most effective drug against all isolates. The MIC ranges and MIC90 values for *G. candidum* and *Ga. Candidum* isolates were 2 - 0.032 µg/ml, 0.5 and 1- 0.032 and 1µg/ml respectively. Following it, caspofungin and itraconazole had the best antifungal activity.

Conclusions: In this study, the identity of 25 and 22 isolates assigned to the *G. candidum* and *Ga. candidum* species by morphological criteria and molecular methods. Antifungal susceptibility of the *Geotrichum* species is rarely investigated but the limited number of available papers indicate are susceptible to systemic antifungals. However, amphotericin B is used as the first-line treatment but due to high toxicity, voriconazole is an appropriate substitute.

Keywords: *Geotrichum*, *Galactomyces*, Dairy products, Antifungal

Topic: Fungal Infections

P170: Epidemiology of ocular infection due to Aspergillus species in patients referred to Farabi Hospital

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Introduction and objectives: Fungal keratitis are the inflammation of cornea due to infection which may result in reduced vision or blindness. In recent years, the incidence of fungal keratitis has increased. The etiologic agents vary as both filamentous fungi and yeasts are implicated as causative agents of fungal keratitis. The aim of this study was to determine the prevalence of Aspergillus infection causing keratomycosis and predisposing factors in Farabi Eye Hospital, Tehran, Iran.

Materials and methods: One hundred corneal scrapings from patients with corneal ulcers were taken by ophthalmologist from 2018 to 2019 were analyzed. Corneal scrapings were subjected to KOH wet mount preparation and inoculated on Sabouraud's dextrose agar (SDA) and blood agar medium. Identification to the species level was confirmed by DNA sequencing of the ITS1/ITS2 rDNA region.

Results: A total of 27 *Aspergillus* isolates comprising *A. flavus* (85.1%) and *A. niger* (14.9%) were identified. According to our result the male (51.8%) were more affected. The mean age of patients was 47 years (range: 33-76 years). The corneal trauma with vegetative matter (75%) was the most common predisposing factor.

Conclusion: Fungal keratitis due to *Aspergillus* spp. is a serious ocular illness in young and middle-aged farmers. Farm activity and related ocular trauma were the principal risk factor of fungal keratitis.

Keywords: Fungi, keratitis, *Aspergillus*

Topic: Fungal Infections

P333: Case report of a female cat infected to *Microsporum canis*

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Introduction and objectives: Dermatophytosis, usually caused by *Microsporum canis*, is one of the most important infectious skin diseases in this species. Poor hygiene is a predisposing factor. *Microsporum canis* is a transmissible fungal skin disease in various animal species and human. Disease is self-limited and not life threating but it can make some problems due to zoonotic aspect especially for pet owners. Humans may be easily infected and develop a similar skin disease

Materials and methods: A female cat were referred to the Veterinary Clinic of Shahid Bahonar Kerman University with signs of erythematous circular plaques in the skin, hyperkeratosis, scaling and hair loss in a vast area. After primary examination, biopsy samples were taken from border of expanding alopecia and fixed in neutral buffered formalin 10%. Scales and hair were cultured for dermatophyte spores and hyphae. Sabouraud dextrose agar and DTM were used to find out the dermatophyte species.

Results: Histopathologic study showed *parakeratotic hyperkeratosis* and variable acanthosis in the epidermis layer. Inflammation was observed as perifolliculitis, luminal folliculitis and furunculosis. Spores and hyphae of dermatophytes infected around the hair shafts that were evident in H&E staining and confirmed by periodic acid–Schiff (PAS). In culture medium, *Microsporum canis* was detected.

Conclusion: *Microsporum canis* is a highly transmissible pathogen in cats that suffer poor husbandry. *Microsporum canis* can be carried mechanically through cats and dogs to other species. The disease has a long-time treatment between 21 to 30 days. Pet owners are in high risk and finding out pathogen and treat it is so important.

Keywords: *Microsporum canis*, Cat, Feline, Dermatophyte, Zoonotic

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P175: Antifungal effects of *Lactobacillus acidophilus* and *Lactobacillus plantarum* against *Candida albicans*

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Introduction and Objectives: Probiotics are live microorganisms that, while consumed in sufficient quantities, can increase the microbial balance in the host's gut and be beneficial to human health. The major probiotics include *Lactobacillus* spp, *Bacillus* spp, *Bifidobacterium* spp, *Escherichia coli*, and *Saccharomyces cerevisiae*. The purpose of this study was to evaluate the antifungal properties of *L. acidophilus* and *L. plantarum* on *C . albicans* isolated from HIV/ AIDS patients.

Materials and Methods: Antifungal properties of both cells and CSFs of *L. acidophilus* and *L. plantarum* against *C. albicans* investigated by Co-aggregation, agar overlay and interference and broth microdilution.

Results: Our finding showed that both cells and CSFs of *L. acidophilus* and *L. plantarum* had antifungal effects against *C .albicans*. For *C. albicans*, the antifungal effects of *L. acidophilus* and *L. plantarum* was higher than FLC.

Conclusion: The antifungal effects of *L. acidophilus* and *L. plantarum* was higher than FLC on *C .albicans*.

Keywords: antifungal effects, probiotic, lactobacilli, *L. acidophilus, L. plantarum, C .albicans*, AIDS/ HIV patients.

Topic: Fungal Infections

P171: In vitro evaluation of antifungal effects of *Lactobacillus acidophilus* and *Lactobacillus plantarum* compared *to* fluconazole against *Candida glabrata* isolated from oral cavity of HIV/ AIDS patients

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Introduction and Objectives: Oropharyngeal Candidiasis (OPC) Known as an opportunistic Fungal Infections in immunosuppression human such as HIV/AIDS patients. *C. albicans* together other *Candida* species such as *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. kefyr*, *C. parapsilosis*, and *C. dubliniensis* have been isolated from infected areas in the mouth. The aim of this study was *in vitro* evaluation of antifungal effects of *L. acidophilus* and *L. plantarum* compared to fluconazole (FLC) against *C. glabrata* isolated from oral cavity of HIV/ AIDS patients.
Materials and Methods: Antifungal properties of both cells and CSFs of *L. acidophilus* and *L. plantarum* compared to fluconazole against *C. glabrata* investigated by Co-aggregation, agar overlay and interference and broth microdilution.
Results: Our finding showed that both cells and CSFs of *L. acidophilus* and *L. plantarum* had antifungal effects against *C. glabrata*. The most intense inhibition was observed at low concentrations of *L. acidophilus* CFS compared to *L. plantarum* CFS and FLC ($p < 0.0001$). In addition, significant difference was detected between antifungal effects of the CFS of two LABs ($p < 0.003$).
Conclusion: The antifungal effects of *L. acidophilus* and *L. plantarum* was higher than FLC on *C. glabrata.* Inhibitory effect of *L. acidophilus* CFS was more than *L. plantarum* CFS and FLC.
Keywords: Oropharyngeal Candidiasis, Antifungal effects, probiotic, lactobacilli, *L. acidophilus, L. plantarum, C. glabrata*, AIDS/ HIV patients.

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P178: A rapid method for vaginal *Candida albicans* detection with germ tube test

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Introduction and Objectives: Vaginal candidiasis is a vaginal mycosis infection caused by species of the genus *Candida*. It is one of the most common vaginal infections in women. As regards germ tube test is the confirmatory test for *Candida albicans*, therefore, speedy diagnosis and management of candidiasis are crucial for these patients. The purpose of this study was to determine the accuracy of rapid vaginal yeast detection assay compared with yeast cultures for the diagnosis of vulvovaginal candidiasis.
Materials and Methods: Examination was done at the Laboratory of Shahid Akbarabadi Hospital (Tehran, Iran). Twenty-five randomly selected yeast isolates were examined in this study. They were isolated from clinical material received by the general laboratory of the Hospital. A sample of vaginal swab was inoculated in the test tubes containing human plasma and serum. The test tubes were incubated for 2 hours at 37°C (modified Test). During the second experiment, samples of vaginal swab was cultured on chocolate agar medium for germ tube assessment, where they were incubated at 37°C for 48 hours (common Test). Then, each sample examined microscopically (under a magnification of 40x) for the presence/absence of germ tube.
Results: Of the 25-specimen isolated from the patients, 10 positive culture samples were obtained. Two patients were positive according to the conventional normal methods. Our new approach also confirmed these results.
Conclusion: Given the importance of the rapid diagnosis of vulvovaginal infections, the new method can help in the diagnosis and treatment of infection.
Keywords: Diagnosis; *Candida albicans*; Germ Tube

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Topic: Fungal Infections

P182: In vitro antifungal activity of silver nanoparticles against resistant *Candida* species

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I**ntroduction and objectives:** *Candida albicans* is a pleomorphic fungus which is a normal commensal in healthy individuals; however, as an opportunistic pathogen, *C. albicans* is the most common etiological agent of candidiasis, now the fourth most frequent infection in the US hospitals, mostly due to the increasing numbers of susceptible compromised patients that are at risk of this systemic fungal infection. Resistance of pathogenic fungi to conventionally available antifungal agents has been increasing and has become a serious problem. On the other hand, nanoscience has emerged as a powerful tool capable of developing and designing new antifungal drugs. The aim of this study was to test minimum inhibitory concentrations (MICs) of silver nanoparticle on 10 fluconazole and itraconazole resistant *C. albicans* strains. These strains were isolated from diabetic patients.

Material & Methods: The study group consisted of 10 *C. albicans* resistant strains isolated from oral cavity of adult diabetic patients. These strains were resistant to antifungal agents (5 fluconazole and 5 itraconazole resistant. The standard strain of *C. albicans* (ATCC 10231) as a control were used in this study. The standard protocol of antifungal susceptibility testing for yeast was performed by the broth micro-dilution method described in the clinical and laboratory standards institute (CLSI) guidelines, documentM27-S3.

Results: MICs of standard and resistant strains were different against silver nanoparticle. MIC was reduced by Ag-NPs in 3 and 2 fluconazole resistant strains by 16 and 4-fold, respectively. About 5 itraconazole resistant strains, MIC were reduced in 2 and 3 fluconazole resistant strains by 8 and 4-fold, respectively.

Conclusion: The results demonstrated that silver nanoparticle had higher activity than azole agents. Therefore, new antifungals are an unavoidable and critical medical need. Consideration of the possibility of oral *Candida* infections in diabetic patients is emphasized for improving patient treatment outcomes and reducing healthcare costs.

Keywords: *Candida albicans*, Silver nanoparticle**,** Antifungal drug resistant strains

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P232: Antifungal Effect of Silver Nanoparticles Synthesized by Rheum turkestanicum Extract against Candida Species

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Introduction: Fungal Infections caused by opportunistic fungi especially candida species are prevalent in human societies today. The most important challenge dealing with different species of Candida is due to their resistance to common antifungal drugs. The occurrence of fungal species resistant to antifungal drugs, convince the researchers to work on new therapeutic methods with minimal side effects for humans. In recent years; the methods of green chemistry for the synthesis of metal nanoparticles have been targeted by many investigations. The aim of the present study was to assess anti-fungal effects of silver nanoparticles (Ag-NPs) synthesized by Rheum turkestanicum extracts against some Candida species.

Materials & Methods: in the current study Rheum turkestanicum was used for synthesis of Ag-NPs and six candida species were studied. Fresh Rheum turkestanicum shoots were collected from Daregaz, Khorasan-e-Razavi province. They were dried and powdered. The shoots extract was prepared by taking of powder with distilled water. To synthesize Ag-NPs, the extract combined with silver nitrate solution. After preparation of the Ag-NPs, their antifungal effects were evaluated by broth microdilution method according to CLSI-M27A3 protocol. The fungal species were associated with different concentrations of the extract and drugs. Finally, MIC and MFC was determined.

Results: Synthesized silver nanoparticles by Rheum turkestanicum showed antifungal effects against candida species. *C.albicans* and *C.glabrata* with MIC=0.4 µg/ml were the most sensitive species to Ag-NPs. The MICs of Ag-NPs for *C.albicans*, *C.glabrata* (2 strain), *C.tropicalis*, *C.krusei* and *C.parapsilosis* were 0.4, 0.4, 0.8, 0.8 and 1.6 µg/ml respectively. *C. albicans* with MIC=2.08×10^{4}µg/ml and MFC=16.67×10^{4}µg/ml was the most sensitive species to Rheum turkestanicum extract alone. This extract did not have fungicidal effects on *C.krusei*, *C.glabrata* and *C.parapsilosis*. The MICs of fluconazole and clotrimazole for *C.albicans* was 8 µg/ml and 0.0625 µg/ml respectively.

Conclusion: Synthesized silver nanoparticles by Rheum turkestanicum were more effective on Candida species than plant extracts alone. *Candida albicans* was the most sensitive species to both extracts. Rheum turkestanicum plant is proper source for synthesized silver nanoparticles and also revealed significant anti-fungal activity against Candida species.

Keywords: Silver nanoparticles, Rheum turkestanicum, Microdilution broth, Candida

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Topic: Fungal Infections

P180: The efficacy of luliconazole against *Fusarium* complex

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Introduction and Objectives: *Fusarium* species are widespread saprophytic fungi that originally considered as phytopathogen, however during the last decades were shifted to humans and animals' diseases. *Fusarium solani* is responsible for the majority cases of fusariosis, and usually causes severe infections with high mortality rates among predisposed patients. *Fusarium* species are inherently resistant to the most available antifungals *in vitro* with considerable high minimum inhibitory concentration (MIC). Luliconazole is a new antifungal that was originally used for the treatment of dermatophytosis. However, some study has shown that it has excellent efficacy against *Aspergillus*, and *Candida* species. The present study was aimed to the evaluation of luliconazole activity against some clinical and environmental isolates of *Fusarium*. Furthermore, this efficacy was compared to other systemic antifungals including; caspofungin, posaconazole, fluconazole, itraconazole, amphotericin B and voriconazole.

Materials and Methods: In our study 48 isolates of *Fusarium* including 2 clinical isolates and 46 environmental isolates were tested against several antifungals. All species were identified using morphology features and PCR sequencing. Antifungal susceptibility was performed according to CLSI M38 A2 guideline against *Fusarium* species with luliconazole, caspofungin, posaconazole, fluconazole, itraconazole, amphotericin B and voriconazole.

Results: Our results showed that luliconazole has very low MIC_{GM} value 1 to 0.0645 µg/ml in comparison with 2.78 µg/ml for posaconazole, 2.14 µg/ml for terbinafine, 0.54 µg/ml for itraconazole, 10.7 µg/ml for amphotericin B, 1.18 µg/ml for voriconazole and 1.39 for caspofungin. Also, the highest resistance to antifungal drugs were observed in amphotericin B (85.1%).

Conclusion: Overall, our finding indicates that luliconazole has a great activity against environmental and clinical *Fusarium* species complex. As well as this drug has a potency to use for fusariosis.

Keywords: *Fusarium*, Luliconazole, Antifungal susceptibility

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Topic: Industrial and Applied Microbiology

P125: Innovative method for the fast removal of *Escherichia coli* from polluted water using electro-Fenton process: Modeling and investigation of the removal mechanism

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Introduction and Objectives: *Escherichia coli (E. coli)* bacteria are commonly used as indicator organisms to designate of impaired surface waters and to guide the design of management practices to prevent fecal contamination of water. Water purification from harmful bacteria using cheap and eco-friendly techniques is very important. Electrochemical advanced oxidation process (EAOPs) have attracted substantial attention owing to their environmental versatility, high efficiency, and safety. Among EAOPs, electro-Fenton (EF) process is one of the electrochemical processes that consist of producing Fenton's reagent (Fe^{2+}/H_2O_2) in acidic medium (pH 3). Therefore, the current study aimed to evaluate the efficiency of the EF process for removing *E.coli* from polluted water.

Materials and Methods: The central composite design employed for efficient removal of *E.coli* as indicator organisms by response surface methodology using Design Expert 11®. The experiments were done under five levels of various operational parameters. The initial concentration of *E.coli* varied among 0.1 to 5×10^7 CFU mL^{-1}, the current density ranging from 2 to 10 mA cm^{-2}, H_2O_2 ranging from 100 to 1000 µL L^{-1}, inter-electrode distance ranging from 1 to 5 cm, and reaction time ranging from 1 to 10 minute.

Results: The maximum removal rate achieved at the initial *E.coli* concentration of 3.5×10^7 CFU mL^{-1}, the current density of 4.5 mA cm^{-2}, H_2O_2 dosage of 750 µL L^{-1}, the inter-electrode distance of 3.5 cm, within the reaction time of 6 min. Regression analysis showed the good fit of the experimental data to the second-order polynomial model with a coefficient of determination (R^2) value of 0.982, adjust correlation coefficient (Adj.R^2) value of 0.9750 and predicted correlation coefficient (pred. R^2) value of 0.956.

Conclusion: Using ordinary radical scavengers demonstrated that hydroxyl radical (˙OH) was the main oxidant species contributed to the removal of *E.coli* under the EF process.

Keywords: Removal; *Escherichia coli;* Electro-Fenton process; polluted water treatment.

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P126: Photo-electro Fenton treatment process as new approach for efficient removal of microbial contamination from aqueous medium: Optimization and inactivation kinetics

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Introduction and Objectives: Microbial contamination of water and the concern for public health are a global problem. Conventional methods for water disinfection such as chlorination and ozonation inevitably form harmful disinfection by-products (DBPs), which probably provide an unintended health hazard. As an alternative method, the photo-electro Fenton (PEF) process which generate hydroxyl radical (˙OH) employed for efficient removal of *Escherichia coli (E.coli)* as representative of microbial contamination from the aqueous medium in the current work.

Materials and Methods: This study carried out experimentally in a laboratory scale. The effect of main variables including initial *E.coli* concentration, solution pH, H_2O_2 dosage, current density, UV intensity, and reaction time on the efficiency of PEF process investigated. The design and process optimization performed using Design Expert 11 software.

Results: Development of treatment model using analysis of variance (ANOVA) revealed that maximum *E.coli* removal efficiency of 99.7% occurred at the initial *E.coli* concentration 5×10^7 CFU mL^{-1}, UV intensity of 12 W cm^{-2}, H_2O_2 dosage of 620 µL L^{-1}, current density of 7.5 mA cm^{-2}, solution pH of 3.5, and the reaction time of 8.4 min. ANOVA displayed the non-significant lack of fit value (0.094), whereas, the predicted correlation coefficient values (Pred. R^2=0.912) were reasonably in agreement with the adjusted correlation coefficient value (Adj. R^2=0.907) demonstrating a satisfactory significant model for *E.coli* removal. The PEF process produces highly oxidizing species, the hydroxyl radical (˙OH), which is mainly responsible for the inactivation of *E.coli*. Kinetics of inactivation process followed first-order kinetics model with the rate constants (K_{app}) of 0.674 min^{-1}.

Conclusion: The obtained results revealed that the photo-electro Fenton process as a green technology was able to inactivate the *E.coli* in aqueous medium effectively.

Keywords: Removal; *Escherichia coli;* Photo-electro Fenton process; Kinetic.

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P157: Isolation and identification of a new strain of *Stappia indica* from the surface layer of a hydrocarbon reservoir from western Masjed Soleyman, Iran, with the ability of propane and/or butane oxidation

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Introduction and Objectives: Short chain alkanes are chemically the least reactive compounds. Despite this, some microorganisms can utilize these compounds as their carbon source for their metabolic activities. A group of them are hydrocarbon oxidizing bacteria which based on the theory of light hydrocarbons leakage from oil/gas reserviors to the surface layer, can be used as indicators for prospecting new oil/gas reserviors. The aerobic oxidation catalyse by soluble diiron monooxygenases i.e. butane monooxygenase and propane monooxygenase which oxidize alkanes to primary or secondary alcohols. The purpose of this study was isolation and identification of a new butane and/or propane oxidizing strain.

Materials and Methodes: The methodology involve the collection and packing of soil samples from oil survey area aceptically and isolation of butane and/or propane oxidizing bacteria by using LPG (Liquefied petroleum gas) as the only carbon source in Mineral base medium. Identification of isolated bacteria carried out by 16S rDNA sequencing and biochemical tests. Polymerase chain reaction was applied for detecting *prmA* (peopane monooxygenase A gene) and *bmoX* (butane monooxygenase X gene) genes in isolate. sequencing of the genes also carried out after successful amplification.

Results: The isolated bacteria identified as a new strain of *Stappia indica* with 99.35% homology of 16S rDNA sequence with *Stappia indica B106*. The *prmA* gene also successfully detected and sequenced in this isolate.

Conclusion: To conclude, the new strain is a new propane and/or butane oxidizing bacterium, the ability that has not been reported for the genus *Stappia*.

Keywords: Short chain alkanes, Butane and/or propane oxidizing bacteria, *prmA*, *bmoX*, *Stappia indica*

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P153: Production and Optimization of Alkaline Protease in the Solid-State Fermentation by *Bacillus licheniformis*

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Introduction and Objectives: Proteases are enzymes that hydrolyze proteins by the addition of water across peptide bonds. Proteases are one of the most important groups of industrial enzymes used in pharmaceutical and food industry for peptide synthesis. These are used in leather industry for dehairing and as an additive of detergent formulation in detergent industry. Alkaline proteases are known to constitute 60-65% of the global industrial market among various types of proteases. The aim of this study was to production and optimization of alkaline protease in the solid-state fermentation by *bacillus licheniformis*.

Materials and Methods: *Bacillus Licheniformis* PTCC 1595 was obtained from the Iranian Research Organization for Science and Technology (IROST). In this work, design of experiments (DOE) methodology using full factorial design was applied to evaluate the influence of different carbon sources (rice husk, rice bran, wheat bran), nitrogen Sources (urea, yeast extract, peptone, casein) and pH (7, 8, 9, 10) on Alkaline proteases production. The fermentation process was held at a temperature of 37 °C for 48 hours. Measurement of the enzyme activity was carried out by measuring optical density at 660 nm.

Results: The highest alkaline protease activity (154.07 U/ml) was achieved when rice husk as carbon source, urea as nitrogen source, at pH 10.

Conclusion: In the present study, the effect of different carbon sources, nitrogen sources and pH on alkaline protease production by *bacillus licheniformis* was studied. The results showed that rice husk, by-product of rice industry can be used efficiently for alkaline protease production under solid-state fermentation.

Keywords: Alkaline Protease, *Bacillus licheniformis*, Solid-State Fermentation, Design of experiments

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P135: Inactivation of *Acinetobacter baumannii* from hospital wastewater using UV/H_2O_2 process as a new approach for oxidation: Modeling and optimization

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Introduction and Objectives: Hospital wastewater by containing multiresistant bacteria and their resistance genes can represent a high biological risk for public health. Multidrug-resistant *Acinetobacter baumannii* (*A.baumannii*) is an emerging pathogen mostly reported as a cause of hospital infections. Because of its high repetition of antibiotic resistance and escaping the biocidal ability of antibiotics, *A.baumannii* emphasized among the ESKAPE pathogens. In the current work, the UV/H_2O_2 process applied for efficient inactivation of *A.baumannii* from hospital wastewater.

Materials and Methods: The central composite design used to enhance the inactivation efficiency of *A.baumannii* by response surface methodology (RSM). The experiments were done under four levels of various operational parameters. The influence of operating parameters such as the initial concentration of *A.baumannii* concentration, H_2O_2 dosage, UV intensity, and reaction time investigated using response surface methodology. The isolated *A.baumannii* determined CHROM agar *Acinetobacter* and several antibiotic discs.

Results: The present study focused on the inactivation of *A.baumannii* from hospital wastewater. The obtained results revealed that the UV/H_2O_2 process was able to inactivation *A.baumannii* effectively. Regression analysis revealed a satisfactory agreement between the obtained experimental results and the predicted inactivation efficiency by the second-order polynomial model. The kinetics of the process follows the pseudo-first-order model.

Conclusion: The UV/H_2O_2 process applied successfully for *A.baumannii* inactivation from hospital wastewater and the predicted model for treatment of synthetic wastewater is in satisfactory agreement with inactivation efficiency of real hospital wastewater treatment.

Keywords: Inactivation; Acinetobacter baumannii; UV/H_2O_2 process; Hospital wastewater; multiresistant bacteria.

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P134: Removal of vancomycin-resistant *Enterococcus faecium* photo electro-Fenton process: reaction mechanism and pathways

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Introduction and Objectives: Multiresistant bacteria and antibiotic resistance genes released into wastewater. However, a large number of sufficient antibiotics used for human therapy. The rapid spread of them in the environment has become an emerging contaminant issue. Vancomycin-resistant *Enterococcus faecium* (VREfm) is a significant nosocomial pathogen all over the world. Therefore, it is very important to develop hospital wastewater treatment technologies for the removal of VREfm. The current study aimed to evaluate the efficiency of photo electro-Fenton (PEF) process for removing VREfm from hospital wastewater.

Materials and Methods: Design of the experiment and investigate the effect of different parameters such as current density, H_2O_2 dosage, inter-electrode distance, UV intensity and reaction time were carried out by application of the response surface methodology (RSM). The VREfm using arabinose agar base and antibiotic discs methods were determined.

Results: In the current study, the removal of VREfm was investigated using PEF process. The experimental design was carried out based on a central composite design with response surface methodology. The significance of the independent variables and their interactions are tested by means of the analysis of variance (ANOVA) with a 95% confidence level. The design results indicated that polynomial equations and response plots soundly justified the interrelationship between the dependent response and the independent variables. Kinetic studies revealed that the pseudo-first order kinetic best fitted to the experimental results of VREfm removal.

Conclusion: PEF process as a promising technique was found to be an efficient approach for removal of VREfm from hospital wastewater.

Keywords: Vancomycin-resistant *Enterococcus faecium*; Removal; Hospital wastewater; Photo electro-Fenton.

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P133: A novel electro-Fenton process for removal of methicillin-resistant *Staphylococcus aureus* from hospital wastewater

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Introduction and Objectives: The presence of antibiotic-resistant bacteria in hospital wastewater and then releasing antibiotic resistance genes in the aquatic environment have become a great threat to human health. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common bacterium in hospital, sometimes can cause fatal infections worldwide. Therefore, the current study aimed to evaluate the efficiency of electro-Fenton (EF) process for removing MRSA from hospital wastewater.

Materials and Methods: Response surface methodology (RSM) under the central composite design (CCD) category of Design Expert 11 software used to achieve efficient removal of MRSA. The main objective of the CCD method is to optimize the response surface and quantifies the relationship between the controllable input parameters and the obtained response surfaces. The isolated and characterized MRSA from hospital wastewater determined with chemical and molecular methods. The effect of various variables including H_2O_2 dosage, current density, initial MRSA concentration and reaction time investigated to achieve the best efficient MRSA removal condition.

Results: Statistical tests (ANOVA and regression) showed that the designed model was in satisfactory agreement with the obtained experimental results. The kinetics of the process follows the pseudo-first-order model. Using ordinary radical scavengers demonstrated that hydroxyl radical (˙OH) was the main oxidant species contributed to the degradation of MRSA under the EF process. The normal plot of residuals demonstrated revealed that the linear curve of normal probability versus the internal residuals was reasonably close to a straight line.

Conclusion: The EF process as an environmentally friendly treatment method was optimized and applied successfully for efficient removal of MRSA from hospital wastewater samples using response surface methodology in the current work.

Keywords: Methicillin-resistant *Staphylococcus aureus*; Removal; Hospital wastewater; Electro-Fenton process; Response surface methodology.

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P290: Preparation of antiserum against rotavirus for use in diagnostic kits based on ELISA and immunofluorescence

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Introduction and Objectives: Rotavirus is a major cause of severe watery diarrhea, vomiting, fever, and abdominal pain. This virus can spread easily among infants and young children. Children who get rotavirus disease can become dehydrated and may need to be hospitalized. It is not easy to distinguish gastroenteritis caused by rotavirus from other enteric pathogens. Therefore, establishing an adequate diagnosis requires testing of fecal specimens with commercially available assays like electron microscopy, immunofluorescence and Elisa. The purpose of this study was to provide antiserum against rotavirus antigens for use in ELISA and immunofluorescence kits.

Materials and Methods: Inactivated virus was injected intravenously into New Zealand White Rabbit on a weekly basis. Blood collection was performed 7 days following last injection. Antibody was purified with different methods like Ammonium sulfate precipitation, Tangential flow filtration and Ion Exchange chromatography.

Results: Results showed adequate quality and quantity of produced antibody. The purity was confirmed by SDS-Page electrophoresis and protein concentration also checked with Bradford method. Purified antibody was used in Elisa after biotinylation. Clinical specimens were checked by designed Elisa kit.

Conclusion: Using antibody and biotinylated antibody in Elisa kit showed acceptable sensitivity and specificity and could provide a convenient, rapid and reliable diagnostic method for rotavirus infections.

Keywords: Rotavirus, Antiserum, Elisa, Immunofluorescence.

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P282: Preparation of Antiserum against Salmonella O Antigen for application in Agglutination and Elisa tests

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Introduction and Objectives: Salmonella infection (salmonellosis) is a common bacterial disease that affects the intestinal tract and usually caused by eating raw or undercooked meat, poultry, eggs or egg products. A few varieties of these bacteria result in typhoid fever, which is more common in developing countries. Several methods like Multiplex or real-time PCR, Elisa, and Agglutination are used to identify these bacteria. However, normally rapid, cost effective and easy diagnostic methods such as agglutination test is recommended. In Iran, positive control antiserum used in diagnostic kits works based on polyvalent agglutination and are against O and H antigens. The purpose of this research was to produce specific anti-sera against O antigen for using in agglutination and ELISA kits.

Materials and Methods: New Zealand white rabbits were immunized by intravenous injections of inactivated bacterial O antigen adjusted to a cell density equivalent to a turbidity of a McFarland number 3 standard. Serum collection was performed 7 days after last injection. Collected Antisera was tested with positive human specimens as well as cross-reaction antibodies. Absorption method was used to obtain specific anti-sera against O antigen. Produced Anti-O antibody was mixed with bacterial H antigen and incubated for 1 hours in 37˚c. The Mixture was centrifuged and supernatant was collected.

Results: Results showed high quality and quantity of mono-specific antibody, which showed no cross-reaction with H antigen.

Conclusion: Agglutination based assays are rapid, cost effective and easy for primary screening of salmonella infections. In addition, anti-sera can be used in Elisa and Immunofluorescence kit after purification.

Keywords: Salmonella, O antigen, specific antiserum, agglutination, ELISA

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P130: Preparation of polyclonal antibody in rabbit against Neisseria meningitidis serogroup A and C antigens used in diagnostic kits

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Introduction and Objectives: Neisseria meningitidis (or meningococci) is a major cause of disease such as meningitis and meningococcemia and it is one of the leading causes of mortality in children in developed countries. Meningococci is classified to 13 serogroups according to serological specificities of their capsular antigens. Serogroups A, B, C, Y, and W135 are responsible for virtually all cases of the human disease. Bacterial identification early in the course of diseases could lead to selection of appropriate antimicrobial therapy and indicate the need for specific immunization for prevention. The purpose of this study was to produce antiserum against meningococcal antigens for use in different diagnostic kits, like agglutination, Elisa and immunofluorescence (IF).

Materials and Methods: New Zealand white rabbits were immunized by intravenous injections of an inactivated suspension of the bacteria adjusted to a cell density equivalent to a turbidity of a McFarland number 3standard. Serum was collected one week after last injection.

Results: The bacterial agglutination test was performed and results showed high quality and quantity of antibody. Furthermore, antibody purification steps were done by using precipitation, filtration and ion exchange chromatography methods. The purity was confirmed by SDS-Page electrophoresis and purified antibody could be used in Elisa and IF tests.

Conclusion: Agglutination tests has always been very popular due to low cost and feasibility of the method. In addition, high purity antibody can be used in clinical diagnosis and serologic identification based on Elisa and IF, where sensitivity and specificity are important issues.

Keywords: Neisseria meningitidis, Antiserum, Agglutination, Elisa and immunofluorescence

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P204: Systematic Study for Synthesis of La^{3+}/α-Al_2O_3 Nanoparticles: Antibacterial Activity Against Pathogenic Microbial Strains

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In this study for the first time, new nanoparticles of the La^{3+}/α-Al_2O_3 were synthesized with the ultrasonic-assisted hydrothermal method in the presence of the honey as an eco-friendly and natural reagent. The as-synthesized La^{3+}/α-Al_2O_3 nanoparticles were characterized using scanning electron microscopy (SEM), transition electron microscopy (TEM), X-ray diffraction spectroscopy (XRD), energy dispersive X-ray (EDX), UV–visible spectroscopy, and Fourier transform infrared spectroscopy (FTIR) techniques. In this work, we report optimum conditions for the synthesis of La^{3+}/α-Al_2O_3 nanoparticles as novel material and as the candidate for antibacterial activity in antibacterial drugs. The XRD and SEM micrograph results demonstrate the formation of pure La^{3+}/α-Al_2O_3 nanoparticles with a sphere like in the size range of 30–80 nm. The synthesis parameters were systematically examined using analysis of variance (ANOVA) through $2k^{-1}$ factorial design and the factors were an assay for product optimization. Results show La^{3+}/α-Al_2O_3 nanoparticles capable considerably inhibited the growth of antibiotic-resistant *Escherichia coli*, *pseudomonas aeruginosa* and *Serratia marcescens* as gram negative bacteria and *Bacillus subtilis* and *Micrococcus luteus* as gram positive bacteria between 64 up to 32 µg/ml. The antibacterial activity was carried out against 7 Gram negative and Gram-positive bacteria using agar well diffusion assay and minimum inhibitory concentration (MIC) was determined.

Keywords: Ultrasound assisted hydrothermal method, La^{3+}/α-Al_2O_3 NPs, Nanocomposites, Antibacterial activity

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P154: Isolation and molecular identification of *Acetonitrile* degrading bacteria from Jahrom city sewage

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introductions and objectives: Environmental pollution is one of the most important problems facing the world today. These contaminations occur as a result of the absorption and accumulation of chemicals in the food chain and damage to the plants and living organisms of that climate. Nitrile is one of the most organic compounds in the Cyan active group, which is increasingly being produced in various natural and synthetic forms. Nitrile is uncontrollably entrained from sewage and sewage sludge and sewage and is considered as an important threat to the health of living organisms. The purpose of this study was to investigate and isolate the Acetonitrile degrading bacteria from Jahrom sewage.

Materials and Methods: At first, the specimens were incubated at ambient temperature with Acetonitrile at 37 ° C for 14 days. After isolation and differential chemistry tests, MIC was used to isolate superior strains. Then strains were evaluated for growth kinematics and chromatographic analysis. At the end of the 16srRNA molecular tests and sequencing to identify the superior strains.

Results: Among the 34 bacterial species of the two isolates, *Pseudomonas otitidis* and Bacillus subtilis were isolated in the shortest possible time, and the superior chromatographic analysis of these two bacteria showed that they were 92.35 and 7.35, Dissolves 80% of 0.1% concentration of Acetonitrile.

Keywords: Acetonitril Biem idation, *Pseudomonas otitidis*, *Bacillus subtilis*, Parenteral bacteria

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P139: Detection of *Coxiella burnetii* in bulk tank milk in dairy cattle farm of Kerman by nested-trans PCR

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Introduction and objectives: Q fever is a zoonotic disease caused by *Coxiella burnetii* that considered a public health problem in different societies. Cattle, sheep and goats are the major reservoirs of this disease; infected animals excrete the bacteria in milk. An effective and rapid method for diagnosis of *C. burnetii* is nested-trans PCR method. The aim of this study was determination of *C. burnetii* in Bulk Tank Milk (BTM) samples from Kerman dairy cattle herds.

Materials and methods: In this study, 48 BTM samples including 4200 industrial and semi industrial dairy cattle was collected. DNA was extracted using a commercial kit and, then, examined by nested-trans PCR method for the presence of *C. burnetii.*

Results: Six of 48 samples (12.5%) were positive. Given the importance of the bacteria in economy of livestock and public health, its rapid and accurate diagnosis is highly important.

Conclusion: Results of this study showed that the cattle milk can be a potential reservoir of *C. burnetii* in southeastern Iran.

Keywords: *C. burnetii*, PCR, milk, Kerman

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P199: Innovation of *Bacillus subtilis* spore display technique to synthesis of Au nanoparticles

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Introduction and objectives: According to the wide applications of Au nanoparticles in electronic and medicine especially in biosensor technology, eco-friendly and cost-effective methods for synthesis are considered. Enzymes are one of the powerful tools in this approach. In this study, tyrosinase was displayed on the surface of *Bacillus subtilis* spores and the spores were used to synthesis Au nanoparticles.

Materials and Methods: We used a protease deficient *Bacillus subtilis* DB104 as a host strain for the surface display of the tyrosinase-histidine$_6$-tag on *B. subtilis* spore. We chose CotE protein as an anchoring motif because it's located in the outer coat layer and has a high abundance. We inserted tyrosinse with histidine$_6$-tag at the C-terminal end of anchoring motif. Western blot confirmed proper expression of the fusion protein. Surface expression of the CotE- tyrosinase-His$_6$ fusion protein was also confirmed using flow cytometry. The production of AuNPs analyzed by transmission electron microscopy and X-ray diffraction technique.

Results: The results revealed that AuNPs were produced due to reducing Au^{3+} to Au^0 by spore displayed tyrosinase. These biogenic nanoparticles showed mixed structures including spherical, triangular and hexagonal with the approximate size 2.5 to 35 nm. Furthermore, purified *Bacillus megaterium* tyrosinase and *Streptomyces* tyrosinase also produced AuNPs.

Conclusion: The supposed mechanism of AuNPs synthesis by tyrosinase, is electron transferring from copper ions to Au^{3+}. The results represent a green environmentally friendly simple method in synthesis AuNPs by spore displayed tyrosinase.

Keywords: Au nanopaerticles, Spore Displayed Tyrosinase, Tyrosinase

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P163: Optimization of protease activity of alkaliphilic bacterium isolated from soil of Ahvaz, Iran

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Introduction and Objectives: Proteases are the hydrolytic enzymes which hydrolyze peptide bond between proteins with paramount applications in pharmaceutical and industrial sector. They produce protein, peptide fragments, and free amino acids. They can be intracellular or extracellular. Extracellular proteases are important for the hydrolysis of proteins in cell-free environments and enable the cell to absorb and utilize hydrolytic products. We isolated a bacterium from soil of Ahvaz, Iran which produces potent alkaline protease. The morphological, biochemical and 16S rDNA gene sequencing studies revealed that the isolated bacterium is Bacillus siralis strain PA02.

Materials and Methods: We used skim milk agar to screen alkaline proteases. A halo (clear zone) around a colony could indicate protease activity. The enzyme's activity was assayed by the caseinolytic method. By this way, the protease used casein as substrate and released tyrosine. We measured the absorbance of the tyrosine at 275nm.

Results: The optimum activity of the enzyme from this organism was observed at pH 11.0, temperature 55 °C and was stable at pH values from 8 to 12 during the 30 minutes of incubation at 35 °C. Temperature stability ranged from 35 °C to 85 °C but the maximum stability was reached at 35 °C retaining 81% of the enzyme's activity after 1 hour of incubation. The enzyme's activity increased in the presence of ions like Ca^{2+}, Zn^{2+}, Na^{+}, and its activity decreased when Fe^{2+}, Mg^{2+} and K^{+} were present.

Conclusion: In conclusion, the activity and stability of this protease in the extreme pH range, its stability at high temperature, makes it attractive for industrial application.

Keywords: Extracellular alkaline proteases, Enzyme stability, *Bacillus siralis*

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P120: Biosynthesis of jarosite by *Acidithiobacillus ferrooxidans* isolated from Sarcheshmeh copper mine

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Introduction and objectives: Jarosite process is one of the most widely used methods in removing iron. *Acidithiobacillus ferrooxidans* are a chemoautotrophic and acidophilic bacterium, which can obtain energy for growth from the oxidation of a variety of inorganic sulfur compounds and have an important effect on formation of jarosite. In the copper bioleaching process, the formation of jarosite on the surface of the biooxidized metal sulfide particle significantly decreases the rate of bioleaching. The main goal of this study to investigate the formation mechanism of ammonium jarosite, by *Acidithiobacillus ferrooxidans* isolated from Sarcheshmeh copper mine.

Materials and methods: *A. ferrooxidans*, has been isolated from pregnant leaching solution (PLS) of the Sarcheshmeh copper mine. The *A. ferrooxidans* was identified using specific amplification of 16S rDNA sequences by PCR. the formation of jarosite was determined by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM) analysis.

Result: The SEM and XRD results showed that biosynthetic jarosite had smooth surface and mainly consisted of ammonium jarosite [$NH_4Fe_3(SO_4)_2(OH)_6$]. The ammonium jarosite crystals were clearly grown by the two-dimensional nucleation mechanism and/or the spiral growth mechanism.

Conclusion: The results will be of significant importance for the further research in the copper bioleaching.

Keywords: *Acidithiobacillus ferrooxidans*; jarosite; biooxidation; pH

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P160: Carbon sequestration by recombinant carbonic anhydrase in *Ralstonia eutropha*

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Introduction and Objectives: Emission of greenhouse gases and plastic wastes are among the most important environmental concerns. The purpose of this study was to use the bacterium *Ralstonia eutropha* as the host for recombinant carbonic anhydrase enzyme. The enzyme is used for carbon sequestration. The by-product of this sequestration is PHB (poly hydroxy butyrate) which can potentially be used as biodegradable plastic.

Materials and Methods: Carbonic anhydrase gene (*CA*) was inserted in expression vector and transformed into *Ralstonia eutropha*. Then PHB production was assayed by FT-IR and GC-MS methods in recombinant and non-recombinant bacteria in different concentrations of LB medium in the presence and absence of CO_2.

Results: The accuracy of cloning was confirmed by PCR using specific primers for the internal region of *CA* gene. Results of FT-IR indicated that extracted bacterial biopolymer spectrum was aligned with the standard PHB spectrum. Production of PHB in LB medium increased 31.94 percent in recombinant bacteria in presence of CO_2. These increases were 18.44 and 34.44 percent compared to non-recombinant bacteria in presence and absence of CO_2, respectively. In 0.5 LB medium, the recombinant bacterium produced 26.57 percent PHB in presence of CO_2 more than in its absence. Non-recombinant bacterium in this medium produced 18.30 and 30.63 percent PHB less than the recombinant cell in presence and absence of CO_2, respectively.

Conclusion: The quantity of PHB production using GC-MS revealed that *CA* gene expression was effective for increasing PHB production in recombinant *R. eutropha*. The recombinant bacterium could produce PHB in the presence of CO_2 by using a simple medium.

Keywords: Carbonic anhydrase, Carbon sequestration, *Ralstonia eutropha*

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P164: Preparation and Purification of specific Antibody against Pneumococcus for application in diagnostic kits

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Introduction and Objectives: Streptococcus Pneumonia is the cause of a wide range of mild infections such as otitis media, sinusitis, and severe infections such as pneumonia, septicemia and meningitis especially in children under 5 years old and old people. Assays of this bacterial infections are based on either antigens or antibodies produced by the host. Detection of the polysaccharide capsule of the bacteria is the most frequently used method for screening of this infection. The purpose of this study was to prepare and purify specific antibody against pneumococci for application in immunofluorescence, agglutination and ELISA-based diagnostic kits.

