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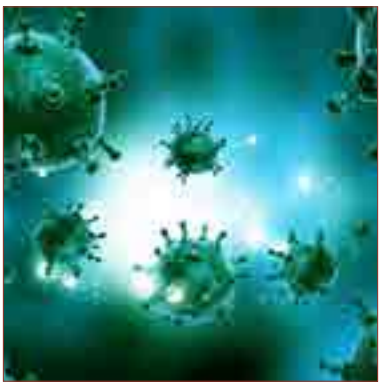
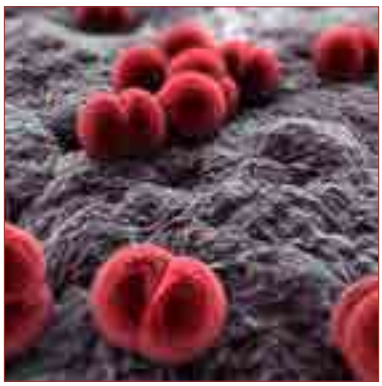
Antimicrobes & Microbial Pathogenesis 2017





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MICROBIAL PATHOGENESIS, INFECTIOUS DISEASE, ANTIMICROBIALS AND DRUG RESISTANCE

August 23-24, 2017 | Toronto, Canada

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Jason Asnis et al., Arch Clin Microbiol, 8:5
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The production of lignin-capped silver nanoparticles with high antimicrobial activity against multidrug resistant bacteria

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Background: One of the most important issues in healthcare today is the development of bacterial resistance to antibiotics which has created a generation of bacteria known as multidrug resistant (MDR) bacteria. Due to antibiotics' inability to treat these MDR bacteria metal and metal oxide nanoparticles have been gaining interest as antimicrobial agents. Among those, silver nanoparticles have been used extensively as broad spectrum antimicrobial agents. Here we describe the production and characterization of silver nanoparticles made from the wood biopolymer lignin as a reducing and capping agent with excellent antimicrobial activity against MDR bacteria. We describe and compare the productions of these particles both through a standard heating procedure and through a microwave irradiation procedure.

Methods: The lignin-capped silver nanoparticles were produced using a simple, one-pot synthesis method and characterized by ultraviolet-visual spectroscopy, dynamic light scattering, x-ray diffraction, and transmission electron microscopy. These particles were then tested for antimicrobial activity against clinical isolates of *S. aureus 700, P. aeruginosa, K. pneumoniae, A. baumannii, and E. casseliflavus*. The bacteria were exposed to the particles overnight in 96-well plates at increasing concentrations (1–20 μg/mL), and their minimum inhibitory concentration (MIC) was recorded for each bacterial strain.

Results: Characterization of the lignin-capped silver nano particles shows uniform spherical nanoparticles with a silver core and a lignin coating with a diameter of 62±1.9 nm for the standard heating and 42.03±0.39 nm for the microwave synthesis, but the microwave method was much faster (10 min vs. 3 days). The MIC of the silver nanoparticles was ≤2.5 μg/mL for S. aureus 700 and *P. aeruginosa*, and ≤1 μg/mL for all other tested strains.

Conclusion: Lignin-capped silver nanoparticles can be successfully produced using both standard heating and microwave irradiation, and show very high antimicrobial activity against a wide range of MDR bacterial strains.

Speaker Biography

Jason Asnis received his BEng in Chemical Engineering (2015) from McGill University, Canada. He is currently an MSc student in the Häfeli Lab in the Faculty of Pharmaceutical Sciences, at The University of British Columbia, Canada. In addition to his research position, he is also the Social Director for the Pharmaceutical Sciences Graduate Society.

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The BceABRS four-component system essential for bacitracin sensing and response is required for biofilm formation and virulence of *Streptococcus mutans*

Yung-Hua Li, David Ropson, Anton Dugandzic and **Xiao-Lin Tian**
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Streptococcus mutans is a primary etiological agent of dental caries worldwide. Natural life of *S. mutans* in dental biofilms often faces life-threatening insults, such as killing by antibiotics or innate defense molecules produced by competing species or by the host. How such insults affect physiology and virulence of *S. mutans* is poorly understood. In this study, we explored this question by analyzing the effects of sub-MIC concentrations of bacitracin and ß-defensin 3 on S. mutans. Microarray analysis showed that both bacitracin and ß-defensin 3 induced differential expression of subsets of genes that were largely regulated by the BceABRS four-component system. The results were further confirmed by examining gene expression profiles of selected genes or genetic loci using qRT-PCR. We then examined the effects of gene deletion of *bceRS* on the peptide antibiotics and virulence. The results showed that a deletion of *bceRS* resulted in a mutant that was sensitive to bacitracin or ß-defensin 3. Introduction of a wild copy of *becRS* in trans (complementation) restored the wild type phenotype of the mutant. In particular, both peptide antibiotics at a sub-MIC induced biofilm formation in the parent but not in the mutant. A competitive fitness analysis showed that the mutant was unable to compete with the parent for co-existence in duel-strain mixed cultures in the presence of bacitracin. In conclusion, the BceABRS four-component system controls a regulon that is required for sensing, response and resistance to bacitracin and ß-defensin. This system may play an important role in adaptation and virulence expression of *S. mutans* in dental biofilms.

Speaker Biography

Yung-Hua Li received his Doctorate in Molecular Microbiology at University of Manitoba. Following his Post-doctoral fellowships in the University of British Columbia and University of Rochester, NY, he worked as a Scientist in the University of Toronto, with his research focus on molecular dissection of microbial biofilms. In 2004, he joined the Faculties of Dentistry and Medicine at Dalhousie University, where he has been directing a research team on genetic analyses of bacterial biofilms, biofilm ecology and pathogenesis.

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A new pheromone-guided antimicrobial peptide HP30 for targeted killing of *Streptococcus mutans* in mixed-culture biofilms

Xiao-Lin Tian, Kayla Cyr, Xingxing Huang and **Yung-Hua Li**
Dalhousie University, Canada

Streptococcus mutans is a leading cariogenic pathogen of dental caries worldwide. Clinically, eliminating *S. mutans* from dental biofilms using antibiotics is not practical, because these agents indiscriminately kill other members of the resident microflora, leading to ecological disruption and other negative clinical consequences. To develop target-specific antimicrobials, we evaluated several fusion peptides and identified a new peptide HP30 that showed a high selectivity for targeted killing of *S. mutans*. In the dual-species cultures, 80% of *S. mutans* cells were killed, but only 20% of *S. sanguinis* were killed following exposure to HP30 (5.0 μM) for 15 min. Similarly, 80% of *S. mutans* cells were killed but only 5% of *Actinomyces naeuslundii* were killed following the same exposure. The peptide-guided killing was also confirmed in the dual-species biofilms and the killing increased with increasing concentrations of HP30. However, a combination of low concentrations of HP30 with EDTA well maintained the killing activity against *S. mutans* in the biofilms. A *S. mutans* mutant lacking the ComD receptor only showed 20% of killing, while a ComD overexpression strain showed 90% of killing, suggesting that HP30 predominantly binds to the ComD receptor before triggering the selective killing. New peptide HP30 displays a high selectivity for targeted killing of *S. mutans* due to an improved binding of the peptide to the ComD receptor.

Speaker Biography

Xiao-Lin Tian has received her MD from Shanghai Medical University. Since 1993, she worked as a Research Technician in Novopharm Biotech Inc. in Winnepeg for six years. She then worked in the Mount Sinai Hospital Lunenfeld Research Institute, Toronto, for another six years. Since 2006, she has been working as a Researcher at Dalhousie University, with expertise in Moleculr Biology, Bacterial Biofilms and Pathogenesis.

