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Pacific Region Infectious Disease Association: Humanity and Microbiology

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Infectious diseases cause significant suffering and death throughout the world. Fortunately, with early diagnosis, appropriate management and good hygiene practices, infectious diseases are also readily treatable. The issue is that these practices require highly skilled staff and access to basic resources, both of which are under developed in the majority of Pacific island countries. The recent emergence of antimicrobial resistance (AMR) throughout the world has made the skilled management of infectious disease even more crucial. Notably, the World Health Organisation has named AMR as a major threat to global health. In the Pacific region AMR is widespread, however, we are currently unable to accurately determine its extent due to the inability to attain reliable laboratory information.

In 2017 Pacific Region Infectious Disease Association (PRIDA) was formally established by a group of like-minded Australian healthcare professionals who had been individually supporting our Pacific island neighbours over a number of years. The infectious disease group consists a broad range of expertise including physicians, nurses, biomedical scientists and pharmacists. As a multidisciplinary team, we provide training at a grass roots level, foster long-term mentoring relationships to support practical and sustainable capacity of infectious disease management in-country. To date we have ongoing projects in the Solomon Islands, PNG, and East Timor as well as close associations with Samoa, Vanuatu, and Marshall Islands.

To support microbiology laboratories in the Pacific, we undertake repeated regular short term in country visits. We work closely with laboratory scientists offering one-on-one training bench training and small group teaching. We collectively identify issues and collaborate to develop the necessary guidelines and protocols to implement best practice. We facilitate donations of basic essential laboratory equipment to ensure appropriate implementation, use and care.

In this lecture, we will present how PRIDA has leveraged the use of microbiology to provide critically needed humanitarian and development solutions in the Pacific region.

2

Mining the microbiota for vaccination and therapy in fungal infections

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The mechanistic role of gut microbes and their microbial metabolites underlying human infections and diseases remains unknown. Fungal commensals coexist in a complex milieu of bacteria within the human body. Severe and recurrent infections tend to manifest more frequently within immunocompromised hosts and microbial dysbiosis, suggesting that the host/microbe environment could significantly influence the infection, the generation of colonization resistance and protective tolerance and the efficacy of therapy. Expanding on these studies will provide insights into how microbial and host metabolism can influence human infections, potentially leading to personalized infection control strategies based on a patient's metabolic state. We have deciphered the contribution of the microbiota at the host/fungus interface and captured the dialogue between the mammalian host and its microbiota via metabolomics. A functional metabolic node by which certain bacteria species contribute to host-fungal symbiosis and mucosal homeostasis in the gut has been discovered in mice and confirmed in humans. A microbial tryptophan metabolic pathway activated through the indoleamine 2, 3-dioxygenase 1 enzyme proved to be capable of correcting dysfunctional host-pathogen interaction. Working through the aryl hydrocarbon receptor (AhR), the indole-3-aldehyde (3-IAld), produced by *Lactobacillus reuteri* upon tryptophan feeding, regulated IL-22 expression and helped maintaining epithelial barrier and intraepithelial lymphocytes function. These activities ensured that commensal bacteria outcompete potential pathogenic fungi, allowing AhR to mediate host-microbe homeostasis through indole sensing. It seems therefore that elucidating the function of microbial metabolites and bacterial chemical signaling systems may contribute to the development of host- and microbiota-directed therapeutics and vaccine efficacy in fungal infections.

This study was supported by the European Research Council (ERC-2011-AdG-293714).

3

Reimagining Antimicrobials and Anti-infective Development Strategies

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According to the World Health Organization, infectious diseases remain the second most common cause of death. Historically, antibiotics have been derived from nature and then chemically manipulated to produce next-generation classes of molecules. These agents are systemically administered to animals or humans and can be accompanied by toxicity, development of antibiotic resistance, and changes to the microflora of the gut. The cost of anti-infective development is prohibitively expensive, and the average time from discovery to completion of the preclinical research phase (in vitro and animal testing) is approximately 6 years. Moreover, even if the anti-infective reaches Phase I clinical trial stage the likelihood of FDA approval is only 19%.

Recent technological and computational advances are accelerating the pace of antimicrobial research. However, we need to characterize host-pathogen interactions to ensure that antimicrobial products tested in vitro will perform as well in vivo and under clinical conditions; this would minimize the chances of failure following the preclinical phase, as well as between phases I and II. Preclinical models that incorporate the host response to pathogens are necessary to create alternative antimicrobial strategies (e.g., suppression of pathogenic potential rather than direct killing of pathogens). Ideally, these models would also accelerate the research and development process.

This presentation will describe new antimicrobials and anti-infective development strategies, such as ex vivo tissue from humans and animals, organoids, and coculture systems. These models more closely reflect host conditions than traditional in vitro experiments, and they can be used to characterize antimicrobial agents that prevent infectious diseases either by suppressing pathogenesis, directly killing microorganisms, or inhibiting host targets.

