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Insights into genetic basis of virulence in Salmonella Dublin

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Calmonellosis is one of the most common foodborne J diseases worldwide that causes a huge burden of morbidity and mortality in humans. Although non-typhoidal Salmonella servoars including Salmonella Dublin are associated primarily with self-limiting gastrointestinal illness they have adapted to cause invasive disease and systemic illness in humans particularly children, elderly and immunocompromised people. Salmonella enterica serovar Dublin is a zoonotic infection that can be transmitted from cattle to humans through consumption of contaminated milk and milk products. Outbreaks of human infections by Salmonella Dublin have been reported in several countries including high-income countries. The genetic basis of virulence and invasiveness of Salmonella Dublin is not well characterized. We apply next generation sequencing and associated bioinformatics analyses tools is characterize the invasome of Salmonella Dublin that enable the bacteria to cause systemic illness in humans. We identified several virulence factors that enable the bacteria to cause

invasive disease in humans however, no genomic markers were detected that differentiate among invasive and non-invasive isolates suggesting that host factors and immune response play a significant role in the disease outcome. There is no vaccine against non-typhoidal *Salmonella* however our understanding of the molecular basis of virulence in invasive *Salmonella* Dublin will provide insights into the development of an effective vaccine through identification of novel virulence-attenuated strains with a potential for use as vaccine candidates for high-risk groups.

Speaker Biography

Manal Mohammed is a lecturer in Medical Microbiology at University of Westminster, UK. She did her PhD at University of Liverpool, UK where she studied the molecular evolution of incurable Japanese encephalitis virus associated with high morbidity and mortality in humans. Her research is focused on application of next generation sequencing technologies and associated bioinformatics analyses tools in investigating the molecular basis of virulence of non-typhoidal salmonellosis in humans and understanding the complex dynamics of bacteria-phage interaction aiming to develop phage therapy as an alternative to antibiotics.

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Clinical application of detection rats in the diagnosis of tuberculosis in key populations

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Background: Tuberculosis disease kills about 1.7 million people worldwide with many deaths occurring in developing countries in south East Asia and Sub Saharan Africa. Children and people living with HIV/AIDS are among key population that STOPTB initiatives aim to enhance TB diagnosis because they contribute significantly to TB burden and they are prone to TB disease because it is difficult to diagnose TB. In countries with high HIV/AIDS prevalence the sensitivity of otherwise sensitive tests may be compromised. We report on clinical application of trained TB detection rats in clinical sputum samples from people living with HIV/AIDS and children.

Methods: Presumptive TB patients produced two sputum samples for TB diagnosis in hospitals and second-line testing by rats. Samples indicated by rats as TB positive were confirmed by concentratedsmear microscopy and bacterial load estimated following guideline/score of the WHO and IUATLD. Sputum from a general presumptive TB population was cultured in 5 different media to determine the various microbes and whether rats could differentiate sputum with *M. tuberculosis* from those with other microbes.

Results: A total of 1,906 PLWHA and 4629 of children within age 1-14 were tested. Conventional TB tests detected 60 adult PLWHA and 331 children whereas rats detected 156 PLWHA and 539 children respectively. Over 50 percent of the

patients detected by rats missed by hospitals had low Bacilli count insputum sample. Application of molecular PCR based confirmation tests showed that missed samples detected by rats' contained Mycobacterium tuberculosis, the pathogen causing TB. Furthermore, rats differentiated sputum containing M. tuberculosis from those with nontuberculous mycobacteria and mycobacteria related Nocardia and Rhodococcus species that are also acid-fast Bacilli often mistaken with TB by microscopy.

Conclusion: Trained TB detection rats have potential for clinical application in detecting TB that could have been missed by conventional TB tests in TB/HIV high-TB burden countries where the diagnosis is still a challenge due to smear negativity that increases in TB/HIV co-infection.

Speaker Biography

Georgies Mgode is a research fellow at Pest Management Centre, Sokoine University of Agriculture (SPMC) heading the vector-borne and zoonotic disease studies section; and is the program manager of APOPO TB involved with novel diagnosis of pulmonary tuberculosis (TB) using trained African giant pouched rats (Cricetomys sp. Swahili: Panyabuku). He has research interest in tuberculosis and rodent borne zoonotic diseases including leptospirosis. He is among the pioneer researchers of tuberculosis detection using rats and he explored the specific odour compounds (volatile organic compounds) of Mycobacterium tuberculosis which are targeted by TB detection rats for doctoral degree studies at the Max Planck Institute for Infection Biology (MPIIB), in Berlin, Germany.