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PCR amplification of genomic *Mycobacterium leprae* DNA by using different gene targets

Vinay Kumar Pathak, Madhvi Ahuja, Ravindra Turankar, Itu Singh, Mallika Lavania and **Utpal Sengupta**
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Several attempts have been made to establish a diagnostic test for leprosy since decades. None of these assays could diagnose more than 60% of PB cases or early cases of leprosy. The present study was attempted to develop a diagnostic test using *M. leprae* specific PCR in clinical samples. The study was aimed to detect *M. leprae* genomic DNA by using two different gene targets. Standardization for sensitivity of PCR for two genes was performed with standard genomic DNA of *M. leprae* strain NHDP-63. The standard DNA was serially diluted in 1:10 ratio up to 12 dilutions in decreasing concentrations. The DNA concentration of first dilution was $1x10^{-1}$ µg/µl or 100 ng/µl to twelfth dilution $1x10^{-12}$ µg/µl or 1 αg/µl. PCR amplification using two gene targets of *M. leprae* namely repetitive element *rlep* and 16S *rRNA* were performed with the same. PCR amplification for *rlep* gene was positive up to concentration of $1x10^{-9}$ µg/µl or 1 fg/µl, similarly it was $1x10^{-10}$ µg/µl or 100 αg/µl for 16S *rRNA* gene target. The sensitivity has been tested with clinical samples of leprosy patients and positivity of result was found 66.0% in case of *rlep* whereas it was 82.0% for 16S *rRNA* gene. In present study, PCR positivity for *rlep* and 16S *rRNA* gene were found efficient in the clinical samples and these gene targets can be further considered to develop a diagnostic tool for detection of sub clinical leprosy.

Speaker Biography

Vinay Kumar Pathak has completed his MSc Biotechnology from Guru Nanak Dev University. Currently, he is pursuing his PhD at Stanley Browne Laboratory, The Leprosy Mission Community Hospital, Delhi. He is working as Senior Research fellow and has published one paper in a reputed journal.

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In-vitro activities of six antifungal drugs against *Candida glabrata* isolates: An emerging pathogen

Nasrin Amirrajab
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Background: *Candida glabrata* is pathogenic yeast with several unique biological features and associated with an increased incidence rate of candidiasis. It exhibits a great degree of variation in its pathogenicity and antifungal susceptibility.

Objectives: The aim of the present study was to evaluate the in vitro antifungal susceptibilities of the following six antifungal drugs against clinical *C. glabrata* strains: amphotericin B (AmB), ketoconazole (KTZ), fluconazole (FCZ), itraconazole (ITZ), voriconazole (VCZ), and caspofungin (CASP).

Materials & Methods: Forty clinical *C. glabrata* strains were investigated using DNA sequencing. The in vitro antifungal susceptibility was determined as described in clinical laboratory standard institute (CLSI) documents (M27-A3 and M27-S4).

Results: The sequence analysis of the isolate confirmed as *C. glabrata* and deposited on NCBI GenBank under the accession number no. KT763084-KT763123. The geometric mean MICs against all the tested strains were as follows, in increasing order: CASP (0.17 g/mL), VCZ (0.67 g/mL), AmB (1.1 g/mL), ITZ (1.82 g/mL), KTZ (1.85 g/mL), and FCZ (6.7 g/mL). The resistance rates of the isolates to CASP, FCZ, ITZ, VZ, KTZ, and AmB were 5%, 10%, 72.5%, 37.5%, 47.5%, and 27.5%, respectively.

Conclusions: These findings confirm that CASP, compared to the other antifungals, is the potent agent for treating candidiasis caused by *C. glabrata*. However, the clinical efficacy of these novel antifungals remains to be determined.

Speaker Biography

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In vitro anti-leishmanial activity of *Artemisia dracunculus* and *Heracleum persicum* extracts in comparison with glucantime

Batool Sadeghi-Nejad[1], Shahram Khademvatan[2], Alborz Eskandari[3] and **Sedigheh Yusef Naanaie[4]**
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[3]Ahvaz Jundishapur University of Medical Sciences, Iran
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Background & Objectives: Cutaneous leishmaniasis (CL) is one of the most common parasitic diseases. It is one of the major public health concerns in developing countries and throughout the world. Pentavalent antimonial compounds like pentostam and glucantime has been used to treat CL for the last 50 years. The use of these compounds has some limitations such as long duration of treatment, high expenses of drugs, and methods of drug use which are intradermal and intramuscular injection. Beside these, lack of response to the treatment in 10-15% of cases and toxic effects on heart, liver, and kidney are other possible side effects [4-6]. Hence, the objective of the present survey was to state the antileishmanial activity of two herbal medicine (*Artemisia dracunculus* and *Heracleum persicum*) extracts were evaluated against *Leishmania major* and *Leishmania infantum* using colorimetric MTT (2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide) assay and compared to the Glucantime as a reference.

Methods: The leaf extracts of selected plants were obtained by maceration. The in vitro assays were carried out on *Leishmania major* and *Leishmania infantum* using colorimetric MTT assay in comparison with Glucantime. The concentration-response curves tested extracts and glucantime solutions were designed and IC50 values were located.

Results and Conclusions: Anti-leishmania effects of *Artemisia dracunculus* and *Heracleum persicum* on *L. major* and *L. infantum* promastigote were revealed with 50% inhibitory concentration (IC50) values of 50.97 and 49.57 mg ml^{-1} for *Artemisia dracunculus*, 29.3 and 14.7 mg ml-1 for *Heracleum persicum*. In comparison with the standard drug, glucantime had IC50 value of 40.2 mg ml^{-1} for *L.major* and 18.5 mg ml^{-1} for L. infantum promastigote after 72 hours incubation, respectively.

Conclusions: These results revealed that compounds from *Satureja khuzestanica* and *Heracleum persicum* have anti-leishmania properties that necessary to survey the effects of these extracts on leishmania genus in animal models in future.

Speaker Biography

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Intradermal trivalent influenza vaccine with and without imiquimod in hemodialysis patients

Sara Abolghasemi, Shabnam Tehrani and **Zahra Doosti**
Shahid Beheshti University of Medical Sciences, Iran

Background: Considering heavy economic burden of influenza, various efforts have been done for better prevention and different vaccination methods employed for expediting immune response. Up to our knowledge, no study has been conducted in patients undergoing routine hemodialysis, so the goal of this study is to evaluate difference between the immunogenicity caused by two different routes of influenza vaccine injection (i.e. intradermal versus intramuscular), and evaluating whether pretreatment with imiquimod could augment and expedite the immune response.

Method: In this prospective randomized, double blind, controlled trial, 120 patents undergoing routine hemodialysis (i.e. for more than 1 month, at least 2 times a week) entered the study and randomly assigned into 3 groups: one experimental, and two controls. For the experimental group (INT-I), 250 mg imiquimod 5% cream (Aldara) was rubbed on deltoid region of right arm, and after 15 minutes, 0.25 cc of trivalent influenza vaccine was injected intradermal. The individuals in the first control group (INT-A), received 0.25 cc trivalent influenza vaccine via intradermal route after rubbing 250 mg aqueous cream in the same region with the same prior interval. For the second control group (IM-A), 0.5 cc trivalent influenza vaccine was injected intramuscular after using 250 mg aqueous topical cream on the same area. The immunogenicity was then measured by serum antibody titers using hemagglutination-inhibition (HI) assays, against two influenza strains: A (H1N1) and B. For comparing antibody titers two blood samples were obtained: the first immediately before and the second 14-21 days after vaccination. The increase in antibody levels against each strain then analyzed for significance.

Results: Among initial 120 participants, 117 persons completed the study. The antibody titers before and after vaccination were measured by hemagglutination inhibition assay. Both increase in antibody titers and means of the antibody increases in intradermal with imiquimod cream (INT-I), intradermal with placebo (INT-A) and intramuscular group (IM-A) were determined. Then the differences between the mean titers of INT-A and IM groups and between INT-I and INT-A groups were analyzed by covariance method (Acova). This study revealed significant response among strain A (H1N1) in intramuscular group (IM-A) comparing with the intradermal with aquas cream (INT-A) (P, 0.05). The subsequent immunogenicity in other groups and for different strains did not show any significant difference (i.e. INTA and IM-A for B strain and INT-A and INT-I for both A and B strains).