Materials and Methods: A total of 10^9 inactivated bacteria were applied for three successive infusions in New Zealand White Rabbit in a week. Blood sample collection and serum separation were performed. Then immunoglobulin purification steps were done using ammonium sulfate sedimentation, tangential flow filtration and DEAE Ion exchange chromatography. Purified antibody was also used for pneumococcal diagnosis in clinical specimens based on agglutination.

Result: The results of quantitative spectrophotometric and measurement in SDS-Page electrophoresis as well as Lowry -Bradford protein assay showed that the purified isolated protein had a high quality and quantity. The results of agglutination test from purified antibody were consistent with the culture method.

Conclusion: Due to low cost and feasibility of the method, agglutination tests has always been very popular in lab for detection of Streptococcus pneumonia. This purified antibody can be used in clinical diagnosis and serologic identification of isolated bacteria.

Keywords: Pneumococcus, Antiserum, Agglutination, Elisa

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P272: Development and preparation of anti-Human globulin (IgG) for detection of brucellosis in Coombs Wright test

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Introduction and Objectives: Brucellosis is a common illness in man and livestock which is caused by the Brucella Species. Animal health is crucial for us for dairy consumption. An important diagnostic test for this bacterium is the Wright test. Because IgG is not a strong agglutinin with its monomeric structure, false negative results of Wright test is probable and therefore, it is better to use the Coombs wright test to detect this type of antibody. Due to the importance of the presence of IgG antibodies in the chronic stages of the disease, and the negativity of the wright tube test, we use anti-human globulin. The purpose of this study was to prepare antiserum against IgG immunoglobulin for use in the coombs Wright test.

Materials and Methods: 250 µg of purified human IgG was mixed with one milligram of alum adjuvant. New Zealand White Rabbit was injected for four times with a week's intervals. Blood collection and serum separation were performed and purification was done using ammonium sulfate precipitation, Tangential flow filtration and DEAE ion exchange chromatography methods.

Results: The results after the completion of anti-human globulin purification steps showed that by using spectrophotometric method and the Lowry and Bradford protein assay methods, as well as the SDS-Page Gel, the purity of the isolated anti-human globulin was quantitatively and qualitatively acceptable.

Conclusion: Purified antibodies against IgG can be immunogenic in the clinical diagnosis of Coombs-Wright tests, the cross-match as well as the detection of blood groups with weak Rh or Du.

Keywords: Brucellosis, Immunoglobulin IgG, Coombs Wright

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Topic: Industrial and Applied Microbiology

P145: Evaluation of non- oxidizing biocides to obtain the consumption of suitable biocide in the cooling tower of Bandar Imam Petrochemical Company

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Introduction and Objectives: Cooling towers provide a unique environment for the proliferation of micro-organisms, including aerobic and anaerobic bacteria, algae, and fungi. In many petrochemical industries chlorine and its derivatives, as well as non-oxidizing biocide as supplementary biocide, are deployed to prevent or inhibit microbial growth. In vitro experiments were undertaken to evaluate the efficacy of chlorine, isothiazoline, quaternary ammonium, and synergistic blend compared to each other.

Materials and Methods: Microbicides were evaluated against sulfate-reducing bacteria, heterotrophic bacteria, algae, and fungi isolated from the cooling tower system under sterile condition. The five non- oxidizing biocides with commercial names ISO1, ISO2, QA1, QA2, SY and chlorine as oxidizing biocide were tested. Antibacterial activity was assessed based on International Standard Test Methods.

Results: The results show that all the non- oxidizing biocides were more efficient for the sulfate-reducing and heterotrophic bacteria, throughout 2 experiments, after 3 and 24 hours. Biocides QA1, QA2 and ISO1 resulted in the destruction of fungi, and only biocide ISO1was shows efficient on elimination of algae.

Conclusion: The final results show that percent kill of biocide ISO1 as supplementary biocide along with chlorine injection has been the higher effect against microorganism, in compared with the other biocides. Many parameters may influence the efficacy of biocides, such as application method of biocide, contact time, target microorganisms, leakage of pollutants (e.g. hydrocarbon and ammonia) and environmental conditions (e.g. pH and water temperature). The choice for a desirable biocide can be helping to improve and prevent biological corrosion in the industrial cooling towers systems.

Keywords: Evaluation, biocide, cooling tower

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Topic: Industrial and Applied Microbiology

P162: The introduction of a new genus as the degrading crude oil from industrial sewage in Shiraz

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Introduction and Objectives: Oil hydrocarbons have the highest environmental pollution. The entry of petroleum hydrocarbons into groundwater pollution also results. Biology is a technology to eliminate oil pollution. The purpose of this study was to identify the molecular components of oil degrading bacteria from industrial waste water from Shiraz.

Materials and Methods: Water and soil samples were collected from industrial waste water from Shiraz. The oil degradation bacteria were identified after Bushnell-Hass enrichment. The identity of the bacteria was determined based on biochemical and molecular tests and by sequencing the 16S rRNA gene. The ability to isolate bacteria for crude oil degradation was studied through gravimetric experiments.

Results: Among the isolated bacteria, *Cellulosimicrobium cellulans* showed the best performance in biodegradation of crude oil. This bacterium was able to degrade a maximum 61% of crude oil after 10 days of incubation.

Conclusion: The results of this study indicate that bacteria can be used in the biological process and reduce the pollution of oil in the environment. The bacteria showed great potential in mixed and single cultures at different concentrations of nutrients, which in turn contributes to increasing the efficiency of eliminating oil pollution**.**

Keywords: Bioremediation, Crude Oil, Industrial sewage, Bacteria

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Topic: Industrial and Applied Microbiology

P143: Screening and evaluation of the alginate production potential of *Azotobacter* from soil in Kerman province

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Introduction and Objectives: *Azetobacter* is one of the most important nitrogen stabilizing bacteria to form a heteropolysaccharide capsule of alginate to protect its nitrogenase enzyme against oxygen. Alginate is widely used due to viscosity, water absorption and elasticity in medicine, food industry, pharmacy, printing industry, paper and even bio-absorption of heavy metals. The purpose of this study was to isolate *Azotobacter* alginate producers from soil in Kerman province.

Materials and methods: 80 samples of agricultural, pasture and forest soils were collected in five districts of Kerman province by cluster samplingFrom the surface to a depth of 10 cm. Initially *Azetobacter* was confirmed using the specific culture media of Manitol agar and Browne N-Free agar and the morphology of the colonies. Then, in order to evaluate the potency of alginate production, strains tested in carbazole solution were used in borate and borate-free conditions. The intensity of the purple color indicated a higher amount of alginate produced by spectrophotometry.

Results: Totally, 38 *Azotobacter* strains were identified. Of the strains mentioned, 23 strains had the ability to produce alginate. Most of the *Azotobacter* strains of Alginate originate in the cities of Baft (47.43%), Kerman (30.43%), Jiroft (8.69%), Sirjan (8.69%) and Bam (4.43%), respectively.

Conclusion: The results of this research showed that the soils of different crop areas with moderate climate in Kerman province have a significant potential for separating alginate generators. Therefore, further research, such as chemical analysis, optimization, purification of alginate, and identification of molecular identity of superior strains, are suggested.

Keywords: *Azotobacter*, Alginate, Carbazolem, Soil

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Topic: Industrial and Applied Microbiology

P214: Introduction of *Arthrobacter citreus* as a Crude Oil degradation from an Oil-Contaminated Soil in Masjed Soleyman

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Introduction and Objectives: The problem of oil hydrocarbons and soil contamination and aquatic ecosystems is a serious global issue. The pollution caused by oil hydrocarbons is considered to be a serious health hazard for all living organisms in the world and according to their inhibitory characteristics, are classified as priority and emerging pollutants. Biodegradation is one of the main biological recovery mechanisms used by degrading microorganisms to remove hydrocarbon pollutants from the environment. In this study *Arthrobacter citreus* bacteria were isolated from an oil-contaminated soil at Masjed Soleyman. *Arthrobacter citreus* Is one of the most important phenol degradations.

Material and Methods: Sampling of oil-contaminated soil was conducted in the Masjed Soleyman district of the foreign school. Initial screening and isolation of bacteria in the Bushnell-Hass salt base were performed and the frequency of bacteria was determined using MPN and CFU methods. In the second stage, screening of bacteria was done by gravimetric method. In the last step, using biochemical and molecular tests and obtaining 16S rRNA sequence, the bacterial genus was identified.

Results*: Arthrobacter citreus* showed a high ability to remove contaminants. Adding nutrients to bacteria, such as carbon and nitrogen sources, reduced the crude oil biodegradation time from 10 days to 2 days. The rate of removal of crude oil by *Arthrobacter citreus* was higher than 68%.

Conclusion: High and rapid degradation potential makes it possible to use this bacterium at the field level to reduce oil pollution in oil-polluted sites. By identifying the genes and enzymes involved in the degradation and transfer of active genes into native bacteria in contaminated areas, rapid elimination and reduction of oil pollution will be possible.

Keywords: Biodegradation, Bacteria, Crude Oil, Bioremediation

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Topic: Industrial and Applied Microbiology

P123: Screening and Characterization of Halotolerant Bacteria Producing Extracellular Lipase Isolated from the Caspian Sea in Mazandaran, IRAN

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Introduction and objectives: Halophilic bacteria are microorganisms that require from 3 to 15% w/v NaCl for sufficient growth. Halophilic microorganisms are potential sources of enzymes with wide application in beverages, pharmaceutical and detergent industries. The aim of the study was to isolate and study the halophilic bacteria producing industrial enzymes. Screening of halophilic bacteria from the coast of Caspian Sea led to isolation of 20 strains and characterization of 9 potential isolates producing industrially important hydrolases.
Materials and methods: Water samples were collected from the Caspian Sea and stored in suitable condition. Sea Water Media was used for screening of halophilic bacteria. Isolates were cultured in a lipase specific media to screen lipase producing bacteria. The strain produced maximum lipase enzyme qualitatively was chosen for further microbial works. Morphology, biochemical and molecular characteristics and production of hydrolase enzymes of the strain was studied. Optimization of growth for the designated isolate was studied in different pH, NaCl concentration and temperature, then determined spectrophotometrically at 600 nm and stated as microbial biomass.
Results: The isolate is a gram - negative strain related to *Psuedoalteromunas sp*. which is able to produce important hydrolases such as protease, Lipase and etc. The optimization of growth conditions determined that the strain is able to grow at 35°C and pH=7 with 4% NaCl concentration which revealed that this isolate is a halotolerant bacterium.
Conclusion: From this present study it can be inferred that the isolate is a strain which can produce lipase enzyme in high quantity.
Keywords: Halotolerant bacteria, Lipase enzyme, Industrial applications

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Topic: Industrial and Applied Microbiology

P155: Effect of Acinetobacter clacoaceticus plant growth-promoting bacterium on the wheat plant

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Introduction and Objectives: Plants growth-promoting bacteria can enhance plants growth by several actions like secretion of phytohormones, ensuring availability of functional nutrients, rescuing plant in abiotic stresses and decreasing pathogenic attacks by releasing antibiotics or toxins. In this study, we did evaluate the effects of Acinetobacter clacoaceticus bacterium on the growth of the wheat plant.

Materials and Methods: Several bacteria were isolated from the rhizosphere of the saffron plant by serial dilution method. NFB medium was used to Identification nitrogen-fixing bacteria. The most resistant bacterium was selected, after salinity and pH tests. 16S rRNA PCR testing was used to identify the bacterium. Formulation of the bacterium was performed with PVA, beet molasses and glycerol to enhance its survival. Agricultural test of bacterium did on the wheat plant and its effects evaluated on the parameter's growth.

Results:16S rRNA Gene sequencing showed that the selected bacterium belonged to the Acinetobacter family. The bacterium was able to grow from ph. 4 to 11 and withstand the salinity of up to 6%. The formulated bacterium had 1.8×10^{8} CFU/ML after six months. The results indicated that the yield of wheat treated with bacteria was up to 20 percent compared to the control sample.

Conclusion: The use of bacteria as biofertilizers can increase the quantity and quality of products and reduce production costs and hazards of chemical fertilizers.

Keywords: *Acinetobacter clacoaceticus*, wheat, biofertilizers

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Topic: Industrial and Applied Microbiology

P156: Isolation, detection and formulation of Serratia odorifera to produce the efficient biofertilizer

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Introduction and Objectives: Agriculture industrial affecting directly on the human's health. To increase the quantity of crops, the applications of fertilizer is a necessary practice. Plant growth promoting bacteria (PGPB) can do a significant role in the supply of plant nutritional needs. This bacterium can soluble of the necessary elements like phosphorous and potassium that trapped in the soil and taking nitrogen of the air. Therefore, uses of this bacterium, increasing the amount of the crops and reduces the usage of chemical fertilizers.

Materials and Methods: To isolation of bacteria, several samples of the soil and saffron plant were collected. Serial dilution method and NFB medium were used to detect of N-fixing bacteria. Then pH and salinity tests were used to selection of suitable bacteria with high adaptability to growth in the natural environment. The most resistant bacteria were selected and determined by 16s rRNA test. Agricultural test performed on the wheat in the natural condition. To increase the bacterial durability, the formulation was performed, and its survival was measured up to nine months.

Results: In total 113 bacteria were isolated in the first stage that was able to grow on the NFB medium. Among them, one bacterium was selected after salinity and pH tests. After the 16s rRNA test, bacterium recognized as Serratia odorifera. The formulated bacterium with Beet molasses, PVA and glycerol after nine months showed 1×10^6 CFU/ML. In the field test, the best result was wheat treatment with bacteria and fertilizer simultaneously.

Conclusion: Attention to bacteria as biofertilizer can help to the decrease amount of the chemical fertilizer usage and increasing of quality of products.

Keywords: biofertilizer, Serratia odorifera, bacterial durability

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Topic: Microbiota and Probiotics

P229: Alteration of Gut microbiota can be a predisposing factor of *Clostridium difficile* infection

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Introduction and Objectives: *Clostridium difficile* (recently *Clostridioides difficile*) is a leading cause of antimicrobial-associated diarrhea (AAD). The present study was carried out to investigate the interactions between the bacterial load of gut microbiota and *C. difficile* in Iranian hospitalized patients.

Material and Methods: This cross-sectional study was conducted from October 2017 to June 2018 in two teaching hospitals in Shiraz, southwestern Iran. During this period, a total of 215 non-duplicated nosocomial AAD samples and 200 random fecal samples from asymptotic patients were collected. Presumptive *C. difficile* isolates were identified by standard microbiologic methods and confirmed by specific PCR primers. The relative bacterial load determined by quantitative real-time PCR (qPCR).

Results: In all, the frequency of *C. difficile* culture-positive samples was 21.4% (n = 46) and 10.5% (n = 21) of diarrheal and asymptotic samples, respectively. Mean log of *C. difficile* load in diarrheal samples was significantly higher than asymptotic samples ($log10^{6.1}$ vs. $10^{2.7}$; $P < 0.001$). Regarding the gut microbiota, *Escherichia coli* was significantly higher in asymptotic samples ($10^{4.1}$ vs. $10^{5.2}$ $P < 0.002$), while *Bacteroides fragilis* was higher in diarrheal samples ($10^{2.8}$ vs. $10^{4.1}$ $P = 0.001$). Also, Mean log of *Lactobacillus casei* was not significantly different among two groups ($10^{1.7}$ vs. $10^{2.2}$ $P = 0.35$). Based on 50th percentile, a mean load of *E. coli* $\leq Log10^{3.9}$ was significantly associated with higher *C. difficile* load in diarrheal samples ($log10^{1.9}$ vs. $10^{1.2}$; $P < 0.001$). While, bacterial load of *B. fragilis* and *L. casei* was not significantly associated with the level of *C. difficile*.

Conclusion: Over-use of antibiotics can suppress the gut microbiota and provide an opportunity for development of *C. difficile* infection (CDI). Our findings, indicates that the level and diversity of the microbiota can be a predisposing factor of CDI.

Keywords: *Clostridium difficile*, Gut microbiota, Antimicrobial-associated diarrhea, Quantitative real-time PCR

Topic: Microbiota and Probiotics

P140: Inhibitory Effect of Probiotic Yeast *Saccharomyces cerevisiae* on Biofilm Formation and Expression of α-Hemolysin and Enterotoxin A Genes of *Staphylococcus aureus*

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Introduction and Objectives: *Staphylococcus aureus* as an opportunistic pathogen is the cause of a variety of diseases from mild skin infections to severe invasive infections and food poisoning. Increasing antibiotic resistance in *S. aureus* isolates has become a major threat to public health. The use of compounds produced by probiotics can be a solution to this problem. The purpose of this study was to investigate the effect of *Saccharomyces cerevisiae* on some virulence factors (biofilm, α-hemolysin and enterotoxin A) of *S. aureus*.

Materials and Methods: Supernatant and lysate extracts were prepared from *S. cerevisiae* S3 culture. Sub-MIC concentrations of both extracts were separately applied to *S. aureus* ATCC 29213 (methicillin-sensitive *Staphylococcus aureus*; MSSA) and *S. aureus* ATCC 33591 (methicillin-resistant *Staphylococcus aureus*; MRSA) strains. Biofilm formation of these strains was measured by microtiter plate assay and expression level of α-hemolysin and enterotoxin A genes (*hla* and *sea*, respectively) using real-time PCR technique.

Results: The supernatant extract has reduced both biofilm formation and expression of *sea* and *hla* genes, while lysate extract only had anti-biofilm effects. In all tests, the MRSA strain has shown more susceptibility to yeast extracts than MSSA strain.

Conclusion: The current study has exhibited the favorable antagonistic effects of *S. cerevisiae* S3 as probiotic yeast on the MSSA and the MRSA strains. By conducting further studies, the compounds produced by this yeast can be used to control *S. aureus* infections.

Keywords: Biofilm, Exotoxins, Probiotic, *Saccharomyces cerevisiae*, *Staphylococcus aureus*

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Topic: Microbiota and Probiotics

P158: Gut Microbiota and Metabolic Syndrome (Diabetes)

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Introduction and Objectives: Metabolic syndrome and its related pathologies are responsible for hundred millions of mortality and morbidity worldwide. Role of gut microbiota (GM) in the emergence of metabolic syndrome (MS) related pathologies including type 2 diabetes mellitus, metabolism of lipids and carbohydrates or obesity have been discussed in recent years. It seems like that MS is multifactorial and is mainly based on environmental factors. So, in this review, we focused on the critical and recent studies of correlation between GM or probiotic bacteria with the emergence of MS and related pathologies from viewpoints of mechanisms of actions and effects of the change in the microbial population on the health and diseases.
Material and Methods: Search method: This review was done using databases including Pubmed and Google scholar search engines with the key words of metabolic syndrome, diabetes gut microbiota, glucose metabolism, hyperlipidemia and mechanism of action.
Results: The first importance of findings was that the microbial floor of metabolic syndrome pathologies was changing in comparison with health state of humans. Majority of studies (except for a few studies) confirmed the beneficial effects of GM restoration in treatment of MS related pathologies. The other important point of this review was that many of previous known risk factors seem like having no scientific and real correlations with emergence of MS. And instead, microbial imbalances should be included as a critical risk factor.
Conclusion: It seems that previously accepted risk factors such as obesity and aging are the results of imbalances in guts beneficial bacteria and not as a risk factor for induction of diabetes. Certainly, comprehensive studies in the future would clearly illustrate the relation of MS pathologies and gut bacteria.
Keywords: Metabolic syndrome, Diabetes, Obesity, Gut Microbiota,

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Topic: Microbiota and Probiotics

P149: Bacteria probiotic's' and immune system

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Introduction and Objectives: Probiotics are living organisms that can have beneficial effects on human health if administered appropriately. Because the healthy digestive system, directly affects the immune system, probiotics increase the immune system by preventing the development of infectious diseases by producing more lymphocytes.

Method and Materials: In this systematic review, after reviewing the Sid information source and the google scholar search engine, and using library searches, Bacteria, Probiotics and keywords were achieved. The criteria for entry of articles include from 2011 to 2019, the full text of articles was accessible.

Result: Probiotics can produce more healthy bacteria that can restore the microbiotatic balance of the intestine. Types of lactic acid bacteria include Lactobacillus species, Bifidobacterium species, Enterococcus Fissure species, Lactococcus lactis, Lconostosteron Mesenteritis, Pseudococcus Syndicacid, and Streptococcus Thermophiles are from probiotic microorganisms. Antibiotics that cause the destruction of flora of beneficial bacteria in the body and avoid pathogenic bacteria that can grow on the internal and external surfaces and lead to the disease. Intestinal flora can contribute to processing of food antigens in the intestine and probiotics can potentiate building of antigens.

Conclusion: It recommends that probiotic strains should be carried out at least with a series of tests, such as antibiotic resistance patterns, metabolic activities, toxin production, hemolytic activity, and so forth. Consumers with probiotic and high quality supplementation Assisted digestion helps accelerate colon detoxification and balance bacteria in your intestine. More efforts are needed on probiotics.

Keywords: Immune system, Bacteria, Probiotics

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Topic: Molecular Diagnosis

P328: Development of quantification kit for bovine viral diarrhea virus

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Introduction and Objectives: Bovine viral diarrhea virus (BVDV), one of the most bovine pathogens, is belong to the genus *Pestivirus* and family of *Flaviviridae*. Infection with this virus is associated with a wide range of clinical or subclinical manifestations, including transient fever, diarrhea, respiratory problems, abortion and teratogenesis. The aim of this research was development of quantification kit for detection of bovine viral diarrhea virus.

Materials and Methods: Total RNA was extracted from BVDV-infected cell culture and reversed to cDNA by TAKARA cDNA synthesized kit. The 5′ untranslated region of BVDV was amplified using PCR method and cloned into T/A cloning plasmid. The recombinant plasmid was propagated and purified from competent cells *Escherichia coli* DH5a. Ten-fold serial dilutions of the recombinant plasmid was prepared and standard curves were generated by TaqMan Real-Time PCR.

Results: The detection limit of cDNA BVDV copy numbers per µl was 1-10 copy and was applied for infected cell cultures and clinical samples.

Conclusion: This method can detect and quantify BVDV RNA in cell culture or samples with higher specificity and sensitivity than conventional RT-PCR.

Keywords: TaqMan Real-Time RT-PCR; Bovine viral Diarrhea virus; quantification kit

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Topic: Molecular Diagnosis

P49: Determination of the prevalence of *Acinetobacter baumannii* isolated from clinical samples by PCR molecular method in Tehran, Iran

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Introduction*: Acinetobacter baumannii* is an opportunistic gram-negative bacterium that is commonly found in hospital infections, especially in the intensive care units (ICU). The present paper aims at Determination of the prevalence of *Acinetobacter baumannii* isolated from clinical samples by PCR molecular method in the city of Tehran.

Materials and Method: In this study, 200 clinical samples from ulcer, pus, sputum, and blood were collected in Mostafa Khomeini, Tohid and Motahari hospitals in Tehran. The identity of the samples was verified by conventional standard biochemical tests and then by PCR and blaOXA-51-like gene amplification methods.

Results: In this study, 60 isolates of *Acinetobacter baumannii* were identified based on biochemical differential tests, the identity of all of which were confirmed by blaOXA-51-like gene amplification through the PCR method. 25 samples were isolated from the ICU, 17 from infectious ward, 13 from the EMC, and 5 from other departments; by which, 25 blood samples (41.7%), 15 pus samples (25%), 12 ulcer samples (20%), and 8 urine specimens (13.3%) were obtained.

Conclusion: *Acinetobacter baumannii* is one of the most important causes of hospital infections. The highest number of *Acinetobacter baumannii*-positive cases was related to ICU and infectious wards.

Keywords: *Acinetobacter baumannii*, hospital infections, PCR

Topic: Molecular Diagnosis

P113: The prevalence of *Fusobacterium nucleatum* among healthy individuals admitted gastroscopy clinic at Tehran, Iran

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Introduction: *Fusobacterium nucleatum* is an anaerobic microbe that was supposed to be a member of normal human commensal flora. Following the great discovery on *Helicobacter pylori* and its reported association with occurrence of gastric cancer, now it claimed that colorectal cancer may be directly associated with individuals colonized with *Fusobacterium nucleatum.* The purpose of our study is to examine the primary existence of this bacterium among the healthy patients using PCR method.

Materials and methods: In a cross-sectional study, we have examined the colorectal biopsy specimen from the eight consecutive candidates who referred to the gastroscopic clinic, Tehran, Iran. PCR approach was used as diagnostic method in this study. A chi-square test was used to analyze the possible association between presence of *Fusobacterium nucleatum* and diagnosed disease. All measurements of significance were two-tailed with a *P*-value of < 0.05 in our analysis.

Results: None of our suspected biopsy samples were infected with *Fusobacterium nucleatum* using our detection approach (P value > 0.05).

Conclusions: *Fusobacterium nucleatum* is an identified pathogenic bacterium implicated in IBD and is overrepresented in colorectal tumors. However, in our investigation *F. nucleatum* was not observed in healthy individuals. Current findings suggested that *F. nucleatum* may be associated with the progression of gastroenterological diseases include colorectal cancer pending new through and accurate experiments confirming current shaped conclusion.

Keywords: *Fusobacterium nucleatum*, colorectal cancer, PCR method, healthy individuals

Topic: Molecular Diagnosis

P236: Rapid differential diagnosis of vaginal infections using gold nanoparticles coated with specific antibodies Rapid diagnosis of vaginal infections

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Introduction and Objectives: Vaginal infections caused by bacteria, Candida and *Trichomonas vaginalis* affect millions of women annually worldwide. Symptoms and signs have limited value in differential diagnosis of three causes of vaginitis. Current laboratory methods for differential diagnosis are either expensive or time consuming. So, in this work development of a method based on gold nanoparticles has been investigated for rapid diagnosis of vaginal infections.

Materials and Methods: Specific antibodies against three main causes of vaginal infections were raised in rabbits. The antibodies were then purified and conjugated to gold nanoparticles and used in an agglutination test for detection of vaginal infections. Finally, sensitivity and specificity of this test for diagnosis of vaginal infections were estimated using culture method as gold standard.

Results: Purification of antibodies from sera was confirmed by electrophoresis. Construction of nanoparticles was proved by TEM and FTIR methods. Conjugation of antibodies to gold nanoparticles was confirmed using XPS method. Sensitivity and specificity of gold nanoparticles for diagnosis of candida species was 100% and for Gardnerella was 100% and 93% respectively.

Conclusion: Gold nanoparticles-based method is a simple, rapid, accurate and cost-effective test for differential laboratory diagnosis of vaginal infections.

Keywords: Gold nanoparticles, Antibody conjugated, vaginal infection, diagnosis

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Topic: Molecular Diagnosis

P286: Diagnosis of Brucellosis Disease in Blood Samples of Patients Suspected to Brucellosis in Kerman city by Real-Time PCR method

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Introduction and objectives: Brucellosis is a common disease among human and animals which is transmitted to human from animals. Diagnosis of this disease is possible in laboratory through cultivating bacteria, serology, molecular methods, each having their own advantages and flaws.
Materials and methods: Regarding the high sensitivity of Real-time PCR method and several reports insisting on the use of molecular methods together with other testing methods, in this study we use Real-time PCR tests and Rose Bengal tests to diagnose the disease in most suspected human samples. So, 100 blood samples and serums were taken from abattoir staff and people returning to medical laboratories to diagnose this disease and the mentioned tests were done for them.
Results: The results coming back showed 39 ones with positive Rose Bengal test and 54 ones with positive Real-time PCR tests.
Conclusion: Based on the results, we can conclude that reliability of Real-Time PCR test is more than Rose Bengal test to diagnose the disease.
Keywords: Real-Time PCR, Rose Bengal, human, Brucellosis, Kerman

Topic: Molecular Diagnosis

P109: Prevalence of *Fusobacterium nucleatum* infection in colorectal cancer patients

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Introduction: Colorectal cancer (CRC) is the third most common cancer in the world and the fourth most frequent cause of death in the world. Recent findings had showed that there is a meaningful association between the report of CRC and presence of *F. nucleatum*. The aim of our study was to examine the prevalence of *F. nucleatum* isolated from biopsy samples taken from CRC patients.

Materials and methods: A total of 25 biopsy samples from CRC patients were tested to investigate the prevalence of this bacterium among the obtained samples. Following the DNA extraction, a specific primers set were used to amplify the *F. nucleatum* housekeeping gene. Statistical analysis was performed with the statistical package PASW Statistics version 18.01. The level of significance was set at 0.05%.

Results: Overall, sixteen out of 25 CRC samples were *F. nucleatum* positive (*P* value=0.046). No significant association was found between resistance and sex, age, were *F. nucleatum* positivity (*P* value>0.05).

Conclusion: In total, *F. nucleatum* is showing the enough potential to at least partially participate in inducing the colorectal cancer. However, in depth analysis of this bacterium using multicenter studies with larger sample size is required before any clearer conclusion.

Keywords: *F. nucleatum, CRC* patients, PCR method, colonoscopy surgery

Topic: Molecular Diagnosis

P100: The Prevalence of Rectovaginal Colonization and Antibiotic Susceptibility Pattern of Streptococcus agalactiae in Pregnant Women in Al-Zahra Hospital, Rasht, Iran

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Introduction and objectives: Maternal rectovaginal colonization with group B streptococcus (GBS) is a main risk factor for vertical transmission of GBS to newborns and life-threatening neonatal invasive diseases. The aim of this study was investigation of the prevalence of anorectal and vaginal colonization with GBS in late of pregnancy by culture-based and polymerase chain reaction (PCR) methods and antimicrobial susceptibility patterns of the GBS isolates in Rasht, Iran.

Materials and Methods: We analyzed 245 anorectal and vaginal swab samples separately from pregnant women at 35 to 37 weeks of gestation. All samples were cultured after enrichment in a selective Todd-Hewitt broth and then assayed by phenotypic characterizations and PCR method for *cfb* conserved gene. Antimicrobial susceptibility was performed using the Kirby–Bauer method.

Results: In total of 245 vaginal samples, 19 (7.8%) were positive based on culture method and 28 (11.4%) by PCR method. Among 245 rectal samples, 24 (9.8%) were positive by culture and 29 (11.8%) samples were positive by PCR. Of 245 pregnant women studied were found to have 9.7% GBS rectovaginal by culture and 15.9% by PCR methods. All GBS isolates were sensitive to ampicillin (77.2%) and vancomycin (72.2%) and were resistant to Penicillin (88.6%), ceftriaxone (75%), clindamycin (95.4%), azithromycin (86.3%), tetracycline (61.3%), erythromycin (47.7%), and levofloxacin (27.2%).

Conclusions: The results of this study indicate that the frequency of GBS isolation from rectal samples was higher than vaginal samples by both culture and PCR. Our study recommended intrapartum antibiotic prophylaxis against GBS infections based on ampicillin or vancomycin for GBS carriers in Rasht.

Keywords: *Streptococcus agalactiae*, pregnant women, antibiotic susceptibility

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Topic: Molecular Diagnosis

P247: Prevalence of *HopQ* gene in patients with gastric ulcer

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Introduction and objectives: There are various virulence factors which play a role in *helicobacter pylori* pathogenesis. One of these factors is the product of hopQ gene. Different researches all over the world indicate that there is a valuable relation between hopQ alleles and clinical diseases. In Iran, some researches have been conducted too. The purpose of this study is to search the prevalence of hopQ alleles in isolated helicobacter pylori from patients with peptic ulcer.

Materials & Methods: Biopsy specimens from 100 patients with gastric ulcer Helicobacter pylori were collected and separated, different allele hopQ, vacA, cagA was determined by PCR (Polymerase Chain reaction). The relationship between genes the chi-square test and Fisher IBM SPSS Statistics version 21.0 was used for statistical analysis.A*P*value of <0.05 was considered as statistically significant.

Results: In this study of 100 gastric ulcer alleles HopQ abundant HopQ I 54 (54%) and HopQ II 46 (46%) was also the genes of CagA and VacA s1 / s2 respectively, with frequency 51 (51%), VacA s1 83 (83%) and VacA s2 17 (17%). The relationship between the gene and gene I HopQ with CagA (P<0.01), the presence of gene II HopQ with CagA (P<0.01), the presence of VacA s1 Gene I HopQ (P<0.026)the presence of VacA s1 gene HopQ II (P<0.026), gene by gene VacA s2 I HopQ (P<0.026) and the presence of VacA s2 with gene HopQ II (P<0.026), were statistically significant. Also, the presence of gene VacA s1 with CagA (P<0.726) and the presence of the gene VacA s2 (P<0.726) CagA statistically significant relationship was not found.

Conclusion: The results can be HopQ impact on virulence genes and CagA and VacA relationship between gene in patients with peptic ulcer to argue. Further studies in this direction would be to identify strategies for physicians and the anti-peptic ulcer disease, gastritis and gastric cancer to improve.

Keywords: Helicobacter pylori, HopQ gene, Peptic ulcer, outer membrane proteins

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Topic: Molecular Diagnosis

P246: Comparison of the Frequencies of *traT*, *ompT*, *hlyD* and *cnf1* virulence genes in *Escherichia coli* Isolates from hospitalized and non-hospitalized Patients with Urinary Tract Infections

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Introduction and Objectives: Global studies revealed that 80-90% of community acquired urinary tract infections (UTIs) and 30-50% of hospital acquired UTIs were due to uropathogenic *E. coli* (UPEC). UPEC have multiple virulence factors that are essential for stability, colonization and pathogenicity in urinary tract. The aim of this study was comparison of frequencies of some virulence genes in *E. coli* isolates from the community acquired and hospital acquired UTIs patients.

Materials and Methods: On hundred *E. coli* were isolated from urines of 49 hospitalized and 51 non- hospitalized UTIs patients that referred to laboratory of Shahid Faghihi hospital in Shiraz. Genomes of these isolates were extracted using the boiling method. Frequencies of *traT, ompT, hlyD* and *cnf1* genes were evaluated by PCR technique. Statistical analysis of the data was performed using SPSS software.

Results: Overall, the highest frequency (71%) was obtained for *traT* gene which encodes conjugal transfer surface exclusion protein TraT and is responsible for serum resistance against phagocytosis. Thereafter, frequency of *ompT* which encodes an outer membrane protease and is responsible for protection of UPEC against antimicrobial protamines of immune system was 54%. Frequencies of genes encoding toxins that contribute to pathogenicity of these strains were 18% and 12% for α-hemolysin (*hlyD*) and cytotoxic necrotizing factor 1 (*cnf1*) genes, respectively. In isolates of hospitalized and non- hospitalized UTIs patients the frequencies of *traT, ompT, hlyD* and *cnf1* genes were 65.3% vs. 76.5%, 51% vs. 56.9%, 16.3% vs. 19.6%, and 12.2% vs. 11.8%, respectively. Comparison of the virulence genes' frequencies in these two groups revealed no significant difference ($P > 0.05$).

Conclusion: Existence of several virulence genes in UPEC has influence on UTIs severity. So evaluation of the frequencies of virulence genes can be helpful for prediction of UPEC pathogenicity and the severity of the resulted UTIs.

Keywords: Uropathogenic *E. coli*, Virulence gene, Hospital and community acquired UTIs.

Topic: Molecular Diagnosis

P116: Prevalence of *Helicobacter pylori* and *Staphylococcus aureus*in human gastric biopsy specimens

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Introduction and objectives: The human stomach is anintolerable microniche ecosystem hosting wide range of persistent bacteria. Regardless the *Helicobacter pylori*(*H. pylori*), there are some clue that *Staphylococcus aureus*(*S. aureus*) can also colonized at this site (1). In current study, we aim to investigate the exact prevalence of these two major bacteria in human gastric biopsies.

Materials and methods: In this study, stomach biopsy samples were collected from patients withgastric disease referring to the gastroenterology section at Mehrad Hospital and Labafi-nejad Hospital, Tehran, Iran. The biopsies obtained in tubescontaining Thioglycollate(4°C) will be shippedto the laboratory for further bacterial analysis. DNA extraction using a commercial genomic extraction kit was conducted before PCR approach. The clinical control samples were also used as positive control in this study.

Results: We analyzed the gastric biopsy samples from 45 patients who presented for endoscopy at Mehrad Hospital and Labafi-nejad Hospital. The patients were 45(71%) and 13 (28.8%) men and women, respectively, with a median age of 48 years (range, 16 to 80 years). In this study, we have identified bacterial species from gastric biopsies of 12 *H. pylori*-positive and 16 *H. pylori*-negative suffering from gastric diseases (95% Cl : 1.03 – 5.1,p value : 0.04)& 9 *S.aureus* positive and 19 *S.aureus* negative suffering from gastric diseases (95% Cl: 0.5 – 5.8, pvalue: 0.3). No association was found between age, gender and certain bacterial colonization.

Conclusion: Current body of evidences in the field of gastrointestinal tract research are suggesting that any effective antibiotic therapy against *H. pylori* should have considered co-existence of *S. aureus*. Further molecular analysis using biopsy samples are needed before final conclusion about current drawn idea.

Keywords: *H. pylori*, *Staphylococcus aureus*, PCR, colonization

Topic: Molecular Diagnosis

P110: The Prevalence of *Pseudomonas aeruginosa* isolates recovered from the gastric mucosa of dyspeptic Patients admitted to the Mehrad Hospital, Tehran, Iran

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Introduction: The gastric acidic condition inhibits the rapid growth of many common digested bacteria. However, our understanding about current composition of gastric biota is limited. Helicobacter pylori is the regular colonizer of human stomach but we are unaware of other bacteria. *Pseudomonas aeruginosa* is one of bacteria listed as potential colonizers of the stomach while we have no data about its exact prevalence in this microniche.
Materials and methods: A total of 25 biopsy samples obtained from dyspeptic patient were examined to determine the prevalence of *P. aeruginosa* using specific PCR method. Our criteria to choose this the samples was being infected with *H. pylori*.
Results: Overall, 16 out of 25 dyspeptic samples were *H. pylori* positive (*P* value=0.005), 15 out of 16 *H. pylori* positive were *P. aeruginosa* positive. A total 9 non-dyspeptic patients obtained 7 *P. aeruginosa* positive samples.
Conclusion: *Pseudomonas aeruginosa* seems a critical member of bacteria colonizing the human stomach when *H. pylori* colonization. We need more studies to identify the exact association between acid suppressive drugs and overgrowth by such opportunist bacteria such as *P. aeruginosa* in dyspeptic patients.

Keywords: *Pseudomonas aeruginosa, PCR method,* Dyspeptic Patients, endoscopy surgery

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Topic: Molecular Diagnosis

P277: Metagenome Extraction of Respiratory Samples and Evaluation of Bacterial Diversity via PCR-DGGE

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Introduction and objective: DNA extraction is a fundamental procedure in any culture independent approach to determine microbial diversity. Methods used for this purpose should accurately reflect the diversity. Due to low microorganism amount in some clinical samples including respiratory samples, DNA extraction method is problematic to get an appropriate yield. In this study, three methods of DNA extraction from pharyngeal swab were evaluated. Then, PCR and PCR-DGGE techniques were carried out for these DNA extractions.

Materials and methods: Pharyngeal swabs were transferred to laboratory in TSB tubes. In the first method, DNA was extracted by regular boiling technique. In the second method, chloroform technique without phenol was used. In the third method, lysis buffer was at first added to pharyngeal samples, and further steps followed for second method. To increase the DNA yield, a physical cell lysis step using glass beads was added to all methods. Afterwards, two sets of universal bacterial primers for 16s rDNA (27f , 1492r and GC-341f , 782r) were used and the final product of the second pair primers surveyed with PCR-DGGE technique.

Results: Highest DNA concentration was obtained from the first method, and lowest DNA concentration was achieved in the third method. However, PCR products were successfully achieved using DNA extraction of the third method when the number of bacterial cells was low. PCR-DGGE result of each sample shows a similar pattern for all methods.

Conclusion: Similar bacterial diversity was reflected by different extraction methods. Although using the first method which is fast, economical and simple, is recommended for samples with high number of bacteria, for samples with low number of bacteria third method is recommended.

Keywords: DNA extraction, Bacterial diversity, PCR-DGGE

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Topic: Molecular Diagnosis

P97: Isolation and Identification of Different Bacterial Species from Bronchoalveolar Lavage of Cystic Fibrosis Patients in Shiraz

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Introduction and objectives: Cystic Fibrosis is the most prevalent autosomal recessive hereditary disease in the world that affects various organs of the body. The most important cause of death in these patients is lung involvement, and 90% of children with CF do not survive due to lung problems. The aims of this study were to evaluation bacteria that presence in bronchoalveolar lavage of CF patients.

Materials and Methods: In these study 30 samples of bronchoalveolar lavage and sputum of CF patients were examined by culture and biochemical method. Then different isolates were confirmed by PCR of 16S rRNA gene and finally sequencing was performed.

Results: In this study, the most bacterial isolate from bronchoalveolar lavage of CF patients were involved in polymicrobial infections, including *Staphylococcus, Pseudomonas, Neisseria flavorans* (according to DNA sequencing), *Delftia acidovorans* (according to DNA sequencing) and *Klebsiella.*

Conclusion: Identifying the bacteria that are colonized the lungs of CF patients is important. Although *P. aeruginosa* is one of the main bacteria, attention to the isolation of other bacteria can be useful in the selection of antibiotics.

Keywords: Cystic Fibrosis,16S rRNA sequencing, *Pseudomonas aeruginosa*

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Topic: Molecular Diagnosis

P299: Recombinant scFv Antibodies against M2e Protein of Influenza A virus

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Introduction and Objectives: The *M* gene of Influenza A virus encodes two conserved proteins, M1 (a capsid protein) and M2 (an ion-channel protein). The M2 protein is an integral membrane protein consisting of an ectodomain (M2e) at the N-terminus. M2e, which consists of 24 N-terminal residues, is remarkably conserved, and the N-terminal epitope SLLTEVET (residues 2-9) is conserved across almost all studied subtypes of Influenza A viruses (99.3%). Recombinant antibodies are new generation of monoclonal antibodies, which are isolated via phage display technology from immune or non-immune phage libraries against target antigens. These antibodies are used for diagnosis of many different antigens and therapeutics proposes. The object of the present study was to isolate recombinant monoclonal antibodies against this important virus.

Materials and Methods: The purified Influenza A virus surface antigen, M2e, was coated to immunotubes and used as a target for selection of antibodies from the Tomlinson I and J phage display libraries of single-chain fragment variable (scFv) antibodies. Clones that were able to recognize antigen were isolated in three rounds of binding, elution and amplification. The specificity of scFv antibodies chosen from the resulting panel, were confirmed using enzyme-linked immunosorbent assay (ELISA), dot blotting and western blotting methods.

Results: Recombinant antibodies capable of recognizing M2e antigen were isolated with high affinity; and their specificity was approved.

Conclusion: Isolated soluble single chain antibodies are good candidates to apply as monoclonal recombinant antibodies in diagnostic kits for detection of Influenza A virus in contaminated samples.