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The structure, function and stability of the sinonasal microbiome during health and chronic rhinosinusitis

Brett Wagner Mackenzie

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hronic rhinosinusitis (CRS) is characterized by persistent inflammation of the sinonasal mucosa. The condition is highly prevalent (~5%) causing significant morbidity and considerable treatment-associated costs to healthcare systems. CRS is a complex, heterogeneous disease resulting from interactions between a patient's genetics, mucosal immune system and sinonasal microbiome. The pathogenic role of the microbiome in CRS remains incompletely understood. With the aim of providing a context for CRSrelated microbiome research, I will present results from a variety of traditional culture-based and molecular techniques that we have employed to explore the roles of both individual bacterial strains and microbial communities. Amplicon sequencing of the bacterial and fungal communities has shed light on the dysbiotic, fragmented CRS bacterial community and the natural temporal variability in healthy subjects. Shotgun metagenomic sequencing was used to provide total

microbial community information, revealing the presence of bacteriophages and bacterial strain-level diversity in patients with CRS. I will highlight the substantial challenges associated with metagenomic shotgun sequencing in low biomass sinonasal samples and describe the advantages of such an approach. This research contributes to our current understanding of the role of the microbiome in CRS and will help inform multifaceted, cross-disciplinary studies that aim to develop more effective treatments for CRS.

Speaker Biography

Brett Wagner Mackenzie recently finished her PhD at the School of Medicine, Department of Surgery at The University of Auckland in Auckland, New Zealand. Her thesis focuses on the role of the human sinonasal microbiome in chronic rhinosinusitis and how to better understand the role of the sinonasal microbiome during health and disease. Her research interests include host-microbiome interactions and microbial community network stability and response to disturbance.

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Amniotic membrane plus conjunctival autograft for pterygia

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Purpose: To compare amniotic membrane associated with conjunctival autograft versus conjunctival autograft alone in the treatment of recurrent pterygia.

Methods: Patients with recurrent pterygia without symblepharon were randomly assigned to undertake pterygium exicision followed by amniotic membrane associated with a small conjunctival autograft (2x3mm) or conjunctival autograft (approximately 5x8mm) alone. The patients were examined after 1, 7, 30, 90, 180 and 360 days after the surgery. Recurrence was considered as a fibrovascular ingrowth of 1.5 mm or more beyond the limbus with conjunctival drag.

Results: Forty eyes of 38 patients with recurrent pterygium were included. Nineteen patients (50%) were female and nineteen (50%) were male. The mean patients' age was 46,8 years old (range between 23 and 69 years old). Twenty one patients underwent amniotic membrane associated with conjunctival autograft and nineteen patients underwent conjunctival

autograft alone. All patients were treated by the same surgeon. The follow up time was 12 months in 30 patients and 6 months in 10 patients. Recurrence was diagnosed in 6 patients [4 in the amniotic membrane group (21,5%) and 2 in the conjunctival autograft group (9,52%)]. Complication (conjuntival granuloma) was observed in one case after 14 days of the surgery.

Conclusions: Our results showed that both, amniotic membrane associated with conjunctival autograft and conjunctival autograft alone, presented low rate of recurrence and complications and are good treatment options for recurrent pterygium.

Speaker Biography

Jose Bonifacio Barbosa Jr is currently working as an ophthalmology consultant in UDI Hospital, Sao Paulo, Brazil. His research interests include cornea, refractive surgery and ocular external diseases etc. He is serving as an editorial member and reviewer in several international reputed journals. He is also a member of many international affiliations. He has authored many research articles/books related to his research interest.

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Intravitreal administration of lysine-acetylsalicylate could be an effective approach to preserve retinal vessels and to inhibit leukostasis in experimental diabetic retinopathy

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Purpose: We explore the effects of lysine acetylsalicylate intravitreal injections to preserve retinal vessels and to inhibit the presence of leukocytes in early stages of streptozotocin induced diabetic retinopathy in rats.

Setting: Inflammation has a central role in diabetic retinopathy development. Oral administration of acetylsalicylic acid (AAS) has reported therapeutic benefits in experimental diabetic retinopathy but controversial in humans.

Methods: Animals were assigned to two groups (intravitreal lysine acetylsalicylate, untreated control) for comparison in between. Intravitreal injections were administered twice (at the weeks 4 and 8 from diabetes induction). Retinal ganglion cell layer (GCL) and outer plexiform layer (OPL) were analysed at central and peripheral retina sections.

Results: Immunohistochemistry assay revealed a significant preservation of central and peripheral retinal vessels in

both layers (P< 0.001) and a significant correlation between leukocytes and preserved vessels in the GCL for both central peripheral retina (P< 0.001) and in the OPL at the central retina (P =0.015).