Conclusion: Although some previous studies among elderly and healthy people showed intradermal route of influenza vaccination more efficacious comparing with intramuscular route, and imiquimod pretreatment expediting and augmenting the subsequent immunogenicity comparing with non-imiquimod pretreated people, this study did not reveal the superiority of intradermal injection over intramuscular route, and also the benefit of imiquimod as premedication. Finally, regarding the acceptable immunogenicity among individuals in intradermal groups (both INT-I and INT-A), we conclude that intradermal route would be an alternative for intramuscular injections.

Speaker Biography

Sara Abolghasemi is an Infectious Diseases Specialist and completed her Fellowship of Infectious Diseases in Immunocompromised patients from Shahd Beheshti University of Medical Sciences, Tehran. She has published few papers about infectious diseases in ISI and PubMed journals and is currently working as an Assistant Professor in Shahid Beheshti University of Medical Sciences.

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The reason for the prevalence of non-toxigenic isolates of *Clostridium difficile* in the clinical samples

Mohammad Moradi, Ebrahim Rezazadeh Zarandi, Shahla Mansouri, Nouzar Nakhaee and **Farhad Sarafzadeh**
Kerman University of Medical Sciences, Iran

Introduction: The non-toxin production variant *C. difficile* $A^-/B^-/CDT^-$ are prevalent in clinical samples. But the reason for their high prevalence of these strains in the clinical diarrhea specimens has not yet been performed.

Materials & Methods: Minimum inhibitory concentration bacteria were performed by micro- dilution technique. About ~10^6 bacteria from 18-hour culture were inoculated to pre-reduced media containing ½×MIC of each antibiotic. After 24, 48 and 96 hours, 1/mL of culture was excluded and heated to killing vegetative forms and pre-activated the spores. The 100 of appropriate dilution are cultured on Columbia blood agar in the form of triplicates. After 72 hours the number of spore were counted based on the colony forming unit.

Results: The results showed that non-toxigenic isolates and historically strain of *C. difficile* (ATCC 9689) and the clinically isolates $A^+/B^+/CDT^-$ produced spore in free antibiotic and ½×MIC media. The spore production non-toxigenic isolates in free antibiotic media was like toxigenic (clinically and ATCC 9689 strain). The VAN, CLI and CAZ inhibited spore production in toxigenic as the same as non-toxigenic isolates ($A^-/B^-/CDT^-$) of *C. difficile* in the similar manner.

Discussion: Since non-toxigenic isolates are common in the clinical samples. Our research showed these isolates capable to produce spore in absence and the presence of antibiotic in similar manner to toxigenic strain. In total, they have lost toxin production ability but they kept the power sporulation and survival in the hospitalized patients who receive antibiotics.

Speaker Biography

Mohammad Moradi completed his PhD in Medical Microbiology, University of Manchester. He is currently working as a Assistance Professor of Medical Microbiology in the Department of Medical Microbiology Medical School, Kerman University of Medical Sciences, Kerman, Iran. He has published numerous research papers and articles in reputed journals and has various other achievements in Molecular diagnosis and anti-microbial resistance patterns. He has extended his valuable service towards the scientific community with his extensive research work.

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August 23-24, 2017 | Toronto, Canada

Comparative assessment of probiotic attributes of *Lactobacillus Plantarum* strains of Ireland and Pakistan

Asma Manzoor[1], Johar Ali[4] , Javed Iqbal Qazi[1], Paul Ross[2, 3] and **Catherine Stanton[2, 3]**
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Fourteen *Lactobacillus plantarum* strains isolated from various food sources and two different climatic regions (Ireland and Pakistan) were genetically characterized at subspecies level with *recA* gene based multiplex PCR amplifications and pulsed-field gel electrophoresis. All the strains were tested *in vitro* for functional probiotic properties, which included the production of bacteriocin against the major food borne pathogens (*L. innocua and L. monocytogenes*), acid tolerance, survival in simulated gastric juice, NaCl tolerance, bile salt hydrolase activity, and antibiotic resistance. The genes encoding bacteriocin (plantaracin 423) were identified from *L. plantarum* strains, and enzymes sensitivity assays to protinease K and pepsin were tested. Results of Genomic fingerprinting following ApaI digestion revealed 10 distinctly different strains of PFGE patterns. Antimicrobial screening revealed, *L. plantarum* AS-4, AS-6, AS-8, AS-13 and AS-14 strains as the potential producers of bacteriocin. The culture supernatants of these strains expressed GIZ up to 12, 12, 14, 11 and 13 mm, respectively against *L. innocua* and the 3932 Da molecular mass was determined by using MALDI-TOF mass spectrometry along with control (*L. plantarum* LMGP-26358) The positive control, previously characterized plantaracin producer strain LMGP-26358, also showed GIZ of 12 mm. On the other hand, all the *L. plantarum* strains were active against a broad range of microorganisms including *L. monocytogenes* DPC 6179, *Enterococcus facelis* 5055 (LMG9737), E. coli DPC EC101, *Bacillus subtilis* LMG 8198, *Clostridium perfringens* LMG 10468, *Clostridium difficile* ATCC 42593 and *Staphylococcus aureus* DPC 6867. Molecular characterization of these isolates was performed by amplification of previously known bacteriocin genes. Polymerase chain reaction analyses revealed that plantaracin genes were present in the genome of *L. plantarum* strains AS-4, AS-6, AS-7, AS-13 and AS-14 along with *L. plantarum* LMGP-26358 and for these bacteria almost similar growth pattern of bacteriocin production was observed. The loss of activity of 13 out of 15 strains confirmed that the antimicrobial substance produced by *L. plantarum* strains was indeed proteinaceous. All the strains showed good *in vitro* functional potential and a significant relationship was found between source of isolation and functional score with promising probiotic potential. Some of the desired characteristics were even better than those of probiotic referenced strains. This study confirmed a high heterogeneity in functional properties of the *L. plantarum* strains and provides insight into optimal screening strategies

Speakers Biography

Asma Manzoor has completed her PhD in Industrial Biotechnology from Govt. College University Lahore with international training from Teagasc Moorepark, Dairy Product Research Center, Fermoy Co. Cork Ireland. Her PhD project at Teagasc Moorepark, was in the area of Comparative assessment of Probiotic attributes of *Lactobacillus plantarum* strains of Ireland and Pakistan. She is currently working as Assistant Professor at Institute of Biochemistry and Biotechnology University of the Punjab Lahore.

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Pseudomonas phage inhibition of *Candida albicans*

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Pseudomonas aeruginosa (Pa) and *Candida albicans* (Ca) are major bacterial and fungal pathogens in immunocompromised hosts, and notably in the airways of cystic fibrosis patients. Bacteriophages of Pa physically alter biofilms, and have been recently shown to inhibit biofilms of *Aspergillus fumigatus*. To understand the range of this viral-fungal interaction, we studied Pa phages Pf4 and Pf1, and their interaction with Ca biofilm formation and preformed Ca biofilm. Both forms of Ca biofilm development, as well as planktonic Ca growth, were inhibited by both phages. The inhibition of biofilm was reversed by the addition of iron, suggesting the mechanism of phage action on Ca is denial of iron. Birefringence studies on added phage showed an ordered structure of binding to Ca. Electron microscopic observations indicated phage aggregation in biofilm extracellular matrix. Phage-fungal interactions may be a feature with several pathogens in the fungal kingdom.

Speaker Biography

Hasan Nazik has completed his undergraduate degree at Istanbul University, School of Medicine-Istanbul/Turkey in 2001, and his Microbiology Residency education at the same University in 2005. He is a Visiting Scholar at California Institute for Medical Research/Stanford University for three years. He has published more than 40 papers in scholarly journals and has been continuing the research on bacterial-fungal interactions.