Keywords: Recombinant antibody, scFv, Influenza A virus, M2e protein.

Topic: Molecular Diagnosis

P273: Diagnosis of *Mycoplasma Pneumonia* in Patients with Respiratory Tract Infections Using Three Methods of Culture, ELISA and PCR Molecular in Tehran Province, Iran

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Introduction and objectives: *Mycoplasma pneumonia* is the most commonly Mycroplasma species cause of human pathogens, including upper respiratory tract infection and atypical pulmonary pneumonia. *Mycoplasma pneumoniae* is also known to be one of the most commonly pathogens causing community-acquired pneumonia (CAP) in some populations, especially in children.
Materials and Methods: In this study, 100 samples of patients with respiratory infections (52% female and 48% male, mean age of 53/62 years and range from 17 to 85 years old) were collected. Selection of suspected cases of *Mycoplasma pneumoniae* respiratory tract infections was conducted with the diagnosis of a super pulmonologist based on clinical symptoms. For the ELISA test, serum samples, PCR and culture in PPLOBroth liquid medium and PPLOagar solid medium from throat swab samples of patients with respiratory tract infections were used. In this study, *Mycoplasma pneumonia* standard strain (ATCC: 29342) was used.
Results: In this study, 17 samples (17%) for Mycoplasma (16SrRNA gene) and 6 (6%) for existence of *Mycoplasma pneumonia* (P1 gene) were reported positive, using specific primers. 37 cases (37%) were positive for ELISA and 14 (14%) colonies related to mycoplasma in PPLOAgar medium.
Conclusion: The results of this study showed that *Mycoplasma pneumonia* is one of the pathogens that cause respiratory tract infections in humans. The PCR molecular method is a quick and sensitive technique which has a high sensitivity and specificity in comparison with other methods such as ELISA and culture.
Keywords: *Mycoplasma Pneumonia*, Culture, ELISA, PCR

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Topic: Molecular Diagnosis

P320: *Yersinia ruckeri* strains Population diversity in rainbow trouts from West Azarbaijan by DGGE technic

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Introduction and Objectives: *Yersinia ruckeri* is a gram-negative stain bacillus and the causative agent of salmonids' enteric redmouth disease. the present research aimed to study the probable strain diversity of *Yersinia ruckeri* by DGGE (Denaturing Gradient Gel Electrophoresis) technic and evaluate the prevalence of the disease in West Azarbaijan' rainbow trout farms.

Materials and Methods: In this regard firstly 124 individual infected fish were obtained from south of West Azarbaijan province. using biochemical tests65 samples were detected as positive samples which were subsequently tested by PCR method of 16S rRNA fragment. using the recent molecular method only 40 samples were confirmed.

Results: Finally, at this study, the prevalence of Yersiniosis in West Azarbaijan' were obtained 31.74 percent. also, the variation of the experiment, from 40 samples, 37 samples (92.5 percent) as biotype 1 and number 3 cases (7.5 percent) were identified as biotype 2.

Conclusion: In a complementary study by DGGE technic showed that the variation of *Yersinia ruckeri* strain not any variation in the population of *Yersinia ruckeri* in all the samples that obtain from West Azarbaijan.

Keywords: *Yersinia ruckeri*, DGGE, rainbow trout

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Topic: Molecular Epidemiology

P323: Survey on presence of *Brucella* genus bacteria in uterine samples collected from referred dogs to the veterinary hospital of Shahid Bahonar University of Kerman

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Introduction and Objectives: Brucellosis as a very important universal zoonotic disease is caused by *Brucella* spp. Dogs, same as many other animals, are considered as a reservoir of this bacteria and can infect human and other animals by shedding this pathogen in their excretions. This is the first investigation to study the reproductive samples related to canine brucellosis in Iran.

Materials and Methods: In this study, the existence of the causative agent of *Brucella* was determined in the uterine samples (70 samples) of dogs attending to the veterinary hospital of Shahid Bahonar University of Kerman. The samples were collected and assessed for the presence of *Brucella* by Real- time PCR.

Result: *Brucella* was identified in 1 out 70 collected samples.

Conclusion: According to the results of this study, dogs should be considered as an important source of this zoonotic pathogen. Detection of *Brucella* in uterine samples emphasizes the need for further investigation of brucellosis in the small animals of this area.

Keywords: Brucellosis, Brucella genus, PCR, Uterine samples, Dog.

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Topic: Molecular Epidemiology

P322: Detection of *Brucella* genus in vaginal samples collected from the dogs of Kerman city by PCR

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Introduction and Objectives: Brucellosis by *Brucella* spp. is a zoonosis disease of public health concern primarily transmitted by ruminants. Dogs has been implicated in the transmission of the infection but their involvement in the epidemiology of brucellosis has been poorly investigated. This is the first investigation to study the reproductive samples related to canine brucellosis in Iran.

Materials and Methods: In this study, we determine the existence of the causative agent of *Brucella* in the vaginal samples of dogs attending to the veterinary hospital of Shahid Bahonar University of Kerman, and also the vaginal swabs collected from a number of breeding kennels. Samples were assessed for the presence of *Brucella* genus by the PCR method.

Result: *Brucella* spp. was identified in 3 out of 70 collected samples from referred dogs and 32 Out of 64 specimens of breeding dogs.

Conclusion: According to the results of this study, dogs should be considered as an important source of this zoonotic pathogen. Detection of *Brucella* in vaginal samples emphasizes the need for further investigation of brucellosis in small animals.

Keywords: Brucellosis, Brucella genus, PCR, Vaginal samples, Dog

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Topic: Molecular Epidemiology

P115: The prevalence of Diarrheagenic *Escherichia coli* and *Shigella* species isolated from patients with gastroenteritis infection referred to Shiraz University of Medical Science hospital (2018-2019)

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Introduction and Objectives: Diarrheal illnesses are a severe public health problem and a major cause of morbidity and mortality in infants and young children, especially in developing countries. A number of different bacterial pathogens such as *Shigella* and diarrheagenic *Escherichia coli* (DEC) with different frequency patterns are isolated as the causes of acute diarrhea in both developed and developing countries. This study was conducted to find the etiology of acute diarrhea in infants and young children.

Materials and Methods: Included in this study were children under the age of 15 who, complaining about diarrhea, referred to the emergency wards of two major hospitals in Shiraz (i.e., Dastgheyb and Namazi). All stool samples were investigated for *Salmonella*, *Shigella*, and DEC. To identify *Shigella* and different groups of *E. coli*, their associated specific primers were utilized.

Results: A total of 300 samples from patients with acute diarrhea were analyzed. The most common (48/190 or 25.2% of positive cases) was DEC and the most prevalent of which were the groups EPEC, EAEC, EIEC, and ETEC comprising 35.4%, 33.3%, 20.8%, and 10.4% of DEC cases, respectively.

Out of the 50 *Shigella* isolates, 34 (68%) isolates were identified as *S. sonnei*, 15 (30%) serotypes were identified as *Shigella flexneri*, 1 (2%) serotypes were identifed as *Shigella boydii*.

Conclusion: This study suggests that EPEC and *Shigella* strains are important contributors to diarrhea in Iranian children. Because of the weakness of routine microbiological tests and poor specifity of serological tests, it is recommended that the EPEC and *Shigella* strains are better detected by molecular methods.

Keywords: Diarrheagenic *Escherichia coli* (DEC), *Shigella*, enteropathogenic, acute diarrhea

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Topic: Molecular Epidemiology

P111: Evaluation of the prevalence of Legionella pneumophila in Iranian clinical samples: A systematic review and meta-analysis

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Introduction and Objectives: *Legionella pneumophila* is the main cause for community-acquired pneumonia especially in hospital environments. In this systematic review and meta-analysis, we evaluated the prevalence of L. pneumophila in clinical samples obtained from Iranian patients.

Materials and Methods: The studies reporting *L. pneumophila* prevalence in Iranian clinical samples that were published between January 2000 and July 2016 were recruited. Comprehensive Meta-Analysis Software (version 3.3.070) was used for quantitative data analysis. Because of high heterogeneity between the studies according to the Cochrane Q and I2 statistics, a random-effects model was used for meta-analysis.

Results: Sixteen studies encompassing 1956 subjects were included in the meta-analysis. The overall prevalence of *L. pneumophila* was9.6% in clinical samples obtained from the Iranian patients. The age spectrum ranged from 6 months to 80 years old. Dyspnea and cough comprised the most common clinical manifestations. In the subgroup analysis, the prevalence of *L. pneumophila* was higher in studies with sample size ≤100 (12.9%) in comparison with studies with sample size>100 (8.4%). In addition, the prevalence of *L. pneumophila* was higher in the years 2009–2016 (9.2%) compared with 2000–2008 (0.7%).

Conclusion: *L. pneumophila* is a major cause of community- and hospital-acquired pneumonia. It is of pivotal importance to implement sensitive and reliable molecular and culture-based techniques to detect and control this infection in healthcare environments

Keywords: Iran, *Legionella pneumophila*, Clinical samples

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Topic: Molecular Epidemiology

P13: Epidemiology investigation of malaria outbreak in some provinces of Iran during 1382-1397: A review

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Introduction and Objectives: Malaria is one of the most important parasitic diseases worldwide, which is characterized by high morbidity and mortality in tropical and subtropical regions. The purpose of this article is a literature search on the epidemiology and outbreak of malaria in some provinces of Iran between 1382 and 1397.
Materials and Methods: From the scientific databases such as the SID, Magiran, PubMed, ScienceDirect, 40 published articles during the years 1382 to 1397 were collected and the epidemiology of malaria in some provinces (including: Qom, Kohgiluyeh and Boyer Ahmad, Homozgan, Isfahan, Hamedan, Mazandaran, Kerman, Kermanshah, Zanjan, Sistan and Balouchestan) was investigated for the following cases: The method of screening, the method of study, the used software and statistical methods, the number of patients with malaria, the percentage of male and female patients, the percentage of Iranian and non-Iranian people, the status of epidemiology, the type and percentage of plasmodium parasite and finally the trend of eradication of the disease.
Results: Malaria is known as the most common transmitted disease by mosquitoes in the south and south-east of Iran, especially the provinces of Sistan and Baluchestan, Hormozgan and Kerman.
Anopheles stephensi, Anopheles delta, Anopheles superpictus, Anopheles fluviatilis, Anopheles coliophasis are the vectors of malaria in Iran. More than 90% of the plasmodium parasites are the type vivax and the rest of the falciparum and mixed species. Most malaria cases are males and aged between 20 and 30, and the outbreak of disease is more than the type of imported transmission. According to the World Health Organization's 2017 survey, there were 57 indigenous malaria cases and imported malaria cases in Iran, indicating a significant reduction in the number of malaria patients in Iran.
Conclusion: Climate conditions, rivers and water resources, stagnant waters, fountains and rivulet, palms, existence of agricultural fields, temperature, precipitation, relative humidity, intensity and direction of wind are the most important climatic factors affecting growth, propagation and the completion of the plasmodium parasite and the anopheles mosquitoes are effective in the onset and outbreak of malaria. Immigration, pilgrimage, tourism attractions and job opportunities and ultimately traveling from other provinces and neighboring countries are effective in the transmission and spread of malaria. According to the WHO, with operational programs, Iran is one of the eastern Mediterranean countries, which indigenous malaria will be completely eradicated by 2020.

Keywords: Malaria, Epidemiology, Anopheles, Plasmodium falciparum, Vivax, Climate condition, Iran.

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Topic: Molecular Epidemiology

P40: Prevalence and Molecular Characteristics of Carbapenemase-Producing *Enterobacteriaceae* in Intensive Care Units: a cross-sectional study in North of Iran

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Introduction and Objectives: Since carbapenems are the last line treatment option for multi-drug resistant *Enterobacteriaceae*, increasing resistance to these antibiotics has been an important concern around the world. Thus, the aim of this study was to investigate the phenotypic and genotypic prevalence of carbapenemase-producing *Enterobacteriaceae*.

Materials and Methods: One hundred non-repeated clinical samples were collected from patients hospitalized in Intensive Care Units of four teaching and treatment hospitals of Sari, North of Iran. The API 20E system was used to identification of the *Enterobacteriaceae*. Also, the minimum inhibitory concentration (MIC) of the carbapenems was detected by *E*-test. The phenotypic Modified Hodge Test (MHT) and Combined Disk Test (CDT) were used to determine the carbapenemase producer clinical isolates, and the polymerase chain reaction (PCR) method was done to study the presence of *NDM*-1, *VIM* and *OXA*-48 genes in chromosomes of the isolates.

Results: The most enterobacterial isolates in the present study included *Escherichia coli* (37%), *Klebsiella pneumoniae* (21%), and *Serratia rubidaea* (10%), respectively. The most and least effective antibiotics against the clinical isolates were amikacin and meropenem with resistance rates of 24% and 73%, correspondingly, while 51% of the isolates were imipenem resistant. Also, 58 isolates were detected as multi-drug resistant (MDR) of which all of them were carbapenem-resistant. Among 73 carbapenem-resistant isolates, 35 (47.9%) and 33 (45.2%) of them were MHT and CDT positive, respectively. Moreover, 30.1%, 31.5%, and 38.3% of these isolates contained *VIM*, *NDM*-1, and *OXA*-48 carbapenemase encoding genes, correspondingly.

Conclusions: This study showed that the MHT is not a reliable test for the study of strains producing OXA-48 or VIM carbapenemases. Also, there was a significant relationship between the presence of carbapenem resistance genes and *Klebsiella pneumoniae* isolates. It seems that the presence of at least two of these genes can be one of the main reasons for resistance to carbapenems in *Enterobacteriaceae*.

Keywords: *Enterobacteriaceae*, Carbapenem, *NDM*-1, *VIM*, *OXA*-48.

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Topic: Molecular Epidemiology

P173: *Aspergillus* diversity in the environments of nosocomial infection cases at a University hospital

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Introduction and Objectives: *Aspergillus* species as opportunistic infections have been increasingly reported in human and immunosuppressive patients per year worldwide. The main object was to use a rapid and cheap molecular method for monitoring of *Aspergillus* infections and epidemiological approaches.

Materials and Methods: Different molecular methods such as restriction fragment length polymorphism based on amplification of ribosomal RNA have been employed to identify Aspergilli at the level of species. The subject of our study was a group of hospitalized patients with clinical and subclinical signs of infection. All of the collected clinical specimens were transported to the medical mycology lab and examined for *Aspergillus* identification.

Results: Environmental specimens were collected from air and surfaces inspected for the *Aspergillus* hospital sources. At first, a growth characteristics and microscopic features on mycological media for the identification of *Aspergillus* species were performed. For the confirmation of *Aspergillus* isolates which similarly found in clinical and environmental sources, molecular method polymerase chain reaction/restriction fragment length polymorphism was carried out. From the mentioned specimens, 102 fungal isolates included *Candida* spp., *Aspergillus* spp. and other fungi. Among the clinical isolates; *Aspergillus flavus* (47%), *Aspergillus fumigatus* (29.4%) and *Aspergillus niger* (23.5%) were the most frequent species respectively. Also, *Aspergillus* isolates from environmental were *Aspergillus niger* (43.7%), *Aspergillus flavus* (41.7%), *Aspergillus fumigatus* (14.6%).

Conclusion: Therefore, polymerase chain reaction-restriction fragment length polymorphism with a single restriction enzyme can be very useful in identification of *Aspergillus spp.*, because of its facility in use, speed, robust, and high sensitivity of diagnosis.

Keywords: *Aspergillus*, identification, molecular, hospital.

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Topic: Molecular Epidemiology

P279: Molecular Typing of *Klebsiella pneumoniae* Using Pulse Field Gel Electrophoresis in Kurdistan province, Iran

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Introduction and Objectives: *Klebsiella pneumoniae* is among the most common causes of hospital-acquired infections and one of the important opportunistic pathogens has occurred as a crucial threat to public health. The perpose of this study was to determine the molecular typing of *Klebsiella pneumoniae* isolates using pulse field gel electrophoresis (PFGE) method.

Materials and Methods: 70 *Klebsiella pneumoniae* isolates were isolated from clinical specimens in hospitals of Kurdistan province, Iran. After conformation of isolates by microbiological standard tests, the genotyping of isolates were performed using PFGE. The calculation of similarities was done by the Dice coefficient, and the unweighted paired group assay was used for cluster analysis (UPGMA).

Results: For all clinical isolates, PFGE was performed. then the bands were analyzed using gels observed with BioNumerics version 7.6. According to the results of the analysis of isolated bands in 39 clusters with similarity greater than 80% (≥80% similarity) classified.

Conclusions: The presence of the isolates with similar genotype may show a common origin and consequently, the dissemination of strains in hospitals. Our results indicate the genotypic variation of *Klebsiella pneumoniae* isolates suggest the different origins for isolates.

Keywords: Molecular Typing; Pulsed-Field Gel Electrophoresis (PFGE); *Klebsiella pneumoniae*; Kurdistan province

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Topic: Molecular Epidemiology

P278: Molecular Typing of *Klebsiella pneumoniae* isolates using Repetitive Extragenic Palindromic Sequence-Based PCR in Kurdistan province, Iran

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Introduction and Objectives: *Klebsiella pneumoniae* is one of the main causes of hospital infection and a member of Enterobacteriaceae family. The main purpose of the present study was determine the repetitive extragenic palindromic polymerase chain reaction (rep-PCR) pattern among *Klebsiella pneumoniae* isolates in Kurdistan province, Iran.

Materials and Methods: During a period from October 2015 to July 2016, 70 *Klebsiella pneumoniae* isolates were collected from different clinical specimens from general hospitals in Kurdistan province, Iran. The genetic relation of isolates was performed using rep-PCR typing assay.

Results: 27clusters distinguished by the result of fingerprinting by rep-PCR. this pattern of isolates showed a wide diversity of clinical isolates in Kurdistan province, Iran.

Conclusion: the present study revealed the emergence and spread of *Klebsiella pneumoniae* isolates with diverse genetic backgrounds in a hospital in Kurdistan province.

Keywords: *Klebsiella pneumoniae*; repetitive extragenic palindromic polymerase chain reaction (rep-PCR) typing; Kurdistan province

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Topic: Molecular Epidemiology

P58: Frequency of biofilm associated genes among *S. aureus* isolated from nasal carriers in two teaching hospitals in Yasuj city by PCR method

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Introduction and Objectives: *Staphylococcus aureus* is one of the most commonly bacterial pathogens can colonize anterior nares. Biofilm formation is one of the most important problem in treatment of S. aureus infection. Biofilm production by *S. aureus* cause more colonization and also development of antibiotic resistance. The aim of this study was to investigate the frequency of biofilm related genes among S. aureus isolated from anterior nare of Health care workers of Shahid Beheshti and Imam Sajjad hospital in Yasuj city in 2016.

Materials and Methods: In this cross-sectional study, 143 isolates of Staphylococcus aureus were collected from the anterior nares of HCW of Shahid Beheshti and Imam Sajjad hospitals in Yasuj city. Polymerase Chain reaction was used to detection of biofilm related genes such as: *bap*, *clf*A, *fnb*A and *ica*D.

Results: The *fnb*A gene was the most frequent gene detected in 125 (87.41%) of *S. aureus*isolates. The *ica*D and *clf*A genes detected in 60.13%and 57.5% of isolates respectively. The *bap* gene was not detected in any isolates. In addition, none of the tested genes were identified in 16 (11.2%) of isolates. Genetic patterns showed that *ica*D / *fnb*A / *clf*A gene pattern with the highest frequency identified in49% of isolates and followed by the fnbA, icaD / fnbA and fnbA / clfA gene patterns detected in 20.3%, 11.2% and 7% of isolates respectively. The *clf*A gene pattern identified in only 1.4% of isolates.

Conclusion: Regarding to the high prevalence of biofilm related genes in *S. aureus* isolates and also the importance of biofilm in colonization, adopting effective treatment and control protocols are necessary. Continues observation for infection control program is essential for spreading of disease and infection control.

Keywords: Biofilm related genes, *Staphylococcus aureus*, Nasal carriers

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Topic: Molecular Epidemiology

P89: Correlation between group B streptococcus GBS capsular typing, tetracycline and macrolide resistance genes in colonizing maternal-newborn

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Introduction and Objectives: Group B streptococcus (GBS) is one of important causative agents of infection in newborns. The distribution of GBS trains with reduced penicillin susceptibility and increasing resistance to macrolides are considered as global concern. The aim objective of the current study was to determine the relationship between GBS isolates of colonizing maternal-newborn and frequency of tetracyclin and erythromycin resistance genes in pregnant women.

Materials and Methods: Samples were collected from recto-vaginal of pregnant women and the ears and noses of their newborns from 2012 to 2014, Semnan province. Capsular types and resistance genes to erythromycin and tetracycline were detect in GBS using PCR and multiplex PCR method.

Results: GBS prevalence was in rectum 9.5%, vagina 27.9%, in the ear & nose infants respectively 28.6% & 26.1%. The highest and lowest antibiotic resistance were observed in tetracycline, erythromycin and gentamicin. The frequency of capsular typing including type III 20 (33.3%), II12 (20%), Ib10 (16.7%), v 9 (15%), IV (8.3%). 6.7% of samples were nontypable. The capsular genes VIII, VII, Ia andVI not detected. There was a significant correlation between tetM, ermB, and ermA gene with type of capsular, but it wasn't relationship between tetK, ermTR andermC with capsular types.

Conclusions: The multi resistance to penicillin, macrolides and tetracycline in type IIa III with correlation between type II, III and tetM, ermB, ermA genes could be as serious problem for treatment of GBS infections.

Keywords: GBS, Antibiotic resistance, Capsular typing

Topic: Molecular Typing

P129: Phylogenic grouping of Avian Pathogenic *Escherichia coli* (APEC) isolated from suspected broiler chickens to colibacillosis in industrial chickens farms in the Hamedan province

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Introduction and Objectives: Avian colibacillosis is an acute systemic disease can cause extraintestinal infections such as aerosacculitis, polyserositis, perihepatitis, salpingitis, pericarditis, osteomyelitis and septicemia in birds. The most considered agent of avian colibacillosis is Avian-Pathogenic *Escherichia coli* (APEC). APEC could be responsible for economic loss in the poultry industry and also has zoonotic importance. Several methods including serotyping, pathotyping and phylothping have been used for *E.coli* typing and assigning. APEC isolate can be assigned by Clermont *E. coli* phylo-typing method a useful and inexpensive genetic tool for bacterial typing. Based on this technique APEC strains are belong to eight phylo-groups (A, B1, B2, C, D, E, F and *Escherichia* clade I).The most prevalent phylogroups associated with colibacillosis are phylogroup A and D. The aim of the present study was to assignment of the phylogenetic group in an APEC isolated from suspected broiler chickens to colibacillosis.

Materials and Methods: The present study was carried out on APEC strains (n= 100) isolated from suspected broiler chickens to colibacillosis in industrial chickens farms in the Hamedan province. The bacterial strains DNA were assigned to phylogenetic typing using the revised Clermont *E. coli* phylo-typing method a Quadruplex PCR based procedure.

Results: The Clermont *E. coli* phylo-typing method results showed that over 81% of APEC isolates can be assigned to eight phylo-groups (A, B1, B2, C, D, E, F and *Escherichia* clade I) and 19% of isolates were not ascribable to any group. Out of 100 APEC strains the predominant phylogroups are phylogroup E (23 isolates) and D (20 isolates).

Conclusion: Our finding indicates that most predominant phylogroups of APEC detected from broilers is E and D phylogroup, however future study and application of new methods for APEC typing is necessary.

Keywords: Avian-Pathogenic *Escherichia coli* (APEC), Colibacillosis, Broiler, phylo-typing.

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Topic: Molecular Typing

P269: Molecular typing of Iranian strains of *Brucella abortus* and *Brucella melitensis* using RAPD-PCR

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Introduction and Objectives: Molecular Typing of pathogenic bacteria are introduced to identify local and regional strains, tracing the source of infection, probable changes in native strains, and even an evolutionary study of phylogenic trees. The objective of this study was to identify DNA polymorphisms and genomic fingerprinting of Iranian Brucella strains using RAPD-PCR technique.

Materials and Methods: 101 strains of Brucella, including seven strains of reference (S.99, 16M., RB51, S.19 544, Rev.1, H.38) and 94 Iranian field strains from different geographical regions during 1340 to 1382 were investigated. Brucella strains were cultured on Brucella broth, Brucella agar according to recommended methods. And the purity of strains cultivated with standard methods was assured. Then, all species were determined by phage-typing. Total DNA was extracted from the strains using three methods, including a phenol, Chlorophorm and Iso amilalchol. Optimization of RAPD-PCR conditions with nine primers of 13 single individual primers and 13 pair individuals primers with two different strains of *abortus* and *melitenesis* were performed separately. The best result was obtained with the AP4 peptide primer with a sequence of 3 \\ 5 \\ -TCA CGC TGC A-A and the primer pair REP- AP4 with 15-mer sequence, 5-CGC TTA TCG GCC TAC-3 and AP4 for Brucella species for the first time.

Results: 101 strains of Brucella strain with AP4 primer were evaluated with optimum RAPD-PCR conditions. The final PCR product in 5.1% electrophoresis gel with ethidium bromide staining revealed 72 different patterns of DNA from 101 strains of Brucella. This method, even among strains within a biotype of the phenomenon of polymorphism, was shown to be repeatable. Within the five genotypes, two to four different biotypes were also included.

Conclusion: RAPD can be useful method to distinguish related bacterial species, Despite the presence of high polymorphism in DNA. in this study, there was a presence of infection among animals of different Brucella biotypes, *Brucella abortus*, Biotype III, and *Brucella melitensis* Biotype 1, which are still dominant and indigenous Iranian strains in the past four decades. This technique appears to be a simple, quick and sensitive for the epidemiological investigation of brucellosis.

Keywords: RAPD-PCR, Brucella, Molecular typing, *Brucella* strains.

Topic: Molecular Typing

P101: Molecular typing of *Acinetobacter baumannii* isolated from ICU patients by ERIC-PCR

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Introduction and Objective: *Acinetobacter baumannii* is an opportunistic Gram-negative pathogen that is being reported with increasing frequency as causes of nosocomial infection. *A. baumannii* is mostly a cause of septicemia, pneumonia and urinary tract infection following hospitalization of patients. This organism has been shown resistance to different antimicrobial agents. Multidrug resistant (MDR) *A baumannii* strains are first line causes of infection, especially in patients hospitalized at intensive care units (ICUs). The aim of this cross sectional study was typing of *Acinetobacteria* and determining the relevant clones that can help in the selection of appropriate antibiotic therapies.

Materials and Methods: *A.baumannii* strains were isolated from a total of 100 samples from ICU. These samples were mainly respiratory samples. Enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) fingerprinting was used to determine the clonal relationship between the different isolated strains.

Result: Out of 100 clinical samples, 62 *A.baumannii* were isolate and confirmed. Using ERIC-PCR fingerprinting genotype analysis, 62 strains of *A. baumannii* were clustered into seven groups. our results revealed that seven main clusters were responsible for the prevalence of *A. baumannii* complex strains at the ICU.

Conclusions: This study found that the increase in the frequency of *A. baumannii* in patients at ICU. The use of molecular epidemiological methods can help us with the detection of the pathogen and preventing from spreading of these resistant strains.

Keywords: *Acinetobacter baumannii*, Typing, ERIC-PCR

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Topic: Molecular Typingy

P96: Genotyping pattern of *Staphylococcus aureus* isolated from nasal carriers among health care workers in two hospitals (Imam Sajjad and Shahid Beheshti) in Yasuj city using coa-typing method.

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Introduction and objectives: *Staphylococcus aureus* can colonizes variety of body sites such as skin, coetaneous membrane and especially anterior nares. The coagulase gene (***coa***) typing is a technique in which the organism is typed based on the polymorphic region of the ***coa*** gene. The aim of the present study was to determine the ***coa*** based typing of ***S. aureus*** isolates from health care workers in two teaching hospitals in Yasuj city.

Materials and Methods: In this cross- sectional study a total of 125 ***S. aureus*** isolates were collected from the nasal nares of healthcare workers in Shahid Beheshti and Imam Sajjad Hospitals in Yasuj city. For typing of *S. aureus* isolates, amplification of the 3'-end region of the coa gene was performed. Finally PCR products were digested with *HaeIII* restriction enzymes and then were electrophoresed on 1.2% agarose gel, and visualized under UV illumination. Bacterial strains were typed according to the DNA bands with diverse size and numbers in each strain. Data were analyzed by descriptive statistics tests.

Results: Amplification of the **coa** gene showed single band in nine different patterns ranging approximately from 400 to 860 base pair. The **coa** gene with 610 bp size was seen in 36 isolates and was considered a predominant type. After digestion of PCR products with *HaeIII* restriction enzymes, totally 15 distinct **coa** gene RFLP patterns, numbered C1 to C15, were observed. The C3 was the most frequent **coa** type with 24 isolates. There is no specific coa types belong to wards or professions in each hospitals.

Conclusion: *S. aureus* isolated from anterior nares of HCWs showed genetic diversity in their coa gene, but only a few coa gene variants were predominant. Hence no prominent coa type was detected according to the wards or types of profession, the sources of bacteria for colonization in anterior nares of HCWs may be related to the hospital environmental outside.

Keywords: *Staphylococcus aureus*, nasal carrier, coa typing, health care workers

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Topic: Mycobacteria and Acid-Fast Bacteria

P102: Current understanding of the *Mycobacterium avium* subsp. *paratuberculosis* genetic diversity in Iran, studied by RFLP-IS*900*

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Introduction and Objectives: In Iran, paratuberculosis has been long a frequently-diagnosed disease in cattle and sheep and goat herds. Only little is known on genotypic variation of the *Mycobacterium avium* subsp. *paratuberculosis* (MAP) population in this country. So the resent study surveyed genetic diversity of an Iranian isolates using RFLP-IS*900*.

Materials and Methods: In the present study, 46 archived bovines, ovine and caprine indigenous MAP isolates (representing four municipal provinces) plus two laboratory strains of MAP 316F and III & V were sub-cultured on plain mycobactin J-supplemented Herrold's egg-yolk slants. All the recovered isolates/strains were subsequently subjected to IS*900* RFLP analysis using *Pst*I.

Results: Totally, ten RFLP patterns were identified with three of them singletons and seven shared types. The identical RFLP type shown by both MAP 316F and III & V strains was categorically different from that of the Iranian field isolates. The largest pattern, represented by 22 isolates, recovered from bovine and caprine. This specific pattern is highly similar to type circulating G, then type B represented by 11 isolates, recovered from all the three animals. Such lack of difference observed in the Iranian environment, is very likely to indicate that the most frequent MAP strains can infect multiple host species.

Conclusion: More study on genetic diversity of MAP in Iran urges using restriction fragment length polymorphism and alternative genotyping system.

Keywords: paratuberculosis، *Pst*I، Restriction Fragment Length Polymorphism

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Topic: Mycobacteria and Acid-Fast Bacteria

P327: Mysterious absence of sheep type strains in MAP Iranian isolates

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Introduction and Objectives: In Iran, paratuberculosis has been long existed in cattle farms and sheep and goat herds. Only little is known on epidemiology of the *Mycobacterium avium* subsp. *paratuberculosis* (MAP) population in this country.

Materials and Methods: in order to better understand of epidemiology, 142 suspicious feces, milk, intestine and lymph node samples from bovine, ovine and caprine hosts from Ahwaz, Alborz, Fars, Isfahan, Golestan, Kerman, North Khorasan, Tehran and Qazvin provinces were collected and cultured on mycobactin-J supplemented Herrold's egg slopes. Then positive culture were confirmed using PCR-IS900 and were analyzed using PCR-Multiplex DMC.

Results: In this study, out of 142 suspicious samples 47 samples were positive culture and were confirmed using PCR-IS900. The result showed that all of the MAP isolated from bovine, ovine and caprine were cattle type strains. This data can mentioned that cattle type among MAP Iranian isolates can infect other host species. However absence of sheep type in Iranian environment is vague because the sheep breeding industry has been existed for long time. This specific type is highly similar to Europe such as Britain. In other hand, animal importation (imported cattle from England to the farm of Abadan Oil Company) is as an assumption which is strongly supported.

Conclusion: Of course, further research is needed to better understand this theory.

Keywords: paratuberculosis، epidemiology، PCR-Multiplex DMC، cattle type strains.

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Topic: Mycobacteria and Acid-Fast Bacteria

P122: First isolation and molecular characterization of *Mycobacterium porcinum* and *Mycobacterium celeriflavum* for potential use in cases of polycyclic aromatic hydrocarbons bioremediation' From markazi province of Iran

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Introduction and Objectives: Polycyclic aromatic hydrocarbons are one of the prevalent oil pollutant. Nowadays these carbohydrates due to their Toxicity, mutagenesis, carcinogenicity, and also environmental stability caused by hydrophobia character and low solubility are considered as one of the preferences of environmental protection agancy. Therefore, clearance of the regions polluted with these compounds are of significance. Biodegradation of these compounds is a safe and affordable method of environmental clearance. In this research, we described the molcular isolation and identification of the mycobacteruim's strains and analyzed their polycyclic aromatic hydrocarbons degration activity.

Materials and Methods: *Mycobacteria* were isolated from a collection of 30 environmental samples from the contaminated sites of Markazi province and identified to the species level using conventional microbiological and molecular methods including the PCR amplification of hsp65 and sequence analysis of, 16S rRNA genetic markers. The growth rate of the isolates in presence of pollutants, chromatography and turbidity were used to assess their biodegradation activity.

Results: A total of 6 mycobacterial isolates (20%) were recovered from 30 samples that belonged to two species of mycobacteruim consisting of *M. porcinum* (4 isolates) and *M. celeriflavom* (2 isolates). The strains of of *M. porcinum* and *M. celeriflavom* could degrade 70% and 90% of 1 mg/l PAH soloution in 7daye.

Conclusion: Our results showed that the of *M. porcinum* and *M. celeriflavom* have a high ability to biodegradation the polycyclic aromatic hydrocarbins. Hence, additional investigations are recomended for isolation and applicationally use of the bacteria's strains for biological deletion of polycyclis aromatic hydrocarbons from contaminated environments.

Keywords: nontubercolous *Mycobacterium*, Biodegradation, 16SrRNA.

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P11: Prevalence and antimicrobial resistance patterns of *Clostridium difficile* isolates in Kerman hospitals of Iran

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Introduction and Objectives: *Clostridium difficile* (*C. difficile*), an anaerobic, spore forming bacillus remains the leading cause of nosocomial-acquired diarrhea that known as *C. difficile* infection (CDI). This study investigated the prevalence and antimicrobial resistance patterns of *C. difficile* isolated from hospitalized patients and patients seen as outpatients suffering from diarrhoea in Kerman, Iran, over a 2-year (2016 to 2018).

Materials and Methods: A total of 151 stool specimens were collected and screened for the presence of *C. difficile* and discovered its toxins by culture, enzyme immunoassay (EIA), and PCR methods. Moreover, the confirmed *C. difficile* isolates were tested against twelve antibiotics (metronidazole, vancomycin, clindamycin, tetracycline, erythromycin, ciprofloxacin, levofloxacin, moxifloxacin, fusidic acid, piperacillin, piperacillin/tazobactam and rifampicin) by disk diffusion method, according to the CLSI, EUCAST, and CA-SFM guidelines.

Results: Out of 151 patients, 66 (43.71%) cases were positive for *C. difficile* by PCR. Two (1.32%) patients were only positive for *C. difficile* toxins based on EIA. A total of 292 clostridial isolates were obtained from specimens by culture, where 133 (45.55%) isolates finally confirmed as *C. difficile* by PCR. One hundred and seven (88.43%) isolates were resistant to at least three antibiotics and defined as multidrug resistant strains. Different and diverse resistant patterns to the antimicrobial drugs were seen among the isolates.

Conclusions: This is the first report of the isolation of *C. difficile* from Kerman hospitals. Results indicate that CDI might be an important nosocomial infection in different hospital wards. Moreover, this study provides a good comprehensive picture of the MDR phenotype characteristics of *C. difficile* isolates in Iran.

Keywords: *Clostridium difficile;* Prevalence; Antimicrobial Resistance; Kerman; EIA; PCR

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P95: Investigating the Causes of Hospital Pneumonia Infection in ICU In the Selected Hospital in Kerman

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Introduction and Objectives: Hospital pneumonia is an important cause of death and the second most commonly reported nosocomial infections due to hospital acquired infections that occur 48 hours or more after admission, and also includes ventilator-dependent pneumonia. The aim of this study was to Investigating the Causes of Hospital Pneumonia Infection in ICU in the Selected Hospital in Kerman.

Materials and Methods: This analytical-descriptive cross-sectional study was performed on 105 patients admitted to the ICU of Kerman-based educational-educational hospital for 6 months from the beginning of December 2018 to the end of June. Data collection was based on a questionnaire designed by the NNIS in the ICU. The collected data were analyzed by SPSS version 22 software.

Results: Of the 300 patients hospitalized in the ICU, 105 were infected with the hospital. 57 (54.3%) were infected with pneumonia, and the remaining 43 (45.7%) were infected with other infections, 17 of them (17 %) Died due to a hospital infection, of which 7 (7%) died due to pneumonia and 88 (83%) others either recovered or died due to other causes. The most commonly occurring strains of acute respiratory infection were Acinetobacter, with a frequency of 59%.

Conclusion: Due to the increased frequency of pneumonia infections compared to other infections in the ICU due to the prevalence of mechanical ventilation, increasing the duration of use of these devices will increase the likelihood of pneumonia infections, which requires awareness And the performance of health care personnel will improve the proper method and principles of disinfection, and by planning and policy on disinfection and sterilization of equipment, the probability of transmitting a hospital infection is minimized.

Keywords: Hospital Pneumonia Infection, Intensive Care Unit, Kerman

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P60: Evaluation of vancomycin Resistance and *MecA* Gene in *Staphylococcus aureus* Isolates of nosocomial and community acquired infections in Bandar Abbas in 2017-2018

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Introduction and Objectives: *Staphylococcus aureus* is a gram-positive coccus, known as one of the most important human pathogens. Methicillin resistant *S. aureus* (MRSA) is a public health concern. Hence, the present study was conducted to determine the pattern of antibiotic resistance and identification of the mecA gene in order to better understand the epidemiology of this bacterium in Bandar Abbas.

Materials and Methods: This descriptive cross-sectional study was conducted on 100 clinical isolates of nosocomial and community acquired infections in Payambar-Azam hospital, Bandar Abbas in 2017-2018. To identify methicillin-resistant isolates, Cefoxitin disc(30µg) was used and antimicrobial susceptibility patterns were performed according to the CLSI method against Azithromycin, Tetracycline, Tigecycline, Linezolid, Clindamycin, Ciprofloxacin, and Gentamicin. Minimum inhibitory concentration (MIC) of vancomycin was measured using the E.test method and the presence of *mecA* gene in MRSA was investigated using PCR method.

Results: The highest resistance rates was to Cefoxitin, Tetracycline, Gentamicin, Azithromycin, and Clindamycin. The MIC of Vancomycin was between 0.75 and 5 µg/mL. Thirty-eight (38%) isolates carrying *MecA* gene, where 50 (50%) isolates were obtained from community and 50 (50%) isolates from hospital-acquired infections.

Conclusion: MRSA are increasing threat faced to society and hospitalized patients. Acquired community infections occur in healthy people without any apparent risk factors and can act as a source of transmission to the community. In present, planning to set up appropriate treatment protocols for hospitals and preventing the distribution of MRSA infections in the community is one of the most inevitable and essential requirements.

Keywords: Staphylococcus aureus ،Methicillin Resistance،Drug Resistance, mecA Protein.

Topic: Nosocomial Infections

P44: Bacterial contamination in concentrate platelate bags sachets that expire dated in Blood Transfusion Center in yasuj city in 2018

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Introduction and Objective: Today in blood transfusion center donors' blood is screening is for some microorganisms. However, since all microorganisms aren't examined Human sepsis associated with blood transfusion is still a major health concern. Therefore, the aim of this study was to evaluate bacterial contamination in concentrate platelate bags sachets that expire dated in Blood Transfusion Center in yasuj city in 2018.

Materials & Methods: Totally 118 units of platelet bags were randomly collected, and with using standard microbiology techniques, the presence of microorganisms, were identified and then confirmed by polymerase chain reaction (PCR) in the presence of 16SrRNA genes. sensivity and resistance of these microorganisms determined by standard antibiotics.

Results: Out of 118 platelet bags, 3 of them showed contamination, that prevalence of bacterial contamination was 2.55%. All of the isolated microorganisms were gram positive such as: (staphylococcus hominis, staphylococcus haemolyticus and staphylocous warneri) and their antibiotic sensitivity and resistance to some antibiotics the most sensitive antibiotic was ampicillin against S. warneri bacteria and the lowest sensitivity was penicillin antibiotic against S. warneri bacteria.

Conclusion: Few microorganisms are tested in blood transfusion. other microorganisms caused blood and blood derivatives contamination including platelets in blood transmission center this research showed that only three contaminations from 118 platelate bags while in another city which are almost consistent with this study the reasons of these contamination can be Tips that were taken during and after blood transfusion and the anticoagulant agents may not be met.

Keywords: Bacterial Contamination - Platelet - Antibiotic Resistance - 16srRNA Gene

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P108: Molecular identification of *Staphylococcus* spp in blood infection patients in Rafsanjan city

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Introduction and Objectives: Bacterial blood infection causes High mortality on patients, so it has a threat role on infected people, so early diagnosis and cure is necessary. *Staphylococcus* are known as the most important factor of hospital infections, which contains %30 of whole hospital infections and %50 of bloodstream infections. In this study, the molecular identification of *Staphylococcus* spp in Suspicious samples to sepsis has been done by PCR method.

Materials and Methods: A total of 200 blood samples were collected from patient's whit suspected sepsis whom referred to Rafsanjan Medical Laboratory. The amount of 100 µl of blood samples were cultivated in Blood Agar and Nutrient Broth respectively. DNA extraction was performed by DNG-Plus kit and PCR method was done to detection of *Staphylococcus* spp bacteria.

Results: Of 200 samples collected, 28 samples were cultured in a liquid culture, after it, 24 of them were discriminated as *Staphylococcus* spp bacteria by molecular mathod. Compared to other works has been done on the frequency of this bacteria in the development of blood infections, our results in Rafsanjan city is similar to other studies.

Conclusion: Molecular techniques such as PCR have a high accuracy and high sensitivity and it is recommended to use this method to detect an infection.

