

Scientific Tracks & Abstracts October 18, 2017

Applied Microbiology & Microbial Tech 2017



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October 18-19, 2017 Toronto, Canada

Kinetic microplate assay reveals lethal and sub-lethal behavior of antimicrobials immobilized on solid substrates

Steven Arcidiacono

US Army Soldier Natick Research Development and Engineering Center, USA

Statement of the Problem: Immobilization of antimicrobials onto surfaces is of great interest, although characterization of activity can be problematic. Traditional assays are designed for determining solution based antimicrobial activity and is incompatible with solid substrates.

Methodology & Theoretical Orientation: A kinetic microplate method was developed to determine the minimum bactericidal concentration (MBC) of the immobilized antimicrobial peptides SMAP and Cecropin P1 through a combination and modification of traditional solution assays, overcoming the difficulties of working with a solid substrate. The microplatebased kinetic assay was used to measure various peptide dose and time-dependent activity at multiple concentrations; viable plate counts were used to determine bactericidal activity and correlated to the kinetic assay results.

Findings: Immobilized peptide activity against both Grampositive and Gram-negative bacteria has been demonstrated, including Acinetobacter baumannii, Bacillus anthracis sterne and Staphylococcus aureus, and correlated to viable plate count results. Compared to peptides in solution, a combination of increased concentration and longer exposure time was required for activity. Immobilized peptide potency was cell-dependent; however, the peptides exhibited activity for all organisms in a dose-dependent manner, reaching a critical concentration

that resulted in complete inhibition. The role of immobilized peptide orientation relative to the solid substrate revealed that orientation is critical to activity.

Conclusion & Significance: This assay successfully determined activity on magnetic beads and planar glass substrates; other substrates such as antimicrobial textiles could also be characterized with this technique. Furthermore, the method yields information regarding sub-lethal concentrations not realized in the traditional assays. This kinetic microplate assay is also useful for testing antimicrobial formulations and reveals both synergistic and antagonistic interactions against clinical isolates and biothreat agents..

Speaker Biography

Steven Arcidiacono has an MS in Microbiology, with significant contributions in the research areas of anaerobic fermentation, antimicrobials, and biopolymer fermentation/fiber spinning. His primary focuses encompass exploratory research and development studies in the following specific areas: 1) colonic fermentation for biotransformation of nutritional and polyphenolic compounds; 2) skin microorganism interaction when in co-culture; and 3) discovery of novel antimicrobials to combat multidrug resistant bacteria and fungi. His prior experience/programs include antimicrobial peptides for detection and microbial protection and aqueous spinning of biopolymers (naturally-derived crystallin proteins and recombinant spider silk). He is Author/Coauthor of >30 peer-reviewed manuscripts, book chapters, proceedings and conference articles (cited >1500 times), numerous presentations, and an inventor on two issued US patents and two patent applications

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Magnetic bead based immuno-detection of *Listeria monocytogenes* and *Listeria ivanovii* from infant formula and leafy green vegetables using the Bio-Plex suspension array system

James B Day US Food and Drug Administration, USA

isteriosis, a disease contracted via the consumption of foods contaminated with pathogenic Listeria species, can produce severe symptoms and high mortality in susceptible people and animals. The development of molecular methods and immuno-based techniques for detection of pathogenic Listeria in foods has been challenging due to the presence of assay inhibiting food components. In this study, we utilize a macrophage cell culture system for the isolation and enrichment of Listeria monocytogenes and Listeria ivanovii from infant formula and leafy green vegetables for subsequent identification using the Luminex xMAP technique. Macrophage monolayers were exposed to infant formula, lettuce and celery contaminated with L. monocytogenes or L. ivanovii. Magnetic microspheres conjugated to Listeria specific antibody were used to capture Listeria from infected macrophages and then analyzed using the Bio-Plex 200 apparatus. As few as 10 CFU/mL or g of L. monocytogenes was detected in all foods tested. The detection limit for L. ivanovii was 10 CFU/mL in infant formula and 100 CFU/g in leafy greens. Microsphere bound Listeria obtained from infected macrophage lysates could also be isolated on selective media for subsequent confirmatory identification.

The method presumptively identifies *L. monocytogenes* and *L. ivanovii* from infant formula, lettuce and celery in less than 28 hours with confirmatory identification completed in less than 48 hours. While FDA focuses its regulatory microbiology methods on development of high throughput techniques, this method is useful for the isolation of *L. monocytogenes* from food samples containing high levels of competitor microorganisms that make it difficult to obtain discrete colonies on plating agars.