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Prevalence of microorganisms and their antimicrobial susceptibility profile in blood samples from an university hospital from Vitória, Brazil

Moraes Rodrigo, Alexandre Coelho Guimarães, Carla Coradini de Mattos Siqueira, Thays Furtado Dias da Mata, Rodrigo Pratte Santos and **Carolina Frizzera Dias**
Santa Casa de Misericórdia de Vitória, Brazil

Due to the elevated number of nosocomial infections and its relation with intra-hospital morbidity and mortality levels, it is crucial to evaluate the responsible agents in order to improve patients' care. This is an observational, retrospective and transversal study performed at the Hospital da Santa Casa de Misericórdia de Vitória, involving 511 patients infected between July 2014 and June 2016. Data on blood culture samples were collected from the database of the Hospital Infection Control Committee (CCIH). For blood culture, sheep blood agar (SBA), chocolate agar and MacConkey's agar (MAC), after culture in an automated blood culture system were used. Microbial identification and susceptibility profile evaluation were performed using the MicroScan auto SCAN-4 (Beckman Coulter®) automated system. The study describes four bacterias in detail: *Acinetobacter* spp., *Klebsiella pneumoniae, Pseudomonas aeruginosa and S. aureus*. The study found that the least active antimicrobials against S. aureus were nalidixic acid and ampicillin, while the most active were amikacin and cefazolin. Secondly, the least active antimicrobials against *Acinetobacter spp*. were aztreonam and ertapenem, whereas the most active were polymyxin and rifampicin. Furthermore, the least active antimicrobials against *K. pneumoniae* were ampicillin and ampicillin/sulbactam, while the most active were amikacin and cefoxitin. Finally, the least active antimicrobials against *P. aeruginosa* were ceftriaxone and cefotaxime, while the most active were erythromycin and polymyxin. Physicians often have difficulty in establishing the susceptibility profile of the etiologic agent of an infection. Thus, knowledge about antimicrobial resistance from the hospital is fundamental, improving clinical management of patients.

Speaker Biography

Moraes Rodrigo has completed his graduation in Pharmacy from Emescam College (2004), Post-graduated in Microbiology from PUC University (2006) and Master's in Biological Sciences (Microbiology) from Federal University of Minas Gerais (UFMG) (2008). He is currently the Coordinator of the Biomedicine Course at PIO XII Faculty and Professor of Microbiology, Cell Biology and Biochemistry, and Professor of Microbiology at Emescam (School of Sciences of Santa Casa de Misericórida de Vitória, ES). He is also a member of the Research Ethics Committee and of the Medical Course Collegiate from Emescam.

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Trend analysis of bacterial uropathogens and their susceptibility pattern: a four-year (2013-2016) study from Aseer region, Saudi Arabia

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Objective: The objective of the study is to analyze the prevalence and resistance rates of bacterial agents causing urinary tract infections (UTIs) in Aseer, Saudi Arabia (2013-2016).

Methods: This was a four year (2013-2016) retrospective study undertaken in Aseer Central Hospital (ACH), Saudi Arabia. A total of 49,779 urine and other UT specimens obtained from patients suspected of having a UTI were analyzed. Urine specimens were inoculated onto CLED agar following standard procedures. Cultures showing significant bacteriuria were subjected to identification and sensitivity testing using VITEK 2 system. Data of patients and uropathogens were assembled, checked and analyzed using SPSS software.

Results: Culture positive samples were 49,779 (59.9% males, 40.1% females; p=0.000). Year trend showed significant variations (p=0.000) and the forecast trend line hypothesized a clear rise. Age groups 70 to 79 years old were the most vulnerable group (22.3%). Gram negative bacilli were 91.8% and the major species were *Escherichia coli* (39.7%), *Klebsiella pneumoniae 15.8%, Pseudomonas aeruginosa* 13.8%, *Proteus mirabilis* 10.6% and *Acinetobacter baumannii* 5%. Antimicrobials with high sensitivity rate were linezolid (99.1%), daptomycin (89.3%), vancomycin (86.7%), teicoplanin (85.5%), ertapenem (85.1%), fosfomycin (82.1%) and tigecycline (80.2%). High resistant rates to uropathogens were encountered with cephalothin (89.8%), nalidixic acid (86.7%) and ampicillin (81.9%).

Conclusions: The majority of uropathogens were resistant to antibiotics commonly used in clinical practice. Linezolid, daptomycin and vancomycin showed the lowest resistance to all uropathogens; this can be revised for empirical treatment of UTIs. Continuous surveillance of uropathogens and their susceptibility is important.

Speaker Biography

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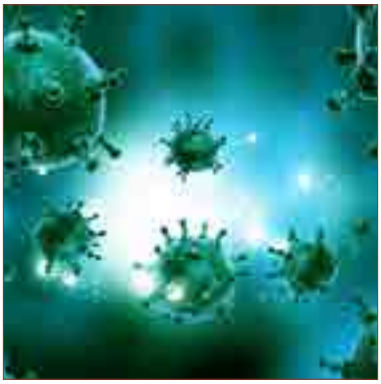

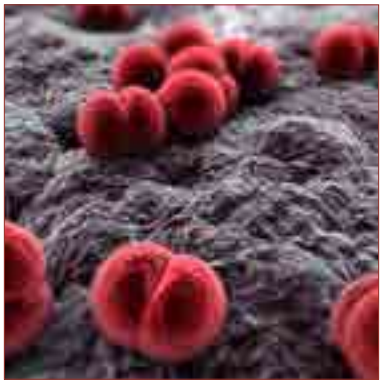

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Characterization and optimization of bacteriocin from *Lactobacillus plantarum* isolated from fermented beef

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Many lactic acid bacteria (LAB) were isolated from 'Shermout', a popular Sudanese fermented beef product intended for long storage. An isolate that demonstrated significant antibacterial activity was identified *as Lactobacillus plantarum* PM4 based on phenotypic, physiological and biochemical characteristics and carbohydrate utilization patterns. The inhibitory activity of the partially purified bacteriocin was completely arrested by the proteolytic enzymes proteinase-k and pepsin but not by α-amylase, asserting its proteinaceous nature. The activity was not due to H2O2 as similar inhibition was obtained by cell-free supernatant (CFS) produced under anaerobic conditions. The bacteriocin showed a molecular weight in the range of 3-5 kDa and had a bactericidal mode of action. No significant reduction in activity was observed on heating at 60°C for 60 min, but activity was lost on heating at 100°C or autoclaving. Highest inhibitory activity was at pH 5.5 and there was appreciable reduction in activity at pH 3, 7 or 9. There was no drop-in activity at -80 or -20°C up to four weeks of storage. However, at 4 and 35°C a gradual decline in activity was observed. *L. plantarum* PM4 exhibited bactericidal activity against *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Escherichia coli* ATCC25922, *Klebsiella pneumoniae and Proteus vulgaris*. Bacteriocin production generally coincided with the phase of maximum growth and the best combination for maximum production of inhibitory activity was at pH 5.5 for 48 hours, further incubation at 25, 30 or 37°C. *L. plantarum* PM4 showed promise as a starter culture in the fermentation of preserved meat products.

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Molecular characterization of glucose-6-phosphate dehydrogenase deficiency specific variants in Amhara region, Ethiopia

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Background: Glucose 6-phosphate dehydrogenase deficiency (G6PDd) is an X-linked hereditary genetic defect, affecting an estimated 400 million people worldwide. Severe clinical manifestations associated with G6PDd (e.g., chronic hemolytic anemia) depends on the type of G6PD molecular variants and exposure to hemolytic triggers (e.g., antimalarial like Primaquine). However, a scarce study on G6PDd renders the use of Primaquine for effective therapeutic treatment of malaria.

Objectives: To determine the availability and characterization of selected molecular variants of G6PDd specific genes among selected populations in malaria endemic area of Amhara region, Ethiopia.

Methods: Using a cross sectional study design, a total of 156 dried blood samples were randomly selected from 360 stored samples of national malaria indicator survey of 2011 starting from July 30/2014 to January 30/2015. Polymerase chain reaction and restricted fragment length polymorphism technique was applied to characterize G6PDd variants as G6PD*A, G6PD*A- and/or G6PD*Mediterranean. Binary logistic regression was applied to see association (P<0.05 is significant) among different parameters.