Keywords: *Staphylococcus* spp, sepsis, PCR

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P307: Optimization of Expression and Characterization of Polytopic Protein as a New Vaccine Candidate against *Pseudomonas aeruginosa* Infections

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Introduction and Objectives: Nowadays, *P. aeruginosa*, the highly regarded opportunistic pathogen, is the leading cause of morbidity and mortality in patients with compromised defenses and chronic diseases such as cystic fibrosis. Due to the emergence of highly resistant strains, at present, aggressive antibiotic therapy is the only choice for management of *P. aeruginosa* infections. Therefore, the development of novel alternative therapeutics including an effective vaccine, is necessary. Several *P. aeruginosa* antigens have been tested for vaccine development, including OprF (major outer membrane protein), ExoA (Exotoxin A), and LecB (fucose-binding lectin) which plays an important role in pathogenesis of *P. aeruginosa.*

The aim of the study was to design a multi-epitope vaccine based on OprF, ExoA and LecB proteins and optimize the expression of polytopic construct contains these genes from *P. aeruginosa* as a vaccine candidate against *P. aeruginosa* infections.

Materials and Methods: In order to design the polytopic construct, we predicted the most probable immunogenic epitopes of OprF, ExoA and LecB using bioinformatics methods. The chimeric gene was introduced into a pET28a vector and expressed in *Escherichia coli* BL21 and its expression was analyzed by SDS-PAGE and western blotting. Finally, in order to optimize the expression of the recombinant protein, cell density, induction time, growth temperature, IPTG (Isopro- pyl β-D-1-thiogalactopyranoside) concentration were studied.

Results: Expression of recombinant fusion protein by *E. coli* using pET22b vector resulted in production of chimeric protein in high concentration. Optimum condition for recombinant protein expression was determined at OD 600 of 0.8, 0.5mM IPTG, six hours incubation time at 30 ºC and BL21 host.

Conclusion: These results suggest that recombinant chimeric protein can be produced in the laboratory and expression can be optimized. Moreover, by purification of recombinant protein and evaluation of its immunogenicity in mice, it can be used as a vaccine candidate against the *P. aeruginosa*.

Keywords: *P. aeruginosa*, OprF , ExoA and LecB, Polytopic vaccine

Topic: Nosocomial Infections

P103: Review of the effect of infection control training on the knowledge of operation room technologists in the Tehran's public hospitals

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Introduction and Objectives: Hospital infections are one of the problems of the last century and ulceration is one of the most important complications after surgery. Failure to comply with health by operation room technologists increases the chances of a patient's infection. Management of wound infection the surgical site is one of the essential skills in this treatment group. The purpose of this study was to determine the effect of infection control training on the knowledge of operation room technologists in the Tehran's public hospitals.

Material and Methods: In this study, valid scientific articles were indexed in ISI, PubMed, Scopus, Sid, Magiran databases using key words (Education, infection control, operation room). The range of time from 2012 to 2018 was considered for the selection of papers. The articles were found in 90 articles, of which 56 articles were included in the study, and then these articles were evaluated in terms of title, abstract, and full text. After removing repetitive and unrelated, 32 articles related to research were selected.

Results: The results of the studies showed that the infection control training was associated with increased awareness, from 48.48% pre-test to 80% post-test. The difference was statistically significant (P <0.001) with paired t-test.

Conclusion: Implementation of training program operation room technologists increased awareness about infection control. The attention of the authorities and the design of the necessary measures to educate this group in the fields of knowledge and practice seems necessary.

Keywords: Education, Infection Control, Operation Room

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P243: Prevalence of Panton valentine, *mecA, SCCmec IV* and *SCCmec V* genes in the *Staphylococcus aureus* hospital isolates of Northern Iran

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Introduction and Objectives: Methicilin-Resistant *Staphylococcus aureus* (MRSA) is one of the most important causative agents of nosocomial infections. The present study deals with frequency of PVL gene and its relationship with *mecA, SCCmecIV, SCCmec V* genes in this bacterium.

Materials and Methods: Ninty two clinical isolates of *S. aureus* were obtained within six months of sampling. Antibiotic sensitivity was conducted by use of Disk Difussion method, and resistance-to-methicilin was tested by Disk oxacillin disk. Those isolates which harbored *pvl* gene were screened for possession of *mecA*, *SCCmecIV* and *SCCmec* genes using specific primers for each gene.

Results: Eight of the isolates harbored *mecA* gene. There were 18 *pvl* postive isolates. 5 isolates of *SCCmec IVa* (27.77), *IVb* and *IVd* each of which 2 isolates and 3 isolates of *SCCmec IVh* were positive, respectively. They were negative in terms of possession of *SCCmec IVc* and *SCCmecV* chromosomal casts. Results of accurate Fisher test showed that, in significance level of 0.05, there was no relationships between *pvl*, *mecA* and *SCCmec IVa,b,c* genes. Approximately, 20% of the samples had *pvl* gene and 50% of them had *mecA* gene. Almost, 60% contained *SCCmec* chromosomal casts. Also, *SCCmec IVa* was the most plentiful trait in the samples.

Conclusion: Our study showed that resistance to methicillin rapidly develops in hospital environment. This can be very worrisome for systems of hygienic and therapeutic cares.

Keywords: *Staphylococcus aureus*, Leukocidin Panton valentine, *SCCmec*, *mecA*.

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Topic: Parasitic Infections

P342: Safety of gentamicin-attenuated *Leishmania infantum* in domestic dogs

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Introduction and Objectives: *Leishmania infantum* (*L. infantum*), a causative agent of canine visceral leishmanias (CVL). The parasite is located visceral organs of dog including in the spleen and liver. There has been much interest in attempts to vaccinate against *L. infection*, because leishmanicidal drugs are cost and drug resistances have been reported. We reported that the attenuated line of *L. infantum* is safe and no clinicopathological changes induced following injection of the attenuated line of L. infantum in German shepherd dogs. In the present study, we showed no clinical signs of CVL were found in the domestic dogs.
Methods and Materials: Parasites: Promastigotes of *L. infantum* were cultivated in complete RPMI (GIBCO) supplemented with 10% FCS and incubated at 25° C in air for 7 days. The stationary phase of promastigotes was harvested and a suspension with concentration of 5 x 10^8 cells/ml in PBS were prepared.
Dogs: Two dogs were injected intravenously (i.v.) with 100 ml of this suspension per kg of body weight. The experiment was terminated after twelve months and the clinical signs of disease were recorded.
Results: None of the dogs showed any abnormalities and clinical signs of disease during the observation period. In addition, no histopathological changes were seen in the liver and spleen of the dogs.
Conclusion: The gentamicin-attenuated line of *L. infantum* is safe in the mixed bread dogs.
Keywords: Gentamicin- attenuated line of *Leishmania infantum*, vaccine, safety, dog

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Topic: Parasitic Infections

P341: Exploring transcription factors in *Echinococcus granulosus*

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Introduction and Objectives: Recent advantages in Next-generation RNA sequencing (RNA-seq) in Echinococcus species has developed a revolutionary opportunity for an in-depth, genome-wide view of cestodes transcriptome and considerably more advantages in the detection of novel transcripts, allele-specific expression, and alternative splicing in compare with other methods. The rate of transcription of genetic information from DNA to messenger RNA is controlled with transcription factors (TF) by binding these proteins to a specific DNA sequence.

Materials and Methods: All proteins sequences of E. granolusus (10,273) was collected from the published genomes of E. granulosus (PRJEB121) in WormBase ParaSite. The FASTA format file was manually uploaded in Animal Transcription Factor DataBase (AnimalTFDB) tool to explore the most comprehensive and accurate information for E. granulosus TFs and cofactors. In order to identify transcription factor in any species, AnimalTFDB does the give-and-take best-hit BLAST between the human and other species with the conditions set as e-value<=1e-4, coverage>=50%, identity>=30%.

Results: Exploring the whole proteins sequences of E. granolusus was classified into 379 transcription factors into 56 Family. Interestingly, three TFs, zf-C2H2, Homeobox, and zf-H2C2-2 have the most domain number with 77, 57, and 24 respectively. These TF Family has less domain number in comparison to other organisms, especially the model helminths organism Caenorhabditis elegans with 100 and 86 for zf-C2H2, Homeobox respectively. However, no significant zf-H2C2_2 TFs was predicted in C. elegans.

Conclusion: Most zoonotic such as Echinococcus species are regularly exposed to a variety of intermediate and definitive host stress conditions. Therefore, they have developed multipurpose systems like transcription factors for accurate signaling transduction. Therefore, identification of these critical regulatory factors could provide fundamental importance to elucidate nature biology of parasitic helminths.

Keywords: *Echinococcus granulosus*, Next-generation sequencing, RNA-seq

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Topic: Parasitic Infections

P14: Diagnose of malaria infection with light microscopy and Nested- PCR methods and phylogenetic Analysis in peripheral blood expansions obtained from suspected patients in Hormozgan and Kerman provinces

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Introduction and Objective: Malaria remains an important public health problem. This infection is a life-threatening disease caused by with *Plasmodium* protozoa. Rapid diagnostic tests (RDTs), for accurate and reliable diagnosing malaria infection is necessary. The purpose of this study was to diagnose of malaria infection with light microscopy (LM) and Nested- PCR methods and phylogenetic Analysis in peripheral blood expansions obtained from suspected patients in Hormozgan and Kerman provinces.

Materials and Methods: 38 and 4 blood smears collected from Hormozgan and Kerman provinces, respectively. These blood smears were evaluated by light microscopy and Nested- PCR methods. Then, nine *Plasmodium*-positive samples were used to genetic diversity determination.

Results: light microscopy results of Hormozgan province show that 4 *P. falciparum*, 33 *p. vivax* and 1 mixed infection with *P. falciparum* and *P. vivax*. For Kerman province, only 4 *P. vivax* was detected by LM. Nested PCR showed there is 2 *P. falciparum*, 31 *p. vivax* and 5 mixed infection with *P. falciparum* and *P. vivax* in Hormozgan province. In addition, Nested PCR confirmed that 100% (n=4) in Kerman province is P. *vivax.* Phylogenic results showed that the isolates H19, H41, H42, H33, A plu 3 and B plu 3 belong to Hormozgan province and isolates f K2, K3 and K4 belong to Kerman province, respectively. Isolate H33 located in subclade I and isolates H19, H42, and H41 were in sub clade II. Isolates of K 2, K3 and K4 placed in sub clade III. All of subclade I to III were *p. vivax.* Subclade IV involved A plu 3 and B plu 3 isolates and were *p. falciparum.*

Conclusion: Use of rapid diagnostic tests such as Nested- PCR for accurate and reliable diagnosing malaria infection especially in endemic areas are recommended.

Keywords: Malaria, Light microscopy, Nested PCR, *Plasmodium falciparum*, *Plasmodium vivax.*

Topic: Parasitic Infections

P338: Investigation of the frequency of Free-Living Amoebae contamination in Swimming Pools of Kerman City using morphological methods in 2018-2019

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Introduction and Objectives: FLA (free-living amoebae) are considered pathogenic for human. These ubiquitous organisms have been isolated from different environment such as water, soil and air. An investigation was conducted to determine the presence of FLA, especially *Acanthamoeba*, *Naegleria* and *Vermamoeba* in swimming pools of Kerman, Iran

Materials and Methods: Sixty-eight water samples were collected from 2 sites 1 meter from the wall, and middle of swimming pools of Kerman in two men and women during 2018 summer. PH, chlorine and water temperature were measured. The samples were filtered using nitrocellulose syringe (0.45 µm); subsequently, they were cultured on 1.2% non-nutrient agar, covered by killed *Escherichia coli* and incubated at 25 and 37ºC. Plates were then monitored for the presence of amoebae daily and positive plates were cloned.

Result: Out of the 68 water samples, 34 (50%) samples were positive for free-living amoebae in the culture method. including 52.9% *Acanthamoeba*, 20.6% *Vermamoeba*, 14.7% *Naegleria* and 11.8% mixed *Acanthamoeba* and *Vermamoeba*. The highest pollution was at sites 1 meter from the wall of the pools (58.8%).

Conclusion: This study indicated the high prevalence of free-living amoebae, especially the pathogenic type, in the water pools of Kerman that could be a source of infection risk for humans. These water sources could be a potential risk factor for the public health. Therefore, the health professionals should prevent contamination.

Keywords: *Acanthamoeba*, Swimming Pool, Free living amoebae

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Topic: Parasitic Infections

P15: Detection of malaria infection with light microscopy and Nested- PCR methods and phylogenetic Analysis in peripheral blood expansion obtained from Sistan- Baluchestan Province

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Introduction and Objectives: Malaria is still one of the most important life-threatening infection disease. According to the WHO reports, there were 215 million cases of malaria in 2015. Malaria cause by the genus *Plasmodium* include *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. The aim of this study was to detect of malaria infection with light microscopy and Nested- PCR methods and phylogenetic Analysis in peripheral blood expansion obtained from Sistan- Baluchestan Province.

Materials and Methods: 55 blood smears were collected from patients with suspected to malaria in a 6-year period in Sistan- Baluchestan Province. Diagnosis of *plasmodium* species was performed by Light microscopy and Nested- PCR methods. In addition, seven *Plasmodium*-positive samples were used to genetic diversity determination.

Results: In total, 55 of 55 (100%) cases were positive by LM and included 3 *P. falciparum* (3.1%), 48 *p. vivax* (49.48%) and 4 mixed infection with *P. falciparum* and *P. vivax* (4.12%). 94.54% (n=52) were positive with Nested PCR and involved 45 *P. vivax* (46.39%) and 7 (7.21%) mixed infection with *P. falciparum* and *P. vivax.* The results of phylogenetic analysis showed that S25, S26, S61 plu 3, S62, S72 plu 3, S74, and S75 isolates belong to Sistan- Baluchestan province. These isolates located three different subclades. These subclades were named I to III. Isolates S26, S75, S62, were in sub clade I, S25 and S74 isolates located in subclade II. All of subclade I and II were *P. vivax.* Subclade III involved S61 plu3 and S72 plu3 isolates and were *P. falciparum.*

Conclusion: Nested PCR had high sensitivity and specificity than microscopy method and was considered as a good approach for malaria detection.

Keywords: Malaria, Light microscopy, Nested PCR, *Plasmodium falciparum*, *Plasmodium vivax*,

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P16: The Prevalence of Parasitic Contamination of Paper Money in Fars Province of Iran

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Introduction: Money is one of the most commonly used microbial carriers. Parasites are no exception to this rule. The aim of this study was to investigate the presence of parasitic species in paper money collected from various sources in Fars province and to suggest ways to improve the health of the community.

Methods: For this study, firstly, banknotes were collected from people between March 2018 and March 2019. Those were randomly collected from different parts of rural and urban areas of Fars province from various sources including butchers, bakers, supermarkets, gas stations and vegetable stores and stored at DW. After those exit from the water the solution was centrifuged at 3000 RPM. Then surface water was drained and expanded from the remaining materials and stained with Giemsa color and was seen under the microscope.

Results: In the urban areas from 54 contamination, 2 (3.7%) of the contaminations were related to Giardia, 22 (40.7%) to E. coli, 8 (14.8%) to Endolimax nana, 4 (7.4%) to Ascaris, 4 (7.4%) to Hookworm, 12 (22.3%) to Unknown larvae, 2 (3.7%) to Hymenolepis nana. In the rural areas, 4 (11.7%) of the infections were related to giardia, 8 (23.5%) to *E.coli*, 6 (17.6%) to Endolimax nana, 2 (5.8%) Ascaris, 4 (11.7%) Hookworm, 10 (29.4%) Unknown larvae.

Conclusion: According to results since Money has many contacts with hands, it can be concluded that hand hygiene is important for promoting community health.

Keywords: infection, paper money, Parasites, health

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Topic: Parasitic Infections

P26: Frequency of Toxoplasma gondii infection among HIV infected clients referred to Shiraz HIV/AIDS research center, 2012-2019

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Introduction and Objectives: Toxoplasma gondii, the obligate intracellular parasite, is responsible for both acute and chronic toxoplasmosis. Immunocompromised patients are at greatest risk for developing acute toxoplasmosis. The prevalence of T. gondii infection depends on geographical areas and population groups. Generally, the seroprevalence in Iran is 39%. The purpose of this study was to determine the frequency of T. gondii infection among HIV-positive patients in Shiraz, Iran.

Materials and Methods: This cross-sectional study was conducted on 600 HIV-positive patients referred to Shiraz Voluntary Counseling and Testing center within 7 years from 2012 to 2019. The diagnosis of HIV-seropositive patients was confirmed by Western Blot test. Toxoplasma gondii antibodies were determined by IgG ELISA. Patients were divided into five age groups and Toxoplasma seroprevalence was evaluated in each age group.

Results: Of total 600 HIV positive patients enrolled in this study, 34.1% were females and 65.9% were males. The mean age of the samples was 40.3 years (SD 10.8 years). Among the HIV-positive individuals, 143 (23.8%) were anti-T. gondii IgG antibody seropositive. The seroprevalence of toxoplasmosis were significantly higher in age groups of 31–40 and 41 -50 years old ($P<0.05$). The seroprevalence of toxoplasmosis in patients with $CD4^{+}<100$ cells/µl was not significantly higher than the other groups ($P=0.48$).

Conclusion: This study showed a lower seroprevalence of latent toxoplasmosis among HIV- positive patients referred to Shiraz Voluntary Counseling and Testing center compared to other studies done in Iran. Advises about preventive behaviors should be considered in HIV infected individuals with Toxoplasma seronegative.

Keywords: HIV, Toxoplasma gondii

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Topic: Parasitic Infections

P326: Experimental *L.infantum* infection in cats, Molecular and histopathological study

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introduction and objectives: Visceral leishmaniosis (Kala-azar) is a fatal sporadic zoonotic disease in many parts of Iran whilst it is endemic in some areas. Dogs constitute the main domestic reservoir for *Leishmania infantum* in Iran but incidence of the disease in cats in some geographical parts, suggested the feline role in the epidemiology of visceral leishmaniosis in our country. In the present study, molecular and pathological findings, were evaluated in cats experimentally infected by *L. infantum*.

Materials and Methods: Eighteen healthy adult cats were divided in three groups, 6 each. Six cats were intravenously inoculated with 10^9 and the other group received 10^8 stationary phase promastigotes of *L. infantum* and the remaining six cats were used as the uninfected control group. During a 24-week period, after clinical examination, blood was collected from jugular vein and PCR was performed. Bone marrow aspirates were obtained at 2 months intervals and at the end of the study, the cats were euthanized and histopathological examination was carried out.

Results: Two weeks after inoculation, nested PCR was able to detect *L. infantum* in the blood samples of the inoculated cats in two groups and remained positive until 24 weeks. PCR of bone marrow became positive two months later but amastigote phase of parasite was observed in bone marrow by cytological examination three months following inoculation. At the end of the study, three of liver and lymph nodes samples were positive by PCR test. In contrast, in histopathological examination, amastigotes were not observed in the liver, kidney, spleen, lungs, intestine, lymph nodes, and brain samples at the end of the study. Splenomegaly, sinus histiocytosis of spleen, lymphoid hyperplasia and depletion, liver congestion, sinusoidal distension and fatty liver change were detected in some cats.

Conclusion: Based on the results of molecular findings, *L. infantun* infiltrated in the bone marrow 2 months after inoculation; however, it was not observed in reticuloendothelial organs. These findings showed probably the natural resistance and slow progression of kala-azar and the considerable sensitivity of PCR assays in cats. Cats may be implicated as a secondary reservoir for *L. infantum* transmission due to long lasting parasitemia. Further studies are essential to describe the immune system function and pathogenesis of *L. infantum* in cats.

Keywords: Cat, Visceral leishmaniosis, Reservoir, Experimental infection, pathology

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Topic: Parasitic Infections

P17: Detection of malaria using blood smear and nested-PCR for suspected patients in south-eastern Iran: A country close to malaria elimination with a high miss diagnosis by light microscopy and RDT

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Introduction and objects: malaria is highly endemic in south-east parts of Iran and the prompt assessment of the malaria cases is dependent on the sensitive and specific malaria identification. The aim of our study is to assess the efficacy and agreement of Light microscopy (LM) and Rapid diagnostic test (*Pf*HRP-2/pLDH RDT) against Nested-PCR.

Materials and Method: In a cross-sectional study, we assessed all malaria suspects that referred to Razi hospital in Saravan city, Sistan and Baluchestan, Iran. The patients' demographics, microscopy data, RDT, and Nested-PCR results were gathered. The Nested-PCR results were set as reference and the other methods were compared against it. All the results were entered in SPSS version 16 and analyzed.

Results: the sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and *Kc* were 55.3%, 100%, 100%, 94.3%, and 0.671 for LM and 55.3%, 99.6%, 95.4%, 94.3%, and 0.602, for RDT, respectively. Sn, Sp, PPV, NPV, and *Kc* of LM were 55.7%, 100%, 100%, 96.2%, and 0.714 for *p. vivax* and 25%, 100%, 100%, 97.9%, and 0.393 for *plasmodium falciparum*, respectively. Furthermore, the Sn, Sp, PPV, NPV, and *Kc* of RDT were 57.7%, 99.6%, 93.7%, 96.2%, and 0.695 for *p. vivax* and 25%, 99.6%, 66.7%, 97.9%, and 0.354 for *p. falciparum*.

Conclusion: both methods were sensitive, specific, and had good agreement in detecting malaria and specifying *p. vivax* specie; however, their agreement was low in case of *p. falciparum* compared to Nested-PCR.

Keywords: Malaria, Nested-PCR, RDT, Light Microscopy, Iran

Topic: Parasitic Infections

P21: Identification of *Fasciola* species Isolated from Zabol by PCR-RFLP Method

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Introduction and objectives: The detection of *Fasciola* species in various geographical regions is essential for health policy making. Here, we aimed to identify livestock (cattle and sheep) related *Fasciola* genotypes by restriction fragment length polymorphism PCR.

Materials and Methods: Seventy adult *Fasciola* flukes were collected from infected livers of cattle and sheep slaughtered in Zabol abattoir. *Fasciola* species were determined based on morphological and molecular features. For molecular detection, *Fasciola* ITS1 region was amplified and sequenced. A 700 bp fragment was amplified. These were digested with Ras1 enzyme. *Fasiola hepatica* specific fragments were 47, 59, 68, 104, and 370, while those related to *Fasciola gigantica* had 45, 55, 170, 370.

Results: Our results showed that the two main species of *F. hepatica* and *F. gigantica* are responsible for fasciolosis in sheep and cattle in our region. No intermediate species were detected.

Conclusion: Genotypic variability of *Fasciola* species was high in our region. It is recommended to assess molecular variation of *Fasciola* isolates in other host livestock.

Keywords: ITS1 PCR-RFLP, Genotyping; *Fasciola hepatica; Fasciola gigantica;* Fascioliasis

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Topic: Parasitic Infections

P24: Antileishmanial activity of spirogyra spp extracts against *Leishmania (L) tropica* [MHOM/IR/NADIM3] promastigotes: An *in vitro* study

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Introduction and Objectives: Leishmaniasis is regarded as a major public health problem in low health literacy and status regions of the world. Because of the toxicity and side-effects of the synthetic drugs, there is growing idea to use of natural sources material. So, the aim of the current study was to determine effect of green prevalent algae extracts against *Leishmania (L) tropica* [MHOM/IR/NADIM3] promastigotes in stationary phase.

Materials and Methods: Hydro alcoholic *spirogyra spp* extracts (SSE) was obtained and stored in –80°C until the date of use. Leishmania major promastigotes (PM) were cultivated in RPMI 1640 to a final concentration of 4×10^6 cells/ml. 11 experimental groups were designed as follows: Group 1 as control: 200 µl of $RPMI_{1640}$ + 4×10^6 cells/ml PMs. Group 2: 200 µl of $RPMI_{1640}$ + 4×10^6 cells/ml PMs + 1 µg SSE. Groups 3-6 were similar to group 2 except 5, 25, 125 and 725 µg SSE were added to the culture. Groups 7-11 were similar to the groups 2-6, except the Glucantime with the same levels added to the culture. Then 24 h after, the anti-Leishmania bioassay were determined by with the Cell proliferation ELISA method.

Results: No difference detected on anti- *Leishmania* activity of the SEE levels compared to the control group (P=0.124). Also, there was no significant difference among various levels of the Glucantime compared to the control group (P=0.248). There was no significant difference on levels of 1-125 µg of SEE compared to Glucantime (P>0.05) but a significant difference detected on anti-*Leishmania* activity of the 725 µg of SEE compared to Glucantime (P<0.05).

Conclusion: These results suggest that SEE have medical potential similar to the Glucantime. Further researches are needed to determine cellular and molecular mechanism of action.

Keywords: *Spirogyra spp*, Hydro alcoholic extract, Glucantime, *Leishmania tropica.*

Topic: Parasitic Infections

P19: Cryptosporidiosis in HIV positive- patients, Bandar Abbas, Iran

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Introduction and Objectives: Cryptosporidiosis is a parasitic disease caused by a small protozoa of the genus coccidia. Due to its communal state between humans and animals, it found to be a significant issue in the medical and veterinary world. Transmission of fecal-oral infections occurs through direct and indirect contact with food and contaminated drinks. Many HIV-positive individuals live in the areas where intestinal parasites are hyper-endemic. Given the significance of the study associated with opportunistic infectious diseases in this group of patients, we decided to study the frequency of the parasite in those individuals.
Materials and Methods: In this descriptive cross-sectional study, we studied the fecal specimen of HIV positive patients under the care of center for Behavioral Disease Counseling (BDC) of Bandar Abbas. The formalin-ether concentration method and then the Ziehl-Neelsen modified staining were used to investigate intestinal coccidial oocysts, including cryptosporidium.
Results: Out of the 133 patients, 80 were males (60.25) and 53 were females (39.8%). The mean age of the patients was 42.15, of which the youngest was 12 and the oldest 82. The tests showed no cryptosporidium or other intestinal coccidia in any of the samples.
Conclusion: Monitoring and evaluating the therapeutic process of the patients lead to less infectious diseases, especially those of opportunistic parasites.
Keywords: Cryptosporidium, Bandar Abbas, HIV

Topic: Parasitic Infections

P230: The In-vitro effects of culture free supernatant and sonicated pellet of *Pseudomonas aeruginosa* on growth of *Leishmania major* amastigotes *and* promastigotes

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Background and Aims: Cutaneous leishmaniasis is an endemic disease with notorious public health effects in Iran. Today, the use of bacterial toxins in the treatment of parasitic diseases is considered. In this study the *In-vitro* effects of the cultured *P. aeruginosa* (cell free supernatant; CFS, and sonicated pellet; SP) on growth of *L. major* was evaluated.

Methods: *P. aeruginosa* strains ATCC 27853, and two different clinical strains of *P. aeruginosa* harboring genes for the four effector proteins (*exo*S,*exo*U,*exo*Y and *exo*T) were used. The presence of *exo* genes was previously confirmed by PCR method in these isolates. CFS and SP were prepared from 24 hours' culture of the bacteria, and their protein content was determined by Pyrogallol Red method. *L. major* strain MRHO/IR/75/ER and murine macrophage cell line (J774.a) were used for evaluation of CFS and SP on growth of *L. major* using the MTT test and Giemsa staining.

Results: The *P. aeruginosa* CSF and SP do not have any significant impact on the *Leishmania* promastigote forms after 24,48 and72 hours of incubation. However, a significant impact of both CFS and SP on the amastigote forms of *L. major* was observed. The CFS was more potent than the SP especially in the case of clinical strains. The time required for complete eradication of amastigotes was between 1to 3 hours for CFS and 3 to 5 hours for the SP, while for standard strain the time for total eradication was between 20 to 24 hours.

Conclusions: The present study showed a good antilesishmanial activity in CFS and SP of strain of *P. aeruginosa* harboring *exo* genes on amastigote forms of L. *major*. Further work on this bacterium and its effect on *L. major* may results in an unexpected treatment option for this parasite.

Keywords: Cutaneous leishmaniasis, *Pseudomonas aeruginosa*, *Leishmania major*, Amastigote, Promastigote.

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Topic: Parasitic Infections

P219: Scolicidal effect and hemolytic activity of chitosan nanoparticles against protoscoleces of hydatid cysts

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Introduction and objective: Surgery is the preferred treatment for Cystic echinococcosis (CE, hydatid cyst), a parasitic infection caused by the metacestode stage of *Echinococcus granulosus* as a major public health concern worldwide. Although most patients can be managed effectively with surgical treatment, up to 20 % experience disease recurrences due to spillage of hydatid fluid cyst (containing metacestode stage). Various scolicidal agents have been applied for inactivation of *protoscoleces* while they are associated with adverse side effects. Thus, the developed sclocidal agents with minimum side effects is an urgent need for surgeons. This study aimed to produce chitosan nanoparticles as a novel scolicidal agent.

Materials and Methods : chitosan nanoparticles were synthesized by ionic gelation method and characterized the physicochemical properties of them by using DLS, FTIR and SEM. Scolicidal and cytotoxicity activities of the synthesized nanoparticles at different concentration (125-1000 µg/ml) and incubation time (10-180 min) were determined by eosin staining and the hemolytic activity, respectively.

Results: FTIR spectra revealed that the chitosan nanoparticles were synthesized. The size of chitosan nanoparticles was in the range of 160–255 nm with spherical shape, the zeta potential and PDI value of the nanoparticles was approximately +42 ± 2.08 and 0.388 ± .034 respectively. The Significant difference between the scolicidal effects of chitosan nanoparticles was observed for all concentrations and various exposure times in comparison to the control group and highest scolicidal activity was observed at 1000 µg/ml after 180 min. Furthermore, the hemolytic activity was negligible at all concentration of the chitosan nanoparticles.

Conclusion: According to the findings of the study, it is anticipated this novel agent has the potential of becoming a safe and efficient natural scolicidal agent. However further studies are recommended to evaluate the in vivo efficacy and cytotoxicity before clinical application.

Keywords: Chitosan nanoparticles, *Echinococcus granulosus*, Hydatid disease, Protoscoleces

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Topic: Parasitic Infections

P281: Designing and making the molecular construct for the production of *Leishmania major* recombinant antigens using pLEXSY-neo2.1 vector

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Introduction and Objectives: The annual incidence of leishmaniasis is increasing and there are some active centers of this disease in different geographic locations of Iran. Yet, no effective vaccine has been made for human. One type of vaccine is a recombinant subunit vaccine consist immunogenic antigens of this parasite. The aim of this study was design and develop of the molecular construct using pLEXSY-neo2.1 vector to produce LACK and KMP11 antigens of *L. major* in *L. tarentulae*.

Materials and Method: The sequences of genes together GFP gene were synthesized within the pLEXSY-neo 2.1 vector. For LACK gene, utr1 vector pLEXSY was used for Trans splicing. Upstream sequences UTR of *L. donuani* alpha-tubulin gene was used for polyadenylation of the LACK gene and trans splicing of the KMP11 gene. As well as, downstream sequences UTR of the alpha-tubulin *L. donuni* were used for the polyadenylation of the KMP11 gene and trans-splicing EGFP gene. The LACK and KMP11 proteins were designed as a secretion. Then, the peptide signal sequence for these two genes was placed within construct. To extract these two proteins after expression, the His-tag sequence for the LACK protein and the S-tag sequence for the KMP11 protein were placed in construct. The vector pLEXSY-neo2.1 / LACK-KMP11-EGFP was cloned in *E. coli* strain Top10 strain. After linearization with the *SwaI* enzyme, transfected into the *L. tarentulae* by electroporation.

Results: After selection of recombinant strains by neomycin, were confirmed by PCR. Recombinant proteins were extracted and purified from the culture medium and approved by Western blot method.

Discussion: *L. tarentulae* is a non-pathogenic parasite that considered as a microorganism producing various eukaryotic proteins, recently. In this study, designed structures capable to produce antigens in this parasite. Due to the convenience and low cost of cultivating this microorganism, as well as the significant amount of production proteins and similarity of the epitopes produced by the antigens Leishmania, this parasite can be used to develop and produce recombinant vaccines.

Acknowledgments: The authors would like to thank the National Institute for Medical Research Development (NIMAD), for financial support (Grant number: 957777).

Keywords: *L. tarentula, L. major***,** recombinant antigens, pLEXSY-neo2.1 Vector

Topic: Parasitic Infections

P25: Synthesis of recombinant KMP11 / LACK proteins of *Leishmania major* in prokaryotic host: an experimental candidate model as a vaccine

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Introduction and Objectives: An attempt to build a vaccine against cutaneous leishmaniasis since 100 years ago. In order to reduce the side effects of dead and or weak live vaccines, studies on the second generation of vaccines, namely vaccine subunits, are ongoing. The aim of this study, construct and purify two KMP11 and LACK antigens of *L. major* in prokaryotic hosts.

Methods: The gene sequences of LACK proteins (NC_007269.2) and KMP11 (NC_007284.2) were obtained from the Gene Bank. These genes synthesized for production of prokaryotic protein inside the pET28a-TEV vector. To extract of these proteins after expression, His-tag sequence for LACK protein and S-tag sequence for the KMP11 protein were inserted in the construct. The pET28a-TEV / LACK-KMP11 construct was transformed into *E. coli* XL1-Blue bacteria. To express of the protein, cells were induced with IPTG. After inducing, the bacteria disintegrated by ultrasound, freezing and thawing processes.

Results: The collapsed body bacteria were separated from the medium by centrifuging. Extraction of LACK protein from His Trap HP affinity columns and KMP11 protein extraction from S-protein Agarose was performed. Concentration of extracted proteins was done by centrifugal filter unit with a limit of 10000 nominal molecular weight limit. Superdex 200 gel-filtration column (GE) columns were used for further purification of the solutions obtained. Finally, pure proteins were confirmed by Western Blot technique.

Discussion: In this study, two LACK / KMP11 antigens were made and purified in *E. coli*. Two KMP11 and LACK antigens of *l. major* are effective immunogenic antigens. The simultaneous use of these two antigens as recombinant subunit vaccines can be considered as a new model in laboratory animals.

Acknowledgments: The authors would like to thank the National Institute for Medical Research Development (NIMAD), for financial support (Grant number: 957777).

Keywords: Recombinant KMP11 / LACK proteins, *Leishmania major*, prokaryotic hosts, vaccine.

Topic: Parasitic Infections

P325: High seroprevalenc rate of Strangles in horses of Northeast and Southeast of Iran

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Introduction and Objectives: Strangles is caused by *streptococcus equi* subspecies *equi*. The bactria typically infect the upper respiratory system and lymph nodes of the head and neck in equidae. Strangles occurs in horses, ponies, donkey and mules worldwide, highly contagious disease that affects horses of all ages, but is most common in young animals. The aim this study was to evaluate the seroprevalence rate of strangles in horses in northeast and southeast of Iran.

Materials and Methods: serum samples from 149 horses were randomly collected in Kerman, Bojnord and Shirvan and were examined by ELISA assay.

Results: seroprevalenc rate of antibody against M protein of streptococcus *equi* was 51.7%.

Conclusion: This study determined that seroprevalene rate of antibody against M protein of streptococcus *equi* in horses in North-east and South-east of Iran, is high and prevention and control measurements should be considered by health authorities**.**

Keywords: Horse, Strangles, ELISA, *Streptococcus equi*, Kerman, North Khorasan

Topic: Parasitic Infections

P98: Inhibition of mouse colon cancer growth following immunotherapy with hydatid cyst fluid and BCG

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Introduction and Objectives: Hydatid cyst located in human and livestock viscera is the larval stage of *Echinococcus granulosus* tape worm. Scientific evidences indicating that certain Parasitic Infections may induce antitumor activities against some types of cancers. In this research work, the effects of a hydatid cyst fluid and BCG on colon cancer tumor growth in BALB/c mice have been studied.

Materials and methods: For this purpose, four groups of mice were injected with colon cancer cells. After 5 days when the sign of tumor growth in mice was seen, group 1&2 were injected with hydatid cyst fluid and BCG respectively. Groups three were injected with only adjuvant and the fourth group left intact without any injection. The size of the tumor was measured and compared in all groups. Then blood samples of mice were evaluated for serum cytokine levels.

Results: In mice injected with hydatid cyst fluid and BCG tumor size were smaller than those of control groups and the difference was statistically significant.

Conclusion: The results of this work indicated that injection of a hydatid cyst fluid and BCG significantly inhibited mouse colon cancer growth and this inhibition may be related to effect of immune response to these antigens.

Keywords: Colon cancer, cytokine, *Echinoccocus granolosus*, hydatid cyst fluid, BCG

Topic: Parasitic Infections

P27: Prevalence and determination type of *plasmodium* in mosquitoes present in the city of Nik Shahr, Southern Sistan and Baluchestan Province, IR Iran, by Multiplex Nested-PCR

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Introduction and objective: Malaria is one of the most important parasitic diseases, and is considered one of the important health issues in tropical and subtropical countries. The aim of this study was to determine the rate and type of infection of Anopheles mosquitoes by malarial parasites using Multiplex Nested-PCR in the South of Sistan and Baluchestan province.

Materials and Methods*:* In the second half of 2017, 400 Anopheles mosquitoes were collected from Zahedan Medical Insecticide Check Centers located in villages around Nikshahr city. The mosquitoes were caught by hand-held methods in domestic (human and animal), natural and artificial outdoor places (Shelterpit). After DNA extraction, molecular analysis was performed using Multiplex Nested-PCR.

Results: Of the 310 samples collected from parts of Lashar, Ahuran and the centre of Nik Shahr city, 6 samples (1.5%) were found to be infected with Plasmodium vivacs, and 90 samples collected from the Fennoj and Bennett sections of the city had no infection. Samples containing plasmodium falciparum and a mixture of Plasmodium vivacs and Plasmodium falciparum were not detected in this study.

Conclusion: The results show that in places where the transmission of both species of Plasmodium vivax and Plasmodium falciparum occur, the detection of malarial parasites by PCR can be a very useful complement to confirm microscopic diagnosis.

Keywords*:* Malaria, Sistan and Baluchestan province, Multiplex Nested-PCR, Anopheles mosquitoes

Topic: Parasitic Infections

P202: Effect of aqua extract Zataria *multiflura* and *Zns* nanoparticle on Tachyzoites of Toxoplasma in Balb/C mice

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Introduction and Objectives: Toxoplasma gondii is one the most important apicomplexan parasite of humans and other warm-blooded animals. We have studied the effect of aqua extract Zataria multiflura on tachyzoite of Toxoplasma gondii in male Balb/C mice.

Materials and Methods: A total of 20 Balb/C mice (control& experiment) were included, and 10000 Toxoplasma organisms of the RH strain Toxoplasma gondii were given oral to each mouse.

Zataria multiflura extract was administered in 4 groups (control, ZM100, ZM500, NZM100 and NZM500). All of the experimental mice except control group were given extract oral (with or without nanoparticles) with 100or 500 mg/kg/day doses 3houres after infection and they received for 7-day single dose per day.

Results: One hundred percent of mice were survived with all of used doses of Zataria multiflura extract with or without nanoparticles at 5 days after infection but one hundred percent of positive control mice were died P=0.015. Comparing of groups, tachyzoites of toxoplasma in the spleen were disappeared in ZM100 (60%) group and in NZM100 (40%) group (P=0.000)

Conclusion*:* The results demonstrate that Zataria multiflura extract are effective on tachyzoites of toxoplasma in mice. Tachyzoites of toxoplasma in the brain of mice were disappeared.

Zataria multiflura extracts with or without nanoparticle were found to be effective for treatment of murine toxoplasmosis.

Keywords: Toxoplasma, Tachyzoites, Zataria multiflura extract

Topic: Parasitic Infections

P20: Study on prevalence of malaria in patients referring to central laboratory in Jiroft city in southern Iran, 2002-2019

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Introduction and Objectives: Malaria is a serious and sometimes life-threatening infectious disease caused by a single-celled protozoan parasite *Plasmodium* species. These parasites are transmitted from human to human through the bite of female adult Anopheles mosquitoes. Four Plasmodium (P) species including: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* are known to infect human. Malaria is usually characterized by several signs and symptoms including: Fever, Chills, Headache, Nausea, vomiting. Muscle pain and fatigue. This disease is a major public health problem in the tropical and subtropical regions. The incidence of this disease is around 270 million per year worldwide. Hot and wet weather conditions favour their Hot and humid weather can make conditions favorable for development and trigger outbreaks of the diseases. Jiroft city has a warm and humid climate. The aim of this study was to investigate the prevalence of malaria in Jiroft city in southern Iran.

Materials and Methods: In this cross-sectional study, referrers from from 2002 to 2019 were studied. Samples were collected from capillary blood. Blood smear was done to detect and diagnose malaria disease.

Results: About 23712 patients were participated in this study. Plasmodium species were found in 979 patients. All positive cases were seen in foreign nationals. All disease caused by *P. vivax*. The most positive cases (215 patients) were seen in 2003. In 2012 no patient was found. The number of positive cases has slowed down between 2002 and 2019.

Conclusion: The present study has shown that prevalence of malaria in Jiroft city has declined dramatically in recent years. In this area, Malaria is not a serious threat today

Keywords: Jiroft, Plasmodium, Malaria

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Topic: Pathogenesis and Virulence Factors

P274: Molecular detection of diffusely adherent *Escherichia coli* (DAEC), isolated from pediatric diarrhea referring to Shahid Dastgheib children Hospital, Shiraz (2019-2018)

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Introduction: The pathogenicity and clinical pertinence of diffusely adhering *Escherichia coli* expressing the Afa/Dr adhesins (Afa/Dr DAEC) in diarrheagenic infections (UTIs). Based on the expression of adhesins, two groups of DAEC strains were identified. Afa/Dr DAEC and AIDA-I DAEC. Afa/Dr family encompasses fimbrial (F1845, Dr) and afimbrial (Afa) adhesins. Afa-I, Afa-II, Ala-III, Ala-V, Afa-VII, Afa-VIII and Dr-2 afimbrial adhesins, as well as Dr and F1845 fimbrial adhesins, constitute the Afa/Dr family. The goals of this study were isolation of *E. coli* from patients with diarrhea in Shiraz (Iran), and detection of DAEC pathotypes in isolates by PCR.

Methodology: Three hundred stool specimens of diarrhea patients were collected from Shahid Dastgheib children Hospital in Shiraz, Fars, Iran. Diarrheagenic *E. coli* strains were isolated by standard biochemical analysis. Conventional PCR were used to detect the Afa/Dr family in the DAEC strains isolated.

Results: A total of 300 samples from patients with acute diarrhea were analyzed. Among 190 isolates were been deteted as *E.coli*, 14/190(7.3%) of the *E. coli* strains were identified as DAEC strains. Afa/Dr family prevalence was *afaE-1* 10/14(71.4%), *afaE-3* 4/14 (28.5%), *afaE-5* 3/14 (21.4%), *daa E* 3/14(21.4%) respectively. *aida/aah* and *afaE-2* genes were not detected in any isolates.

Conclusion: Our analysis indicated that DAEC strains may be considered as potential pathogens in Shiraz, southern Iran. Further, although the prevalence of DAEC is low, prevention of infection caused by this bacterium among diarrheagenic patients is crucial. Therefore, further characterization of the different virulence aspects of DAEC strains is required.

Keywords: Diarrheagenic *Escherichia coli* (DEC), acute diarrhea, DAEC

Topic: Pathogenesis and Virulence Factors

P252: The role of CTX and satellite RS1 phages genomic arrangement in *Vibrio cholerae* toxin production in two recent cholera outbreaks (2012 and 2013) in IR Iran

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Introduction and Objectives: The objective of the present study was to investigate the genomic arrangement of CTX/RS1 prophages in 30 *V. cholerae* from 2 consecutive year's outbreaks and to compare the role of different CTX/RS1 arrangements in cholera toxin expression among El Tor strains.