Speaker Biography

James B Day is a Research Microbiologist at the U.S. Food and Drug Administration in College Park, Maryland, where he is involved in developing detection methodologies for bacterial pathogens in contaminated foods. He has developed techniques for rapid identification of *Francisella tularensis, Salmonella enterica* and *Listeria monocytogenes* in various food matrices and recently established a novel macrophage-based assay for enrichment of intracellular bacterial pathogens for enhanced identification. He earned his PhD from the University of Miami School of Medicine (UM), where he worked on bacterial pathogenesis of *Yersinia pestis*. At UM, he developed a widely used system to measure virulence protein secretion and host cell translocation. He worked on type III secretion mechanisms of *Salmonella enterica* as well as regulatory factors that control virulence protein induction.

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Large-scale molecular detection of microorganisms

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ith the advent of high-throughput molecular techniques in molecular microbiology (Next Generation Sequencing, NGS) it became possible to explore microbiomes of rather complicated niches, such as microbiome of the gut, genitourinary tract, skin, or environmental microbiomes. The NGS technology enables to decipher even the most complex biological samples and analyze the DNA contents thereof. We have developed a large-scale 16S rDNA (panbacterial) and 18S rDNA (panfungal) NGS approach, combined with quantitative Real-Time PCR, to identify the microbiome and fungome in their complexities, in a quantitative way. This technological approach allows us to assess the microbial flora of a given anatomic location in full extent, with direct relevance to the rational selection of antimicrobial agents, if relevant. This high-throughput approach also allowed us to identify novel microbial agents, previously not suspected

to infect humans – e.g. *Candidatus Neoehrlichia mikurensis* in two immunocompromised individuals, or several zoonotic *Chlamydia* and *Mycoplasma species* directly transmissible to humans. The presented data will cover a unifying concept of molecular microbiology, entailing both human and veterinary infections, as the overlap between both is greater than previously anticipated.

Speaker Biography

Sona Pekova has completed her graduation as MD from the Medical Faculty, Charles University in Hradec Kralove, Czech Republic. She continued her PhD studies at the Academy of Sciences of the Czech Republic in Prague and the 1st Medical Faculty, Charles University in Prague, Czech Republic. She has her PhD in Molecular Biology, Virology and Immunology. She has extensive expertise in molecular hematology, molecular hematooncology, molecular microbiology and molecular genetics, both in human and veterinary medicine, with tens of peer reviewed scientific papers documenting her medical and scientific history.

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Rhizobial inoculation increases soil microbial functioning and gum Arabic production of 13-years old *Senegalia senegal* (L.) Britton trees in the north part of Senegal

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hizobial inoculation has been widely used in controlled old K conditions as a substitute for chemical fertilizers to increase plant growth and productivity. However, very little is known about such effects on mature trees in natural habitats. In this study, we investigated the effect of rhizobial inoculation on soil total microbial biomass, mineral nitrogen content, potential CO2 respiration, fluorescein diacetate (FDA), acid phosphatase activities and gum arabic production by 13-years old Senegalia senegal (Syn. Acacia senegal) under natural conditions in the north part of Senegal during two consecutive years. Rhizobial inoculation was performed at the beginning of the rainy season (July) for both years with a cocktail of four strains (CIRADF 300, CIRADF 301, CIRADF 302 and CIRADF 303). Rhizospheric soils were collected in both dry and rainy seasons to a depth of 0-25 cm under uninoculated (UIN) and inoculated (IN) trees. Trees were tapped in November (beginning of dry season) using traditional tools. Gum arabic was harvested every 15 days from December to March. The results obtained from both years demonstrated that rhizobial inoculation increased significantly the percentage of trees producing gum arabic,

gum arabic production per tree, soil microbial biomass, FDA and acid phosphatase activities. However, there was no significant effect on C mineralization and mineral nitrogen (N) content. Gum arabic production was positively correlated to rainfall, soil microbial biomass and mineral nitrogen content. Our results showed a positive effect of rhizobial inoculation on soil microbial functioning and gum arabic production by mature *S. senegal trees*. These important findings deserve to be conducted in several contrasting sites in order to improve gum arabic production and contribute to increase rural population incomes.