Result: Of 156 studied dried blood spot samples, 10 (6.4%) had G6PD genotype available. G6PD*A (100%) was the only genotype characterized, while neither G6PD*A- nor G6PD*Mediterranean genotypes were detected. There was no statistical significant difference between G6PDd and other socio demographic and risk related variables (P>0.05).

Conclusion: G6PD*A variant was the only G6PDd genotype detected in this study. G6PD*A variant has almost (90%) the same enzymatic activities with the wild type. Therefore, this result supports the safe use of primaquine, especially the single low dose for transmission interruption of Plasmodium falciparum gametocyte and radical cure of *Plasmodium vivax*, as a part of malaria elimination toolkit, among selected populations in malaria endemic areas of Amhara region.

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Microbial pathogenesis, drug development and patent related court cases

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While some bacteria, such as *Helicobacter pylori* and *Agrobacterium tumefaciens*, are known to cause cancer in human beings and plants, it has also been known for over hundred years that when certain bacteria infect human beings with cancer, the cancer regresses, often totally. It is also well-known that many pathogenic bacteria with long term residence in the human body as biofilms consider the human body as their habitat and try to protect it from outside invaders such as cancers, viruses and parasites through secretion of protein weapons. In one instance, *Pseudomonas aeruginosa*, an opportunistic pathogen, secretes a protein azurin on contact with cancer cells. Upon release, azurin enters preferentially to cancer cells and interferes in cancer cell growth through multiple mechanisms involving complex formation with various cellular proteins in cancer cells that promote cancer cell growth. Such complex formation then leads to loss of function of such cancer growth promoting proteins. Thus azurin is known to induce apoptosis in cancer cells, as well as interfere in rapid cancer cell growth, through stabilization of tumor suppressor protein p53. Azurin also forms complexes with vascular endothelial growth factor receptor (VEGFR) and cell surface associated receptor tyrosine kinases such as EphB2 to inhibit angiogenesis and cell signaling in cancer cells to inhibit their growth. A chemically-synthesized 28 amino acid fragment (Azurin 50-77), termed p28, has completed a phase I trial in 15 stage IV cancer patients with metastatic tumors that were resistant to all conventional drugs and these patients had a life expectancy of about 6 months. P28 not only showed very little toxicity but also significant beneficial effects including partial and complete regression of the tumors in four patients, significantly prolonging their lives. P28 has also shown similar lack of toxicity but good efficacy in several pediatric brain tumor patients. The University of Illinois at Chicago holds many patents on azurin/p28 as anticancer and anti-infective agents and the patent eligibility issues on such products of nature will be discussed.

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Cross regulation of intracellular metabolism and virulence in *Listeria monocytogenes*

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Intracellular bacterial pathogens are metabolically adapted to grow within mammalian cells. While these adaptations are fundamental to the ability to cause disease, we know little about the relationship between the pathogen's metabolism and virulence. My lab focuses on studying the bacterial pathogen *Listeria monocytogenes*. We investigate the intracellular metabolism of this bacterium during infection and how it senses host derived metabolites as localization signals. We recently discovered that *L. monocytogenes* responds to low availability of BCAAs within mammalian cells by triggering virulence gene expression. This response is dependent on the nutrient global regulator CodY, which directly activates the major virulence regulator, PRfA. Furthermore, we reported that L-glutamine, an abundant nitrogen source in host serum and cells, also serves as an environmental indicator and inducer of virulence gene expression. Rapid intercellular uptake of L-glutamine is the signal as listerial intracellular concentration of L-glutamine had to cross a certain threshold to activate virulence gene expression, acting as an on/off switch. The mechanisms behinds these metabolic signals were identified, revealing how intracellular pathogens gouge for host derived metabolic cues and use them to cross-regulate metabolism and virulence.

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Impact of the renutrition milk on the clinical profile and the intestinal microbiota of malnourished children

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Child malnutrition represents an insidious plague that causes the death of 3.1 million children aged less than 5 years in the world every year. Recently, several studies have looked at the effect of intestinal flora on weight regulation. Our own objectives are to characterize the clinical profile and the composition of the intestinal flora of malnourished children and the healthy ones who are residents in the city of Mascara (Algeria). And the other one is to specify the impact of the administration of the renutrition milk on intestinal microbiota composition of malnourished children. In total, 40 children of both genders aged between 2 months and 36 months were selected for this study. A clinical examination with Pediatricians and microbiological analysis of the fecal matter was carried out. The first results revealed that malnourished children included in the study suffer from severe malnutrition characterized by stunted growth and remarkable underweight and their intestinal flora is quantitatively and qualitatively different from that of healthy ones on one hand and on the other hand the administration of the milk of renutrition has no significant influence on the composition of the intestinal flora in these malnourished children.

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Prevalence and evaluation of drug-resistant urinary tract infections caused by *Enterococcus* and biotypes in a multi-center study in Tehran

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This study was performed to determine the frequency and drug resistance and biotypes of *Enterococcus*-related urinary tract infections in a multi-center study in Tehran. In this observational cross-sectional descriptive study, 39991 consecutive patients suspected to have UTI attending to hospitals were enrolled and were evaluated for frequency, drug resistance and biotypes of *Enterococcus*-related urinary tract infections. In this study it was seen that 2589 subjects (6.5%) had established UTI among them 87 subjects (3.4%) had *Enterococcus*-related urinary tract infection. Among them 70 cases were evaluated for biotype and drug resistance showing all cases were *faecalis* biotype. The Nitrofurantoin and Gentamicin showing 1.6% and 85.9% were those with least and most drug resistance, respectively. According to the results obtained in this study, it may be concluded that *Enterococcus* is responsible for three percent of urinary tract infections with dominant biotype of *faecalis*. The most sensitivity and resistance were related to Nitrofurantion and Gentamicin, respectively.

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Molecular characterization of Carbapenem resistant *Enterobacteriaceae* from intensive care units of a tertiary care hospital of Islamabad

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Enterobacteriaceae are Gram-negative rods causing serious infections in intensive care units (ICUs) of hospitals. These organisms are showing resistance to several classes of antimicrobials and resistance genes are spreading by acquired plasmids in bacterial population. Resistance to carbapenem group of antimicrobials is an emerging problem for clinicians and surgeons. Isolation, identification and molecular characterization of carbapenem resistant *Enterobacteriaceae* (CRE) from patients admitted in intensive care units of tertiary care hospital. This research is a prospective, non-randomized, descriptive study. In 9 months, 83 isolates of CRE from ICU samples were processed in Department of Pathology, SZABMU, PIMS Pakistan. Out of 83 CRE samples, 26.5% were from urine, 26.5% were from endotracheal tube tip, 14% were from blood, 13% were from pus, 13% were from tracheal secretions, 4% were from fluids and 3% were from catheter tip. 75% were *Klebsiella pnemoniae*, 17% were E. coli, 2.25% were *Klebsiella* specie, 2.25% were Enterobacter agglomerans, 2.25% were *Enterobacter cloacae* and 1.25% was *Klebsiella oxytoca*. CRE are 100% resistant to imipenem, meropenem and ertapenem. Tigecycline is the only parental drug which is found effective against CRE isolates. 14.5% of CRE isolates was sensitive to amikacin. MIC of imipenem showed 100% resistance for CRE isolates. NDM gene was present in 28 (56%) samples. VIM gene, KPC gene, IMP gene were not detected. NDM positive isolates were 48% *Klebsiella pneumoniae*.

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Antibiotic practice for pneumonia among under-five children in inpatient department at a private pediatric teaching hospital in Dhaka city, Bangladesh

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Background: Pneumonia is the leading cause of morbidity and mortality among under-five children for more than three decades, particularly in low income countries like Bangladesh. World Health Organization (WHO) developed pneumonia case management strategy which included use of antibiotics for both primary and hospital based care. This study aims to describe antibiotic practice for treating pneumonia among children in a private pediatric teaching hospital in Dhaka city, Bangladesh.