Materials and Methods: A total of 30 *V. cholerae* strains from cholera patients of Iran 2012 and 2013 outbreaks were included in this study. The identity of strains was confirmed by Biochemical and molecular methods. southern blot technique was used for determining Copy number of CTX and satellite RS1 phages. cholera toxin expression was evaluated by Real- time PCR method.

Result: Profile A with TLC-RS1-CTX-RTX arrangement was observed in 46.7% of total isolates with RS1 phage locating adjacent to TLC element. Fifty percent of isolates showed profile B with TLC-CTX-RS1-RTX arrangement and one single isolate (3.3%) revealed TLC-CTX-RS1-RS1-RTX arrangement (profile C). No RS1 element was detected adjacent to TLC element in B and C profiles. No truncated CTX phage genome was detected among isolates of 2 years.

Different CTX-RS1 arrangement profiles (A, B and C) with different RS1 copy numbers and locations, uniformly showed low cholera toxin production level in El Tor strains with no significant differences, which reveals that different RS1 copy numbers and locations do not affect cholera toxin production level (P-value>0.05). Instead, increased cholera toxin expression was observed for control Classical biotype *V. cholerae* strain.

Conclusion: variations in RS1 prophage did not affect CT expression level in related El Tor *V. cholerae* strains. CTX genotyping establishes a more valuable database for epidemiologic, pathogenesis and source tracking purposes.

Keywords: *Vibrio cholerae*, CTX phage, RS1, Iran, Toxin Production

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Topic: Pathogenesis and Virulence Factors

P250: Evaluation of biofilm formation in *Pseudomonas aeruginosa* isolates

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Introduction and objectives: *Pseudomonas aeruginosa* is an opportunistic pathogenic bacterium responsible for both acute and chronic infections. Beyond its natural resistance to many drugs, its ability to form biofilm, a complex biological system, renders ineffective the clearance by immune defense systems and antibiotherapy. The aims of this study was to determine the biofilm formation ability by *P. aeruginosa* clinical isolates.
Materials and Methods: in this study, 99 clinical isolates of *P. aeruginosa* were studied. The ability of producing biofilm was examined by crystal violet microtiter plate assay and multiplex polymerase chain reaction (mPCR).
Results: Among 99 isolated, ۸ isolates formed strong biofilms, 12 isolates moderate, 47 isolates were formed weak biofilm and 31 isolates did not form biofilm. The PCR results on 8 isolates with strong biofilm showed that 8 isolates had genes *cupA* and *lasR*, 5 isolates had *rhlR* and 3 isolates had *rhlI*.
Conclusion: The presence of genes encoding cell surface organelles, such as flagella and fimberial structure, and the genes that involved in quorum sensing system have a key role in biofilm formation. Infections with *P. aeruginosa* are a major problem for human health, and the understanding of the adhesive mechanisms used by the bacterium to colonize tissues as well as abiotic surface such as catheters or other medical devices is necessary to understand the infection process.
Keywords: *Pseudomonas aeruginosa*, Biofilm, *cupA* gene, PCR

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Topic: Pathogenesis and Virulence Factors

P248: Prevalence of biofilm associated genes in clinical isolates of *Staphylococcus aureus*

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Introduction and Objectives: *Staphylococcus aureus* is the most important etiological agent of biofilm associated-infections. It is one of the Gram-positive pathogens causing a wide range of nosocomial infections. Genes involved in biofilm formation is a defensive mechanism of this pathogen to combat the host immune response and remain stable in hostile environment. The aim of this study was to investigate prevalence of biofilm associated genes (BAGs).

Materials and Methods: Eighty samples of *Staphylococcus aureus* isolates from human infections referred to the Microbiology Laboratory, Medical Research Center of Namazi Hospital, Shiraz University of Medical Sciences were collected. The isolates were confirmed by phenotypic and molecular tests as *S. aureus*. Thirteen BAGs including *rbf, SigB, SasG, icaA, sarA, icaR, icaD, clfA, clfB, fib, fnbpB, bap* and *fnbpA* were investigated by PCR assay.

Results: The results showed that *sigB* (93.7%) and *SarA* (90%) were the most prevalent BAGs followed by *rbf* (83.7%), *fib* (80%), *sasG* (78.7%), *icaR* (78.7%), *clfB* (78.7%), *clfA* (78.7%), *fnbpA* (73.7%), *icaD* (66.2%), *icaA* (50%), *fnbpB* (22.5%). However, *bap* was not detected in any isolate.

Conclusion: Prevalence of biofilm-associated genes were relatively high among the isolates. As staphylococcal biofilms are associated with a wide variety of infections in the human body, more genotyping of the isolates is underway.

Keywords: *Staphylococcus aureus*, Biofilm, Biofilm-associated genes, Iran

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Topic: Pathogenesis and Virulence Factors

P112: Distribution of Mycoplasma hominis in Prostate Cancer Reffered to Hamadan Shahid Beheshti Hospital in 2018

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Introduction and Objectives: Mycoplasma hominis is an opportunistic pathogen in the human genital tract and is capable of causing chronic infection in the prostate gland. The long-term relationship between bacteria and host cells can have reciprocal effects, the most important of which is the effect of bacteria on the cell growth cycle. Considering the importance of prostate cancer, the association of this bacterium with prostate cancer has been investigated in this project.

Materials and Methods: In this study, thin sections of 61 prostate biopsy blocks of patients with prostate cancer and 70 prostate biopsy blocks were obtained from patients with Benign Prostatic Hyperplasia. DNA were extracted from all samples and amplified by real-time PCR targeting 16S rRNA and yidC genes.

Results: Out of 61 samples of prostate biopsy in patients with prostate cancer, 7 cases (11.4%) were positive for Mycoplasma hominis infection; while the bacterium was not detected in any sample of patients with Benign Prostatic Hyperplasia.

Conclusion: However, further studies are needed to prove the role of Mycoplasma hominis in the development of prostate cancer, but due to the significant relationship between the associations of this bacterium in patients with prostate cancer, it is necessary to detect this organism in the genitourinary tracts of men and infected people should be treated immediately.

Keywords: Mycoplasma hominis, Prostate Cancer, Benign Prostatic Hyperplasia

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Topic: Pathogenesis and Virulence Factors

P104: Seroprevalence of anti-*Helicobacter pylori* and anti-CagA IgG antibodies in dyspeptic patients of Kerman, Iran

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Introduction and Objectives: *Helicobacter pylori* (*H. pylori*) infection is common worldwide and is the main pathogenic factor in the development of peptic ulcer disease. It has also been associated in the development of gastric cancers resulting from the cytotoxin-associated gene A (CagA). *CagA*-positive *H. pylori* strains has been more closely associated with gastric disease than *CagA*-negative isolates. The aim of this study was to evaluate the seroprevalence of anti-*H. pylori* and anti-CagA IgG antibodies in in a population of dyspeptic patients from Kerman, Iran.

Materials and Methods: In this prospective epidemiological survey, a total of 659 people, ages 17 to 72 years, were evaluated for the presence of general anti-*H. pylori* IgG, and then 91 serum samples (70 seropositive for *H. pylori* IgG and 21 seronegative for *H. pylori* IgG, as control) for anti-CagA IgG by two commercial ELISA kits, according to manufacturer's instruction.

Results: The prevalence of general anti-*H. pylori* IgG was 58.1% (383 of 659 patients) that increased progressively with age ($p<0.05$) and was not appreciably influenced by the sex ($p=0.08$). Older patients showed a higher seroprevalence of *H. pylori* infection, in the age range from 51 to 72 years. The prevalence of anti-CagA IgG antibody in seropositive and seronegative patients for general *H. pylori* IgG was 52.9% (37 of 70) and 61.9% (13 of 21), respectively.

Conclusions: The findings suggest a relative high prevalence of *H. pylori* infection in dyspeptic patients of Kerman that was related to several risk factors as socioeconomic status and traditional living conditions. This is the first report of the high prevalence of anti-CagA IgG in both seropositive and seronegative patients for general IgG, indicating the importance of this antibody in diagnosis of *H. pylori* positive patients after seroconversion of the general IgG.

Keywords: *Helicobacter pylori*, Seroprevalence, CagA, Dyspeptic patients, Kerman

Topic: Pathogenesis and Virulence Factors

P263: Evaluating the prevalence of virulence genes of *Escherichia coli* in patients affected by urinary tract infection

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Introduction and Objectives: Urinary tract infection due to *Escherichia coli* remains to be a major health problem worldwide. UPEC is one of the major causes of urinary tract infections among the general public. These bacteria have various virulence factors, including adhesion and toxins. Pilli P and FimA are the main members of *E. coli* adhesion in the pathogenesis of urinary tract infections. Due to high prevalence of urinary tract infections in the community, the aim of the present study was to evaluate the prevalence of fimA, hly-A and papG virulence genes in Uropathogenic *Escherichia coli* in Iranian female adults with urinary tract infections.

Materials and Methods: From March to October 2016, 200 urine samples were collected from 200 inpatient and outpatient women. The *Escherichia coli* bacteria was detected and isolated, using biochemical techniques and supplementary tests in the Microbiology Laboratory of Shahrekord University of Medical Sciences. Then virulence factors were identified by PCR method.

Result: The prevalence of papG, fimA and hlyA were 44, 74 and 26% of in inpatients and 52%, 76% and 28%, in outpatients, respectively.

Conclusion: There was a significant correlation between papG and pyelonephritis in inpatients. There was no relationship between the frequency of papG, fimA and hlyA genes, and cystitis. The results of this study indicated that the fimA gene was the most frequent among the general public, and the hlyA gene was the least frequent and the papG gene was associated with pyelonephritis.

Keywords: UPEC, Virulence factors, UTI, *Escherichia coli*, Cystitis

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Topic: Pathogenesis and Virulence Factors

P239: Phenotypes and genotypes analyzing of biofilm forming *Staphylococcus epidermidis* isolated from clinical specimens

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Introduction and Objectives: *Staphylococcus epidermidis* as an important opportunistic pathogen and a major reason for foreign body infections owing to antibiotic resistance to the greatest of the antibiotics is looked upon as a health threat in hospitalized patients. The present study aimed to investigate the *in vitro* biofilm formation capacity, the presence of biofilm related genes (*icaA, icaB, icaC, icaD, sdrG* and *atlE*) and antibiotic susceptibility patterns of clinical *S. epidermidis* isolates.

Materials and Methods: Following the isolation of coagulase-negative staphylococci taking advantage of traditional biochemical procedure, *S. epidermidis* isolates were identified by molecular approaches. The frequency of biofilm related genes was assessed by multiplex colony PCR. Biofilm formation and antibiotic susceptibility testing were assessed by microtiter plate and disk diffusion methods, respectively.

Results: 54 isolates of *S. epidermidis* were identified according to molecular detection of *sesC* gene. Biofilm formation phenotype was detected in all *S. epidermidis* isolates; 45 (83.33%) of isolates produced strong biofilm and 5 (9.26%) of isolates formed the moderate biofilm and 4 (7.41%) strains as weak biofilm producer. The most frequent biofilm related gene was *sdrG* (98%), followed by *atlE* (84%), *icaC* (80%) and *icaD* (70%). Basing on the susceptibility testing, Cefamandole and Amikacin are the most effective antibiotics against and the least effects belonged to Methicillin and Amoxicillin/Clavulanic Acid.

Conclusion: Because of the increasing frequency of biofilm forming *S. epidermidis* and antibiotic resistance, a prompt determination is essential for eradication of introduced strains that have the ability of biofilm-forming and resistance to an antibiotic for avoiding other medical devices related infections.

Keywords: *Staphylococcus epidermidis*, Biofilm-related genes, Multiplex Colony PCR, *atlE*, *sdrG*, *icaADCB*

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Topic: Pathogenesis and Virulence Factors

P105: Bacterial infection in male infertility and its relationship with semen quality and seminal plasma components

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Introduction and Objectives: Bacterial infections are associated with production of reactive oxygen species which could affect DNA integrity in spermatozoa, thereby reducing sperm fertilization capability. The aim of this study was to evaluate the bacterial infections of seminal plasma and its effect on sperm characteristics.

Materials and Methods: Semen samples were collected from 56 infertile men and 35 fertile, referred to infertility center and subjected to standard bacterial culture. Semen analysis were assessed according to fifth edition of World Health Organization (WHO) laboratory manual for the examination and processing of human semen. Also sperm DNA integrity were carried out in each samples.

Results: The prevalence of bacterial infections in semen samples was 25.4 % and 5.7% in infertile and control group, respectively. Significant differences was observed in two groups ($p<0.001$). Among all bacterial infections inform infertile patients 36.3% was *Escherichia coli*, 30.1% was *Staphylococcus aurous*, 18.8% was *Streptococcus agalactiae*, and 14.9% was mix bacteria. These numbers in control group were, 15.7%, 21%, 16.5% and 46.8%, respectively. The sperm motility, morphology and motility were significantly lower in *E.coli* and mixed species groups than others in infertile patients. This data was similar in control group. Also sperm DNA integrity was significantly lower in *E.coli* group than other in both infertile and fertile men.

Conclusion: There our results showed that, there was a significant correlation between seminal bacterial infection and reduction of sperm quality and DNA integrity, which in turn affect spermatozoa fertility capacity. Therefore the results of the present study suggest that special seminal bacterial infections possibly affect the quality of semen in infertile patients, and that antibiotic therapy may be recover fertility potential.

Keywords: Bacterial Infection, Infertility, Seminal plasma, Sperm DNA integrity.

Topic: Pathogenesis and Virulence Factors

P99: White Blood Cell (WBC) Count and C-Reactive Protein Value as Predictors of Bacterial Infections in Febrile Outpatient Children at Tabriz, Iran

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Introduction and Objectives: Infections remain the major cause of unnecessary antibiotic use in pediatric outpatient settings. Complete blood count (CBC) is the essential test in the diagnosis of infections. C-reactive protein (CRP) is also useful for assessment of young children with serious bacterial infections. The purpose of the study was to evaluate leukocyte populations and CRP level to predict bacterial infections in febrile outpatient children.

Materials and Methods: The values of CBC by Advia Siemens 2120i auto analyzer and serum CRP levels were evaluated in 60 febrile patients with documented infections (n:36 bacterial, n:24 viral) and 31 healthy controls at Tabriz Children's Hospital.

Results: The mean CRP, neutrophil and Immature granulocyte (IG) values were significantly higher in bacterial infections than in viral infections and controls ($p<0.05$). C-reactive protein was significantly correlated with neutrophil level in bacterial infections (r: 0.76, $p<0.05$). Specificity of IG was greatest at 93%, only a modest 56% for neutrophil and mild 18% for CRP, whereas 100% for combination of IG, neutrophil and CRP.

Conclusion: Acute bacterial infection seems to be very unlikely in children with normal leukocyte populations and CRP values, even if clinically signs and symptoms indicate acute bacterial infections.

Keywords: White Blood Cell populations, C-reactive protein, Febrile children

Topic: Pathogenesis and Virulence Factors

P287: Survey of Polymerase Chain Reaction Efficiency in the Detection of Mycoplasma, Listeria and Brucella in Culture Negative Samples Obtained from Women with Abortion

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Introduction and Objectives: Misabortion is a disorder resultant of numerous causes such as infections. It is clear that bacterial infections due to genital *mycoplasma*, *Listeria* and *brucella* can cause septic abortion. A right time diagnosis of these infections can improve women fertility. This study was conducted to survey the efficiency of PCR in detecting the specimens with negative culture results in women suffering from septic abortion. We also did this by considering individual variables.

Material & Methods: In this analytical and descriptive study, appropriate samples were collected from a total of 87 women with septic abortion who referred to Karaj's hospitals. The samples were cultured into *mycoplasma, Listeria* and *brucella* specific media. Then, the bacterial genus was verified by different biochemical tests. PCR test is performed on negative culture cases and SPSS-18 software is used for the statistical analysis of data.

Results: From the total of 87 blood samples, 37 samples (42.5 %) were positive for *mycoplasma* and *ureaplasma* (25 cases) and *Listeria monosytogenes* (12 cases) with both culture and PCR method. Our result showed no positive cases for *brucella*. From the total cultured specimens, 12 cases (13.8%) were positive and 75 cases (86.2%) were negative. We performed PCR test for negative cultured results. With PCR method, 25 cases (33.33 %) showed positive results and 50 cases (66.67%) showed negative results. The results also showed a significant statistical relation between PCR test results with recurrent abortion and level of education ($P< 0.05$).

Conclusion: The results show that PCR is a more sensitive, easier and faster method in comparison to culture for detecting bacterial cause septic abortion. It is obvious that quick diagnostic and starting antimicrobial therapy at the right time can prevent and decrease abortion's complications, so it recommended then that using PCR in detecting this bacterial cause septic abortion can be more effective.

Keywords: Septic Abortion, Culture, PCR

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Topic: Pathogenesis and Virulence Factors

P318: *In vitro* and *in vivo* expression of virulence genes in *Trueperella pyogenes* based on a mouse model

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Introduction and Objectives: *Trueperella pyogenes* is an important opportunistic pathogen causing a number of pyogenic infections in ruminants and other animals. This microorganism expresses several extracellular virulence proteins that contribute to its pathogenic potential. Co-infection with other bacterial species such as *Escherichia coli* or *Fusobacterium necrofurom* increases the persistence of bacteria and the severity of the diseases. The aim of this study was expression of some pathogenesis genes including *plo*, *nanH*, *fimA* and *cbpA* and co-culturing of *S. dysgalactie*a, *E. coli*, *S. aureus*, *F. necrophorum* and *L. plantarum* in experimental and mice models in clinical samples collected from cattle with metritis, mastitis and skin abscess symptoms.

Materials and Methods: Nine isolates with outstanding clinical symptoms including 3 metritis, 3 mastitis and 3 cutaneous abscess isolates with all virulence encoding genes were separated and cultured in TSB broth for 48 hours reaching 0.5 × 10^8 bacterial count forwarding RNA extraction. In the next step, co-culturing of *S. dysgalactiae*, *E. coli*, *S. aureus*, *F. necrophorum* and *L. plantarum* strains for 48 hours reaching 0.5 × 10^8 bacterial count forwarding RNA extraction were done separately to the *T. pyogenes* isolates. 0.5 × 10^8 CFU/mL of *T. pyogenes* co-culturing with *S. dysgalactiae*, *E. coli*, *S. aureus*, *F. necrophorum* and *L. plantarum* strains was formulated, injected and 48-hour intraperitoneal incubated by 0.5-mL volume into the mice categorized in 6 separately groups. So, after anesthesia and intraperitoneal cutting side of the experimental mice, liver, heart, spleen and intraperitoneal fluid of mice were collected in sterilized containers following tissue homogenization and RNA extraction according to the Kit manufacturer instruction.

Results: By Livac formula, *plo*, *NanH*, *cbpA* and *fimA* genes expression observed 17, 8, 15 and 16 times more in metritis, mastitis and cutaneous abscess samples respectively. Change in *plo*, *nanH*, *fimA* and *cbpA* genes expression in co-culture in comparison with pure-culture of *T. pyogenes* in mice model indicated that, *E. coli* and *F. necrophorum* lead to increase and *L. plantarum* contributes to decrease in genes expression. There is not any significant increase observed in genes expression in co-culturing of *T. pyogenes* with *S. dysgalactiae* and *S. aureus*. Co-operative functions of this bacterium with other pathogens leads to enhance the expression of pathogenesis gene consequently increasing in the symptoms of the disease.

Conclusion: Antagonistic effect of using of some functional bacteria and their metabolites can be very helpful for declination in expression of virulence encoding genes and pathogenesis of mentioned disease bacterial agents.

Keywords: *Trueperella pyogenes*, gene expression, Co- infection, Real time PCR

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Topic: Pathogenesis and Virulence Factors

P265: Comparsion of virulence gene between *Escherichia coli* isolates from Urinary Tract Infections and normal fecal flora and their relationship with phylogenetic groups

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Introduction and Objectives: *Escherichia coli* is the most prevalent facultative gram-negative bacillus in the human fecal flora, usually inhabits the colon as commensal. It's the most common cause of the Urinary Tract Infections (UTI). UTI isolates have several virulence factors which may be different from the common normal fecal flora. The phylogeny of the *E.coli* species, with the identification of eight phylogroups (A, B1, B2, C, D, E, F and clad1) is linked to the lifestyle of the strains. The aim of present study was determination of new phylogenetic groups of UTI and normal fecal flora isolates *E.coli* and to compare the presence of several virulence factors between UTI and normal fecal flora.
Materials and Methods: Totally 50 *E.coli* isolates from UTI and 50 *E.coli* isolates from normal fecal flora were tested. The PCR method was used for identification of virulent genes *vat, chuA, fyuA, yfcv, iroN, iucD*. The phylogenetic groups was performed using universal primers for quadruplex genotype (*arpA, chuA, yjaA, tspE4.C2*) for groups A, B1, B2, C, D and additional primers for identification of clade 1, E, F.
Results: Virulence factor genes (*vat, chuA, iroN*) in UTI isolates was significantly higher than the isolates derived from normal fecal flora ($P \leq 0/001$). Virulence factor gene *vat* was only isolated in UTI isolates and was not seen in fecal samples. In addition, the isolates from the UTI was mostly in Group D and isolates from the normal fecal flora was in clade1. Group E was not detected neither in fecal nor in UTI isolates.
Conclusion: In conclusion, the isolates from UTI have more virulent factors that in fecal normal flora and the phylogenetic grouping showed the Group D to the more common in UTI and clade1 mostly in normal fecal flora.
Keywords: *Escherichia coli*, Virulence factors, Phylogenetic groups

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Topic: Pathogenesis and Virulence Factors

P241: Relationship of biofilm formation with *csg*, *afa*, *kpsMT* II and *fyuA* virulence genes in uropathogenic *Escherichia coli* isolates from southwestern Iran

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Introduction and Objectives: Urinary tract infection (UTI), an inflammatory disease occurs to a high multiplication of pathogenic microbes in the urinary system. *Escherichia coli* (*E. coli*) are the most frequent cause of UTI. The virulence factors responsible for pathogenesis outside of the gastrointestinal tract belong to various functional groups. The formation of biofilm one is pathogenic mechanism, allowing *E. coli* to persist in the genitourinary tract and hindering the eradication of microorganisms. Biofilms are group of microorganisms which are embedded within a self-produced matrix of extracellular polymeric substance which adhere to each other. This study was conducted to analysis the relationship of virulence genes with biofilm forming *E. coli* isolated from patients with suspected UTI Collected from the medical diagnostic laboratories of Yasuj city in southwestern Iran.

Materials and Methods: A total of 130 *E. coli* isolates isolated from patients with UTI were collected and characterized by routine bacteriological methods. Biofilm detection by Congo red agar (CRA) and Microtitre plate method (MTP) and and the presence of *csg*, *afa*, *kpsMT II* and *fyuA* virulence genes was examined by polymerase chain reaction (PCR) assay. Data analysis was performed using SPSS 16.0 software.

Results: From 130 isolates of *E. coli* isolated from UTIs, the biofilm formation rate by MTP and CRA was 75.38% and 86.92%, respectively. About 28.46% (37 of 130) of the isolates harbor afa gene. csgA gene was found in 100% (130) isolate, fyuA was found in 85.38% (111 isolate) and kpsMT II genes were found in 84.61% (110) isolate. In this study, the frequency of virulence genes csg, fyuA, kpsMT II and afa in isolates that were able to produce strong biofilms were 100%, 78.6%, 85.7% and 14.3%, respectively, in isolates with moderate biofilms of 100%, 93.5 %, 90.3% and 22.6%, respectively, and isolated in weak biofilms were 100%, 86.8%, 84.9% and 34%, respectively. There is no significant relationship between the presence of fyuA, kpsMT II and afa virulence genes with biofilm formation. ($P<0.307$, $P<0.609$, $P<0.421$).

Conclusion: PCR molecular method was more reliable for the detection of biofilm forming UPEC as compared to phenotypic methods MCRA and MTP methods. The results of this study show the importance of virulence genes in the isolates of the biofilm producing *uropathogenic E. coli*. All the positive biofilm producer strains could be expressed in the csg gene. The results also indicate a high prevalence of virulence genes of csg, fyuA and kpsMT II among *uropathogenic E. coli* (UPEC) isolates from patients with UTI in Yasuj, southwest of Iran. There was no statistically significant correlation between presences of virulance genes with biofilm formation.

Keywords: Antibiotic resistance, Biofilm, Virulence, genes, Uropathogenic, E. coli, Urinary Tract Infection

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Topic: Pathogenesis and Virulence Factors

P195: Evaluation of the Effect of Heracleum persicum Extract on the Quorum Quenching of Biofilm Production Disease Genes in *Staphylococcus aureus*

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Introduction and Objectives: Bacteria by regulating and communicating through the microcells of the n-acyl hemocerin lactone messenger (AHL) regulate and express the synchronized genes. This phenomenon is called Quorum Sensing, which is done in response to bacterial cell density. QS regulates important processes, including biofilm production, antibiotics and pathogens. Disturbance and quenching in this process Quorum Quenching cause the lack of gene expressions responsible for biofilm formation. The aim of this study was to evaluate the anti QS effect of Heracleum persicum extract on biofilm production of Staphylococcus aureus isolates.

Materials and Methods: 23 strains of Staphylococcus aureus isolated from dental implant infection by molecular method (PCR) were isolated and identified by amplification of the COA gene. After extraction, to evaluate the effect of QSI by *Chromobacterium violaceum CV026* strain of anti QS test, and flask culture method was used to determine the inhibitory effect of violacin production and CV026 growth on the extract. The microtiter plate method (MTP) was also used to determine the inhibitory effect of the extracts on biofilm formation.

Results: The results of the tests showed that the extract (17.5±0.3) significantly (P>0.05) had a significant sensory impedance effect, and the flask test prevented the production of violacein in The concentration of 0.25 was found in *CV026* strain, and the effect of this property on inhibiting biofilm formation in Staphylococcus aureus was reported at 35µg / ml.

Conclusion: According to the effect of QSI extract of Heracleum persicum and due to the multi drug resistance of bacteria in the present age, these compounds can be used as non-synthetic drugs in the route of prevention and treatment of chronic infections.

Keywords: S. aureus, Heracleum persicum, AHL, QS, *CV026*, Biofilm

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Topic: Pathogenesis and Virulence Factors

P249: Frequency of Panton- Valentine Leukocidin among nasal methicillin resistant *Staphylococcus aureus* isolates colonizing burn ward staff, Yazd-2018

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Introduction and Objectives: *Staphyococcus aureus* is one of the most common cause of nosocomial infections in burned patients. The ability to produce different toxins as well as antibiotic resistance, make difficulties in treatment of *S. aureus* infections. Panton- Valentine Leukocidin (PVL) is a pore- forming protein which can kill white blood cells of humans. The aim of this study was to determine the frequency of PVL among methicillin resistant *S. aureus* (MRSA) colonizing burn ward staff.

Materials and Methods: Non- duplicated nasal swabs were collected from anterior nares of 159 individuals working at burn ward of Shahid Sadoughi hospital. Detection of *S. aureus* isolates was performed using conventional biochemical tests. All *S. aureus* identified isolates were screened for methicillin resistance using cefoxitin disk. Phenotypically methicillin resistant isolates were investigated for the presence of *mec* (A) gene. PCR experiment was carried out for detection of PVL- encoding gene (*pvl*).

Results: Totally, 37 isolates were identified as MRSA, of which 78.4% (29 of 37) carried *pvl* gene.

Conclusion: according to the results, high prevalence of pvl^+ MRSA was found. Since these isolates are more virulent compared to pvl^- ones, detection of nasal carriers of pvl^+ MRSA in such high risk burn wards can be helpful in effective reduction of health care associated infections.

Keywords: MRSA, PVL, nosocomial infection, nasal carriers

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Topic: Pathogenesis and Virulence Factors

P266: Molecular survey of *slyA, stn, sopB, Phop/Q and spvc* genes of *Salmonella typhimurium* isolated from clinical samples by multiplex PCR

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Introduction and Objectives: *Salmonella* is a Gram-negative intestinal organism and causes food poisoning in human. *Salmonella* has five virulence genes, *stn, Phop/Q, spvc, slyA* and *sopB*. These genes encode proteins in different parts of the bacteria that can confront with immune system, and the complement system and can cause death in the cell. The aim of this study was to detect *slyA, stn, sopB, Phop/Q* and *spvc* genes in *Salmonella typhimurium* strains isolated from clinical samples by the multiplex PCR method and to determine antibiotic resistance patterns.

Material and Methods: In this descriptive cross-sectional study, in 2018, 60 stool samples in order to identify *Salmonella typhimurium* from Alborz-Karaj Hospital were collected. After confirmation of the strains by using standard biochemical and microbiological tests, an antibiotic susceptibility test was performed on a Muller Hinton Agar medium and based on Clinical and Laboratory Standards Institute (CLSI) guidelines. Multiplex PCR assay was performed to detect virulence genes using specific primers.

Results: The results of the antibiotic susceptibility test showed that all isolates were sensitive to imipenem, gentamicin and amikacin. Also, molecular findings showed that the prevalence rates of *Phop/Q, slyA* and stn genes were 100%, 98.3%, and 91.6%, respectively. While *sopB* and *Spvc* genes were not observed in isolates of *Salmonella typhimurium*.

Conclusion: The results of this study indicate that the prevalence of virulence genes in clinical *Salmonella typhimurium* isolates can serve as an alarm for the prevalence of these genes to the other *Salmonella* serotypes.

Keywords: *Salmonella typhimurium*, Virulence genes, Multiplex-PCR

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Topic: Pathogenesis and Virulence Factors

P309: Effect of whole cell Vaccination on major virulence factor expression of *Bordetella pertussis* isolates in Iran

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Introduction and Objectives: Bordetella pertussis is the causative agent of whooping cough, a highly contagious respiratory disease most severe in infants and young children. Notably the resurgence of pertussis has recently been associated with the emergence and spread of pertussis toxin promoter (*ptxP3)* isolates and lacking the production of vaccine virulence factors. In This study we have been screening Fha and Prn production from 100 isolates collected during 2005-2018, in Iran with WCV immunization program.

Materials and Methods: We select 100 *B. pertussis* isolates from Pertussis Reference Laboratory of Pasteur Institute of Iran. Real-time PCR were used to confirm *B.pertussis.* To evaluate the expression of major virulence factors Pertactin (PRN) and filamentous hemagglutinin (FHA), SDS-PAGE and western-blot were used.

Results: The western blot analysis showed that all tested *B. pertussis* isolates expressed Prn and all but one expressed Fha and one isolate, was identified with severely reduced express Fha. We have sequenced genomes of these strains and identified differences compared with genome reference B. pertussis Tohama I.

Conclusion: In conclude, in many countries reporting Prn and Fha-deficiency due to vaccine influence but in Iran it does not looks seems like this. Because, ACV-induced immunity is focused on just a few proteins, creating a stronger selection pressure for strains expressing antigenic variants of these proteins. These results demonstrate surveillance of *B. pertussis* provide a better understanding of the effect of WCV on the evolution of the pathogen deficiency and emphasis the importance of continued surveillance of other major pertussis virulence factors and optimize strategies to reduce the incidence of pertussis.

Keywords: Bordetella pertussis, Filamentous hemagglutinin, Whooping cough

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Topic: Prevention and Control of Infectious Diseases

P311: Conventional and molecular characterization of *Clostridium perfringens type B* vaccine strain

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Introduction and Objectives: Clostridium perfringens is a spore-forming gram-positive bacterium which is found in the intestines of humans and animals. The best way to control these diseases is through vaccination. The purpose of this study is characterization of vaccine strain, using microbiological and biochemical properties, molecular approach and toxicity titration.

Materials and Methods: Vaccine strain (CN228) was cultured anaerobically in liver broth for 24 h. Afterwards, characterization of strain was done using microbiological (including Gram staining, culture on Blood agar and LB agar and motility) and biochemical tests (including fermentation of sugars, lecithinase, gelatinase, indol, and catalase). Then genomic DNA was extracted using phenol and chloroform extraction method. The target gene was amplified through PCR by specific primer which retrieved from gene bank and designed using OLIGO software. Finally, MLD of toxin was performed using intravenous injection into white mice tail vein. The challenged mice were observed daily over 3 days to determine toxin activity.

Result: *C. perfringens* colonies are smooth, round, and grayish which are surrounded by a double zone of hemolysis. In Gram staining was observed gram positive straight or curved rod shape. The other microbiological and biochemical properties of vaccine strain are also found similar to those of *C.* perfringens type B. On the other hand, Vaccine strain was confirmed using PCR analysis.

Conclusion: The result showed that vaccine strain is confirmed with reliable references and could be used as vaccine strain.

Keywords: *Clostridium perfringens type B*, PCR, Diagnosis, toxin

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Topic: Prevention and Control of Infectious Diseases

P291: *In silico* Prediction of Pneumococcal truncated forms of DnaJ (hsp40) as Multivalent Pneumococcal Vaccine Antigens

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Introduction and Objectives: *Streptococcus pneumoniae* colonizes the human nasopharynx and can cause diseases, including pneumonia, otitis media, septicemia and meningitis in pediatric, elderly, and immunocompromised populations. Presently, two types of pneumococcal polysaccharide-based vaccine are available. These vaccines have limitation of poor immunogenicity in children under two years old and limited serotype coverage. These limitations have provided the impetus for development of novel vaccines based on broadly representative, serotype-independent, highly-conserved pneumococcal protein antigens. DnaJ is a member of heat shock protein40 family (hsp40) and plays an important role in the pathogenesis of pneumococcal infection and is highly conserved in different serotypes of pneumococcal strains. Since after innate immune responses, humoral immune system has key defensive role in nasal and salivary mucosal sites against pneumococcal.

Material and Methods: We used in-silico methods for predicting continuous antibody epitopes from DnaJ protein sequence. Using Immune Epitope Database(http://tools.iedb.org/bcell/), six prediction including bepipred linear epitope, Chou & Fasman Beta-Turn, Emini surface accessibility, Karplus & Schulz flexibility, Kolaskar & Tongaonkar antigenicity and Parker hydrophilicity were performed.

Results: Our results were shown that according to the four first parameters including linear epitope, flexibility, surface accessibility and antigenicity predictions together and two another parameters (antigenicity and hydrophilicity), we could divide the DnaJ to two truncated forms. According to results of analysis of four first parameters, amino acids 1 to 139 at N-terminal of DnaJ had the highst scores and according to analysis of two parameters including antigenicity and hydrophilicity predictions, the truncated form of DnaJ (amino acids 140 to 378) can be prefer as proper truncated forms of DnaJ.

Conclusion: Finally based on results, we can use truncated forms of DnaJ in stimulating immune system as immunogenic forms and in multivalent pneumococcal vaccines design. In future we will design fusion truncated forms of DnaJ with other pneumococcal proteins and will investigate in-vivo immunogenicity of these truncated forms.

Keywords: Hsp40, *Streptococcus pneumoniae*, Multivalent Pneumococcal Vaccine, Bioinformatics, Iran.

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P315: *In silico* Prediction of Pneumococcal truncated forms of Neuraminidase, A as Multivalent Pneumococcal Vaccine Antigens

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Introduction and Objectives: Streptococcus pneumoniae is the most frequent cause of bacterial meningitis in children and is particularly associated with severe disease with a high mortality rate and brain damage leading to neurological sequela. All currently available polysaccharide-based pneumococcal vaccines have limitations and elicit only serotype-specific immunity. Alternative technology is the use of pneumococcal proteins for producing independent serotype-based vaccines. Pneumococcus has several proteins that are well-conserved among different serotypes, are surface-displayed and thus antibody accessible. One of them is neuraminidase A (NanA) that has important role in nasopharyngeal colonization of pneumococcus, acute otitis media and pneumococcal meningitis. Since after innate immune responses, humoral immune system has key defensive role in nasal and salivary mucosal sites against pneumococcal infection.

Materials and Methods: thus, we used in-silico methods for predicting continuous antibody epitopes from NanA protein sequence. Using Immune Epitope Database(http://tools.iedb.org/bcell/), six prediction including bepipred linear epitope, Chou & Fasman beta-turn, Emini surface accessibility, Karplus & Schulz flexibility, Kolaskar & Tongaonkar antigenicity and Parker hydrophilicity prediction were performed. We divided the NanA amino acids sequence to four truncated forms and investigated above parameters for each truncated forms.

Results: Our results were shown that according to analysis of linear epitope scores, the truncated forms of NanA (amino acids 1 to 258 and 776 to 1035) had the highest scores. Results of flexibility, surface accessibility, beta-turn and antigenicity analysis showed the truncated forms of NanA (amino acids 259 to 517 and 518 to 775) had the highest scores and analysis of hydrophilicity showed the highest score for the truncated form of NanA (amino acids 776 to 1035).

Conclusion: According to results, we can use two or three truncated forms of NanA to design multivalent pneumococcal vaccines. In future we will design fusion truncated forms of NanA with other pneumococcal proteins and will investigate in-vivo immunogenicity of these truncated forms.

Keywords: Neuraminidase A, Streptococcus pneumoniae, Multivalent Pneumococcal Vaccine, Iran.

Topic: Prevention and Control of Infectious Diseases

P283: Iran's first domestically developed ELISA Kits for diagnosis of Paratuberculosis and Brucellosis in bovids, a major step forward in response to the fast-extending local market for veterinary diagnostics

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Introduction and Objectives: In clinical perspective, early warning and diagnosis of infection is a pivotal step in successful implementation of prevention and control measures. This is where serological methods come first in provision of highly reliable results in epidemiological context. In the Iranian environment veterinary-used ELISA kits are all imported from abroad leaving a 200 million Euros market open to normally European suppliers. In 2016 when the "Science and technology park" initiative came into action at Razi institute, Mehr Nam ZistFanavar (MeNaZiFa), a science–based start-up in collaboration with Razi institute focused on development of its first diagnostic kit, Paratuberculosis ELISA kit. At the beginning things seemed not that straight forward but in the end the effort appeared to be a total success as in technical side principle difficulties such as non-specific reactions that typically challenge ELISAs accuracy, were well addressed.

Materials and Methods: In patenting and licensing, MeNaZiFa managed to attract positive response from all referees employed by the Iranian Veterinary Organization. MeNaZiFa is now therefore among the few recognized world companies active in the production field of paratuberculosis ELISA kits holding the technical know-how. In marketing, signals from cattle industry professionals received since June 2017 when the kit was commercially lunched first, are in support of the MeNaZiFa-Razi kit compered to its competing rivals both in cost and in application out come. Production of this kit covers variety of different phases including preparation of the antigen, effective coating and blocking of the plates, preparation of diluents as well as conjugates and establishments of Cutoff points which is software (ROC)-assisted process.

Result: In clinical trial, sensitivity and specificity of the kit have been tested against a huge cattle serum collection accommodating over 10,000 samples of fresh and frozen serums from different farms.

The work on this large KMF (Kit Master File) resulted in achieving a 99% level of specificity with an impressive high score of CV (9.5) compared to other available products in the Iranian market.

Conclusion: The MeNaZiFa later decided to use its constructive experiences with paratuberculosis to develop a further kit, the Brucellosis ELISA kit using the *B. abortus* S99 strain. This time things move forward quite smoothly and hassle-free with the production and marketing licenses granted to them by IVO to cover the national market. Thanks to God, if things go ahead as they do now, we will soon welcome new locally-made diagnostic kits, ELISAs of course, for many of animal infectious diseases.

Keywords: ROC- Paratuberculosis - ELISA - Brucellosis

Topic: Prevention and Control of Infectious Diseases

P12: The Importance of *Clostridiums* and Clostridial Toxins in the Prevention and Treatment of Diseases of Animals and Human

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Introduction and Objectives: bacteria, as living beings and complementary to human life, have always been the focus of attention of specialists and manufacturers of biological products. *Clostridium* bacteria have different species and the toxins produced by these species are one of the most dangerous and specific bacterial toxins. The production of toxins has a different constructional and functional nature, which makes them extremely hazardous and also very useful.

Materials and Methods: various uses of toxins and economic look at these toxins have led to more applied studies on these toxins for treatment and various cases. For example, the use of *Clostridium novyi* NT for the treatment of cancer or the use of *Clostridium botulinum* neurotoxin in treatment and cosmetic products applications are economical interest of biological producers.

Results: research on the functional and economic aspects of *Clostridium* bacteria and their toxins can create, in addition to creating targeted research projects for researchers and students, producing highly biotechnological products with high added value in the country. Research area into the isolation, screening and production of bacteria, as well as the production of various toxins, isolation and purification are very important and economical for researcher and manufacturers.

Conclusion: these activities, in addition to improving the quality of research on bacterial products, can be a good opportunity for the country to produce knowledge and the therapeutic and biological products of this bacterium for the purpose of treatment and economics.

Keywords: *Clostridium*, toxin production research, cancer, cosmetic products

Topic: Prevention and Control of Infectious Diseases

P107: Prevalence of Helicobacter pylori infection by Ab Detection in Kermanshah, s Referance Laboratory in year 1397

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Introduction and Objectives: Helicobacter Pylori is one of the important agent associated causally with a diverse spectrum of gastric disorders. The aim of this study was to determined of Prevalence of Helicobacter Pylori infection patterns in Kermanshah,s Province.

Materials and Methods: Total of 3214 Patients with IgA, IgG & IgM request sample (413 female, 302 male) was evaluated by Elisa assay in Reference Laboratory of Kermanshah.

Results: Results shows that, 4 cases was positive for 266 IgA request, 9 cases was positive for 1441 IgM request and 666 cases was positive for 1850 IgG request.Also 249 Positive cases was for 993 H.p stool Ag test.

Discussion: Results showed the chronic Helicobacter Pylori infection is very high Prevalence in female in Kermanshah Province . IgA and H.p stool Ag test less than IgG And IgM requested by Physician . So,The Physician should pay more attention about the current requests for Patients, with gastric disorders by the more important Antibodies detection.

Keywords: Helicobacter Pylori, Antibodies evaluate, Gastric disorders

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Topic: Prevention and Control of Infectious Diseases

P212: Application of the more affordable nanoparticles against the main bacterial infectious diseases in rainbow trout rearing industry

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Introduction and Objectives: Emerging of Infectious outbreaks caused by bacterial agents in the aquaculture industry, expansion of bacterial resistance to antimicrobial agents, and trouble of drug residuals in the products have led to the application of novel, safe and affordable alternatives such as Nanoparticles (NPs). The present study intends assessing the toxicity of CuO and ZnO NPs at *In vitro* conditions on the main bacterial pathogenic agents for the reared fish, including *Lactococcus garvieae*, *Streptococcus iniae*, and *Yersinia ruckeri*.

Material & Methods: First, the physical features of NPs crystals were determined by X-ray diffraction (Shimadzu, XRD-6100). Then, the sensitivity of them to the mentioned NPs was assayed with compared florfenicol, as a reference antibiotic, through the well diffusion method. Also, MIC/MBC were determined by the microdilution technique. We use one-way ANOVA in order to data analysis.