Speaker Biography

Dioumacor Fall completed his PhD in 2009 from Cheikh Anta DIOP University (Dakar-Senegal). He pursued his Post-doctoral studies at the Common Laboratory of Microbiology IRD/ISRA/UCAD in Dakar. He joined the Senegalese Institute of Agricultural Research (ISRA) as a Researcher in 2011. His work focuses on plantmicroorganism-environment interactions and how they can contribute to improve plants production particularly in a climate change context. He is working as the Head of the Microbiology Laboratory of the National Center for Forestry Research (CNRF) at ISRA. He has more than 23 peer-reviewed publications.

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Reproducibility of four identification methods of antibiotic-resistant *Mycobacterium tuberculosis* isolated from displaced and nondisplaced Iraqi patients with reference to QuantiFERON

Mohemid M Al-Jebouri and Burhan A Ali University of Tikrit, Iraq

Background: The first and major step in the diagnosis of TB is its accurate and early detection. To fulfill this objective, many methods have been developed and reported that obtain early growth of *M. tuberculosis*. For exactly detection of the TB cases, recently a novel polymerase chain reaction (PCR) based diagnostic kit has been developed. It is based on the nucleic acid amplification (NAA) of specific region of *Mycobacterium* DNA. QuantiFERON-TB test, (QFT) an *in vitro* diagnostic test that measures a constituent of cell-mediated immune reactivity to M. tuberculosis was approved by Food and Drug Administration (FDA) as an aid for identifying *Mycobacterium tuberculosis* infection.

Methodology: In the current study, there were 50 patients (18 displaced and 32 nondisplaced TB patients) and 40 healthy controls. The patient was examined for the presence of TB utilizing QuantiFERON-TB Gold In-Tube assay, polymerase chain reaction(PCR), AFB smear and TB culture. Drug susceptibility of isolates to first-line anti-tuberculosis drugs was performed using the proportion method on Lowenstein Jensen medium (L J medium) within 2-4 weeks.

Results: It was found that the frequency of positivity of acid-fast stain, culture and QuantiFERON for displaced and non-displaced patients was 36, 33.3 and 100 and 64, 66.7 and 100% respectively. The positivity towards polymerase

chain reaction for primers IS6110 and MPB64 for displaced patients was 37.5 and 100% respectively, whereas for nondisplaced patients was 14.3 and 100 % respectively too. The present study revealed that 20 isolates out of 34 tested were resistant to one or more of anti-tuberculosis drugs tested which were isoniazid, streptomycin, rifampicin and ethambutol. Statistically, there was a significant difference between types of drug and frequency of resistance among displaced and nondisplaced Iraqi patients (P<0.05).

Conclusions: The PCR test for the presence of primer MPB64 and QuantiFERON test were 100% positive for all mycobacterial isolates tested from displaced and nondisplaced patients, whereas other identification tests revealed variations in reproducibility. The present study showed that all the mycobacterial isolates tested for antimycobacterial drugs were resistant to at least one antibiotic used and most of them were multiple-resistant. Statistically, there was a significant difference between types of drug and frequencies of resistance (P<0.05).

Speaker Biography

Mohemid M Al-Jebouri is Professor at Department of Microbiology, College of Medicine, University of Tikrit, Tikrit, Iraq. His research interest includes: Infectious Diseases, Pharmaceutical Microbiology, Allergy and Immunity, Clinical Diagnosis and Management by Laboratory Methods.

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Detection of sequences of novel insect *Flaviviruses* from *Uranotaenia macfarlanei*, known as frog feeding mosquitoes, in Okinawa, Japan

Mika Saito¹, H Kise^{1, 2} Y Kushida^{1, 2} M Mizuyama^{1, 2} Y Sato^{1, 2} and C Oyakawa³ ¹University of Tokyo, Japan ²University of the Ryukyus, Okinawa, Japan, ³Nansei Environmental Laboratory Co. Ltd., Japan

Statement of the Problem: Infectious diseases caused by mosquito-borne Flaviviruses, including *Dengue virus*, *Japanese encephalitis virus*, *Zika virus*, and Yellow fever virus, represent a worldwide public health threat. According to ecological changes and rapid increases in their incidence and geographic distribution, *Flaviviruses* are classified as emerging or re-emerging pathogens. The mechanisms underlying winter period maintenance and sylvatic transmission remain unclear. Okinawa is in the southern part of Japan, includes small islands, and has a subtropical climate, which allows some vector mosquitoes to survive year-round. Based on the vulnerability of isles against external stimuli including the invasion of known and unknown pathogens, we initiated a project for the comprehensive and highly sensitive detection of pathogens from field-caught mosquitoes.