Conclusions: Intravitreal use of lysine acetylsalicylate has never been reported but its ability to reduce leukocytes and to preserve retinal vessels in early stages of diabetic retinopathy avoiding side effects associated to oral AAS makes it a tool that deserves to be explored.

Speaker Biography

Cristian Fernandez Martinez is currently working in Hospital General Universitario de Elche, Spain.

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Management of Helicobacter pylori gastric infection via surface-grafted antimicrobial peptides

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elicobacter pylori chronic infection is associated, among $oldsymbol{\Pi}$ other severe gastric disorders, with intestinal-type gastric carcinogenesis, being the fifth most common cancer and the third leading cause of cancer-related death worldwide. Classical H. pylori eradication treatment, combining two antibiotics and a proton pump inhibitor, reduces the risk for gastric carcinoma development, but treatment of H. pylori infection is challenged by a dramatic fall in eradication rates all over the world. Currently, this bacterium is listed among the 16 antibioticresistant bacteria that pose greatest threat to human health according to the World Health Organization. Antimicrobial peptides (AMPs) present an alternative to conventional antibiotic therapies, being their most striking feature the low tendency to induce bacterial resistance, since AMPs selectively damage the bacterial membranes through mechanisms that bacteria find difficult to evade. In an in vivo scenario, "unbound AMPs" can undergo proteolysis and peptide aggregation, leading to efficiency decrease. AMP grafting onto nanoparticles has been reported as a good strategy to protect peptides from aggregation and enzymatic degradation in vivo, therefore increasing long-term stability and avoiding cytotoxicity associated with application of high AMP concentrations. In

this study we demonstrated that the AMP MSI-78A could be surface-grafted without compromising its activity. Moreover, MSI-78A-decorated surfaces were highly effective against *H. pylori*, killing bacteria by contact in a short time span, since after 2h only 2% of *H. pylori* remained viable in suspension. These results encourage the utilization of grafted MSI-78A on biocompatible nanoparticles as an alternative to the currently available therapy against *H. pylori*, opening new routes for gastric infection management.

Speaker Biography

Paula Parreira graduated in Microbiology from the Universidade Católica Portuguesa (Portugal) in 2007. In the same year, joined the team of Prof. M Cristina Martins at the Institute of Biomedical Engineering of University of Porto (INEB) and from 2007 to 2013, conducted her PhD studies under the guidance of Prof. M Cristina Martins and Prof. Deborah Leckband (University of Illinois, at Urbana-Champaign, USA). After finishing her PhD, Paula Parreira's post doctoral research has continued to focus on development of non-antibiotic strategies against microbiological human pathogens, namely against the gastric pathogen *Helicobacter pylori*, with emphasis on natural molecules coupled with bioengineered approaches. Currently, Paula Parreira is a research assistant in the Bioengineered Surfaces Group at Instituto de Investigação e Inovação em Saúde (i3S; Portugal) and has published several papers in first quartile journals, book chapters and participated in several international conferences.

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The nature of microbial involvement in the development of adenotonsillar hyperplasia

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bstructive sleep apnoea (OSA) has become a more common indication for tonsillectomy than recurrent tonsillitis (RT). Few studies have addressed possible differences in pathogenesis between these two conditions. Children with RT and OSA are often being treated in the community with multiple courses of antibiotics before surgery. Current understanding of the role of bacteria in disorders of the tonsils is mainly based on the culture of tonsil swabs. Swab cultures reflect only a very small fraction of the bacteria present on the mucosal surface and may not represent the bacterial communities within the tonsil crypts. Culture-independent methods, based on bacterial 16S rRNA gene sequencing, have been used to increase understanding of the tonsillar microbiome. We utilised these techniques, combined with histology, to evaluate the local lymphocyte response and associations with bacterial community composition of the tonsils removed from children for either RT or OSA. We also aimed to investigate potential differences in adenotonsillar

microbiota according to sampling location, both on and within the adenoids and palatine tonsils. Finally, a randomised control trial (RCT) was undertaken to evaluate whether a course of amoxicillin-clavulanate altered the tonsil microbiome in children with recurrent tonsillitis immediately before tonsillectomy. These results demonstrate significant differences in the local lymphocyte response and bacterial community composition in tonsil tissue between RT and OSA patients. We observed variations in bacterial diversity and composition based on sampling sites in the tonsils but not the adenoids. Finally, no variation in bacterial diversity of the tonsils following a course of broad-spectrum antibiotics was noted, suggesting antibiotics have minimal impact on the tonsil microbiota. Accordingly, the liberal use of antibiotics for this condition should be challenged.

Speaker Biography

James Johnston is in the final year of his PhD at The University of Auckland, Auckland, New Zealand. He is a trainee in otolaryngology-head and neck surgery with the Royal Australasian College of Surgeons.

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