Results: The diameter of Nano CuO and ZnO crystals was estimated 41.48 and 49.6 nm, respectively. Evidence indicates the positive gram bacteria had intermediate sensitivity to ZnO, but they were resistant to CuO. However, enteric redmouth disease agent was absolutely sensitive to ZnO. Nano zinc oxide and copper oxide could significantly inhibit the growth of *Streptococcus iniae* or kill it at 0.18 and 0.24 µg/ml and more, respectively. Also, MIC/ MBC of ZnO NP were obtained for *Lactococcus garvieae* and *Yersinia ruckeri*, in order equal to 0.18 and 0.06 µg/ml.

Conclusion: Antibacterial potency of Nano ZnO was more than another NP and florfenicol at same concentration. Therefore, the application of it will be advised if there are not the observable toxic effects of this NP at the suggested concentration for reared fish.

Keywords: Nano copper oxide (CuO), Nano zinc oxide (ZnO), *Lactococcus garvieae*, *Streptococcus iniae*, *Yersinia ruckeri*.

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P211: *In vitro* study on Nano zinc oxide's bactericidal effect against the pathogenic agents of Iranian sturgeon infectious outbreaks

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Introduction and Objectives: Previously, two bacteria, including *Aeromonas veronii* and *Chrysobacterium joostei*, were isolated from diseased beluga and stellate sturgeon which identified as the agents of severe mortality, and infectious symptoms. The present assay targets assessment of Nano zinc oxide (ZnO)'s bactericidal effects against these bacterial pathogens in the form of *In vitro* study.

Material & Methods: For this purpose, the physical features of Nano ZnO crystals were determined by X-ray diffraction (Shimadzu, XRD-6100). Afterward, the sensitivity of bacteria to the nanoparticle was measured via the well diffusion method. At the final stage, MIC was determined by the microdilution technique, whereas MBC was evaluated through cultivating of the samples of transparent wells on Muller Hinton agar.

Results: XRD analysis demonstrated that Nano ZnO crystals' diameter was estimated equal to 49.6 nm. According to the findings, both bacteria were sensitive to Nano ZnO, whereas they had intermediate resistance to florfenicol (reference antibiotic). The obtained MIC of ZnO for *A. veronii* and *Ch. joostei* were 0.06 and 0.03 µg/ml, respectively. Inhibitory effect of the assessed nanoparticle for our motile aeromonad was equivalent to the bacteriocidal trait (MBC). However, MBC of ZnO for the cytophaga was recorded twice as much as its MIC (about 0.06).

Conclusion: The bactericidal/ inhibitory impact of Nano ZnO recognized well-suited for control of these pathogenic agents at *In vitro* level. It seems that necessary to use this nanoparticle in the sturgeon farms, after pass to *In vivo* and toxicity tests.

Keywords: Nano zinc oxide (ZnO), Sturgeon, *Aeromonas veronii*, *Chrysobacterium joostei*, XRD analysis.

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Topic: Prevention and Control of Infectious Diseases

P38: The role of microbiological laboratories in the prevention of hospital infections by implementing quality control management

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Introduction and Objectives: Hospital infections are one of the major causes of mortality in hospitalized patients. Considering the importance of proper diagnosis of bacteria in the hospital infection, important and fundamental steps in the microbiology lab are to identify and identify the bacteria correctly by implementing quality control in the processes before, during and after the bacterial identification. Also, the rate of infection control principles at hospital levels, the average infection rate varies from 5% to 25% on average worldwide. Therefore, considering the importance of the subject, the aim of this study was to evaluate the bacteria by sampling the medical, medical and other environment with the implementation of the above programs.

Materials and Methods: In a 3-year period from 1398 to 1396, using standard Haas method and implementing quality control management in the above triple processes, sampling from different areas of the hospital and medical equipment and other areas, identification of bacteria, confirmatory diagnosis Bacteria, response, interpretation, and expression of the importance of isolated bacteria and preventing the spread of bacteria.

Results: Out of 4320 samples, 531 isolates of pathogen and peripheral bacteria were isolated from which 95 (17/80%) cases of enterobacteriacea, 120 (22.59%), gram-positive bacilli, 253 (64.6% 47%), micrococcus, 56 (10.54%) non-fermented bacillus and 7 (31.1%) streptococci.

Conclusion: This study showed that isolated bacteria in each year had a significant decrease compared to previous years due to the proper attitude of hospital personnel involved with the importance of hospital infection. It can also be noted that the role of quality control meriset in identifying and identifying bacteria in the control and monitoring of possible adverse reactions is denial-of-pump. Increasing the non-standard capacity of the hospital is likely to result in hospital infections in hospitalized patients requiring training and supervision in this regard. Is. Quality control in pre-testing, testing and post-test procedures in microbiology departments of hospitals is carried out repeatedly and accurately, and all records, such as non-compliance and corrective actions, are documented.

Keywords: Hospital Infection, Microbiology, Qualitative Control

Topic: Prevention and Control of Infectious Diseases

P33: Bacterial Contamination of Computer Keyboard Computers of Imam Khomeini Hospital, Ardebil

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Introduction and Objectives: There are many reports about the environmental pollution of hospitals such as doors, pressure gauge, ventilator, bedside and many other devices with pathogenic bacteria such as Staphylococcus aureus and enterobacteria, which leads to hospital infections. In recent years, there has been a significant increase in the use of computers in different parts of the hospital. The work of the hospital staff with the computer and then the referral to the patients is part of their daily routine. It can also be said that the hands of the staff and the hospital environment play a role in the transmission of hospital infections. The purpose of this study was to determine the contamination of computer keyboards with the organisms producing infectious diseases.

Materials and Methods: Samples were collected from physiologically-stained swabs from 25 key computers in hospitals. Aerobic and anaerobic cultures were prepared using standard anoximet extraction system. The colonies were identified according to microbiological methods.

Results: All computers studied were infected with at least one bacterium. Known pathogenic bacteria colonized 9.3% of computers, while 80.8% of them were infected with organisms that could, in certain clinical conditions, be able to develop a hospital infection. Staphylococcus aureus was the most commonly isolated bacterium.

Conclusion: The results of this study indicate that the amount of contamination is higher than that of other studies, which may be due to the inappropriate use of disinfectants. Keyboard and mouse Computers are cloned with pathogenic bacteria and should be regularly sanitized and disinfected. The use of plastic sheets on computer keyboards is recommended.

Keywords: bacterial contamination, computer keyboard, Staphylococcus aureus, Ardebil

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Topic: Prevention and Control of Infectious Diseases

P324: Assessment of antibiotic prescription and application in small animal dentistry

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Some dental treatments may lead to oral infections although there were no previous signs of infection. Therefore, preventing the infection and acknowledging infectious complications should be one of the main priorities. Prescribing antibiotics as prophylaxis can minimize the probability of infection.

In this study, field surveys were conducted in Tehran veterinary clinics and Kerman veterinary clinics. These surveys were conducted considering initial conditions for antibiotic administration. After analysis of empirical data, a survey for correct antibiotic selection factors, right dosage of antibiotics and appropriate period for treatment was conducted. In the following, complications that can threat patient's life in case of antibiotic nonconformity and prophylaxis were expressed.

The main consumption of antibiotics is limited to treatment process (for conditions such as cellulite, acute dentoalveolic abscess, pericoronitis, osteomyelitis and etc.) and prevention process (preventions needed for post operation wounds, general or partial weakness of immune system and etc.). Non-principled prescription of antibiotics can lead to gastrointestinal symptoms, more infection, drug resistance, drug interaction and etc.

The disadvantage of unnecessary consumption is that the doctor won't notice that with prescribing a lower dosage, the effect will remain but the side effect will decrease. Unnecessary consumption of antibiotics is a common issue. However, not only it does not reduce the possibility of infection, but also is harmful. Unfortunately, there is no specific strategy based on previous evidence for prescribing antibiotics in oral and dental disease specially in small animals. Therefore, prescriptions are based on experience, expenses, application methods used by owners and etc.

Keywords: Antibiotic, Small Animal, Dentistry

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Topic: Prevention and Control of Infectious Diseases

P261: The relation of serum *Helicobacter pylori* CagA antibody and biomarkers of gastric cancer in dyspeptic patients in Kerman, Iran

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Introduction and Objectives: *Helicobacter pylori* (*H. pylori*), a worldwide infection, is associated with chronic gastritis, gastric ulcer, peptic ulcer, gastric atrophy, intestinal metaplasia and gastric cancer resulting from the cytotoxin-associated gene A (CagA). This study was evaluated the association between the serum levels of *H. Pylori* CagA antibody and gastric cancer biomarkers in dyspeptic patients of Kerman, Iran.

Materials and Methods: A total of 53 serum samples (38 seropositive and 15 seronegative for *H. pylori* IgG) from dyspeptic patients of Kerman were tested for anti-*CagA* IgG by a commercial ELISA kit (Euroimmun, Germany). Serum concentration of CA125, Ca19-9 and CEA cancer biomarkers were measured by a commercial kit (Roche, Germany) with Cobas machine (Cobas-e411). Cancer biomarker serum levels were compared in *H. pylori* CagA+ and CagA- patients.

Results: The prevalence of anti-CagA IgG was 92.45% (49 of 53 patients). The mean titres of anti-CagA IgG antibodies in CagA+ and CagA- patients were respectively 115.78 ± 78.69 and 5.50 ± 6.45 RU/mL that was statistically significant between groups ($p = 0.008$). The positivity of CA125, Ca19-9 and CEA biomarkers in CagA+ patients were 11.3% (6 person), 5.7% (3 person), and 24.5% (13 person), respectively and other patients shown a normal CA125, CA19-9, and CEA test. The Chi-square test did not show significant relationship between anti-CagA IgG antibodies and cancer biomarkers.

Conclusions: According to the results of this study on dyspeptic patients of Kerman, the serum levels of *H. Pylori* CagA antibody could not used as an appropriate biomarker for early diagnosis of gastric cancer.

Keywords: *Helicobacter pylori*, Cytotoxin-associated gene A (*CagA*), Biomarker, Gastric cancer

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P244: Vitamin D Levels and its Relation to Peripheral Blood Inflammatory Markers in Patients with Urinary Tract Infection

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Introduction and Objectives: The vitamin D status affects inflammatory responses. Vitamin D deficiency and its relationship with infections or autoimmune diseases have been found in many studies. We aimed to examine the association of 25(OH)D levels with inflammation markers in patients with urinary tract infection (UTI): neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), lymphocyte ratio-to-monocyte ratio (LMR), and the systemic immune-inflammation index (SII).

Materials and Methods: In this study, women (65 in the pateint group and 35 in the control group), who were between 18-60 years old, were studied. Serum 25(OH)D levels was measured by ELISA. The value of inflammation markers were all derived from complete blood counts. The correlation between these variables was also assessed by Pearson analysis.

Results: Serum 25(OH)D levels significantly decreased in patients compared to the control group. Patients had significantly higher white blood cell, neutrophil counts and NLR than the control group; it was found a lower count of lymphocyte and no significant difference for the monocyte count in patients group. When both MLR and LMR were compared between the two groups, there was no statistically significant difference in their values. However, the two parameters had a significant correlation with 25(OH)D levels in patients. The mean platelet count did not significantly differed between the two groups; the PLR significantly increased in patients group. The PLR was also inversely correlated with 25(OH)D levels. The SII (N×P/L) of patients was significantly different from those of the control group and had an inverse correlation with 25(OH)D levels in patients group.

Conclusion: The vitamin D might independently modulate response to infection and provide easily available new predictors of infection as compared to the commonly used parameters such as WBC, neutrophil count, and CRP requiring to be evaluated further.

Keywords: Vitamin D; Inflammation; Urinary Tract Infection; Inflammatory markers

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Topic: Prevention and Control of Infectious Diseases

P238: Investigation of Retrograde group B Streptococci infection and still-birth in Rat

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Introduction and Objectives: Group B Streptococcus (GBS) still catches infants live in the world. Ascending GBS infection during pregnancy involves bacterial trafficking from the vagina, ultimately leading to bacterial invasion of placental membrane, the amniotic cavity, and the fetus. It causes different complications like stillbirth and neonatal mortality. So, GBS experimental infection of rats was done and investigated their complications.

Materials and Methods: Up to 24 female Wistar Rats were mated and intra vaginally infected after pregnancy.The control group was received intra-vaginally 100 uL sterile normal saline and treatment group was inoculated with 100 uL of 10^8 CFU/ml of GBS(ATCC 13813) in the third week after mating.Five rats were mated and infected but had normal delivery. Before delivery (20^{th} day) uteruses were removed and pups were cultured in Thioglycolate broth. Microbiological identification were confirmed by Beta hemolytic on sheep Blood agar, Gram staining. Catalase test and CAMP test.

Results: In the control groups there were 89 pups vs. infected group had 51 pups. Culturing showed negative (100%, 89/89) for control groups and 48 pups (from 51) 94.11% had positive culture and GBS were recovered consequence. Two of five mothers that were infected and normally delivered, did not have any pups. One rat delivered 2 pups and another three ones. After removing uterus, four dead pups in uterus branch of one mother was observed. Two rats delivered dead pups after 20^{th} days of mating.

Conclusion: These effects showed that GBS is still one of the delivery defects that causes abortion and can causes to infect the amniotic sac and embryo in the form of retrograde that results to fetus death. and this study confirmed the Vornhagen′s study in 2017. Also, in the infected group as control, it was observed that GBS causeS to both preterm delivery and fetus death.

Keywords: GBS, Experimental Infection, Retrograde, Stillbirth

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Topic: Prevention and Control of Infectious Diseases

P221: Antibacterial silver Nano-composite hydrogel based on polyurethane-polyethylene glycol (PU-PEG) for wound healing applications

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Introduction and Objectives: The medical researchers have paid considerable attention to wound as a serious dilemma in sores or burn wounds. The aim of this study was to synthesis a novel Nano-composite hydrogel via click reaction chemistry followed by introducing of silver nanoparticles (Ag-NPs).

Material and Methods: Dialkynyl PEG was synthesized using PEG-2000 in the presence of NaH and propargyl bromide. Then, 2,2-bis(azidomethyl) 1,3-propane diol was synthesized using 2,2-bis(bromomethyl) 1,3-propane diol in the presence of NaN_3. Finally, polyurethane with pendant azido groups was synthesized using hexamethylene diisocyanate, dialkynyl PEG and 2,2-bis(azidomethyl) 1,3-propane diol in the presence of dibuthyl tin dilorate catalyst in THF solvent. Thereafter, Hydrogel was synthesized via coupling reaction between azide and alkyne groups of prepolymers in the presence of $CuSO_4.5H_2O$ and sodium ascorbate catalyst system. Green synthesis of the Ag-NPs was performed using oak fruit hull (Jaft) extract and then the NPs with concentrations (100, 200, 400 or 800 ppm) were loaded in the hydrogel. Various analysis methods such as NMR and FTIR spectroscopy, FE-SEM and XRD were used for characterization of hydrogel silver nano-composite. Finally, antibacterial activity of the silver nano-composite hydrogel was investigated against *Psudomonas aeruginosa* PAO1 and *Staphylococcus aureus* ATCC 13565 using disk diffusion and macrobroth dilution methods.

Results: FTIR and NMR analysis showed that the prepolymers and hydrogel were synthesized successfully. FE-SEM revealed the porous microstructure of the hydrogel. XRD results showed the crystallinity of the Ag-NPs. According to disk diffusion test on Mueller Hinton agar, 10 mm and 12mm inhibition zone around the Ag-loaded hydrogels was observed against *P. aeruginosa* and *S. aureus*, respectively. Also, minimum inhibitory concentration (MIC) of Nano-composite hydrogel against *P. aeruginosa* and *S. aureus* in macrobroth dilution was in NPs concentration of 200 ppm.

Conclusion: Based on these data, our PU-PEG based Nano-silver composite hydrogel can be used in the field of wound dressing or burn related infections management.

Keywords: Green synthesis, Silver Nano-composite, Antibacterial hydrogel

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Topic: Prevention and Control of Infectious Diseases

P46: Prevalence and antibacterial sensitivity of pathogens isolated from blood culture from Sections patients received in Afzalipour Hospital, Kerman lab, 1396

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Introduction and Objectives: Blood stream infections are a vast range of disorders, that can differ from limited bacteremia to mortal septicemia. According to the increasing antibiotic resistance among strains of **bacteria**, the purpose of this study was to determine the pattern in the antibiotic resistance patterns of bacteria strains **in the blood stream** isolated in patients, referring to **in Afzalipour Hospital, Kerman**.

Materials and Methods: Clinical data was collected from patients with positive blood cultures for one-year period. Standard laboratory methods were used for blood culture. Antibiotic sensitivity was tested using Kirby-Bauer disc diffusion method.

Results: Of the 262 bacterial pathogens isolated from patients, 56.1% were gram-positive and 43.9% were gram-negative. The common isolates were: Staphylococci species 141 (54.1%), Streptococcus species 3 (1.1%), Escherichia coli 20 (7.6%), Klebsiella spp. 45 (17.1%), Pseudomonas spp. 30 (11.4%), Enterococcus 3 (1.1%), Acinetobacter spp. 20 (7.6%). The most effective antibiotic against Gram positive isolates was vancomycin and the most effective antibiotic against Gram negative isolates was ciprofloxacin.

Conclusion: This study provides information on antibiotic resistance of blood isolates. It may be a useful guide for physician antibiotic therapy and will help in formulation of antibiotic therapy strategy in this part of the country.

Keywords**:** Blood stream infections, Antibiotic resistance, Bacterial pathogen.

Topic: Prevention and Control of Infectious Diseases

P29: Bacteriology and antimicrobial susceptibility patterns in children with congenital nasolacrimal duct obstruction in Isfahan, Iran 2018

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Introduction and Objectives: Dacryocystitis is one of the most common eye diseases due to inflammation of the lacrimal sac. It can be of two types: acute and chronic forms. An acute form of this disease is presented as inflammation of lacrimal sac with burning and erythema of overlying tissues and a lacrimal abscess can be seen in more than 20% of cases. The aim of this study is identification of common bacteria causing nasolacrimal duct infection and determination of their antimicrobial susceptibility profiles in children with congenital nasolacrimal duct obstruction.

Materials and Methods: This cross-sectional and analytical study was done in the ophthalmology department of Isfahan University of Medical Sciences (center of Iran) from January to February 2017. Identification of specimens was done using phenotypic and genotypic methods. Disc diffusion method with MAST antibiogram discs was used for antibiotic susceptibility tests, according to the Clinical and Laboratory Standards Institute, 2017 .

Results: All of 59 isolates from culture of specimens belonged to gram positive cocci. Staphylococcus epidermidis was the predominant species (n=51, 83%) followed by Staphylococcus aureus (n=5, 8.1%), Staphylococcus haemolyticus (n= 2, 3.2%) and each of Staphylococcus saprophyticus, Staphylococcus hominis and Streptococcus pneumoniae (n=1, 1.6%). Totally, highest resistance was found against erythromycin and tetracycline while vancomycin, chloramphenicol, ciprofloxacin and imipenem showed the highest susceptibility.

Conclusion: The present study is useful for determining the appropriate antibiotic for systemic treatment of dacryocystitis in our region. vancomycin, chloramphenicol, ciprofloxacin are the most sensitive antibiotics against the most common isolated microorganisms. Since the bacteriology of nasolacrimal duct infections differs in different regions, more studies in other parts of our country are recommended to detect bacterial pathogens involved in acute infections.

Keywords: Dacryocystitis, antibiotic resistance, congenital nasolacrimal duct obstruction, bacteriology

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Topic: Prevention and Control of Infectious Diseases

P119: The impact of the journal club on nurses' awareness of blood culture contamination

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Introduction and Objectives: Contamination of blood cultures is common from microorganisms outside the bloodstream. This contamination delayed or imprecise treatment, increased risks of morbidity, extended the length of hospitalization, increase laboratory work, and increased costs of care. The aim of this study was to develop staff professionalism in drawing blood culture and promote effective change by journal club.

Materials and Methods: This hospital-wide educational intervention study was conducted with a pretest and posttest design, 100 nurses working in three hospitals affiliated to Tabriz University of Medical Sciences were enrolled in the study in 2019. The journal club based on problem and evidence was done two hours per hospital. Of the 25 articles that were searched for specific terms during the review, two articles were selected. The content of the journal club included a pretest, presentation of two selected articles, question session, and posttest. Data analysis was performed using descriptive statistics a paired t-test.

Result: The mean age of the participants was 30.15 ± 7.18 years and %75.5 had more than 5 years' work experience. The mean score of pretest and posttest was found to be 7.90±3.02 and 13.88±3.27, respectively. %90 of the participants stated that the information provided was up-to-date and practical. The result showed that nurses' awareness of blood culture contamination has a significant statistical difference after the presentation of the journal club ($p< 0.001$).

Conclusion: Contamination of BCs can never be completely crossed out but there is evidence by increasing the knowledge and skills of nurses based on the best practice BC collection techniques can minimize BC contamination. This study led to the onset of Clinical Audit of Blood Culture contamination in hospitals affiliated to Tabriz University of Medical Sciences to reduce it below 3% and according to global statistics.

Keyword: blood culture contamination, journal club, nursing

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P224: Isolation, morphological characterization and cross-host determination of lytic phages infecting multidrug resistant *Pseudomonas aeruginosa* from different sources

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Introduction and objectives: *Pseudomonas aeruginosa* colonization is one of the major complications of burn wound infection leading to higher risk of morbidity and mortality. Unfortunately, antibiotic resistance among *Pseudomonas aeruginosa* is increasing due to irregular and overuse of antibiotics. Phage therapy, eradicating of infecting bacteria with bacteriophages,could be a future alternative to antibiotic treatment of bacterial infections. This study was aimed to isolate bacteriophages against MDR-*Pseudomonas aeruginosa* isolated from burn wounds from hospital sewage, soil, and sewage polluted rivers in Iran and determination of their cross-host by EOP.

Material and Methods: Samples were collected from hospital sewage, soil and polluted rivers in Rasht and Hamadan four times. Suspensions of various strains of *Pseudomonas aeruginosa* isolated from burn wounds were mixed with samples in double LB broth. Lytic phages isolated after centrifugation, filtration and serial dilution. Finally, host range against 12 MDR-*Pseudomonas aeruginosa* were determined by EOP to evaluate the effect spectrum of the bacteriophages. Furthermore, morphological characteristics were determined using TEM (Transmission electron microscopy).

Results: In this study 18 lytic bacteriophage were isolated after four times sampling . 5,9 and 4 phages isolated from sewage, polluted rivers and soil respectively. Six of eighteen phages were selected as highly effective phages that infect wide range of burn wound infecting MDR-*Pseudomonas aeruginosa* by EOP. TEM imaging revealed all isolated phages belonged to *Myoviridae* family.

Conclusion: EOP showed that more *Pseudomonas aeruginosa* strains were lysed by phages isolated from soil compared to other sources.

Keywords: MDR-*Pseudomonas aeruginosa*, Bacteriophages, EOP, TEM

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P207: The effect of *Satureja* essential oils on *Pseudomonas aeruginosa* virulence factors

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Introduction and Objectives: *P. aeruginosa* is one of the most common opportunistic bacteria in nosocomial infections, which has a significant resistance to antimicrobial agents. Due to restrictions in the use of antibiotics, the tendency to replace them with natural products is increasing. In this study, evaluated the antimicrobial effects of 4 species of Satureja essential oils (*S. mutica*, *S. bachtiarica*, *S. rechingeri jamzad* and *S. khuzestanica*) on virulence factors of *P. aeruginosa*.

Materials and Methods: The minimum inhibitory concentration of Satureja essential oils was determined by microdilution broth method against standard strains of *P. aeruginosa* PAO1 and *P. aeruginosa* 8821M. In the following, the effects of Sub-MIC concentrations of essential oils were investigated on motility, biofilm formation, alginate, elastase and alkaline protease production of these two strains.

Results: Each of the four Satureja essential oils had antimicrobial effects against standard strains of *P. aeruginosa*, and also sub-MIC concentrations of the extracts significantly reduced the virulence factors production of these strains. Meanwhile, *S. khuzestanica* had the most antimicrobial activity.

Conclusion: In this study, the antagonistic effects of Satureja essential oils were observed against *P. aeruginosa*. By further study, these essential oils can be used as an antimicrobial compound against this bacterium.

Keywords: Essential oils, *Pseudomonas aeruginosa*, Virulence factors, *Satureja*.

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P203: Inhibition of *Pseudomonas aeruginosa* quorum sensing by subinhibitory concentrations of metal-ciprofloxacin complexes

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Introduction and Objectives: *Pseudomonas aeruginosa* quorum sensing (QS) system is a cell to cell signaling mechanism that regulates virulence factors and pathogenicity. Therefore, the QS system in *P. aeruginosa* may be an important target for pharmacological intervention. The present study aimed to investigate the effects of sub-minimum inhibitory concentrations (sub-MIC) of metal-ciprofloxacin complexes against *P. aeruginosa* quorum sensing related virulence factors.

Materials and Methods: After synthesis and structural confirmation of metal- ciprofloxacin complexes (cu-cip, zn-cip, mn-cip and co-cip) using NMR, FTIR and X-ray, the MIC of ciprofloxacin alone and metal complexes was detected against *P. aeruginosa* PAO1 using broth microdilution method. The effect sub-MIC (1/4 and 1/16 MIC) concentrations of these compounds on motility and biofilm formation was also determined.

Results: MIC of ciprofloxacin and metal-ciprofloxacin complexes was 0.125 µg/ml. A significant decrease (ranged from 15% to 53.6%) in biofilm formation was observed when PAO1 strain was grown with 1/4 and 1/16 MIC of all compounds ($P< 0.05$). The inhibitory effect was concentration dependent. The concentration of 1/4 of co-ciprofloxacin demonstrated greater inhibition of biofilm formation with 53.6%. Furthermore, these components with 1/4 and 1/16 of MIC reduced swarming and twitching motilities ranged from 15-57%.

Conclusion: The results indicate that sub-MIC of metal-ciprofloxacin complexes exhibited inhibitory effects against *P. aeroginusa* QS related virulence factors. These compounds could lead to the development of alternative therapeutics against *P. aeruginosa.*

Keywords: Metal –ciprofloxacin complexes**,** *Pseudomonas aeruginosa***,** Quorum sensing**.**

Topic: Prevention and Control of Infectious Diseases

P201: Synthesis of a quercetin-loaded hydrogel based on Hyaluronic Acid-Polydimethylsiloxane (HA-PDMS) with antibacterial activity

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Introduction and Objectives: One of the recent applications of the hydrogels is wound dressing to promote the wound healing process. In this study we synthesized a quercetin-loaded HA-PDMS composite hydrogel in which quercetin antibacterial activity was stabilized.

Materials and Methods: Cross-linked HA hydrogel was synthesized using PDMS-DG in alkaline condition, for 2 hours at 37 °C. Cross-link between HA and PDMS was performed based on OH (from HA) and epoxy (from PDMS) reaction leading to ether bond formation. After that, the room temperature (RT) dried hydrogel was immersed in quercetin solution (4 or 2 mg/mL) for 24 hours in dark and RT. Quercetin-loaded hydrogel was characterized using NMR analysis. Antibacterial activity of the quercetin-loaded hydrogels was investigated against *Psudomonas aeruginosa* PAO1 using disk diffusion and bactericidal efficiency (40 mg of quercetin-loaded hydrogels was dissolved in a 1ml medium. Then 150 µL mixture of bacterial suspension at 10^6 CFU/ml and hydrogel solution (1:1) was cultured for 24 hours at 37 °C.

Results: HA-PDMS hydrogel was synthesized as a transparent gel with vial inversion property. Loading with quercetin, changed the color of the hydrogel to yellow. NMR analysis showed that the HA-PDMS hydrogel and quercetin-loaded hydrogels were synthesized successfully. According to disk diffusion test on Mueller Hinton agar, 12 mm inhibition zone around the quercetin-loaded hydrogels was observed against *P. aeruginosa*. Also, bactericidal efficiency test revealed 66.76% and 15.75% inhibition in *P. aeruginosa* growth in the case of 4 and 2 mg/mL quercetin immersed hydrogels, respectively.

Conclusion: Based on our results, quercetin-loaded hydrogel has a good potential in bacterial infections and wound healing management.

Keywords: Hyaluronic acid, PDMS-DG, Quercetin, Antibacterial hydrogel

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Topic: Prevention and Control of Infectious Diseases

P262: High immunogenicity of *Streptococcus pneumoniae* IgA1 protease

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Introduction and Objectives: IgA1 protease is one of the virulance factors in Streptococcus pneumoniae and plays an important role in bacterium pathogenesis. In the present study, the immunogenicity of IgA1 protease was evaluate in healthy human sera**.**

Materials and Methods: IgA1 protease truncated gene (705bp) was amplified by PCR and following digestion with restriction enzymes, ligated with pET28a expression vector. Recombinant protein expression was carried out in E. coli BL21 strain and recombinant protein purified by affinity chromatography. Antibody titer against recombinant protease in three groups of age 0-2, 2-40 and over 40 years old healthy individuals was measured with ELISA assay.

Results: Recombinant protein was expressed and purified successfully. Antibody titer against IgA1 protease showed a significant correlation (p-value<0.05) between groups under two and over two years at titers of 20, 40, 80 and 160.

Conclusion: According to the results, an appropriate antibody titer against recombinant protease was obtained especially in people with over two years old. It concluded that the high levels of antibody against recombinant IgA1 protease may be resulted from permanent stimulation of immune system by IgA1 protease which naturally produce by streptococci in oral cavity.

Keywords: IgA1 protease, *Streptococcus pneumoniae*, ELISA assay

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Topic: Prevention and Control of Infectious Diseases

P308: Rubella Immunity in Pregnant Iranian Women: A Systematic Review and Meta-Analysis

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Introduction and Objectives: Rubella infection within the first trimester of pregnancy may lead to adverse pregnancy outcomes. The present study was conducted to evaluate the immunity against rubella among the pregnant Iranian women.

Materials and Methods: The steps of meta-analyses were conducted based on the MOOSE protocol and reported according to the PRISMA guidelines. To review the associated English and Persian literature, a comprehensive search was conducted among the international databases such as Scopus, PubMed/Medline, Science Direct, Embase, Cochrane library, Web of Science and Google Scholar search engine as well as Iranian databases, until April 1, 2018 using the following medical subject headings (MeSH) keywords: 'Pregnant', 'Gestational','Complications of pregnancy','Rubella infection', 'Prevalence, 'Epidemiology', 'Immunity', 'Immunization', 'Antibody', 'Immunogenicity' and 'Iran'. Cochran's Q test and I2 index were used to investigate heterogeneity in the studies. Random effects model and fixed effects model were respectively used to estimate the rate of rubella immunity and the effect of different variables on immunity against rubella. The obtained data were analyzed using Comprehensive Meta-Analysis Ver.2.

Results: Fifteen studies constituting 7,601 pregnant Iranian women met the inclusion criteria. The overall pooled rubella immunity rate was 90.1% [95% confidence interval (CI): 86.1-93.1]. Rubella immunity rates were respectively 88.6% (95% CI: 80.6-93.6) and 91.5% (95% CI: 88.1-93.9) before and after national vaccine program. Rubella immunity rates were 91.4% (95% CI: 87.8-94.0) and 87.2% (95% CI: 74.3-94.1) based on the enzyme-linked immunosorbent assay (ELISA) and haemagglutination-inhibition (HAI) methods, respectively. There was no significant association between rubella immunity and vaccination program (P=0.398), diagnostic methods (P=0.355), geographic regions (P=0.286), quality of the studies (P=0.286), occupation (P=P=0.751), residence (P=0.801), and year of the studies (P=0.164), but it was significantly associated with age (P<0.001).

Conclusion: Despite high rubella immunity among the pregnant Iranian women, anti-rubella antibody screening is recommended for all women of childbearing age.

Keywords: Immunity, Iran, Meta-Analysis, Pregnant Women, Rubella

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Topic: Prevention and Control of Infectious Diseases

P294: Prevalence of Human Immunodeficiency Virus (HIV) and Hepatitis C in drug abusers referring to methadone clinics at Fasa city in 2014

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Introduction and Objectives: In Iran, drug-users especially intravenous drug users are considered among high-risk groups for HIV and HCV infection. Given the key role of preventive measures to control these disease, ongoing surveillance is essential in high-risk groups. The aim of this study is to determine the prevalence of these infections in drug-users admitted to MMT centers in Fasa and their relation with some risky behaviours among them.

Material and Methods: This study is a cross-sectional study performed on 252 drug-users admitted to MMT clinics in Fasa during 7 weeks in the months of June and July 2015 with venous blood sampling, completing a check list of high risk behaviors and risk factors for HIV and HCV infection, and testing blood samples for Anti-HIV Antibody and Anti-HCV Antibody titer with ELISA kit. The data was analyzed using SPSS 21 and p-values less than 0.05 were considered as significant.

Results: Of the 252 subjects, 30 (11.9%) were HCV positive and 9 (3.6%) were HIV positive. The prevalence of HIV and HCV in individuals with a history of injection was 19.2% and 50% respectively and in individuals without a history of injection was 1.8% and 7.5% respectively. As well as in those with a positive history of injecting drug use ($p < 0.001$), history of injecting drug use with shared equipment ($p<0.001$), and history of imprisonment or detention ($p<0.001$), the prevalence of HCV, and in people with a history of injecting drug use ($p<0.001$), a history of injecting drug use with shared equipment ($p<00.01$), history of imprisonment or detention ($p=0.008$) and a history of unprotected extra-martial sex ($p=0.047$) HIV prevalence was significantly higher.

Conclusion: Given the notable prevalence of these diseases in Fasa city in drug users, particularly injecting drug users and probable lack of awareness of some patients about their disease, while taking measures to raise awareness and reduce harm, is felling the need to more widespread surveillance and screening.

Keywords: Prevalence, HIV, HCV, Drug users, Methadone maintenance treatment

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Topic: Prevention and Control of Infectious Diseases

P213: Investigation of the inhibiting potency of common antibiotics in aquaculture for *Lactococcus garvieae* isolated from the reared rainbow trout in Fars Province

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Introduction and Objectives: The present study was intended to assessing the in vitro efficacy of some used antibiotics in aquaculture for inhibiting *Lactococcus garvieae* isolated from the reared rainbow trout (*Oncorhynchus mykiss*) in Fars Province.

Materials and Methods: For this purpose, the antibiotic susceptibility test (AST) was performed by disk diffusion on the Fars isolates, which previously were identified and reported. Then, the minimum inhibitory/bacteriocidal concentration (MIC & MBC) were determined by microdilution methods via reading the opacity degree of each dilution (by microplate reader) and recultivating them on plates.

Results: According to findings, the isolated *Lactococcus garvieae* from the diseased fish of Fars province was susceptible to enrofloxacin (ENR), erythromycin (E) and florfenicol (FFC). In general, this bacterial pathogen showed resistance to 54.5% of the studied antibiotics. Regarding results, MIC and MBC of FFC for the assessed bacteria were recorded 1.2 and 5 µg/ml, respectively.

Conclusion: It seems that for treatment and control of lactococcosis in fish, use of FDA-approved antibiotic (FFC), would be more efficient. As if, fortunately, based on evidence, FFC is still effective on both pathogen species in Fars Province and other parts of the country.

Keywords: Minimum inhibitory concentration (MIC), Minimum bacteriocidal concnetration (MBC), Rainbow trout (*Oncorhynchus mykiss*), *Lactococcus garvieae*, Florfenicol (FFC).

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P312: Evaluation of the immune responses following co-administration of PilQ and type b-Flagellin from *Pseudomonas aeruginosa* in the burn mouse model

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Introduction and Objectives: Considering the increased antibiotic resistance of *Pseudomonas aeruginosa*, the evaluation of immune response against the antigens of this bacterium seems necessary. In this study, the protective efficacy and immunological properties of *P. aeruginosa* recombinant PilQ (r-PilQ) and type b-flagellin (FLB) proteins was evaluated in the burn mouse model of infection.

Materials and Methods; The inbred BALB/c mice were immunized with r-PilQ and FLB antigens. To investigate the type of induced immune response, sera were analyzed by ELISA for total IgG, IgG1, and IgG2a isotypes. After the final immunization, the IL-4, IFN-γ, and IL-17 cytokines level were examined in the spleen of non-challenged mice. Fifty days after lethal challenge, the survival rate and bacterial burden in the skin and other internal organs of experimental mice were assessed.

Results: The *in vivo* administration of r-PilQ, FLB and combined antigen resulted in a significant increase in the survival of mice (66%, 75%, and 83%, respectively) infected by the PAO1 strain of *P. aeruginosa* in the burn model of infection. Immunization of mice with r-PilQ and FLB mixture induced high titers of IL-4 and IL-17 cytokines compared to control groups ($P < 0.05$). The high titer of antisera raised against combined antigen was able to inhibit the systemic spread of the PAO1 strain from the site of infection to the internal organs.

Conclusion: We concluded that the parallel role of IL-4 and IL-17 is necessary for elimination of the bacteria and promotion of survival in the immunized burn mice.

Keywords: *Pseudomonas aeruginosa*; PilQ; Flagellin; burn; immunization; cytokine

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Topic: Prevention and Control of Infectious Diseases

P259: Characterization of the Outer membrane vesicles (OMVs) obtain from *Bordetella pertussis*

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Introduction and Objectives: Pertussis is a respiratory infectious disease that has been resurged during the last two decades despite high vaccine coverage worldwide. The increase in pertussis cases has been mostly attributed to waning vaccine-induced immunity, the switch from whole cell vaccines to acellular vaccines (aP) and pathogen adaptation in the face of the selection pressure induced by the vaccine that results in changes in the *B. pertussis* bacterial population strains that do not have a perfect match with the vaccine.

The epidemiological situation of pertussis points out the need to develop new generation of vaccines that capable of conferring both long-lasting immunity and protection against different strain genotype in this regard we characterize the properties of OMVs obtained from *Bordetella pertussis* as a good vaccine candidate.

Material and Methods: The isolate of *B. pertussis* was grown at 36 ◦C for 72 h on Bordet Gengou agar (BGA) supplemented with 10% defibrinated sheep blood, and subcultured 24 h in the same medium before use.

The OMVs obtained from *Bordetella pertussis* strain were negatively stained and examined with an electron microscope.SDS-PAGE, immunoblot techniques were used to describe OMVs properties.

Results: the size range of OMV was consistent from batch to batch and similar to previously described OMV preparations. To further characterize the OMVs immunoblottings were performed using specific antibodies against Ptx and Prn and Fha. OMVs studied expressed all three main protective immunogen.

Conclusion: Altogether according to the results OMVs derived from *B. pertussis* express 3 virulence factors, it is tempting to speculate that protective efficacy observed with all OMVs may be attributed to common factors that are expressed in all bacterial strains used. since OMVs reported here contain main immunogens used in currently acellular vaccines but expressed in the context of the pertussis membrane, allowing the immune response to preferentially target surface-exposed epitopes in their native conformation it might be considered a possible basic material for the development of acellular pertussis vaccines.

Keywords: *Bordetella pertussis*, outer membrane vesicles, Vaccine candidate

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Topic: Prevention and Control of Infectious Diseases

P268: Overproduction of the pro-inflammatory cytokines by peripheral blood mononuclear cells from *Helicobacter pylori*-infected patients with peptic ulcer

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Introduction and Objectives: *H. pylori* is one of the most prevalent infection in human and chronic infection with this bacterium may lead to peptic ulcer (PU), gastric mucosa-associated lymphoid tissue (MALT) lymphomas and gastric cancer. The host immunologic and genetic parameters play a critical role in the expression of the bacteria-related clinical symptoms. This investigation aimed to evaluate the expression pro-inflammatory cytokines IFN-γ and IL-12 by peripheral blood mononuclear cells (PBMCs) from *H. pylori*-infected peptic ulcer (PU) patients and asymptomatic (AS) subjects.

Material and Methods: The fresh PBMCs were obtained from 20 PU patients, 20 AS subjects and 20 non-infected individuals and were cultured for 32 hours without stimulation, in the presence H. pylori-derived crude extract or PHA. Then levels of the IFN-γ and IL-12 in the supernatants were measured using ELISA method.

Results: No significant differences were observed between HP-stimulated PBMCs and non-stimulated PBMCs from non-infected individuals concerning the IFN-γ and IL-12 production. In both PU and AS groups, the levels of IFN-γ and IL-12 production by HP-stimulated PBMCs were significantly higher than non-stimulated PBMCs. The IFN-γ and IL-12 similarly expressed in non-stimulated PBMCs and PHA-stimulated PBMCs from PU patients, AS subjects and non-infected individuals. The amounts of IFN-γ and IL-12 production by HP-stimulated PBMCs from PU were significantly higher than equal cultures in AS subjects and non-infected individuals.

Conclusion: The HP-stimulated PBMCs from PU produce higher amounts of IFN-γ and IL-12 than AS subjects and non-infected individuals that represent the possible contribution of these cytokine in the pathogenesis of PU.

Keywords: *H. pylori*, Peptic ulcer, IFN-γ, IL-12

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Topic: Treatment and New Drugs

P216: In vitro survey of Antibacterial Effect of *Myrtus Communis, Satureja Hortensis* and *Ocimum Basilicum* Essential Oils on *ESCHERICHIA COLI, BACILLUS CEREUS, BACILLUS LICHENIFORMIS* and *PSEUDOMONANS AEROGINOSA*

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Introduction and Objectives: In this study, the antimicrobial effect of *Myrtus communis, Satureja hortensis* and *Ocimum basilicum* essential oils was studied on four food pathogens, namely *Escherichia coli, Bacillus cereus, Bacillus licheniformis* and *Pseudomonas aeruginosa* in vitro.

Materials and Methods: Minimum inhibitory concentration (MIC) and the constituents of the essential oils were identified by gas chromatography (GC) and gas chromatography linked to mass spectrometry (GC/MS). The micro-dilution method was used to determine the MIC of the essential's oils on the pathogens.

Results: Generally, it was found that the *Ocimum basilicum* essential oil had a stronger antibacterial effect than *Myrtus communis* and *Satureja hortensis* on the four mentioned bacteria, whereas the *Satureja hortensis* essential oil had the weakest inhibitory effect.

Conclusion: Results showed that for inhibiting the growth of *Escherichia coli*, *Bacillus cereus, Bacillus licheniformis* and *Pseudomonas aeruginosa*, the concentrations of 78.125 ppm and 156.25 ppm of the *Ocimum basilicum* essential oil were the best choices, respectively.