Methodology & Theoretical Orientation: In 2015 and 2016, we collected 3396 mosquitoes from 33 sentinels in Okinawa Island. These mosquitoes were morphologically and genetically identified and pools were made (max 20). RNA was extracted from each pool, RT-BT-PCR was conducted with *Flavivirus* universal primers targeting the NS5 protein gene (Kuno), and positive PCR products were sequenced.

Findings: We detected 4 nucleotide sequences of novel *Flaviviruses* from *Uranotaenia macfarlanei* spp, known as frog-feeding mosquitoes, near a cave in Okinawa Island in June, Sept, and Oct. 2016. A BLAST search and phylogenetic

analysis of the NS5 protein using the Neighbor Joining method, showed that the sequences created Okinawan-specific clusters within the clade of insect-specific *Flaviviruses*, and showed the highest homology with Nakiwogo virus isolated from Mansonia Africana, Uganda. Attempts to isolate the virus were unsuccessful.

Conclusion & Significance: Four sequences were detected at different times in one location; therefore, viral circulation was established in the area, possibly between frogs and *Uranotaenia*. These are distinct from other pathogenic agents of mammals including humans. Pokilotherms may play important roles in maintaining sylvatic forms of *Flaviviruses*.

Speaker Biography

Mika Saito is a Veterinarian and Assistant Professor in the Department of Virology, Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan. She has graduated and received a PhD from the Graduate School of Veterinary Medicine, Hokkaido University in 1986 and 2009, respectively, and received an MA in International Development from Nihon Fukushi University, Aichi, Japan in 2002. Her major research interest includes risk assessments of infectious diseases caused by mosquito-borne *Flaviviruses* such as Japanese *encephalitis* virus and virus, including human and environmental factors, such as socio-economics and human lifestyles, and relationships with wild life. She is a Group Leader of mosquito research group in the project "development for the control strategy of vector-borne and zoonotic diseases in Okinawa and formulation of networking among stakeholders Okinawa Communicable Disease Research Hub Formation Promotion Project since 2015". She is attempting to combine IT and Al for risk assessments and the control of diseases.

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Continuous wastewater treatment contaminated with heavy metals by coupling a microbial fuel cell and a microbial electrolysis cell

Chansoo Choi, Guorong Xie and Bonsu Lim Daejeon University, Republic of Korea

he main objective of this study was to find the feasibility of continuous removal of mixed heavy metal ions from wastewater by coupling a microbial fuel cell (MFC) and a microbial electrolysis cell (MEC) in a continuous mode (Fig. 1). This research focused on a mixture of chromium (VI) ions, zinc (II) ions, copper (II) ions, and nickel (II) ions, as a typical plating solution, to be removed using the MFC-MEC coupled system. The electrode material was graphite felt. To compare parameters the system was also run in a batch. In the batch mode, the effects of Cr (VI) initial concentration on removal efficiency of Zn (II) and Ni (II) in MEC has been first studied. The initial concentrations of Cr (VI), Cu (II), Ni (II), and Zn (II) were all 10 ppm in MFC, and the concentrations of Ni (II) and Zn (II) were 10 ppm in MEC. As Cr (VI) concentration increased from 10 ppm to 100 ppm, the voltage supply to MEC was increased, and Ni (II) and Zn (II) reduction rate was also increased. EIS has been applied to investigate the effect of experimental conditions on electrochemical reactions. The impedance of different Cr (VI) concentrations from 10 ppm to 100 ppm in MFCs showed that, compared with low initial Cr (VI) concentrations, higher initial Cr (VI) concentrations exhibited much lower resistance (Ohmic resistance; 19.3~13.4 ohms, charge transfer resistance; 28.2~21.5 ohms), and thus the MFCs were able to deliver more power toward MEC. The initial Cr(VI) concentration increased power generation by both increasing the cathode potential and decreasing the resistance of MFC. A typical current density and power density at the maximum power point were 1.08 Am-2 and 863 mWm-2 respectively. The