Methodology: We conducted this cross-sectional study during November, 2012 in a private pediatric hospital in Dhaka city and the study participants were <5 children admitted with pneumonia.

Findings: We enrolled 80 children during the study period. Among them 28 (35.4%) were underweight, 14 (17.7%) moderately underweight and 13 (16.5%) severely underweight. Based on WHO classification (2005), 43 (54%) had severe and 37 (46%) had very severe pneumonia, diagnosed by research physician. Among the prescribed antibiotics in the hospital, parenteral ceftriaxone was the most common 40 (50%) followed by cefotaxime plus Amikacin 14 (17.5%), cefuroxime 7 (8.8%), ceftazidime plus amikacin 6 (7.5%), ceftriaxone plus amikacin 3 (3.8%), meropenem 2 (2.5%), cefepime 2 (2.5%) and cefotaxime 2 (2.5%).

Conclusion: Despite WHO pneumonia treatment strategy, use of higher generation cephalosporin and carbapenem was high in the study hospital. The results underscore the non adherent use of antibiotic to WHO guidelines the importance of antibiotic surveillance and enforced regulatory policy implication for the rational use of antibiotics in treating hospitalized children with pneumonia.

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Over prescribing- not all the doctor's fault: The impact of public understanding

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It has been broadly reported that antibiotics are over-prescribed and it is likely contributing to increasing bacterial resistance. Over 80% of all antibiotic prescriptions are written empirically i.e., without knowledge of the causative pathogen or its susceptibility pattern. There have been various national programs implemented to reduce antibiotic prescribing which are based either on a penalty or reward system. However, despite these efforts there is often an underlying and almost primal influence in this process, the patient. It is clear from several studies that the public do not fully understand bacterial resistance, how it develops and what may be done to possibly change it. The World Health Organization and others have implemented surveys to understand better how patients think and what their attitudes are towards the problem. In this presentation, a summary of the various recent findings will be presented alongside some alternative approaches to raising public perception and understanding of the global crisis.

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Effects of *Bifidobacterium* breve feeding strategy and delivery modes on experimental allergic rhinitis mice

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Background: Different delivery modes may affect the susceptibility to allergic diseases. It is still unknown whether early intervention with probiotics would counteract this effect.

Objectives: The effect of different delivery modes on immune status and nasal symptoms was investigated on established allergic rhinitis (AR) mouse model. In addition, the immunoregulatory effects and mechanisms of different feeding manners with *Bifidobacterium breve (B. breve)* were examined.

Methods: Live lyophilized *B. breve* was orally administered to BALB/c mice born via vaginal delivery(VD) or cesarean delivery (CD) for 8 consecutive weeks, after which they were sensitized by ovalbumin (OVA) to establish experimental AR. Nasal symptoms, serum immunoglobulins, cytokines, splenic percentages of CD4+ CD25+ Foxp3+ regulatory T(Treg) cells and nasal eosinophil infiltration were evaluated.

Results: Compared with VD mice, mice delivered via CD demonstrated more serious nasal symptoms, higher concentrations of OVA-specific immunoglobulin (Ig) E, more nasal eosinophils and lower percentages of splenic CD4+ CD25+ Foxp3+ Treg cells after establishing experimental AR. These parameters were reversed by administering *B. breve* shortly after birth. However, the effect of *B. breve* did not differ between different delivery modes.

Conclusion: CD aggravates the nasal symptoms of AR mice compared to VD. This is the first report that oral administration of *B. breve* shortly after birth can significantly alleviate the symptoms of AR mice born via both deliveries, probably via activation of the regulatory capacity of CD4+ CD25+ Foxp3+ Treg cells.

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Carbon dots: A new class of light-activated antimicrobial agents

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Infectious diseases caused by bacterial pathogens have been a serious threat to public health for decades and remain one of the major concerns of our society. Control and prevention of pathogen contamination are effective ways to reduce the risk of such disease. Photo-activated antimicrobial technology is a rapidly developing field in response to the demand in development of effective treatments, control and prevention of bacterial infectious diseases. While colloidal TiO2 has been the traditional photo-activated antimicrobial agent for years, novel materials are discovered and added to this field rapidly. Recently, carbon dots have been demonstrated for their great potential in serving as effective light-activated antimicrobial agents. Carbon dots (CDots) are generally small carbon nanoparticles with various surface passivation schemes, with their unique optical properties and photocatalytic functions. This study reported CDot's photoinduced bactericidal functions, with the results suggesting that the dots were highly effective in bacteria-killing with visible light illumination. Several important factors that are associated with the light-activated bactericidal efficiency, including surface modification, fluorescence quantum yield and others have been investigated. Mechanistic implications of the results will be discussed. Challenges and opportunities in further development of CDots into a new class of effective, low cost, low to non-toxicity visible/natural light-responsive bactericidal agents for bacteria control and other potential antimicrobial applications will be discussed.

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Antibiotics and antibiotic resistance genes in landfill

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Antibiotics and antibiotic resistance genes (ARGs) are big challenges for human beings. Use and disposal of antibiotics results in the release of large amounts of antibiotics into environment via different ways, including discharge from municipal sewer systems, application of biosolids onto agricultural fields and aquaculture etc. Landfills receive unused and unwanted antibiotics and ARGs in municipal solid waste (MSW) and are supported to be important reservoirs of antibiotics and ARGs. However, the extent of antibiotic and ARGs present in landfills has not been fully characterized. Here, we investigated the distribution and dynamics of antibiotics and ARGs in several landfills in China. Our results showed that (1) antibiotics and ARGs are widely distributed in refuse and landfill leachate and (2) physiochemical parameters of refuse and leachate impact the distribution of antibiotics and ARGs in landfill.

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The role of *Staphylococcus aureus*, related bacteria and their virulence factors in pathogenesis of multi organ infections: Future molecular targets in prevention and treatment, in addition to already available antibacterial drugs

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Staphylococcus aureus and related bacteria such as *Streptococci* cause Millions of fatal human infections annually around the globe with sequelae of complications and of which some are very difficult to treat with cheap and widely available antibacterial drugs especially in Uganda, East Africa. There is also an increasing incidence of bacterial resistance against various categories of antibacterial drugs causing chronicity of some infections. The following are the examples of virulence factors; lipases, collagenases, super antigens, exfoliatin A and B, haemolytic toxins e.g. beta toxin which is a sphingomyelinase and protein A. The major will be in; Osteomyelitis, toxic shock syndrome, sepsis and necrotizing fasciitis. The purpose of this study is to analyze these virulence factors in detail which will subsequently lead to the discovery (innovation) of polyvalent antitoxins (e.g. in vaccine form or a gamma globulins) that will be used to prevent such fatal infections in groups at high risk. The study will also reduce future antibiotic abuse and eventually also reduce antibiotic resistance.

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The impact of large changes to human populations on the presence of ESBL-producing *Enterobacteriaceae* in a wastewater treatment plant

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Introduction: Wastewater treatment plants (WWTP) could be a crucial point in the spread of Extended Spectrum Beta Lactamase (ESBL)-producing *Enterobacteriaceae* in the environment. *bla*CTX-M groups (1, 2, 8/25, 9), *bla*SHV, *bla*TEM and *bla*OXA enzymes have rapidly become the most important ESBL, with increase in many countries during the last decade. Large changes to human populations due to different social and climatic events could exacerbate this issue.

Objectives: Bangor is a small city, with a very high proportion of its population being students, many of whom are international. The aim of this work was to compare the presence of ESBL-producing faecal coliforms bacteria in Bangor's WWTP before and after the week of students' arrival to the city ("welcome week").

Methods: Over a five-week period (two weeks before students' arrival and three weeks after), water samples were collected twice a week, from the influent, primary sedimentation tank, aeration tank and treated sewage throughout the WWTP that serves Bangor city. Counts of E. coli and other faecal coliforms (OFCs) were performed on selective (primary UTI) agar and then isolates were confirmed by biochemical and PCR tests. ESBL producers were screened by combination disc method and identified genes by multiplex PCR.