Keywords: *Myrtus communis*, *Satureja hortensis*, *Ocimum basilicum*, Essential oil, MIC, GC/MC

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Topic: Tkreatment and New Drugs

P200: Antibacterial effects of nanochitosan-gelatin film including *Cumin Cyminum L* essential oil on growth of *some bacterial pathogens* by disk diffusion method

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Introduction and Objectives: The use of biodegradable biopolymer films can cause increase the shelf life of food in industrial packaging. These films via thin layers of different materials on the surface of foods can control microbial hazards. Chitosan, as a cationic polysaccharide, can form gel and Gelatin that is a protein derived from collagen and a gel producer that has a hydrophilic nature and can delay the growth of molds. Also, *Cumin Cyminum* is one of the most popular spices that has antimicrobial properties in active packaging. *Staphylococcus aureus*, *Listeria monocytogenes*, *E. coli* O_{157}*:* H_7 and *vibrio parahaemolyticus* are important foodborne pathogens which lead to infectious diseases and dangerous intoxication in humans. So, in this study antibacterial effects of nanochitosan (2%) gelatin(4%) film including *Cumin Cyminum L EO*(0;0.3;0.6;0.9)% on growth of some bacterial pathogens by disk diffusion method, have investigated.

Material and methods: After steam-distillation and GC/MS of *Cumin Cyminum EO;*and according to protocol of *(Sigma-Aldrich)*, nanochitosan 2% and Gelatin 4% was prepared.So;10^7 *cfu/ml* inoculums from each bacterial pathogens cultured on Muller Hinton Agar and done disk diffusion.

Result: Analyzing of SPSS software (Version:21-ANOVA) showed the inhibitory effect of gelatin-nanochitosan ,only, with 0.9% *E.O* on *E. coli O157: H7* (ATCC: 18776) (12 ± 1 mm). The inhibitory effect of *Listeria monocytogenes* (ATCC: 87946) on gelatin-nanochitosan film with different concentrations of *E.O* indicates a significant difference between each concentrations of *EO* ($p<0.05$). In the case of *Staphylococcus aureus* (ATCC: 36454), this inhibitory effect was observed as well as increasing of *E.O* concentrations.Also, *Vibrio parahaemolyticus* (ATCC: 43996), in comparison with others has more inhibitory effect by gelatin-nanochitosan, with and without *E.O* ($p<0.05$).

Conclusion: Application of *Cumin Cyminum EO*(.0.9%)- nano-chitosan -gelatin films inhibit the growth of these bacterial pathogens and have potential to extend shelf life of food.

Keywords: Nanochitosan, gelatin, *Cumin Cyminum EO*, film, antimicrobial effects, disk diffusion.

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Topic: Treatment and New Drugs

P208: Designing of anti-virulence compounds to combat *Pseudomonas aeruginosa* pathogenicity by disrupting the quorum-sensing system

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Introduction and Objectives: Increasing antibiotic resistance requires immediate novel therapeutic decisions to battle microbial infections. One of the alternative approaches to overcome resistance is intervening with the bacterial pathogenicity without affecting the cell viability. Here, we selected PqsR, one of the virulence regulator proteins of *Pseudomonas aeruginosa*, as an attractive target in its quorum-sensing network.

Materials and Methods: Based on the reported antimicrobial effects of Henna (*Lawsonia inermis*), and the chemical structure relationship of Lawsone and PqsR ligand, a series of Lawsone derivatives were synthesized to assess against *P. aeruginosa* PAO1. After determination of MIC (Minimum Inhibitory Concentration) values, one of the compounds was selected and evaluated against biofilm formation, virulence factors production (including pyocyanin, pyoverdine and protease), acyl homoserine lactones (AHLs) accumulation, and Tobramycin effectiveness potentiation. Additionally, its interaction with the active site of PqsR was studied by molecular modeling.

Results: The results indicated that the selected compound can be used as a potential inhibitor in preventing biofilm formation and virulence factors production which are important factors in antibiotic resistance. It is also a good candidate to increase Tobramycin potency against *P. aeruginosa*.

Conclusion: This study presents a novel insight into ligand-based drug design and affords a chemical scaffold to inhibit *P. aeruginosa* pathogenicity by targeting PqsR.

Keywords: *Pseudomonas aeruginosa*, PqsR, *Lawsonia inermis*, Lawsone, Biofilm

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Topic: Treatment and New Drugs

P321: Propolis ethanol extract effect on the cytoplasmic membrane in *Yersinia ruckeri* causative agent of salmonids' enteric red mouth disease

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Introduction and Objectives: Propolis is a resinous substance produced by honeybees which collected from different plants. *Yersinia ruckeri* is a gram-negative stain bacilli and the causative agent of salmonids' enteric red mouth disease.

The aim of this article is to identify the effect mechanism of propolis ethanol extract on the cytoplasmic membrane in *Yersinia ruckeri.*

Materials and Methods: After collecting propolis from beehives, propolis ethanol extract was prepared; and analysis of constituent materials was carried out by **gas chromatography-mass spectrometry (GC-MS)** method. Then propolis ethanol extracts antibacterial activity against *Yersinia ruckeri* Was performed by microdilution technique. In the next step, bacteria face with the 1× concentration of inhibitory concentration in growth level and rate of ATP production, membrane potential and the ratio of $NAD^+/NADH$ the rate was *measured.* statistical survey of data was done with the **analysis of variance** (**ANOVA**) method.

Results: Results showed the minimum bacteriostatic and bactericidal dose of propolis ethanol extract against *Yersinia ruckeri* equal 62.5 and 125 μg/ml by sequence; and ATP production rate and *Yersinia ruckeri* membrane potential in effect of propolis ethanol extract were significantly reduced.

Conclusion: Based on the results, it can be concluded propolis ethanol extract applies its antibacterial action in *Yersinia ruckeri* by inhibiting peptidoglycan synthesis and cytoplasmic dysfunction.

Keywords: Propolis, salmonid, *Yersinia ruckeri*

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Topic: Treatment and New Drugs

P223: Evaluating the synergistic effect of cinnamon extracts and honey against multidrug-resistant strains of *Pseudomonas aeruginosa* isolated from the burn ward

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Introduction and Obejectives: The rapid increase of drug resistance and failure of existing antibiotics to treat infections caused by *Pseudomonas aeruginosa* MDR strains, introducing the new therapeutic combination is needed. This study aimed to evaluate the synergistic effects of cinnamon extracts and honey against multidrug-resistant strains of *Pseudomonas aeruginosa* isolated from burn ward.

Materials and Methods: The minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of each antibiotic (Gentamicin and Ciprofloxacin) alone or in combination with the aqueous and alcoholic extracts of cinnamon and honey was determined by broth microdilution method for clinical and environmental isolates of *P.aeruginosa* based on the CLSI guidelines. The synergistic effect of combinations was evaluated using a checkerboard test and FICi evaluation. Data analyzed using SPSS software by Chi-square and Fisher's exact tests.

Results: The means MIC for Cinnamomum zeylanicum Alcoholic extract-Honey(CZ.A.H), Cinnamomum zeylanicum watery extract-Honey(CZ.W.H), Ciprofloxacin-Cinnamomum zeylanicum watery extract(CP.CZ.W), Gentamicin-Cinnamomum zeylanicum watery extract(GM.CZ.W), Ciprofloxacin-Cinnamomum zeylanicum Alcoholic extract(CP.CZ.A), Gentamicin-Cinnamomum zeylanicum Alcoholic extract(GM.CZ.A), Ciprofloxacin-Honey(CP.H), Gentamicin-Honey(GM.H) against MDR strains were as 6.80, 15.63, 18.62, 5.84, 10.70, 3.84 and 36.88 µg/mL, respectively. The means MBC for CZ.A.H, CZ.W.H, CP.CZ.W, GM.CZ.W, CP.CZ.A, GM.CZ.A, CP.H, and GM.H against MDR strains were as 13.17, 30.25, 29.63, 31.52, 11.32, 21.28, 60.11and 70.03µg/ml, respectively. According to the FICi results, 14.1, 1.3, 3.8, 19.2, 32.1, 29.5, 9 and 9% of isolates had the synergism for CZ.A.H, CZ.W.H, CP.CZ.W, GM.CZ.W, CP.CA.A, GM.CZ.A, CP.H and GM.H combinations, respectively.

Conclusion: This study showed the acceptable synergistic effect of aqueous and alcoholic extracts of Cinnamon and honey in combination with Ciprofloxacin and Gentamicin.

Keywords: Synergistic effect, cinnamon, honey, *Pseudomonas aeruginosa*, burn ward

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Topic: Treatment and New Drugs

P235: In vitro Anti-bacterial, anti-biofilm formation activities and urease inhibitory effect of four medicinal plants of Lamiaceae family against *Klebsiella pneumoniae*

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Introduction and Obejectives: *Klebsiella pneumoniae* is among the most frequently recovered from nosocomial infections. This opportunistic pathogen has a high potential for antibiotic resistance and also it can generate a thick layer of biofilm. Antibiotic resistant strains were emerging and widely spread worldwide. Thus, it is necessary to combat drug resistant strains through the use of novel drugs. Some medicinal plants possess remarkable activity against bacterial agents. Among them, Lamiaceae family are in pharmaceutical interest. So, the aim of the study was to evaluate the in vitro antibacterial and anti-biofilm formation activities of *Satureja rechingeri*, S. *khuzestanica*, *S. bachtiarica* and *S. mutica* essential oils against *K. pneumoniae*.

Materials and Methods: The Essential oils were obtained from Medicinal Herbs Research Center of Tehran. The tested strain was a standard strain of *K. pneumoniae* ATCC700603 For evaluation of the minimum inhibitory concentration (MIC) of essential oils, the microdilution method was used. Also standard method was used for evaluation of anti-biofilm activities of Sub-MIC value of essential oils. Colorimetric and Iodometric assays were used for determination of urease and betalactamas activity.

Results: According to our data, the MIC value of essential oils was 4096µg/ml. Sub-MIC value of essential oils were inhibited biofilm formation of K. pneumoniae. But *S. khuzestanica* had more activity and *S. mutica* had less activity than others. Also, urease activity of *K. pneumoniae* was inhibited by sub-MIC value of all tested oils.

Conclusion: According to our analysis *S. khuzestanica* had a good antibacterial, anti-biofilm formation activities and urease inhibitory effects against *K. pneumoniae*, but additional studies and researches is required to explore the exact mechanisms of the antibacterial action and functions of this phytocompound.

Keywords: *K. pneumoniae*, Lamiaceae, *Satureja*, *S. khuzestanica*, anti-biofilm

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Topic: Treatment and New Drugs

P218: Antibacterial effects of combination of silver nanoparticles and extracts of Ghalghaf gall against *Escherichia coli* and *Staphylococcus aureus*

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Introduction and Objectives: *Escherichia coli* and *Staphylococcus aureus* are the most common causes of hospital- and community-acquired infections and antimicrobial resistance among them has become a major health problem**.** Recently nanoparticles are among the compounds that have been most noticed due to their antimicrobial properties. It has been shown that the production of nanoparticles by using plant extracts leads to the acquisition of nanoparticles with increased antimicrobial activity. The purpose of current study was to evaluate antibacterial effects of combination of silver nanoparticles and extracts of Ghalghaf gall against the mentioned bacteria.

Materials and Methods: In this study silver nanoparticles were synthesized using extracts of Ghalghaf gall. Antibacterial activity of these nanoparticles against the standard strains of *Escherichia coli* and *Staphylococcus aureus* were evaluated by determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values by broth macro-dilution method according to Clinical and Laboratory Standards Institute (CLSI) procedures.

Results: The MIC and MBC values of the combination of silver nanoparticles and extracts of Ghalghaf gall obtained for *Escherichia coli* were 78 µg/ ml and 156 µg/ ml respectively. The observed MIC and MBC values of the mentioned combination for *Staphylococcus aureus* were 156 µg/ ml and 1250 µg/ ml.

Conclusion: According to the results of current study, the combination of silver nanoparticles and extracts of Ghalghaf gall showed remarkably higher antibacterial effects compared to silver nanoparticles alone especially against *Escherichia coli*.

Keywords: Antibacterial activity, Silver nanoparticles, Ghalghaf gall, *Escherichia coli*, *Staphylococcus aureus*

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Topic: Treatment and New Drugs

P225: New derivatives of amidoalkyl-2-naphthols as a novel series of antibacterial agents

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Introduction and Obejectives: Amidoalkyl-2-naphthols are an important group of molecules as they can easily convert to oxazine derivatives with different possible medical applications such as antitumor, antimalarial and antibiotic properties. In this study, antimicrobial activity of new derivatives of amidoalkyl-2-naphthols was assessed using disk diffusion method.

Materials and Methods: Antimicrobial activity of the derivatives was assessed against two medically important bacteria: *Staphylococcus aureus* and *Acinetobacter baumannii* (Gram positive and Gram negative, respectively). Disk diffusion method was performed according to the latest version of the CLSI Method using 100 mg/ml concentration of each compound. After 24 hours of incubation, antibacterial activity was measured as inhibition zone diameter. An inhibition zone diameter of 10 mm was arbitrarily chosen to represent bacterial susceptibility to the compounds.

Results: Out of 53 recruited compounds, 17 displayed antimicrobial activity. While three compounds were effective against both *S. aureus* and *A. baumannii*, 13 were only effective against *S. aureus* and one only against *A. baumannii*.

Conclusion: Derivatives of amidoalkyl-2-naphthols could be effective antibacterial agents. As *S. aureus* and *A. baumannii* can cause nosocomial infection, further studies are needed to evaluate the therapeutic potential of the studied compounds in infectious diseases.

Keywords: amidoalkyl-2-naphthols, antimicrobial agents

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Topic: Treatment and New Drugs

P234: The use of bacteriophage in aquatice disease treatment

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Introduction and Objectives: Bacteriophages or phages are bacterial viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism. Phage therapy is widely being reconsidered as an alternative to antibiotics. Bacterial resistance to antibiotics has now become a big issue, so the focus on the development of bacteriophage therapy has now increased. Replacing phage instead of antibiotics is a new option in the aquatic world.

Materials and Methods: In this research, after separating bacteriophages of *Aeromonas hydrophila* with Chloroform and dual layer culture, bacteriophages was concentrated. After determining the minimum concentration of bacteria growth inhibition by bacteriophage under external conditions they were also used in in vivo and in vitro conditions or intra peritoneal injections and immersion.

Results: The effect of bacteriophage on the survival rate of rainbow trout was when the bacterial concentration of 1×10^8 cfu and 1×10^3 pfu of the bacteriophage were immersed. There was no significant difference between the control and positive control groups in any of the treatments, but there was a significant difference between the control group and the treatments (intra peritoneal injections and immersion) ($P < 0.05$). Data analysis also showed this The results of this study showed that one of the ways to increase the survival rate of trout fish was to increase the survival rate of trout fish and reduce the mortality rate of $1 \times 10^8 ml^{-1}$ of bacteria to direct contact with bacteria and bacteriophage in the treatment group had the most effect on the survival rate of trout and eventually reduced mortality.

Conclusion: The results of this study showed that bacteriophages are one of the ways to increase the survival rate of trout fish.

Key words: *Aeromonas hydrophila*, Bacteriophage, survival rate

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Topic: Treatment and New Drugs

P166: Evaluation of Antifungal effects of TiO_2 and Amphotericin B on different *Candida* species *in Vitro* condition

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Introduction and Objectives: *Candida* species are known as cause serious fungal infections that produce cutaneous, mucosal and systemic infections. Nowadays, mortality and morbidity due to Candida species especially in immunocompromised patients was increased. Therefore, finding new approach to treat and control of these infectious agents necessary. Nanotechnology is science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nanometers. The aim of this study was to evaluate the antifungal effects of titanium dioxide nanoparticles and Amphotericin B on different *Candida* spp.

Materials and Methods: In present study, Minimum Laboratory Concentration (MIC) of titanium dioxide Nano particles and Amphotericin B were determined by broth microdilution (BMD) methods according to the guideline of Clinical and Laboratory Standard Institute (CLSI) (M27-A3 document). In Addition, Minimum fungicidal concentrations (MFC) was defined as the lowest concentration of the TiO_2NPs and or AmB that killed 98%-99.9% of each *Candida* spp.

Results: The results showed that the TiO_2-NPs had antifungal activity against pathogenic *Candida* spp. and could inhibit the growth of all the tested *Candida* spp. The MIC and MFC of TiO_2NPs against *Candida* spp. was 128 to 256 µg/ml and 256 to 512 µg/ml, respectively. MIC and MFC of AmB were 8 to 16 µg/ml and 16 to 32 µg/ml, respectively.

Conclusion: Our finding showed that the TiO_2 had antifungal potential against pathogenic *Candida* spp. and could inhibit the growth of all tasted *Candida* spp. However, their antifungal properties was significantly less than AmB.

Keywords: *Candida* spp, TiO_2 Nanoparticles, Amphotericin B.

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Topic: Treatment and New Drugs

P198: In vitro Eradication of *Pseudomonas aeroginosa* Persister Cell Producers by Herbal Medicine Running title: Anti-persister of *Peganum harmala*

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Introduction and Objectives :Unfortunately, today multidrug resistant (MDR) bacteria are very common in communities and could serious threat to human health. However, new mechanism of survival of bacteria without any resistance is debatable. One of the most important mechanisms is persister cell formation in bacteria. Persister cell can be one of the important causes in chronic infections. Nevertheless, there are many challenges in the treatment of persister cell. The discovery of new drugs and appropriate therapies in this area is very necessary. So, the purpose of this research was discovering an antipersister drug in *Pseudomonas aeruginosa* clinical isolates.

Materials and Methods :Thirty *P. aeruginosa* clinical isolates were used. Antibiotic susceptibility testing was done and persister cell assay was performed. Finally, *P. harmala* was used as an anti-persister cell drug for in this isolate.

Result : Our finding demonstrated that concentration of 35ug/ml completely has the anti-persister properties.

Conclusion :So, it can be herbal candidate against persister cells but further analysis including in vivo study and finding the main component should be done to commercial this herb as new anti-persister drug.

Keywords: persister cell, *Peganum harmala, pseudomonas aeruginosa*

Topic: Treatment and New Drugs

P220: The Comparison between Pimpinellaanisumand Grisofulvin in the treatment of Dermatophytosis Caused by MicrosporumCanisin cats

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Introduction and Objectives: Dermatophytosis, usually caused by Microsporumcanis, is the most common fungal infection in cats worldwide, and one of the most important infectious skin diseases in this species.Grisofulvin as antifungal tab has side effects such as: nausea and diarrhea so we decided to investigate the therapeutic effects the Pimpinellaanisumas one of the herbal medicinal plants of Tehran and grisofulvin as antifungal agent in the treatment of cats with dermatophytosis.

Materials and Methods:10 number of two years oldcats with dermatophytosis in Tehran were randomly selected by wood lamp and randomly divided into 2 groups 5. The first group received twice daily orally every 12 hours, 1 tab of grysofulvin at dosage 10mg/kg as control group and the second group (treatment) was also, every 12 hours 5mg/kg were fed by Pimpinellaanisum autoclaved powder.

Results: After a week of prescribing these drugs results were as follows: Pimpinellaanisum recipient group like the group receiving oral antifugal tablets of grisofulvin recovered their health and appetite also returned to normal. At the end of the two groups of dermatophytosis were negative.

Conclusion: Because of the many advantages of traditional medicine and herbs and its lower price compared to chemical drugs, it's better to replace these drugs with recent like chemical drugs. It must be noted that we earlier found the same findings in using this herbal drug on gogs in Tehran province, too.

Keywords: Pimpinellaanisum, Grisofulvin, MicrosporumCanis, Cat

Topic: Treatment and New Drugs

P300: Immunomodulatory evaluation and computational modeling of quercetin-3-O-α-L-rhamnopyranoside from *Rapanea melanophloeos* against influenza A virus

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Introduction and Objectives: Influenza A virus (IAV) is still a threat for human and animal health. The clinical manifestations of this infection are related to immune dysregulation, which causes serious risk factor for morbidity and mortality. The usage of traditional medication with immunomodulatory properties against influenza infection has been increased recently. Our previous study has shown the antiviral activity of quercetin-3-O-α-L-rhamnopyranoside (Q3R) isolated from *Rapanea melanophloeos* (RM) (L.) Mez (family *Myrsinaceae*) against H1N1 (A/PR/8/34) infection. This study aimed to confirm the immunomodulatory effect of Q3R on selective pro- and anti-inflammatory cytokines against IAV *in vitro*, also, to evaluate the physical interaction of Q3R with virus glycoproteins using computational docking.

Materials and Methods: The MDCK cells were exposed to the Q3R and 100$CCID_{50}$/100µl of H1N1 in combined treatments (co-, pre- and post-penetration treatments). Cell-free supernatants treated for 48 hr were collected and stored at -80°C until further processing. TNF-α, IL-6 and CCL-2 as pro-inflammatory cytokines and IL-27 and IFN-β as anti-inflammatory cytokines were measured by ELISA. Computational molecular docking as a virtual screen of the potential anti-influenza activity of Q3R was also performed.

Results: The expression of cytokines proteins was significantly affected by Q3R treatment. It was shown that Q3R was much more effective against IAV when it was applied in co-penetration treatment. The molecular docking results showed the strong binding ability of Q3R with neuraminidase/hemagglutinin from H1N1 (A/PR/8/34).

Conclusion: Quercetin-3-O-α-L-rhamnopyranoside was significantly effective against influenza infection outcome by immunomodulatory properties and binding ability to the viral receptors. Further research will focus on detecting the detailed specific mechanism of Q3R in virus-host interactions.

Keywords: Influenza A virus, Quercetin-3-O-α-L-rhamnopyranoside, Cytokine, Molecular docking

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Topic: Treatment and New Drugs

P189: Investigating the effect of Myrtus Communis extract on Trichomonas Vaginalis parasite

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Introduction and Objectives: Given the significant effects of the extract of Myrtus Communis and considering its being a natural flora of Iran, and considering chronic and recurrent cases of trichomoniasis and the necessity of trying to find more efficient medication with less complications , the present study was conducted to determine the effect of Myrtus Communis extract on Trichomonas vaginalis.

Materials and Methods: The present study is a descriptive, cross-sectional study, the subjects of which were selected through simple sampling method, was conducted on patients with genital infection including discharge, itching, burning and smelling bad, referring to gynecologist from September 2018 to April 2019; after being examined and documented by physicians, the subjects were referred to laboratory for evaluation to see if they would be diagnosed with vaginal infection or not. A drop of the sample was placed on a slide and examined by light microscope in order to provide direct swap testing. The samples were cultured in Dorset and evaluated after 24 to 48 hours to check the growth of single-celled organisms. The effect of the extracts on Trichomonas vaginalis trophozoite was evaluated based on the minimum inhibitory concentration.

Results: The efficacy of the studied extract was 99.44% at a dose of 300 µg 24 hours after intervention; this rate was 100% 48 and 72 hours after intervention. For 600 µg, this rate was 99.67% in 24 hours and 100% in 48 and 72 hours after intervention.

Conclusion:The extract of Myrtus Communis in two concentrations of 300 and 600 micrograms showed a high potential for killing Trichomonas vaginalis parasite, indicating the necessity of further studies especially in vivo, in order to introduce this plant as an efficient medical intervention with much fewer side effects in comparison with other chemical medications used to treat this common form of infection.

Key words: Myrtus Communis, Trichomonas Vaginalis, trichomoniasis

Topic: Treatment and New Drugs

P124: Evaluation of the Effect of Lactobacillus planetarium Probiotics Produced from Broad Bean Seed in Prevention of Helicobacter pylori in Stomach Tissue of C57BL/6 Mice

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Introduction and Objectives: Helicobacter pylori is one of the most common human infections, which colonizes more than half of the world's population. This causes chronic stomach inflammation diseases without clinical syndromes, gastric and duodenal ulcer, and stomach cancer. Nowadays, the use of probiotics has received much consideration as one of the common therapeutic methods, which prevents bacterial colonization by creating a balance in the microbial gastrointestinal tract.

Materials and Methods: This experimental study was conducted on 30 rats in five groups from August 2016 to June 2017 in the Microbiology and Animal Laboratory of Shahrekord University. First, the rats were infected with H. pylori bacteria. PCR method was used to confirm the presence of bacteria in the stomach to ensure that the rats were inoculated with H. pylori. After inoculation, the infected rats were treated with probiotic product, and then gastric tissue of the infected group was evaluated by haematoxylin and eosin stain.

Results: The absence of Cag A and Ure C genes in fecal specimens of the group receiving probiotic products before and after H. pylori incubation showed a positive effect for this product on the prevention and treatment of H. pylori infection. Also, in stomach histology specimens, the effects of mild inflammation were observed in treated group with the probiotic product before and after H. pylori inoculation compared to the control group.

Conclusion: The results of this study showed that the addition of probiotic to a non-dairy product (broad bean extract) can be effective in preventing and treating H. pylori infection in the animal model.

Keywords: Helicobacter pylori; Probiotic; Broad bean; Haematoxylin; Gastrointestinal tract

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Topic: Treatment and New Drugs

P270: Molecular detection of *AdeFG* efflux pump genes in clinical isolates of *Acinetobacter baumannii* and their role in antibiotic resistance in Tehran

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Introduction and Objectives: *Acinetobacter baumannii* is one of the most important causes of hospital bacterial infections in the world. recently antibiotic-resistant strains of *A. baumannii* have proliferated, which may be due to the efflux pumps.

Materials and Methods: In this study, 200 clinical Isolates from ulcer, pus, sputum, and blood were collected in Mostafa Khomeini, Tohid and Motahari hospitals in Tehran, and their identity was verified by conventional standard biochemical tests. Then, the antibiotic-sensitivity pattern was determined by the disc diffusion method in the presence and absence of efflux pump inhibitors in samples according to the CLSI instructions. *AdeFG* efflux pump genes in samples were identified by PCR method.

Results: In this study, 60 isolates of *A.baumannii* bacteria were identified based on biochemical differential tests. The identity of all samples was verified by PCR and *blaOXA-51-like* gene amplification methods. Investigation of the antibiotic-resistance of samples showed that 98.34% of the samples were resistant to the three antibiotics of ciprofloxacin, norfloxacin and levofloxacin. According to results the of PCR method, 100% of the 60 *A. baumannii* samples involved the *AdeF* gene and 76.66% involved the *AdeG* gene.

Conclusion: The results of this study indicate that resistance to all three antibiotics of ciprofloxacin, norfloxacin and levofloxacin exists in *AdelaFG* gene-carrying strains due to the efflux pump and they have a significant relationship. The role of other factors and mechanisms involved in inducing the resistance should not be ignored.

Keywords: *Acinetobacter baumannii*, efflux pump, antibiotic resistance, *AdeFG*

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Topic: Viral Infections

P295: Detection of Canine Herpesvirus in vaginal samples collected from dogs in Kerman city by Real Time-PCR

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Introduction and Objectives: Canine Herpesvirus is belonged to the family of herpesviridae and has been detected in various countries worldwide. This virus can result in reproductive disorders, such as abortion stillbirth and week puppies. Virus transmission is mainly through the placenta, birth canal, sexual contact and respiratory tract. Considering the global prevalence of this virus and its economic importance due to abortion and stillbirth for dog breeders, this research was conducted to determine the prevalence of CHV1 in dogs of Kerman.

Materials and Methods: In this study, the presence of herpes virus in vaginal swab specimens and biopsy of the uterus of dogs referring to Veterinary Hospital of Shahid Bahonar University of Kerman and some breeding kennels of Kerman city were determined. Samples were collected and evaluated using Real-Time PCR.

Result: Canine Herpesvirus type 1 was detected in 21 samples from a total of 140 collected samples (7/70 vaginal swabs and 14/70 uterine biopsies) from referred dogs and 21 out of 64 vaginal samples of breeding dogs.

Conclusion: Considering the significant prevalence of this virus, it is necessary to carry out management measures in control and prevention this disease. Developing of proper diagnostic method for rapid screening of infected dogs is highly recommended.

Keywords: Canine Herpesvirus, Reproductive samples (Uterine and vagina), Dog, Kerman

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Topic: Viral Infections

P288: Frequency of Active Cytomegalovirus infection in Patients with STEMI

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Introduction and Objectives: Various risk factors for the expression of coronary arteries have been reported, which the role of infections has been considered. Cytomegalovirus (CMV) is one of the viruses suspected of causing endothelial damage and one of the highest prevalence rates in human populations. The aim of this study was to determine the prevalence of active cytomegalovirus infection in patients with ST-Elevation Myocardial Infraction (STEMI). There are different ways to elucidate the acute and/or recurrent form of cytomegalovirus infection. In this study we have evaluated the presence of CMV specific IgM antibody in serum as a marker of acute or recurrent CMV infection.

Materials and Methods: This descriptive study was conducted among patients admitted to Shahid Madani Hospital in Tabriz, East Azerbaijan. Overall one hundred patients were included in our study; test group (fifty patients with STEMI) and control group (fifty patients with STEMI with normal angiography). Blood samples from patients in both groups were examined for CMV IgM antibody titers and CRP levels. Serological markers were evaluated by using ELISA method.

Results: 55 (55%) men and 45 (45%) of patients were female. The mean age of participants was 57 ± 1.3. In terms of CMV IgM antibody titer we have shown that sixteen percent of test group were positive, but only two percent of the control group were positive. Therefore, there was a significant CMV IgM level among test group patients (PV <0/05). *Remarkably, 72 percent of patients in test group were CRP positive.*

Conclusion: Based on our data we could consider the possibility of association of the acute CMV infection in the process of the formation of coronary artery atherosclerosis, further research like the detection of the virus genome is needed to confirm the relationship between CMV and STEMI.

Keywords: Frequency, Cytomegalovirus (CMV), STEMI, CRP

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Topic: Viral Infections

P297: Molecular identification of Sheeppox virus (SPV) in Kerman province, Iran

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Introduction and Objectives: Sheeppox virus (SPV) is belongs to Poxviridae family, Chordopaxvirinae sub-family, and capripoxvirus genus. SPV is an endemic disease in Iran and has very important role in agricultural economy. It is included in the notifiable diseases of Office International des Epizooties (OIE). The purpose of this study was molecular identification of SPV in Kerman province of Iran.
Materials and Methods: A total of forty-five biopsy samples from skin lesions of sheep suspected to SPV were collected from different districts of Kerman province. A previous developed capripoxvirus specific PCR assay was applied to identify the P32 gene encoding capripoxvirus immunodominant antigen to identify SPV.
Results: Eleven samples (24.44%) were shown positive results for 390bp fragment of P32 gene. Spatially, the disease was recorded in 8 out of 20 districts.
Conclusions: Our results revealed that SPV is endemic and dispersed in Kerman province of Iran. Hence, ring vaccination should be undertaken for a period of two to three years, to try to eradicate the SPV. The study also highlights high sensitivity of this PCR in detection of SPV.
Keywords: Sheeppox virus, PCR, P32 gene, Kerman, Iran

Topic: Viral Infections

P301: Phylogeny and characterization Of Iranian Fowl Adenoviruses based on the penton gene, 2019

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Introduction and objective: Since 2012 Inclusion Body Hepatitis (IBH) has happened as an economically important disease in Iran and the number of IBH cases has been increased. Disease consequences and induced sudden deaths threaten poultry industry; thus, characterization of species and serotypes is helpful to implement preventive strategies.

Material and Methods: PCR is a quick and appropriate method for identification and characterization of IBH virus. IBH was routinely recognized based on parts of the hexon gene, but in this survey, the penton gene has been studied for the first time in Iran. Six viruses were identified from broilers of north, west, and center of Iran during 2019. Sequence alignment and phylogenetic analysis based on 1406 bp region of the penton gene were done.

Results: Results indicated that detected viruses belonged to species D mostly serotype 11.

Conclusion: Similar results about cluster patterns obtained in the current study with those from previous studies, supported the hypothesis that the penton gene can be replaced with the hexon gene in phylogenetic analyses. Although hexon protein comprises most of the capsid proteins, penton protein may be conserved in the IBH virus. Therefore, the penton gene can be utilized in next studies instead of the hexon gene.

Keywords: Inclusion Body Hepatitis (IBH), PCR, Penton gene, Iran, Broiler, Avian Adenovirus, phylogenetic analysis.

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Topic: Viral Infections

P296: The frequency of EBV in inflammatory lesions of periapical cysts using PCR techniques

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Introduction and objective: One of the major problems of the periapical cyst is incomplete root canal treatment (incomplete endodontic treatment) that can be seen in some patients as holes in the gums using X-ray. Microorganisms not only play a role in the creation of apical lesions but also cause the diseases reaches the chronic stage. This study aimed to investigate the effect of Epstein-Barr virus *(*EBV*)* on the exacerbation of these diseases by using PCR techniques.

Material and Methods: Twenty-one formalin fixed paraffin wax embedded tissue blocks of inflammatory periapical lesions, which were previously diagnosed, were gathered from patients who had referred to the Department of Pathology, Faculty of Dentistry of Islamic Azad university, Isfahan (Khorasgan) Branch during 2011-2018. DNA extraction from formalin fixed paraffin wax embedded tissue blocks was performed using Gene All extraction kit and Aaron gene. Extracted DNAs were confirmed using β-globin gene. In terms of EBV frequency, the samples were evaluated by Nested- PCR and PCR.

Results: In the two experimental groups, the frequency of EBV was found respectively at 4 % of periapical cyst. Fisher's test did not show a statistically significant difference (P value> 0.05).

Conclusion: Based on the obtained results it can be claimed that EBV may possibly be involved in the pathogenesis or development of periapical lesions. Since limited researches have been done in this field and the results of the previous studies differ and apparently contradict each other, conducting further studies with larger sample sizes is recommended.

Keywords: Epstein-Barr virus (EBV), periapical cyst, Nested- PCR and PCR.

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Topic: Viral Infections

P298: The prevalence of Human T-lymphotropic virus in 20 to 60 years in khoy city

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Introduction and Objectives: The HTLV virus known as first human Retrovirus and it's a part of the Oncoviridea family. An important feature of virus is geographic prevalence in our country. About 20 million of people infected in the world. This virus is a leading cause of advanced T-cell leukemia in adults. The purpose of this study was to investigate the prevalence of this virus in the population of Khoy city.

Material and Methods: In this cross – sectional study, 327 individuals who referred to the Diagnostic Laboratory of Qamar Bani Hashim Hospital in Khoy city from Jan 2017 to Jan 2018, were provided with peripheral blood samples. After sampling ELISA tests were done for HTLV-1 antiviral antibodies for all samples. Positive samples confirmed by western blot test. For data analysis we used SK test (Kolmogrov-Smirnov) for non-normalization data. All of these analysis was analyzed by SPSS V.23 software. $P < 0.05$ was considered all meaningful.

Results: HTLV-1 were positive 0.6% (2/327) of the participants according to the results of ELISA. These samples confirmed by western blot testing.

Conclusion: The results showed that Khoy city is a non-endemic. Also, our result showed that no significant coralation with age and sex in Khoy city. However, education are recommended to prevent the spread of the virus. Since there is no interventional therapy for HTLV-1 infection, education is the most important principle for preventing the transmission of infection to other people, especially in the blood transfusion organization, which is more at risk due to the nature of the work.

Keywords: HTLV-1 virus, Elisa, antibody, T cells, Khoy city

Topic: Viral Infections

P314: Anti - whole Influenza A/H1N1 Virus Polyclonal Antibody for passive immunization against influenza

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Introduction and Objectives: Acute respiratory viral infections are the main cause of outbreaks all over the world. The efficacy of medical interventions such as vaccination is generally failed due to antigenic shifts and drifts among virus strains and the lack of long-lasting effective immune responses. Following the recent influenza virus pandemic, and the absence of enough efficacious vaccines for prevention, it is an outmost need to have access to strategies which provide enough periods to prepare a new vaccine. One of the practical ways to prevent the spread and prevalence of the viral diseases is intranasal passive immunization using antiviral antibodies.
Materials and Methods: Influenza virusA/H1N1/PR8 was inactivated using UV radiation and then inactivated whole virus with Freund's adjuvants (Complete and Incomplete) was injected into New-Zealand white male rabbits. The polyclonal antisera of rabbits were evaluated by HI. Then IgG was purified using DAEA-cellulose column chromatography. The quality and properties of purified IgG were evaluated using SDS-PAGE and ELISA. Finally, using Virus Neutralization Test (VNT), virus capturing using antibody was evaluated.
Results: The HI result showed that the produced anti-whole virus antibody was able to recognize whole virus epitopes. The HI results confirmed anti-virus pAb reached reasonable titers after three injections. The Purified polyclonal IgG was evaluated using ELISA and VNT. The results showed IgG reacted with the virus antigen up to 1:32000 dilution in ELISA and 2048 titer in VNT.
Conclusions: The data showed that inactivated whole virus was able to stimulate immune response to produce antibody at satisfactory level.
Keywords: ELISA, Influenza Virus, VNT, Polyclonal antibody, HI.

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Topic: Viral Infections

P304: A combination analysis of the drug resistance mutations in HIV protease and Gag genes in Iranian HIV infected patients

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Introduction and Objectives: Recently Protease inhibitors (PIs) are commonly used as a first-line regimen in HIV-1-infected patients. However, drug resistance mutations in protease gene as well as Gag gene reduce the efficiency of these inhibitors. This study aimed to investigate the drug resistance mutations in both genes in Iranian infected patients.

Material and methods: RNA of the sera of 15 patients were extracted and then were used to amplify the protease and Gag regions by using the Nested PCR method. After sequencing, the sequences were analyzed to define drug resistance mutations. The physicochemical properties, post-modification positions, structural analysis, and subtyping were evaluated by using several reliable Bioinformatics tools.

Results: While several mutations were found in both genes in comparison with reference sequences, we could not find any drug resistance mutations in the Gag and protease genes. The most prevalence subtype among samples was AD and serval post modification potions were identified. The secondary and tertiary structures for Gag and protease genes were constructed by several bioinformatics tools.

Conclusion: Our findings showed, in spite of numerous mutations in enrolled samples, protease inhibitors could still be effective to inhibit HIV infections in Iranian patients. In addition, this study has estimated comprehensive data of Gag and protease proteins which could be useful for further studies to introduce novel inhibitors to inhibit HIV infections.

Keywords: Gag, protease, HIV, resistance mutations

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Topic: Viral Infections

P92: Detection of the drug resistance mutations in HIV reverse transcriptase and GP41 genes in Iranian HIV infected patients

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Introduction and Objectives: Reverse transcription plays a key role in the life cycle of the HIV virus and is responsible for the synthesis of double-stranded (ds) DNA from a viral single-stranded (ss) RNA genome. HIV envelope glycoprotein is responsible for viral attachment to target cells and the subsequent fusion of viral and target cell membranes. Several inhibitors have been introduced to inhibit RT and GP41 functions. However, recently, several drug resistance mutations were found in both regions. The aim of this investigation was to define the drug resistance mutations in both RT and GP41genes and define the effect of these mutations on the interaction between both proteins and inhibitors.

Material and methods: RNA of the sera of 30 patients were extracted and then were used to amplify the gp41 and RT genes by using Nested PCR method. After sequencing, the results were analyzed to define drug resistance mutations. The physicochemical properties, post-modification positions, structural analysis, and subtyping were evaluated by using several reliable Bioinformatics tools.

Results: In spite of several mutations which were found in the GP41 gene, we could not find any drug resistance mutations. However, the analysis of RT sequences showed a high level of resistance to RT inhibitors in several samples. Subtyping analysis showed AD was the most prevalent subtype among Iranian patients .

Discussion: Our results showed the possibility of Gp41 inhibitors to control HIV infections in Iranian infected patients.

Keywords: Reverse transcriptase, GP41, HIV

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Topic: Viral Infections

P303: Study of possible interactions between HIV V3 protein and cell chemokine receptors

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Introduction and Objectives: HIV-1 gp120 contains several domains and five surface-exposed mobile loops (V1 to V5). Among all mentioned loops, V3 loop binds to chemokine receptors which is required for viral entry. This study aimed to define the possible interactions between V3 regions and receptors.

Materials and Methods: V3 protein sequences were obtained from the gene bank, and then by various software, physical and chemical characteristics, post-modifications sits, changes in amino acid and second and third-dimensional structures were determined. Also, the interactions between V3 and chemokine receptors were examined.

Results: Results showed there are numerous phosphorylation, glycosylation sites, and disulfide bonds in the V3 protein. Docking results indicated the probable interaction between the V3 protein and different receptors. The results showed the effect of mutations on the interactions between V3 and receptors.

Discussion: The high energy values of the interaction between V3 and receptors indicated the high binding potential of proteins. Analysis of the tertiary structure of the V3 protein showed different conserved regions which have great potential to bond with receptors.

Keywords: V3, gp120, HIV

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Topic: Zoonotic Diseases

P329: Association analysis of IL-12, IL-13 and TNF-α polymorphisms with susceptibility to brucellosis

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Introduction & Objectives: The cytokine gene polymorphism is important for the genetic predisposition of infectious disease. The present study was aimed at identification the associations between IL-12 (+1188 A/C), IL-13 (-1512 A/C), IL-13 (-1112C/T) and TNF-α (-238 G/A) genotyping in Brucellosis patients.

Materials & Methods: This case-control study was conducted on 107 Brucellosis patients and 107 healthy controls. The SNPs of TNF-α (-238 G/A) and IL-12 (+1188 A/C) were done by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) and IL-13 genotyping at positions -1512 (A/C) and -1112 (C/T) were analysis by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) methods.

Result: IL-13 (-1512A/C) was associated with Brucellosis risk in dominant model (OR (95% CI) =2.17 (1.02–4.62)), P-value=0.041. However, there was no difference in allele and genotype frequencies of TNF-α (-238 G/A), IL-12 (+1188 A/C) and IL-13 (-1112 C/T) between patients and controls.

Conclusion: In conclusion the gene variation may be useful for identification of genotype as biomarkers for identification of people at high risk.

Keywords: Cytokines; Polymorphisms; Brucellosis; Iran

Topic: Zoonotic Diseases

P319: Investigating the frequency of Leptospira serotypes in stray dogs

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Introduction and Objectives: Leptospirosis is one of the important infectious disease that spreads throughout the world, which is caused by Leptospira. A wide range of domestic and wild animals are the important reservoirs for Leptospira; and these animals are the source of infection for humans. Direct contact with infected animals or exposure to contaminated water with the urine of the animal reservoir results in the transfer of leptospirosis to humans. Stray dogs seem to have a high risk of exposure to Leptospira species and may be contaminated with some of the leptospiral serovars.

Materials and methods: The aim of this study was to determine the leptospirosisseroprevalence among stray dogs. In this study, blood samples were obtained from 120 stray dogs in Chaharmahalobakhtiariprovince; none of the dogs had clinical symptoms of leptospirosis at the time of sampling. Then, DNA extraction was performed from blood samples. Finally, PCR method was accomplished.

Results: The results showed that nine dogs (7.5%) were infected with Leptospira. Moreover, stray dogs can be infected through hunting of contaminated rodents or contacting with Leptospira infected water and soil. As a consequence, the disease can be transmitted to other animals and humans by contaminating the environment.

Keywords: Leptospira, Stray dogs, Chaharmahalobakhtiariprovince

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Topic: Zoonotic Diseases

P285: Designing of Dot-Elisa system for diagnosis of Glanders

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Introduction and Objectives: Glanders is one of the most dangerous and oldest zoonotic diseases that is caused by *Burkholderia mallei*. Iran has been determined as the center for glanders for many years because of annual outbreaks. Risks of transmission of disease is also very high. In other hand, horses cannot be kept for long time especially in the border areas so quick detection is very urgent. Therefore, the aim of this study is designing of Dot-Elisa system for rapid detection.