typical removal efficiency for chromium ion by reduction of Cr (VI) in the MFC was in the range of 96.9%~100% for 10 ppm after 8 hours. That of Cu (II) in MFC was only in the range of 29.0%~29.7% for 10 ppm. On the other hand, the removal efficiencies of Ni (II) and Zn (II) in the MEC were in the range of 55.0%~59.9% and 76.2%~77.6% for 10 ppm, respectively. The removal efficiencies of Zn (II) and Ni (II) in the MEC were slowly increased with the initial concentration of Cr (VI) in the MFC increased. In the continuous mode, effects of the hydraulic retention time (HRT from 2h to 12h) on the removal efficiency of 10 ppm solution have been studied. HRT had a little impact on removal efficiency of each ion. The removal efficiencies were 55.0%~78.5% for Cr (VI), 30.6%~32.4% for Cu (II), 55.0%~59.0 for Ni (II), and 75.3%~75.8% for Zn (II), respectively. Even if the initial concentration of Cr (VI) significantly changed, the other three ions showed only a little change as HRT increased because (1) the concentrations of Ni (II) and Zn (II) in MEC were as low as ppm range, and (2) remaining un-reacted Cr (VI) and Cu (II) flew into MEC, interrupting the Ni (II) and Zn (II) reduction in MEC. With one train the remaining concentrations of Ni (II) and Zn (II) were 4.5 ppm and 2.5 ppm, respectively for HRT of 2h.

Speaker Biography

Chansoo Choi has his expertise and passion in development of microbial fuel cells. He approaches this method for removal and/or recovery of heavy metals from wastewaters, simultaneously generating electrical energy. He also has been developing storage batteries for use to store alternative energy sources, such as solar cells. He developed gold, copper, and silver recovery models, and mercury, cadmium and lead removal models after years of experience in research and teaching in universities.

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Developing a novel green feed additive as alternative to feed antibiotics in poultry

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Administration of antibiotics in animal feed at sub therapeutic levels has been associated with growth promotion; however their indiscriminate use has led to a significant increase in the emergence of drug resistant strains. Additionally, antibiotics are also being identified as emerging environmental contaminants. Hence, it has become critical to develop effective green alternatives to feed antibiotics, without affecting livestock productivity. In this work, we propose a novel formulation based on a combinational approach comprising of a pyroligenous liquid, enzyme, organic acid and yeast proteins that can be used in lieu of feed antibiotics in poultry. The enzyme, organic acid and yeast biomass have been produced by solid state and submerged fermentation using agriculture wastes as substrates, thus making the process cost effective. Qualitative tests by well

diffusion method have confirmed the antibacterial activity of the pyroligenous liquid against several Gram positive and gram-negative bacteria, with maximum activity against *Salmonella enterica* and *Listeria monocytogenes* which are two of the most common bacterial pathogens associated with poultry. Antibacterial activity was also observed at very low concentrations of the pyroligenous liquid. The other components are being tested for their antimicrobial activity against common poultry pathogens.

Speaker Biography

Gayatri Suresh is currently pursuing her PhD in Water Science from Institut National de la Recherche Scientifique (Eau, Terre et Environnement) at Québec city in Canada. She has Master's in Microbiology from University of Pune, India, and is currently working on developing an alternative to antibiotics in poultry.

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Clostridium difficile infection: Prevention is in your hands

Sarbjeet Sharma SGRDIMSAR, India

Costridium difficile has emerged as a quintessential nosocomial pathogen by multidrug resistance and its spore forms persisting in the environment, surviving the routine disinfectants. It is responsible for at least one fourth of antibiotic-associated diarrhea in hospitalized patients and almost all cases of life-threatening pseudo membranous colitis. Recent epidemics of a hyper virulent strain were associated with significant morbidity and mortality, highlighting the need for better prevention treatment of *Clostridium difficile* infections. Preventive measures proven effective are judicious use of broad spectrum antibiotics plus selective restriction of clindamycin, an agent strongly responsible for suppressing gut flora allowing multiplication

of *C. difficle*, personal disinfection by preferring soap and water hand washing over alcohol based scrubs that are ineffective against spores and environmental control by isolation of patients and use of sodium hypochlorite on surfaces of equipment. These, along with prompt diagnosis and alerting can go a long way in curbing outbreaks.

Speaker Biography

Sarbjeet Sharma is a Clinical Microbiologist to the work of diagnostics for over 20 years, with keen focus on HIV and Tuberculosis. She has contributed many international and national publications and presentations, one of the latest being at ICAAC 2012, San Francisco, USA: "Comparative evaluation of Conventional techniques and Nested PCR in the diagnosis of Extra-pulmonary TB.