Results: The mean counts of E. coli and OFCs before welcome week were higher than after. This is probably due to dilution factor by heavy rainfall that occurred subsequent to student arrival. Treatment by the WWTP eliminated 98.8-99.2% of total E. coli and OFCs, respectively. Of the E. coli isolated, a greater proportion was ESBL-producing before welcome week (4.3%) than after (2.0%). However, there was effectively no difference in the proportion of ESBL-producing OFCs recovered before or after welcome week (10.3% and 10.9%, respectively). In the E. coli, genotyping found that *bla*CTX-M group 1 was the most common enzyme-producing gene in both periods. However, the most frequently detected ESBL gene among OFCs was *bla*TEM and then *bla*SHV before welcome week, while *bla*SHV and then *bla*TEM predominated thereafter.

Conclusions: Treatment of wastewater significantly reduced counts of faecal indicator bacteria from the influent to effluent stages. In this study, large population changes were not found to affect the presence of ESBL at WWTP.

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H3N2 influenza vaccine rates and other protective behaviors amongst college students

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Influenza infections can result in seasonal outbreaks and epidemics in the USA. The 2014-2015 influenza outbreak was attributed to the H3N2 influenza A strain. This outbreak was partly attributed to the mismatch between the causative H3N2 influenza A strain and the annual influenza vaccine. The aim of this study was to determine if the mismatch between the causative influenza strain and the vaccine impacted vaccine rates or other protective health behaviors amongst college students. In this study, an online survey was used to determine the influenza vaccination rates and any changes in student hygienic behaviors during the 2014-2015 influenza season amongst college students. Survey responses were collected from Jan 15, 2015 to Feb 15, 2015 and elicited 265 responses from undergraduate students. The total vaccine rate among respondents was 23%, but compared to the previous year (2013-2014) the overall vaccination rate among respondents decreased by 10%. Regardless of vaccination, 53% of total respondents reported a slight change or more in the protective health behavior of hand-washing. The influenza vaccination rate amongst college students is within the range of the national CDC vaccination rate of 31% for this age group. The decrease in vaccination rates from 2013-2014 to 2014-2015 was consistent with the mismatch between the influenza strain and vaccine targets. Beyond vaccination, protection against influenza also involves enhanced personal and hand-hygiene behaviors. Such behaviors are very important in a college campus due to close living conditions and other social and casual behaviors.

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Precision medicine for infectious diseases at the MIF locus

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Human genetic studies together with data from mouse models indicate that variations in the expression of Macrophage Migration Inhibitory Factor (MIF) affect the severity of different infections. MIF is expressed by the innate response to promote pathways necessary for pathogen clearance. Functional polymorphisms in the MIF gene (MIF) occur commonly, with the lowest expression promoter variants present in 45-78% of studied populations. In community-acquired pneumonia, high genotypic MIF expressers show a 50% increased survival benefit when compared to low genotypic MIF expressers. There is population stratification at the MIF locus and evidence for allelic selection in regions endemic for malaria, where low genotypic MIF expression appears to protect from the lethal inflammatory sequelae of infection. Evidence of MIF's role in protection from Mycobacterium infection has prompted examination of the potential contribution of MIF alleles to the high prevalence of TB in Africa. In an HIV+ cohort, genetic low expressers of MIF were 2.4 times more frequently identified among patients with Mycobacterium bacteremia than those without. A higher prevalence of low expression alleles among TB cases than controls without active TB also was observed. As South Africans show the highest global prevalence of low expression MIF alleles, this finding suggests a contribution of functional MIF polymorphisms to the high prevalence of TB in this population. Insights into the structure-function relationship between MIF and its receptor have enabled the design of first-in-class small molecule MIF agonists that enhance MIF binding and signal transduction. One MIF agonist (MIF20) shows beneficial action in mouse models of Mycobacterium and S. pneumoniae infection. Pharmacologic augmentation of MIF, which is in pre-clinical development, may be a useful strategy in low genotypic MIF expressers. Such an approach may be especially beneficial as adjunctive therapy in resistant or difficult to treat infections as in MDR and XDR TB.

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Antibiotic resistance in lactic acid bacteria isolated from human dental plaque

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Dental caries is a significant public health problem which results in destruction of the calcified tissues of tooth. *Lactobacillus* has been reported to occur in high numbers in dental caries. The aim of this study was to screen for the incidence of *Lactobacillus* from cases of dental caries and to determine the antibiotic resistance profile of them. Specimens from dental plaque were collected from 30 patients. Phenotypic and molecular methods were used for identification of *Lactobacillus*. The antimicrobial sensitivity test was performed to determine their resistance to 7 antibiotics. Then molecular detection of antibiotic resistance genes was carried out using Multiplex PCR method. Out of 30 dental plaque samples, *Lactobacillus* was isolated from 14 (46/6%) of them. *mecA* gene was the most important determinant responsible for penicillin resistance. Also bla TEM gene had greater role in Cefazolin and Cefixime resistance than *bla SHV. bla SHV* and *bla OXA-1* genes had the same part in Amoxicillin-Clavulanic acid resistance. Also tetracycline resistance caused by both *tetK* and *tetM* genes identically. In conclusion *Lactobacillus* have important role in the formation of dental plaque which usually shows multidrug resistant patterns to commonly used antibiotics.

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Perceptions and practices of community pharmacists towards antimicrobial stewardship

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The objective of this study was to assess the perception and self-reported practices of community pharmacists towards antimicrobial stewardship. A cross-sectional study was conducted among community pharmacists between March-April, 2015, using a self-administered, pre-tested questionnaire in the State of Selangor, Malaysia. A simple random sampling approach was used to select pharmacy sites. Descriptive and inferential statistical methods were used to analyse the data. A total of 188 pharmacists responded to the survey, giving a response rate of 83.5%. The majority of participants (n=182, 96.8%) believed that antimicrobial stewardship program helps healthcare professionals to improve the quality of patient care. However, more than half of pharmacists were neutral in their opinion about the incorporation of antimicrobial stewardship programs in community pharmacies (n=102, 54.2%). Though collaboration was often done by pharmacists with other health professionals over the use of antibiotics (n=104, 55.3%), a significant proportion of participants (n=102, 54.2%) rarely/occasionally participate in antimicrobial awareness campaigns. Pharmacists having postgraduate qualification were more likely to held positive perceptions of, and were engaged in, antimicrobial stewardship than their non-postgraduate counterpart ($p<0.05$). Similarly, more experienced pharmacists (>10 years) held positive perceptions towards antimicrobial stewardship ($p<0.05$). The study highlighted some gaps in the perception and practices of community pharmacist towards antimicrobial stewardship. Development of customized interventions would be critical to bridging these gaps and improve their perception and practices towards antimicrobial stewardship.

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Bactericidal auxotrophy as new drug target space to eliminate persistent human pathogen *Mycobacterium tuberculosis*

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Tuberculosis is a dreadful disease caused by a successful human pathogen *Mycobacterium tuberculosis* (*M. tb*). Strict compliance to long chemotherapy, lack of diagnostics and vaccines has led to emergence of MDR (multi drug-resistant) and XDR (extensively drug-resistant) strains. Therefore, better understanding of metabolic relationship and interactions among host and pathogen are of fundamental importance for better design of effective vaccine and drug therapies. Availability of essential nutrients, cofactors, and metabolites are of prime importance for successful survival and proliferation of any pathogen. Many pathogens like *Legionella, Coxiella, Francisella, Salmonella* and Listeria have acquired the ways to sustain them by acquiring nutrients, cofactors and essential metabolites from the host. However *M. tuberculosis* lives autonomic life style and is equipped with its own biosynthetic pathways for most of the nutrients, which along with being boon also makes *M. tb* more vulnerable. Most of the auxotrophs of *M. tb* are not proliferate but they survive and persist. Here, using genetic approach, we have discovered novel bactericidal auxotrophies, which rapidly kill *M. tb* in vitro as well as in vivo without the appearance of any suppressor mutants. *M. tb* auxotrophs in these pathways got rapidly killed in immunocompetent, immunodeficient mouse models and in macrophages, despite recruitment of macrophage amino acid transporter on phagosomes. Time course metabolomic and transcriptomic studies on starved cells showed multifactorial mechanism of death involving perturbances in envelope integrity and redox imbalance leading to oxidative stress followed by rapid cell death. Furthermost excitingly, screening fragment library we have obtained fragment inhibitors which cause allosteric inhibition of enzymes in the pathway. Thus, our findings identify a novel attractive target for antimycobacterial therapy and may be for other pathogens also.