Materials and Methods: So, 40 serum samples were collected from different provinces, along with reference strain of *Burkholderia mallei* and 3 healthy horses was used as positive and negative control respectively. The antigen and antibody optimum dilutions was determined by checkerboard titration. After optimization of Dot-Elisa system, Antigen (1.2 McFarland dilution) was prepared and coared into wells and was incubated at 37 ° C for 3 hours then it was washed with PBS containing tween (pH 6.4). In the next step diluted blocking (100µl) was poured and incubated for 3 hour. Repeated washing with PBS containing tween, then diluted serum samples (1/64 dilution) was poured into wells and incubated for 30 minutes in the refrigerator. After that the wells were washed with PBS, the diluted Anti-horse (100µl) (1/50 dilution) was added and incubated for the same time in the refrigerator. After washing steps, TMB (50µl) was added. Finally, the color reaction was observed.

Result: In this study, out of 40 samples, 6 cases were positive. Considering that mallein test as a standard test and comparing with Dot-Elisa, the sensitivity and specificity were 100% and 91.9% respectively.

Conclusion: Therefore, Dot-Elisa is suggested as a rapid diagnostic method for disease cases.

KeyWords: *Burkholderia mallei*, Glanders, Dot-Elisa, Diagnosis

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Topic: Zoonotic Diseases

P306: Cellular Immune responses of *Brucella melitensis* recombinant vaccine consisting of outer membrane protein conjugated with D-LPS in mice

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Introduction and Objectives: Brucellosis is a zoonotic disease of economic-hygienic importance caused by at least 12 different *Brucella* species. They cause infection in several animal species and humans. An effective and safer vaccine against human and animal brucellosis is in a pressing demand. *Brucella melitensis* is the global pathogenic species of *Brucella*. The recombinant outer membrane protein 31 KDa (Omp31) from *B. melitensis* is considered as a protective immunogen and an important candidate vaccine against human brucellosis. The objective of this study was to evaluate cellular immune responses of *B. melitensis* recombinant vaccine consisting of outer membrane protein conjugated with detoxified lipo-polysaccharide (D-LPS) in mice. It could be designed as an antigen candidate for development of prevention and diagnosis human brucellosis programs.

Materials and Methods: In this study, groups of BALB/c mice were immunized and boosted (two weeks intervals) with the purified recombinant Omp 31 protein conjugated with detoxified LPS of *B. melitensis*. One control group was received PBS only. Prior to immunization, all mice were then sacrificed and their spleens were aseptically harvested for in vitro cytokine production using the ELISA method. Statistical analysis was used for evaluation of significant differences between the groups and a P value ($P < 0.05$) was considered to be statistically significant.

Results: The results of this study showed both T cell populations CD4 and CD8 T-cells play a role in *Brucella* immunity. that the ratio concentration of IFN-γ to IL-4 in supernatant cell culture of immunized mice with recombinant Omp31 protein conjugated with D-LPS were higher than the control group.

Conclusion: These results suggest that the rOMP31protein conjugated D-LPS may induce a specific CMI response in mice, particularly in the production of Th1-type cytokines such as IFN-γ. suggested that CD8+ T cells play a more critical role than CD4+ T cells in controlling brucellosis. It induces a strong Th1 dominated immune response with production of IFN-γ and CD8+ specific cytotoxic cells, but not IL-4 after immunization in mice. Further investigations and focusing on the efficacy of Omp31 against oral challenges are underway.

KeyWords: Omp31, D-LPS, *Brucella melitensis*, IL-4, IFN- γ

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Topic: Zoonotic Diseases

P146: Prevalence of Salmonella spp. In egg producing layer farms in South Khorasan province in 2019

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Introduction and Objectives: Salmonella contamination of food, especially eggs, is one of the major causes of digestive disorders in humans. Contamination of Salmonella in laying hens is important for the transmission of contamination to the production and consumer food chain. The aim of this study was to investigate the contamination of laying hens in southern Khorasan province with Salmonella spp. using fecal culture methods.

Materials and Methods: This cross-sectional study was carried out in all Layer hen farms in south Khorasan Province, from April to June 2019. A 150 g weight fresh stool sample from at least 10 points of each farm was sent to central veterinary laboratory under cold chain procedure. Samples were cultured according to the protocol number CVL/DM/P/010 of the IVO. Positive results were confirmed with specific anti-serum. Positive and suspected samples were sent to Zahedan Veterinary Laboratory for PCR. Also, by sampling blood from positive fields, a rapid test was performed using the SP antigen.

Results and Conclusion: Of the total 35 samples submitted, 3 samples (8.5%) were positive for fecal culture and anti-sera. These specimens were sent to another laboratory for PCR testing, which were all positive again. Salmonella species in one of positive was diagnosed Salmonella cholerasuiss using specific anti-serum. The result of a rapid test with SP antigen was negative in two samples and suspicious for one sample, which was also reported negative at a dilution of 1/16.

The rate of infection from fecal culture in this study was 8.5%. This rate was reported in Bokai et al., 3.5%, Morshed et al., 9% and Akbrian et al., 6.5%. So, the prevalence of salmonellosis in South Khorasan province was not significantly different from other studies in the country. Basically, the presence of Salmonella in a producing layer hen farm is a major risk factor for public health. Therefore, continuous monitoring of these farms by high-sensitivity laboratory methods and further identification of Salmonella species and its effective contamination should be done by related government offices. Another issue that the findings of this study showed, were the difference between the results obtained from the three methods of testing and their different specificity and sensitivity. So, it is necessary to find the best screening and confirmation methods for Salmonella monitoring based on more detailed studies.

Keywords: Salmonella, Prevalence, Fecal culture, Layer hens, south Khorasan

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Topic: Zoonotic Diseases

P302: A case report of Distemper Virus in a human specimen in Iran

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Introduction and Objectives: Canine distemper virus (CDV) of morbillivirus, severe and highly contagious disease in dogs from Paramyxoviridae family is remarkably similar to the measles and Bovine rinderpest are two species of this family. Due to the mutation in the virus, its hosts are increasing. Respiratory, digestive and nerve problems are a hallmark of the disease. The purpose of this survey is to report a case of diagnosis of serum and molecular Distemper in a human sample.

Materials and Methods: At first, the sample of saliva was tested by a CDV diagnostic chromatography kit with 2 weeks interval, and then the positive samples of the blood were conducted to the Molecular Detection Laboratory.

Case report: Person's 31-year conflict respiratory symptoms of cough, leaving the confusion of a piece with fever, malaise, anorexia and diarrhea and cramps are severe and interfere with disturbances in vision and concentration to the local clinic without testing، The possibility of the flu was diagnosed but did not respond to treatment and was suspected due to a history of frequent contact with distempered pets. This case was examined and improved after antibiotic treatment and supportive treatment and the symptoms were discontinued and completely treated.

Results: The results of serological and molecular tests of the blood sample of the suspect were declared positive and confirmed by molecular test (PCR).

Conclusion: this is the first human distemper virus in Iran. according to emerging evidence distemper virus shows clinical signs in human. It is recommended that vaccination against distemper virus in domestic animals be taken seriously.

Keywords: distemper, Human, Blood, Serology, PCR

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Topic: Zoonotic Diseases

P132: *Outbreaks of brucellosis related to Brucella abortus* contamination *in unpasteurized camel milk* of two Iranian regions

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Brucella spp. commonly infect humans in various regions worldwide. Human brucellosis mainly spreads through the consumption of contaminated raw dairy products and meat from domestic livestock (water buffalo, goats, sheep, cattle, pigs and camels). In this regard, the origin and routes of transmission of this bacterium should be carefully determined in order to control the source of infection. This study aimed to evaluate the rate of *Brucella spp.* contamination of milk samples from dairy camels in Iran. For this purpose, 96 milk samples from 96 dairy camel herds were collected from two provinces and investigated for the presence of *Brucella spp* contaminations by both bacterial culture method and polymerase chain reaction (PCR). No clinical manifestation of brucellosis was reported in camels from which milk samples were collected. Using the culture method, three milk samples (3%) originating from two camels of Isfahan province (4%) and one camel from the Semnan province (2%), were contaminated with *Brucella abortus*. According to PCR analyses, *B. abortus* gene was detected in 14 (14.5%) milk samples, including 9 and 5 samples from Isfahan (18%) and Semnan (11%) province, respectively. PCR method revealed significant differences (p=0.02) in the level of contamination with *B. abortus* between milk samples collected from two regions. These results represent the first report regarding the isolation of *B. abortus* from raw camel milk in Iran and highlight the importance to screen apparent healthy camels. Therefore, the consumption of raw camel milk may contribute to the spread of human brucellosis in endemic regions.

Keyword: *Brucella abortus;* camel; culture, milk; PCR

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Topic: Zoonotic Diseases

P75: The identification and in vitro antimicrobial susceptibility testing of human *Brucella* spp. isolated from Iranian patients

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Brucellosis is a widespread zoonotic disease with high prevalence in both animals and humans. It is endemic in many parts of the world including Mediterranean areas. The aim of this study was to evaluate the susceptibility of *Brucella* strains isolated from human clinical specimens to nine antimicrobial agents commonly used for the treatment of infection disease. Bacteria were cultured and identified by molecular typing and *Brucella* biotyping. Minimal inhibitory concentration (MIC) and disk diffusion test were used to compare the efficacy of antimicrobial agents against and to define the susceptibility profile for each strain. In this study, all 54 *Brucella* isolates were identified as *B. melitensis* biovar 1. Our results showed that the majority of the tested antibacterial drugs, excepting Colistin and Ampicilin sulbactam had effective activity against *B. melitens* in both MIC and disk diffusion methods and could be implemented in therapeutic regimens with confidence. Moreover, probable resistance to colistin, rifampin, ampicillin-sulbactam and imipenem were reported in 54 isolates (100%), 1 isolate (1.9 %), eleven isolates (20.4 %) and 2 isolates (3.7%), respectively. These results suggest that the efficacy of regularly used antibiotics for brucellosis treatment should be commonly monitored. In conclusion, based on the present study, appropriate precaution should be exercised in the context of antibiotic administration to prevent future antibiotic resistance.

Keywords: Antimicrobial susceptibility; brucellosis; *Brucella melitensis*; Iran

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Topic: Zoonotic Diseases

P276: The comparison of Multiplex PCR with serological method for determination of occurrence of Brucella in Sheep blood

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Introduction and Objectives: The genus Brucella consists of 10 species, of which *Brucella abortus* (*B. abortus*), and *Brucella melitensis* (*B. melitensis*) are pathogenic for cow and sheep. Also, they are the main pathogenic species in human. Developing countries have still problem with brucellosis. Since the clinical feature of the disease is nonspecific; serological tests that were replaced to culture methods but, they have little sensitivity, especially in the early stages of the disease that production rate of the antibody is low. Thus, recently molecular methods have been used to distinguish the pathogen. This study aimed to determine the occurrence rate of Brucella in sheep blood through comparison of serological test and Multiplex-PCR.

Materials and Methods: A total of 70 blood samples were taken from 70 individual sheep. Rose Bengal test (RBT), Wright and 2ME were performed on the isolated serum. DNA was extracted from the whole blood. Multiplex-PCR was used for determination of *B. abortus* and *B. melitensis*.

Result: Overall, 4 (5.7%) of the serum samples were positive according to RBT test, while 9 (9.9%) of the serum samples were positive according to Wright and 2ME tests. Twenty-five (35.7%) blood samples were found positive on PCR-amplified IS711 gene for detection of *B. abortus*, while none of the samples were positive for *B. melitensis* gene. The differences of serological test and PCR-IS711 were highly significant ($P<0.05$).

Conclusion: Interestingly, sheep bloods have a high level of contamination with *B. abortus*. Molecular method is more sensitive than serological tests for detection of Brucella spp.

Keywords: Brucella, PCR, sheep.

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Topic: Zoonotic Diseases

P317: Serological and molecular evaluation of brucellosis in farm dogs in Kerman

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Introduction and Objectives: Canine brucellosis mainly caused by *Brucella canis* however *B. abortus*, *B. melitensis* and *B. suis* also cause brucellosis in dogs. Infection of dogs with these organisms usually occurs through contact with or ingestion of infected fetal membranes or aborted fetuses and raw dairy materials.
Materials and Methods: In this study, blood samples were gathered from 50 adult dogs that kept in cattle, sheep and horse breeding farms, regardless of their gender and clinical status. PCR, Rose bengal and Wright tests were used to determine the contamination. GeneAll Exgene™ DNA extraction kit was used and confirming of Brucella genus was accomplished with the specific primer of IS711.
Results: Rose bengal test was positive in 28% of samples and 6% showed 1/160 and higher titer in Wright test. Two dogs (4%) showed *B. melitensis* infection in the PCR test which kept in the sheep farms with the history of abortion in ewes. Clinical findings showed discospondylits and orchiditis in a 6 years old male breed mix dog and the other case was a 6 years old female German shepherd with stillbirth history.
Conclusion: This molecular study determined the definite role of dogs in epidemiology of Brucellosis in our country. The possibilities of disease transmission to farm animals and public health hazards must be considered. Dogs in Brucella-infected flocks, proposed to be tested by serological tests annually and the reactors dogs must be depopulated for for better fulfilment of eradication programs.
Key words: Brucellosis, Zoonosis, Dog, PCR, Serology

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Topic: Zoonotic Diseases

P316: Importance of canine brucellosis in breeding kennels

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Introduction and Objectives: *Brucella canis* is the most prevalent *Brucella spp* in canine population and has been reported as an important cause of reproductive abnormalities such as abortion and fetal reabsorption in dogs. In infected males, normal morphology of sperms has been changed and sometimes epididymitis, orchitis and scrotal edema occurs. Reproductive disorders result in economic loss in breeding kennels. Other species that cause brucellosis in dogs includes *B. abortus*, *B. melitensis* and *B. suis* which commonly occurring through consumption of non-pasteurized dairy product in kennels. This study was aimed to determine the brucellosis frequency and its relationship with clinical findings and reproductive disorders in kennel dogs.

Materials and Methods: Blood samples were taken from 90 adult dogs that were kept in breeding kennels, regardless of sex and clinical status. PCR, Rose bengal and Wright tests were used for screening. Exgene TM®GeneAll DNA extraction kit was used and PCR was performed using specific, IS711 primers.

Results: Based on the results, 30 percent of the samples were Rose bengal positive, but all Wright tests reported negative. PCR was positive in 2 samples which first one belonged to a 3 years old female Rottweiler with infertility history and the other case was a 6 years old male Doberman with mating disorder, which infected by *B. abortus* and *B. canis* respectively.

Conclusion: Result of this study indicates that canine brucellosis could be considered as a causative agent of reproductive disorders in breeding kennels. On the other hand, reports of *B. canis* infection in human population in Iran, shows that the possibilities of disease transmission to kennels personal and veterinarians and public health hazards of disease must be further considered.

Key words: Kennel, Iran, *B. canis*, *B. abortus*, PCR

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P28: Study of sarcocyst contamination rate by using histopathology and molecular method in carcasses of cattle slaughtered in Isfahan

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Introduction and Objectives: Sarcocystis is one of the most common parasites in domestic animals. The parasite is important in terms of economic and pathogenicity in domestic animals.

Materials and methods: In this study, the esophageal muscles, diaphragm and the heart of 100 slaughtered cows at the isfahan slauphter-house were collected which contamination was investigated based on age, sex, season and infected organs. macroscopic cysts were found by studing of the carcasses and specimens in 7 cows. Microscopic cysts of Sarcocystis were detected by histopathologic method and by staining with hematoxylin-eosin and bradyzoites under a microscope.

Results: The rate of infection was found as below: in the heart (44%); in the esophagus (30%); and in the aperture (12%).

Conclusions: Heart was recognized as the most contaminated tissue. Also, the highest infection was between the ages of 2-7 years old and mostly appear in the summer. Femal are most faced with contaminations.DNA extracting and refining was performed by using kits and PCR conditions were provided for replication of 18 s R-rna fragment was provided. The PCR evaluation showed that microscopic cysts belong to the genus sarcocyst.

Keyword: sarcocyst, slaughter-house, PCR, contamination organ

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P18: *Toxoplasma* and Toxoplasmosis in camels: a scoping review and research gaps

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Introduction and Objectives: *Toxoplasma gondii* is one of the most prevalent protozoan parasites causing morbidity and mortality in humans and various livestock. Camel is an important livestock in the Middle East and North Africa (MENA), however camel toxoplasmosis has been neglected in these countries. Using literature mining, in this article, we have provided a comprehensive overview of the literature on the current status of toxoplasmosis in camel. Research gaps and the required fields of information are identified in this scoping review.

Materials and Methods: All documents on *Toxoplasma* infection of camels were extracted in May 2019 from three principal databases, Google Scholar (title only), PubMed (title/abstract) and Scopus (title/abstract/keywords) using ("camel") and ("*Toxoplasma*" or "Toxoplasmosis") as the keywords, without any time limit. Two independent investigators monitored the eligibility of the articles and resulting data. The documents were organized in an Excel dataset according to different features of the publications including the first author, corresponding author, year and country of publication, study location, parasite genotype, journal title, citations and article type and topic.

Results; In total 67 articles were found in Scopus, 18 in PubMed and 12 documents in Google Scholar. More than 77% of the articles were original and 64% were epidemiological investigations. In recent years, "camel toxoplasmosis" attracted a lot of attention, as 54% of the articles have been published in the last 8 years. Geographically, the largest number of the articles were conducted in Iran (18%) and Saudi Arabia (17%). Mean citation per paper was calculated as 11± 15.2. The most-cited article was published in 2006 with 77 citations.

Conclusion: Although North Africa, especially Sudan, holds a large population of camels, little information is available from this region. More research attentions are needed towards clinical works and molecular epidemiology of camel toxoplasmosis.

Keywords: Camel, Toxoplasmosis, *Toxoplasma gondii*, Scoping review, Research gaps

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Topic: Zoonotic Diseases

P334: Current status of *Lophomonas blattarum* in human and animals: a scoping review of the literature

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Introduction and Objectives; *Lophomonas blattarum* is a multiflagellated protozoan, parasitizing arthropods, especially the intestine of termites and cockroaches. Human infection with this flagellate was first reported in China in 1993. Recently this protozoan has been frequently encountered in patients with pulmonary diseases. The pathogenesis of this protozoan in humans is still debatable. Since little is known on the epidemiology and pathobiology of this protozoa, we conducted a comprehensive scoping review to investigate and integrate available information on this emerging protozoan.

Materials and methods: All documents on *L. blattarum* were extracted in May 2019 from three leading scientific databases, Scopus, PubMed and Google Scholar (title only) using the keyword "*Lophomonas blattarum*" with no time limits. Two individuals studied the data independently and the documents were reviewed and organized in a spreadsheet based on different article features including first author, corresponding author, publication year, country, journal title, citations, article type and topic, host and patient population.

Results: As of 1933, we found 43 documents in Scopus, 30 in PubMed and 79 articles in Google Scholar. *Lophomonas blattarum* has attracted a lot of attention in the last decade so that 64% of articles have been published in this period. Half of the publications were performed in China, mostly published in the Chinese Journal of Parasitology and Parasitic Diseases. Only five investigations including two case reports have been published from Iran. The most studied patient/host population were humans with bronchopulmonary infections. The mean±SD citations per paper was 4± 4.4.

Conclusion: Obviously further studies are required on the epidemiology and pathobiology of this flagellate in different regions of the world. Our results indicate *L. blattarum* has not yet been widely investigated in Iran.

Keywords: *Lophomonas blattarum*, Scoping review, Iran, Bronchoalveolar lavage

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Topic: Zoonotic Diseases

P271: Rapid identification of *Mycoplasma pneumoniae* cell culture contamination using PCR

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Introduction and Objectives: *Mycoplasma pneumoniae* is one of the mycoplasmas species which could contaminate cell cultures. Identification of *M. pneumoniae* is so important. The Rapid identification of these contaminations is the objective of this study. It could be significant role in preventing and controlling of contamination in cell cultures.

Materials and Methods: In this study, the isolation and detection of *M. pneumoniae* from 82 cell culture samples being sent to the Mycoplasma Reference Laboratory using PCR method were performed. Briefly, M1F and M3R primers that have the ability to identify mycoplasma genus from the 16S rRNA gene were used. The P1 adhsein gene and MPF and MPR specific primers were used to initiate the PCR reaction to detect *Mycoplasma pneumonia.*

Results: Of the 82 samples, 48 (58.53%) negative and 34 (41.47%) of the samples were positive using mycoplasma genus PCR as diagnostic method. *M. pneumoniae* did not detect from those sample by using those primers and there is not any *M. pneumoniae* detected in this study.

Conclusion: The results of this study indicate that *Mycoplasma pneumoniae* is not a factor contributing to cell cultures in Iran. PCR could be an alternative method instead of the culture because according to the results of this study, PCR has high accuracy, speed and cost-effective for detecting *M. pneumoniae*.

Keywords: *Mycoplasma pneumoniae*, Cell culture, PCR

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P331: Prevalence of Leptospira spp in students of Veterinary Faculty Medicine and raw milk of cattle and sheep in Shahr-e-Kord, Iran

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Introduction and Objectives: Leptospirosis is a zoonotic disease, and in humans, primarily as a disease-related occupation that clinical symptoms variety, such as Weil's syndrome, flu-like symptoms, meningitis, meningoencephalitis, pulmonary hemorrhage with respiratory failure, the form of skin disease is characterized by red bumps, light scarring, diarrhea, and the risk of abortion in pregnant women.
Materials and Methods: In this study, 40 urine specimens (30 samples of veterinary students and 10 veterinary hospital staff), 50 samples of cow's milk and 50 samples of sheep's milk were surveyed in Shahrekord during one year. In order to detect leptospira spp bacteria in the samples, the PCR test was used to detect the flaB gene.
Results: Following the PCR test, 10 urine specimens (25%), 12 cow's milk samples (24%) and 6 sheep's milk samples (12%) were infected with Leptospira. 10 urine specimens were divided into 3 samples (30%) and 7 (38.33%).
Conclusion: The necessary steps to control and enforce health issues are essential.
Keywords: Leptospira, urine, students, milk, cattle, sheep, Shahrekord

Panels

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Panel 2: Mycobacteria & Acid-Fast Bacteria

Isolation and Identification of Mycobacterium from Captured Cats and Mice Belonging to Tuberculosis Infected Farms

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Introduction and Objectives: *Mycobacterium bovis* is a major cause of zoonotic diseases. The present study was aimed to evaluate the potential role of cats and mice in the transmission of *Mycobacterium* in cow dairy herds.
Materials and Methods: A total of 7 cats and 5 mice were screened from an animal husbandry. Gastric acids of captured cats were cultured on LJ (Löwenstein–Jensen). The liver, spleen, and lung of mice were cultured on LJ medium followed by sterilization. The acid-fast staining and also PCR were used for detection of *Mycobacterium* spp.
Results: Five from 6 cats were positive by acid-fast staining. PCR and 16S rRNA sequencing methods were confirmed the *Mycobacterium tuberculosis* Complex (MTB). Three out of 5 mice were positive by acid-fast staining. PCR and 16S rRNA sequencing methods were confirmed the *Mycobacterium tuberculosis* Complex (MTB). Currently, we are conducting PCR-RFLP and RP Typing to identify this bacterium more precisely.
Conclusion: We indicated that the mice and cats are potential source for *Mycobacterium* spp. Thus, they can infect dairy cattle farms.
Keywords: *Mycobacterium tuberculosis* Complex, *Mycobacterium*, PCR *IS*6110, 16S rRNA

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Panel 2: Mycobacteria & Acid-Fast Bacteria

Isolation and purification of ESAT6/CFP10 complex antigen secreted by *Mycobacterium tuberculosis* strains C

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Introduction and Objectives: Tuberculosis (TB), caused by *Mycobacterium tuberculosis*,is one of the most prevalent infectious diseases. The pathogenesis of *Mycobacterium tuberculosis* is related to its proteins; the early secretory antigenic target (ESAT6) and culture filtrateprotein-10 (CFP10), expressed from the region of deletion-1 (RD1). These proteins are highly specific and potentially useful for the diagnosis of tuberculosis.This research focused on purification of low molecular weight proteins from *M.tuberculosis* C vaccinal strain by ammonium sulphate precipitation.

Materials and Methods: *M.tuberculosis* C vaccinal strain grown in Löwenstein-Jensen medium and Dorset Henley liquidmediumwere inactivated by subjecting to 68ºC for 90 min, filtered, and the proteins in the supernatant fluid precipitated with ammonium sulphate at 4ºC. The obtained precipitates were dialyzed and subjected to gel chromatography (G-50) and the obtained fractions analyzed for protein concentrations by Lowry method. The approximate molecular weight in the purified fractions were determined by SDS-PAGE. Finally, ESAT6 and CFP10 protein complex in the purified fractions were confirmed by western blot analysis using ESAT6 specific antibody.

Results: Maximum amount of the proteins were precipitated in the presence of 20 %**ammonium sulphate. During SDS-PAGE analysis, protein bands of approximately 10-15 kDa were observed.** Purity of the proteins preparations was ≥95%, as judged by sodium dodecyl sulfate (SDS)-PAGE and subsequent staining with Coomassie blue**. The presence of ESAT**-6/CFP10 complex in the partially purified fractions were confirmed by Western blot analysis.

Conclusion: Sufficient amounts of ESAT-6/CFP-10 purified by the mentioned method might be suitable for the development of diagnostic kit for *Mycobacterium tuberculosis***in future.**

Keywords: *Mycobacterium tuberculosis,* ESAT-6/CFP10, purification, chromatography, SDS-PAGE, Western blotting

Panel 2: Mycobacteria & Acid-Fast Bacteria

Molecular Characterization of Epidemiology of MDR Mycobacterium tuberculosis isolated from tuberculosis patients resistant to Ofloxacin and Ciprofloxacin

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Introduction and Objectives: Multidrug-resistant tuberculosis (MDR-TB) is anescalating problem, particularly in the developing countries such as Iran. Continuous surveillance of drug resistance is required for effective management of TB patients.

Materials and Methods: The susceptibility patterns of clinical*Mycobacterium tuberculosis* strains were retrospectively analyzed from April2012 to March2018. Identification and drug susceptibility testing (DST) were performed using conventional and molecular methods.

Results: A total of 3012 clinical specimens were collected form TB suspected patients. Of them, 100 (3.3%) were culture positive and assigned as *M. tuberculosis* by phenotypic and molecular methods. According to DST, 62*M. tuberculosis*strains were pan-susceptible and 38 were resistant to at least one anti-TB drug. Seventeenisolates were also assigned as MDR-TB.

Conclusions:there wasa relatively high rate of MDR-TB in our study. Hence, improved diagnosis and treatment of MDR-TB should be highly prioritized.

Keywords: Multidrug-Resistant Tuberculosis, MDR, Iran

Panel 3: New Laboratory Methods for Diagnosing Infectious Diseases

Innovations in *salmonella* detection

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Salmonella is a major food-borne pathogen and a diverse genus. The majority of *salmonella* cause gastroenteritis. Infection typically occurs after the ingestion of contaminated food or water. It is estimated 95% of *salmonella* infections are due to consumption of contaminated foodstuffs. Rapid detection and identification of this food-borne and zoonoses pathogen is key to be safe in the food supply. Culture as a gold standard is not suitable for foodstuffs so this traditional detection besides molecular biology improved the speed of detection.

The methods for this purpose divided in two categories, culture dependent and culture-independent approaches. These methods include PFGE, Traditional serology, Phage-typing, PCR/qPCR, MALDI-TOF MS, LC-MS, WGS and Metagenomics.

PFGE use for subtype and 1-3 days need to complete process, Traditional serology use for serotype and need non-motile strain and up to 3 days need, Phage-typing use for serotype and can detect *salmonella* typhi, *salmonella* paratyphiA, *salmonella* typhimurium, *salmonella* enteritidis, also 1-2 days need to perform, PCR/qPCR use for genus to serotype but it depend on primers and 4-6 hours complete the process, MALDI-TOF MS use for species and in less than 5 minutes you can reach the result, LC-MS use for serotype to sub serotype level and in less than 1 day you can reach the result, WGS use for detection of strain and it depend on analysis time and 3-4 days is enough to get the result, Metagenomics use for detection of genus to strain and it depend on analysis time and 3-4 days is enough to achieve the result.

In conclusion it is important to know that not every method will be recommended or even suitable for every specimen. Also use of these methods depend on sensitivity, specificity, matrix driven effect, technical complexity, user skill, technical process, cost of equipment and cost of per sample.

Keywords: *Salmonella*, food-borne, detection, identification

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Panel 4: Zoonotic Disease

Design and optimization of diagnostic glanders cassette using immunoblotting method based on immunoreactive proteins of *Burkholderia mallei*

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Introduction and Objectives: Glanders is one of the oldest contagious and dangerous zoonotic diseases manifesting ulcerative granulomatous lesions on the skin and mucous membranes. Early methods possessing desirable sensitivity and specificity is important to diagnose the disease considering the just only one case report and preventing disease by identification and eradication. The present study was aimed to design and optimize Dot-blot ELISA and Western blot methods using immunoreactive antigens of *Burkholderia mallei.*

Materials and Methods: Three farm horses were subcutaneously immunized with a crude suspension of heat-inactivated *B. mallei* adjuvanted with incomplete Freund's adjuvant (IFA) to achieve a hyperimmune sera panel. The immunization was done for 1, 14, and 28 days with 1 dose of 1 ml antigen containing 10^6 cfu/ml. The hyperimmunity of sera was confirmed by ELISA and CFT. *B. mallei* whole-cell proteome was precipitated by trichloroacetic acid (TCA) followed by sonication method and quantified by Dot-blot ELISA and Western blot using HRP-conjugated rabbit anti-horse IgG. A comprehensive set of positive and negative horses' sera validated the test.

Results: 11 out of 121 sera samples were positive by Dot-blot ELISA and Western blot. A ladder pattern of the *B. mallei* immunoreactive proteins was seen within the region of 20-90 kDa and scored positive. The immunoblotting assay indicated a noticeably higher diagnostic specificity for positive or negative sera of glanders.

Conclusion: Trustful methods possessing desirable sensitivity and specificity are important to diagnose the disease and eradicate infected cases. We assume the immunoblot assay was adaptable for serodiagnosis of glanders in endemic areas and typically in less-developed countries.

Keywords*: Burkholderia mallei,* glanders, Dot-blot ELISA, antibody

Panel 4: Zoonotic Disease

The role of Veterinary Medicine in controlling of Crimean-Congo Hemorrhagic Fever (CCHF)

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Introduction and Objectives: Crimean-Congo hemorrhagic fever (CCHF) is one of the most important and the most widespread tick-borne viral disease of human beings in the world. CCHF is thought to have originated in Africa 1000-5000 year ago, although strain Ap92 found in Greece is also considered an ancient lineage. Since 2000 the infection has caused epidemics in Turkey, Iran, Russia, Uganda and Pakistan. Since 1999, CCHF has been reported in 26 of the 31 provinces of Iran. The data also indicated viremic livestock act as the main routes of CCHF virus transmission in Iran, as those are dangerous for transmission of CCHFV to human. The hosts of the CCHF virus include a wide range of wild and domestic animals such as cattle, sheep, and goats. Viremic mammals can transmit CCHFV to ticks. Many birds are resistant to infection, but ostriches are susceptible. Reptiles rarely affected. In domestic animals, the infection is usually sub-clinical and lasts from a few days to a few weeks. So, shepherds, campers, agricultural workers, veterinarians, abattoir workers, and other persons in close contact with livestock and ticks are at risk of infection.

Materials and Methods: In Iran, antibodies to CCHFV in sheep and cattle were first detected in 1970. The detection of IgG in livestock revealed that 35.8% of 5842 sera were positive for CCHFV IgG. However, the first confirmed human case of CCHF was diagnosed in Iran in August1999.

Results: The control of CCHF include: Reduce ticks in the environment, Quarantine for animal, Wear mask, gloves and gowns when slaughtering and butchering animals in slaughterhouses or at home to prevent skin contact with infected animal tissue or blood.

Conclusion: In this paper the role of Veterinary Medicine in controlling of CCHF and the latest information about the disease will discuss in details.

Keywords: CCHF, Iran, Animals, Control.

Panel 4: Zoonotic Disease

Epidemiological situation of crimian congo haemorrhagic fever in 1998-2018 in Iran

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Introduction and Objectives: Crimean-Congo hemorrhagic fever (CCHF) is the most significant and widespread tick-borne virus disease that has spread to more than 30 countries in the continent of Africa, Europe and Asia. The widespread distribution of the Crimean -Congo hemorrhagic fever (CCHF), as well as the ability of the virus to cause severe human disease with a high rate of fatalities, has led to this disease being taken into consideration. In Iran, after diagnosis in 1999, patients are diagnosed and reported on an annual basis in different parts of the country on the basis of the definition and care plan for the disease. Crimean-Congo hemorrhagic fever (CCHF) can be transmitted through bite, contact with the livestock and contaminated secretions and contact with tissue, blood and contaminated human blood

Materials and Methods: The data collection method through the summary forms of disease information is defined by the definition of suspicious, probable and definitive cases as a telephone and emergency report on a daily basis by health centers in the portal system of the Center for the management of communicable diseases.

Patients are reported according to a disease-control plan with three symptoms including fever, thrombocytopenia (platelet loss), and bleeding from across the country. Blood samples were collected from patients in three stages (diagnosis, five days after diagnosis and ten days after diagnosis) and serologically tested IgM and IgG antibodies, as well as molecularly RTPCR.

Results: The geographical distribution of the disease in the country and its epidemiological status indicate that definite patients are dispersed in most parts of the country. Between 1999 and 2018, there were 1424 cases of confirmed cases, of which 190 were death. The highest rate of fecundity was in 1999 (50%) and the lowest was 1385 (2%). The highest number of cases is in the age group of 20 to 40 years, most of them were identified in June and July, and in urban areas (63%) more than rural areas (37%) and males (79%) more than females (21%) occurred. The most occupations of the farmer-livestock group (321 cases) were 22.5% of the slaughterhouse worker (241 cases) and 17% were housewives (240 cases). Regarding the route of transmission, most cases are reported through direct contact with the blood, secretions and infected tissues of the affected animal during slaughter, and its analysis will be fully presented. Report of probable and definite cases from different regions of the country that most of the reporting provinces in the country are Sistan and Baluchestan province with 851 cases, Khorasan Razavi 118 cases and Kerman 68 and the number of deaths in Sistan and Baluchestan province with 71 cases, Khorasan Razavi has 18 cases and Fars has 13 most cases in the country.

Conclusion: Considering the trend and the state of the epidemiology of disease in the country, physicians' awareness of timely diagnosis and treatment for identifying patients and reducing the mortality rate should be strengthened.

Keywords: Iran-Epidemiology - Crimean-Congo hemorrhagic fever (CCHF)

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Panel 4: Zoonotic Diseases

Molecular detection of *Borrelia burgdorferisensulato* in tick infested dogs in Isfahan province, Iran

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Introduction and Objectives: Lyme borreliosis is caused by *Borreliaburgdorferi*and is one of the most common vectors borne diseases transmitted by ticks and has a worldwide geographic distribution the current study was carried out to evaluate the presence of *B. burgdorferisensulato* in blood samples of ninety-seven guard and sheep dogs in Isfahan, Iran using PCR technique. Dogs are infected to this spirochete by blacklegged tick or Ixodesricinus bite. The tick has horizontal transmission and tick Maternal transmission of parasite is not approved yet.

Material and Methods: Ninety-seven blood samples of guard and sheep dogs in districts of Isfahan, Iran with average age of 3.5 years were selected randomly and examined. PCR was applied to analyze the extracted DNA from blood samples.

Result: Out of ninety-seven guard and sheep dogs whose blood samples were examined using PCR, 12 samples were positive (12.37%) for *Borreliosis*. The possitive samples consist of 10(10.30%) male and 2 female dogs (2.06%). Complete blood count (C.B.C) was performed for each samplebutHematologic changes in infested samples were not recognizable. In the survey of *Borreliaburgdorferi*prevalence with SPSS (version 24.0) and by Fischer exact test there was a significant difference between age groups 1-2 years old (P=0.029) and this age group with age group of more than 3 years old (P=0.032)and also there was a significant relation between sex and rate of infection by using chi square method.

Conclusion: The results demonstrate that the prevalence of *Borreliaburgdorferi*is relatively high in dogs from the Isfahan province, Iran.

Keywords: *Borreliaburgdorferi*, Dogs, Isfahan, PCR, Iran

Panel 5: Emerging & Re-emerging Infections

Leptospirosis in Iran

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Leptospirosis is one of the most wide spread zoonosis worldwide which caused by Pathogenic *leptospires*. In humid tropical and subtropical areas with high rainfall (Such north of Iran), it is a greater problem than in those with a temperate climate. The disease is regularly prevalent in poor urban areas in developing countries.

The clinical syndromes in human are difficult to distinguish. The presentations can differ from a moderate fever likes flu to more serious illnesses considered by hemorrhage, jaundice, myalgia, renal dysfunction, and aseptic meningitis leads to death in some infected patients.

After infection, leptospires can colonize the proximal renal tubules of an inclusive different of wild and domestic animals for a longtime and are secreted through the urine into the environment. Humans most commonly become infected through occupational, recreational, or domestic contact with the urine of carrier animals, either directly or via contaminated water or soil. Due to the large number of pathogenic serovars (more than 250) and widespread of animal reservoirs in the environment, eradication of Leptospirosis is problematic. In spite of vaccination, the disease still survives in some regions of the country. The incidence of leptospirosis is probably grossly underestimated, because of limited diagnostic capacity in the regions where the burden of disease greatest.

To guide in diagnostic procedures in order to obtain early diagnosis so that prompt and appropriate management can be instituted and prevention and control measures can be carried out at the earliest possible stage to reduce morbidity and mortality. The clinical diagnosis should be confirmed by laboratory tests. Therefore, reliable and rapid tests are adequate for the diagnosis of leptospirosis. Samples may be also sent to higher centers with more facilities to do specific tests such as MAT, PCR and culture.

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Panel 9: Food, Industrial and Applied Microbiology

Verotoxigenic *E. coli* as an emerging foodborne infection

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Verotoxigenic *Escherichia coli* (VTEC) or Shiga toxin-producing *Escherichia coli* (STEC) is an important pathogen that can cause diarrhea or hemorrhagic colitis in humans. Hemorrhagic colitis occasionally progresses to hemolytic uremic syndrome (HUS), an important cause of acute renal failure in children and morbidity and mortality in adults. In the elderly, the case fatality rate for HUS can be as high as 50%. *Escherichia. Coli* O157: H7 has been recognized

as a cause of this syndrome since the 1980s. The reservoirs for STEC O157: H7 are ruminants, particularly cattle and sheep, which are infected asymptomatically and shed the organism in feces. Other animals such as rabbits, pigs, and poultry can also carry this organism. Infections with STEC in other serogroups, including members of O26, O91, O103, O104, O111, O113, O117, O118, O121, O128, and O145, are increasingly recognized as causes of hemorrhagic colitis and HUS. Humans acquire STEC by direct contact with animal carriers, their feces, and contaminated soil or water, or via the ingestion of underdone ground beef, other animal products, and contaminated vegetables and fruits. The infectious dose is very low, which increases the risk of disease. There are many studies that showed the prevalence of *E. coli* O157: H7 is not high in Iran not only in human but also in animals and food animal origins (meat, milk,). But other verotoxigenic *E. coli* strains than O157 H: 7 are more prevalent in Iran.

Thorough cooking of raw meats, pasteurization of milk, treatment of private water supplies, and the avoidance of cross-contamination from raw meats or cattle feces to other foods are the most effective ways of preventing STEC infections. Generally, the detection of STEC is laborious, and currently, there are no simple, inexpensive methods available for routine isolation of all STEC strains. Good hygiene practices at processing plants including monitoring for microbiological indicators (Enterobacteriaceae and in generic *E. coli*) to determine the effectiveness of those practices is likely to be the most an effective method for reducing the public health risks for STEC infection.

Keywords: *Escherichia coli*, Verotoxin, Foodborne infection.

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Panel 10: Medical ethics

Training of research ethics

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Ethical thoughts are essential basis for conduct of biomedical research, particularly those involving human subjects. The mandatory research ethics training has been considered as an integral part of the "good science" in several western countries. Teaching research ethics is a requirement within modern health science.

It is clear, that simply recommending new students that ethical behavior is a requirement in a modern environment of medical research and evidence-based practice is not acceptable.

Students will advantage from learning the background, issues, benefits, concepts, theory and skills associated with research ethics. On the basis of experience of designing, developing and integrating the teaching of research ethics in a new, fully integrated medical school curriculum, delivered using Problem Based Learning (PBL) and the recent literature relating to the teaching of research ethics to produce the following 12 Top Tips to training of research ethics.

Tip 1: The learning environment. (A key requirement is to undertake the teaching of research ethics within a positive and supportive learning environment. It is essential that the School or Department has a learning philosophy that supports the teaching of research ethics within the curriculum), Tip 2: Students should understand why they need to study research, ethics Tip 3:Start early and integrate throughout course, Tip 4:Establish the course outcomes and structure,(It is important to have learning outcomes that are clear, can be seen to have demonstrable value, can be mapped onto the curriculum, fulfil the requirements of the curriculum and reflect the ethos of the program), Tip 5 Where possible integrate with other aspects of teaching and learning,(Developing a program of research ethics education into a taught program will be more successful if it is viewed as an integrated part of the course and not as an additional educational requirement.) Tip 6: Course material used to deliver research ethics teaching,(Maintaining student interest in a subject that may appear to have little, immediate clinical impact can be achieved if a wide variety of different teaching materials are offered and used) Tip 7: Consider using active teaching and group discussion,(There is a clear emphasis within the literature that teaching research ethics can be facilitated by encouraging students to undertake active learning) Tip 8: Use alternative and varied sources of expertise,(Research ethics is a multi-disciplinary field and it is important to involve the expertise and experience of lawyers, philosophers, ethicists, educationalists and the public.) Tip 9: Additional learning opportunities. (Seek additional opportunities outside the course and the core curriculum to expose students to research ethics instruction even if this is infrequent and minimal) Tip 10: Offer training and support to a broad-based faculty not only research ethics teachers,(It is important to recognise that the attitudes students will develop towards research ethics will be influenced by every teacher that they meet throughout their course and not just the faculty members who deliver the research ethic component of the curriculum.) Tip 11: Develop an effective assessment strategy.(Assessing students understanding of research ethics can provide challenges. It is clearly not as easy to assess as other parts of the core curriculum, and there is a danger of over concentrating assessment on the mechanics of applying and achieving ethical approval from ethics committees rather than the understanding of the paradigms of research ethics) Tip 12:Evaluate and adapt. (It is important that the teaching of research ethics within a curriculum is evaluated and reviewed.)