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Detection of ER stress after infection of human macrophages by Mycobacterium tuberculosis

Samuel Eguasi Inkabi Linköping University, Sweden

Post infection, macrophages, the first cells in the lungs that propel defense against pathogen invasion and play crucial activity in the onset and maintenance of immune responses against Mtb. The macrophages play this crucial defense role by phagocytosis, which have the macrophages "eat" up the Mtb bacilli. Macrophages therefore become infected with mycobacteria and may undergo apoptosis (programmed cell death) to destroy pathogens and prevent further spreading. Apoptosis, which results in the elimination of Mtb can be triggered by endoplasmic reticulum (ER) stress which is the physiological or pathological processes that disturb protein folding in the endoplasmic reticulum caused by the phagocytosis of the Mtb bacilli by the macrophages. The dysregulation of ER homeostasis can cause chronic diseases in humans and it is crucial to study ER stress using mammalian cells to understand ER-stress related diseases such as Tuberculosis. Here, we studied the ER stress induction and the extent of ER stress induction using human monocytes derived macrophages (hMDMs). We used the ER stress inducers tunicamycin and thapsigargin, and also infecting the macrophages with different doses of Mtb and

analysing CHOP and ATF6-alpha expression by western blot. This indicated that both inducers triggered CHOP activation, that a low dose of Mtb suppressed the expression of these ER-stress markers in most donors, and that infection with a higher dose of Mtb stimulated expression of both markers in 4 out of 6 donors. Alternatively, live microscopy was also performed on Raw macrophages and 16HBE epithelial cells after transfection with the ER stress plasmid sensor pEGFP-XBP1dDBD-STOP-tagRFPt and stimulation with tunicamycin and purified protein derivative of tuberculin (PPD). We have here confirmed the detection of ER-stress in human monocyte derived macrophages using positive inducers, and shown that low doses of Mtb decreases induction of ERstress whereas, high dose of Mtb induces ER- stress

Speaker Biography

Samuel Eguasi Inkabi has completed his MSc in Medical Biology from Linkoping University, Sweden. He also holds a Bachelor's in Biochemistry from Kwame Nkrumah University of Science and Technology, Ghana. His research focuses on infectious diseases, cancer and avian genetics. He has co-authored a publication and authored peer review papers in reputed journals.

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Study of antioxidant activity in extracts and alcohol (methanol, ethanol) Saffron petals

Elahe Khani Shiraz University of Medical Science, Iran

Medicinal plants are an important source of antioxidants; natural antioxidants increase plasma antioxidant power and reduce the risk of some diseases such as cancer, heart disease and stroke. Due to the toxicity and malnutrition effects of synthetic antioxidants in foods, the need for natural antioxidants from plants, which is less toxic and more effective, is a significant need. Saffron petals plantrich sources of polyphenols have not been considered yet. The aim of this study was to evaluate the antioxidant and phenolic compounds of saffron petals. In this study, saffron petals extract was extracted separately with the solvents such as ethanol and methanol with 30, 70 and 90% and water. The concentration of phenolic compounds was evaluated by Folin Ciocalteau and its antioxidant activity by reduction of free radical DPPH. The results showed that the type and

amount of solvent have effected on the phenolic compounds and antioxidant, and a significant relationship between the number of phenolic compounds, and inhibitory effect was observed. Also with increasing concentration, anti-oxidants effect and polyphenol extract increases.

Speaker Biography

Elahe Khani completed her education in Occupational & Environmental Medicine in 2017 and Medical degree from Shiraz University of Medical Sciences int. branch, Somayeh High School i n Sep 2002 - Jul 2006, Seminar on Developmental disorders in children in shiraz university of med school . 2017, Research in renal stone patient. Jan 2007 - Jan 2014 and evaluate the relation between calcium, phosphorus level, pth hormone, cratinine and urea level in blood and urine in the patients with renal stone (adults and also children). she is familly doctor in sina clinic and hospital Arsanjan (june 2016-Present).

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Molecular and phenotypical characterization of *Mannheimia haemolytica* isolated from goats in Baghdad province

Waffa A Ahmed Baghdad University, Iraq

annheimia haemolytica (M. haemolytica) is a Gramnegative bacterium, which can infect humans and animals. It's commensal as a normal flora of the nasopharynx and tonsils in cattle, sheep and goats, pneumonic pasterellosis is one of the most economically important infectious disease in goats with a worldwide prevalence. This study aimed to investigate the incidence of M. haemolytica by bacteriological and molecular characterization in goats. One hundred nasopharyngeal swabs were collected from apparently healthy farm goats, seven lung tissue specimens and five nasal mucus swabs from slaughtered goats in Baghdad. All samples were cultured on Blood and MacConkey agars. Biochemical tests and EPI20E kit were used for identification of the suspected colonies. 5(4.46%) isolates of *M. haemolytica* were identified phenotypically and confirmed diagnosis by polymerase chain reaction (PCR) technique using two primers 16s rRNA and 12s rRNA

genes. The results of this study concluded that identification of *M. haemolytica* by PCR was in accordance with those of phenotypic test and use of more than primer could improve identification of isolates.