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Direct evidence of viral infection and mitochondrial alterations in the brain of fetuses at high risk for schizophrenia

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There is increasing evidences that favor the prenatal beginning of schizophrenia. These evidences point toward intra-uterine environmental factors that act specifically during the second pregnancy trimester producing a direct damage of the brain of the fetus. The current available technology doesn't allow observing what is happening at cellular level?, since the human brain is not exposed to a direct analysis in that stage of the life in subjects at high risk of developing schizophrenia. In 1977, we began a direct electron microscopic research of the brain of fetuses at high risk from schizophrenic mothers in order to finding differences at cellular level in relation to controls. In these studies we have observed within the nuclei of neurons the presence of complete and incomplete viral particles that reacted in positive form with antibodies to herpes simplex hominis type I [HSV1] virus, and mitochondria alterations. The importance of these findings can have practical applications in the prevention of the illness keeping in mind its direct relation to the Aetiology and Physiopathology of schizophrenia. A study of amniotic fluid cells in women at risk of having a schizophrenic offspring is considered. Of being observed the same alterations that those observed previously in the cells of the brain of the studied foetuses, it would intend to these women in risk of having a schizophrenia descendant, previous information of the results, the voluntary medical interruption of the pregnancy or an early anti HSV1 viral treatment as preventive measure of the later development of the illness.

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The preventive effect of date palm (*Phoenix dactylifera*) seed and fruit hydroalcoholic extracts on Carrageenan-induced inflammation in male rat's hind paw

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Background & Objective: The side effects of NSAIDS drugs have caused increasing interest of scientists in herbal medicines as alternative treatment. In this study, the anti-inflammatory effect of seed and fruit of date palm hydroalcolic extracts, due to having antioxidants, was studied.

Materials & Methods: In this study, the extracts of date palm seed and fruit were prepared by maceration method in 70% alcohol. Eighty (80) male rats Wistar, divided into 10 groups of eight (8) in each, 4 groups received different doses (100, 200, 400 and 600 mg/kg) of seed extract and 4 other groups different doses (100, 200, 400 and 600 mg/kg) of fruits extract of the palm and the positive control aspirin (300 mg/kg) and the negative control group saline (5 ml/kg) via injection intraperitoneally. Half an hour later all animals received 100 µl of 1% carrageenan into the rats' hind paw subcutaneous. The changes in rats paw edema was measured by plethysmometer every hour for five hours.

Results: The effect of all of the doses of date palm seed extract on edema were less than Aspirin ($P<0.05$). But there was no significant difference between the group that received 400 and 600 mg/kg date palm fruit extract when compared with aspirin group. The dose 400 mg/kg of fruit extract showed the most anti-inflammatory effect and it was assigned as the best dose.

Conclusion: It is likely that with further studies on different model of animals and also on human model the palm fruit extract could be used for pain treatment.

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The tick gut microbiome - critical gatekeepeers

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B. burgdorferi colonization and transmission involve interactions between the tick gut and the spirochete and are orchestrated spatially and temporally by molecular changes in the spirochete and the tick gut. While several studies have defined global changes in *B. burgdorferi* genes during spirochete colonization of the tick and transmission to the host, little is known of *I. scapularis* gut genes. The realization that the tick gut is also co-habited by diverse indigenous microbiota brings a new correlate to tick-spirochete interactions in the context of colonization and transmission. We show that PIXR, a secreted tick gut protein, inhibits bacterial biofilm formation and maintains tick gut bacterial homeostasis. The tick gut bacterial composition shapes the metabolite milieu of the gut, as seen by changes in the gut metabolome upon PIXR abrogation. The gut metabolome may influence the spirochete entering the tick gut by providing: (i) molecular cues that present the spatial context critical for *B. burgdorferi* to prepare for colonization and (ii) a nutrient milieu essential for spirochete survival. This study underscores the functional significance of the three-way interactions between the tick, its microbiome and the spirochete and offers a new insight into how the tick vector modulates *B. burgdorferi* colonization.

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Phytochemical screening of the exudate of *Aloe otallensis* and its effect on *Leishmania donovani* parasite

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Objectives: The objectives of the study is to evaluate antileishmanial activity of methanolic extract of *Aloe otallensis* (*A. otallensis*) on the promastigote stage of *Leishmania donovani* (*L. donovani*) as compared to standard drugs and to screen its phytochemical constituents.

Methods: Phytochemical screening was done by using the method mentioned by Evans and Trease on methanolic extract of the exudates of *Aloe otallensis* leaves. The extract was also evaluated for in vitro antileishmanial activity against L. donavani which is found from the Parasitology Unit of Black Lion Hospital. The result was compared to standard drugs of sodium stibogluconate, milfostin and paramomycin.

Results: The extract has a good antileishmanial activity with an IC50 of 0.123 0 µg/mL on *L. donovani* (AM 563). The experimental data showed that relatively it had better activity than paramomycin and milfostin but less activity than sodium stibogluconate. The data analyses were done by GraphPad Prism version 5 software after it was read by ELISA reader at the wave length of 650 nm. The phytochemical screening of the exudates of *A. otallensis* showed the presence of phenol, alkaloid and saponin.

Conclusions: The methanol extract of the exudates of A.otallensis has a good anti- leishmaniasis activity and this may be attributed to phenol, alkaloid and saponin present in the plant. But it needs further analysis for the conformation of which constituent presents in high concentration to know which one has the strongest effect.

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Effect of antimicrobial peptides against methicillin resistant *Staphylococcus aureus* isolates

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Staphylococcus aureus is a major pathogen responsible for both nosocomial and community-acquired infections. The severity of *S. aureus* associated infections ranges from benign localized skin abscesses to life-threatening diseases, such as arthritis, osteomyelitis, and endocarditis. *S. aureus* can adapt rapidly to the selective pressure of antibiotics, and this has resulted in the emergence and spread of methicillin-resistant *S. aureus* (MRSA). The extensive use and misuse of antibiotics have created the antibiotic resistance problem. Multi-drug resistant MRSA may enzymatically degrade the antibiotic, alter the antibiotic target site, or pump out the incoming antibiotic from the cell. This leap in the antibiotic resistance impairs the successful treatment of pathogenic infections. This progression leads to the fatal outcome and affects the economic burden of the country. Formation of biofilms by MRSA is another significant issue to control. It is estimated that biofilms account for up to 80% of microbial infections in the body. Biofilms also underlie importunate infections of implanted medical devices. Within a biofilm, bacteria display differential gene expression and are upward of 1000-times more resistant to conventional antibiotic treatment. Bacteria embedded in biofilms are often difficult to eradicate with standard antibiotic regimens and inherently resistant to host immune responses. As a result, treatment of many chronic *S. aureus* biofilm related infections, including endocarditis, osteomyelitis and indwelling medical device infections is hindered. Therefore a novel solution must be approached to curb this growing trend of drug resistance and formation of biofilms in MRSA. Antimicrobial peptides (AMPs) a growing class of natural and synthetic peptides, also presents a broad-spectrum activity. AMPs are small molecules and play an important role in innate immune system and are effective against multi-drug resistant organism due to unique mode of action. Hence, AMPs would be attractive targets against potential biofilm forming MRSA.

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