Speaker Biography

Waffa A Ahmed is from the University of Baghdad. She has worked as the head of Department of Quality Control, Veterinary State Company, Ministry of Agriculture, (2005-2006). She has worked for eight years as Assistant Professor and Scientific Researcher in Unit of Zoonotic researches, (2006-2014), also in Department of Microbiology (2014-2017), College of Vet. Medicine, University of Baghdad. She had participated in several scientific conferences in Iraq and global conferences in USA, Canada, Germany and Egypt. Also, she had many invitations from many conferences. She has participated in many examining committees for Post-graduate students in Baghdad University and other universities in Iraq. She has taught more than seven courses for Under and Post-graduate students (Diploma, MSc and PhD) in pathology, microbiology, and zoonotic diseases in college of Vet. Med. She has published more than 30 papers in reputed journals and has been serving as an Editorial Board Member of Research Journal of Biology, and reviewed more than 100 articles in about seven journals and more than 20 theses (scientific evaluation).

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Nancy S Miller

Boston Medical Center, USA

A point of view about point of care testing for diagnostic microbiology in the US: What we have, what we need, what is on the way?

he last decade has seen an explosion of new technology and diagnostics in clinical microbiology. So, where are all the tests for infectious diseases at the point of care? Can we find the Holy Grail of diagnostics or does something else define its success? What does the US point of care quest have in common with the global healthcare community and vice-versa? This presentation looks at near patient testing for infectious diseases as it evolves in the US, both because of and despite the influence of global medicine and ex-US markets. A view from both ends of the telescope is presented to provide up-close and wide-angle perspectives. To that end, clinical case-based examples introduce and illustrate a system-based interrogation of considerations: the mythology of laboratory decentralization, want versus need, technology versus innovation, barriers to implementation, biosafety, governance, quality management, and outcome analysis.

Speaker Biography

Nancy S Miller is a board-certified Pathologist and a Clinical Microbiologist-Laboratory Director with more than 15 years' experience in infectious disease diagnostics and patient care. She has earned an MD with distinction in research from SUNY-Stony Brook and completed her residency in Anatomic and Clinical Pathology and fellowship training in Medical Microbiology, all at the Johns Hopkins Medical Institutions. Currently, she is the Medical Director of Clinical Microbiology and Molecular Diagnostics at Boston Medical Center, where she is immersed in the daily challenges of diagnostic microbiology and process improvement. Also, she is an Associate Professor in the Department of Pathology and Laboratory Medicine at Boston University School of Medicine. These responsibilities complement her translational research in innovative and improved diagnostics for infectious diseases, method comparisons, and outcome studies. Recent work includes PI-initiated commercial grants and academic collaborations with Boston University colleagues including NIH-supported projects.

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Video Presentations October 19, 2017

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October 18-19, 2017 Toronto, Canada

Effects of the biological life and mankind on the Earth (mainly between 1778 and 2015)

Béla Ralovich Ministry of Human Resources, Hungary

omponents and events of timeless and endless Universe are consequences of permanent flow of energy and substance in accordance with the laws of eternal Nature which will never be totally known by mankind. All components of the Universe have lived their own physical life. The life of our Earth is exceptional because of the presence of biological life. The biological life is a biological phenomenon of a living unit, which lasts till its own death. The living unit is a substantial matrix, which is bordered by a permeable membrane/wall. Inside the unit and through its membrane/wall, an organized and directed energy and substance transport flows. The living unit has been affected by the outside effects and it can accommodate to those only between the borders of its own life requirements. In the same time, it influences on its environment, too. Now it seems that the living unit is only present in the Bio-sphere of our Earth which is a closed system for it. In the case of any kind of closed systems, it is obligatory permanently to ensure the specific life conditions

which are necessary for a continuous life and reproduction of a living unit. It seems that the appearance of biological life and mainly that of men have fundamentally effected on the Earth. In consequence of the effects different periods can be determined in the life of our Earth. It is necessary to know about these periods because each of them had/ have special energetic and substantial processes which had/ have biological consequences. Now, we shall only deal with a short piece of the period of the life of our Earth which has started in 1778.

Speaker Biography

He is Working in Institute of Microbiology of UMSP, Institute of Public Health and Epidemiology of UMSP, he also worked at National Meat Research Institute of Budapest, Ministry of Welfare, Budapest, he got Scholarship in Wellcome in Nottingham, British Council in Oxford, he did his Ph.D. in 1973 and D.M.S. in 1986 from the Hung. Academy of Sciences (HAS); His research field includes Bacteriology, Infections, Immunology, Epidemiology, Food hygiene, Environmental protection. He is Adviser of WHO for Listeriosi; he Published 165 articles, 14 books and paper-books.

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A stem bark extract from *Khaya grandifoliola* (*Meliaceae*) C DC. stimulates the chemotactic and phagocytic activity of rat peritoneal macrophages exposed to CCl4

Florence Pare Ngoungoure University of Yaoundé I, Cameroon

Khaya grandifoliola is a plant species used in traditional medicine in Cameroon for the treatment of many diseases. This study determines the capacity of chloroform/ methanol (CH2Cl2/CH3OH) (1:1v/v) extract of stem bark of the plant to boost the chemotactic and phagocytic activities of rats macrophages in response to challenge with Saccharomyces cerevisiae. In vitro study showed that Khaya grandifoliola extract (KGE) caused a non-dependent concentration increase in NO production, reduced NBT dye by 75% and enhanced the activity of lysosomal enzyme by 47% at 1 µg/ml. The results of the *in vivo* study shows that after seven consecutive days of CCL4 exposure, the dose 100 mg/kg of body weight, KGE significantly (p< 0.05) increased the activity of NADH oxidase (1.3 folds), lysosomal enzyme (2 folds); production of H2O2 (2 folds) and NO (7 folds) compared to control group. We conclude that KGE stimulates the phagocytic activity of macrophages both *in vitro* and *in vivo* making it a candidate substance for strengthening the immune system.

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Detection of carbapenem resistant Gram-Negative Bacilli from infected wounds in Khartoum state-2014

Reem AbdElmoniem Dahab Khalil

University of Medical Sciences and Technology, Sudan.

Background: Carbapenem family are from the recently synthesized beta-lactam antibiotics which used as last resort antibiotics for treating infections caused by multidrug-resistant Gram-negative bacilli and the resistant to them by Gram-negative bacilli have been developed, due to production of variety of carbapenemase enzymes and other mechanisms that significantly limits treatment options for life-threatening infections.

Objective: This study aims to detect carbapenem resistant Gram-negative rods from infected wounds in Khartoum state and the production of carbapenemase enzymes by the resistant isolates using phenotypic methods.

Method: 100 wound swabs were collected. All samples were cultured directly on blood and MacConkey agar, Cultures were examined macroscopically and microscopically, different standard biochemical tests were performed for identification of Gramnegative bacilli. Standard antimicrobial susceptibility testing to Meropenem antibiotic was done for all Gram-negative bacilli isolates, and Modified Hodge test was performed for the resistant isolates.

Results: 77 Gram-negative bacilli were isolated from 100 samples, the commonest pathogenic isolates were Proteus species (28%) followed by Klebsiella species (24%), Escherichia coli(20%), Pseudomonas species (17%), Enterobacter species(10%) and

Acinetobacter species(1%). 13% of the isolates were Carbapenem resistant, and 50% of the resistant isolates were positive for carbapenemase enzymes production using Modified Hodge Test.

Conclusion: the percentage of Carbapenem resistance is high. Pseudomonas species followed by Escherichia coli were the most carbapenemase producers. Modified Hodge test is simple method for detection of carbapenemase enzymes that can detect many types of carbapenemase but not all types and it does not specify the types. Further studies should be performed using larger sample size and other specific methods especially PCR.

Speaker Biography

Reem AbdElmoniem Dahab Khalil is a 24years old medical laboratory specialist (microbiologist), studied at UMST, Sudan and completed the master degree by the age of 23,both by excellent degree, and cGPA 4.85 out of 5 in the BSc. Now she is a lecturer in International University of Africa, Sudan and at the same time working at a hospital, she is a beginner researcher, and she is very interested in the research work, especially antimicrobials.

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