4

Microbes that have changed the world: Influenza in the vulnerable elderly

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The continued importance of influenza in modern day society will be illustrated by discussion of the epidemiology of ageing and frailty, our personal lifetime risk of being in an Aged Care Facility, and the importance of ACFs as incubators for viral disease. This will be illustrated by data from the cluster randomized controlled trial of influenza control (Booy et al PLoS ONE 2012; 7(10) e46509). Key lessons included:

1. The complexities of research in a vulnerable population in ACFs (very topical given the current Royal Commission).
2. The importance of laboratory investigations, with only 9/23 influenza like illness outbreaks proven to be influenza, 5/23 with other viral aetiology, 9/23 no viral agent identified.
3. A policy of treatment and prophylaxis (T&P) with the antiviral agent oseltamivir (compared to only treating symptomatic residents –"T") was found to have modest benefits in reducing influenza outbreak duration (T&P 10.8 days versus T only 24 days, p = 0.04), attack rate (T&P 22.9% versus T only of 36.5%, p = 0.002), hospitalisation (T&P 3.5% versus T only 4.7%, p = 0.7) and deaths (T&P 2.5% versus T only 3.5%, p = 0.7).
4. ACFs acted as local reservoirs of influenza infection, with staff and visitors vectors to the local community.

Public health policy for ACFs can influence overall community health emphasising the importance of "difficult" research involving vulnerable people in our community.

5

HIV: still a global problem requiring prevention and cure

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Despite the rollout of antiretroviral therapy (ART) around the world HIV continues to produce a major global health burden. Although ART has resulted in a 50% reduction in AIDS related deaths since the peak in 2004, in 2017-18, WHO estimated there were 1.8 million new infections globally and that only 21 million of 37 million people currently infected are on ART. ART can also be given to healthy individuals at high risk of acquiring HIV as pre-exposure prophylaxis (PrEP) which has been successfully deployed in Australia but not in high HIV incidence countries. Prior infection with Herpes simplex virus type 2 enhances HIV acquisition three fold. Therefore, a vaccine against HIV (and possibly HSV2) is required to prevent HIV transmission and a cure is needed so people can safely stop ART. For vaccine development, we need a better understanding of how HIV (and HSV) initially penetrate both inflamed and uninflamed anogenital mucosa and interact with resident (innate) immune cells.

We recently demonstrated that HIV and HSV first encounter and replicate in Langerhans cells and a new type of (CD11c+) dendritic cell (DC) in human genital mucosa (Nature Comms 2019). These DCs can transfer HIV to CD4 T cells which infiltrate the dermis leading to rapid systemic spread or initiation of a latently infected T cell reservoir which persists for life. Type I IFNs are induced early in HIV/SIV infection in humans, and macaques and can limit HIV spread and the size of the reservoir. However HIV inhibits IFN-I production in the initial target dendritic cells (DCs), but this may be restored after subsequent infiltration of plasmacytoid DCs. HIV then develops resistance to IFNs. Preventing the initial IFN inhibition by HIV is a strategy to reduce the likelihood of HIV obtaining a 'toehold' in its target cells. HSV differs in being transferred to dermal DCs before stimulating CD4 and CD8 cells. Further definition of this pathway will assist HSV vaccine development

6

Not available

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Not available

7

characterisation of influenza viruses circulating in Australia during a high inter-seasonal period in 2018-9

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Introduction

Influenza causes seasonal epidemics in temperate regions of Australia, with peak activity occurring in winter-spring seasons (July-September). Here we analyse influenza viruses that have been circulating in unusually high numbers Australia-wide during the most recent inter-seasonal period (Dec 2018- April 2019)

Methods

We analysed influenza positive samples sent to the WHO Collaborating Centre for Influenza (WHO CC) and notifications of laboratory-confirmed influenza from the Australian National Notifiable Diseases Surveillance System (NNDSS).

Results

During the influenza inter-seasonal period (December 2018-April 2019), the WHO CC received 1890 samples compared to the combined inter-seasonal periods of the previous **three** years, of 1669 samples. This data was reflected in the NNDSS reporting of laboratory-confirmed cases of influenza, with 35,257 (to 15 April 2019) cases reported in the current inter-seasonal period, compared to 49,275 for the previous three years combined. The current inter-seasonal period has been dominated by the A(H1N1)pdm virus, with 63% of viruses analysed here belonging to this subtype, followed by A(H3N2) (31%), and influenza B (5%). Antigenically, the A(H1N1)pdm viruses were similar to the virus included in the 2019 southern hemisphere vaccine though there was considerable genetic heterogeneity. The A(H3N2) viruses also fell into several distinct genetic clades, with the predominant subclade being 3C2a1b with a 131K substitution in the HA, similar to A(H3) viruses circulating in Japan and China during this period.

Conclusion

Summer influenza activity in Australia has been at record levels in 2018-19, with both A(H3N2) and A(H1N1)pdm09 viruses co-circulating. The causes of this dramatic increase in influenza activity are unclear at this stage but may be related to a late epidemic of H1N1pdm09 in Northern Australia in 2018 combined with a late and extended influenza 2018-9 season in Asia and elsewhere, that may have resulted in A(H3N2)-infected tourists returning to Australia.

8

Role of *IRGM* polymorphism in *Helicobacter pylori* related Gastric cancer.

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Introduction: Globally, gastric cancer (GC) is the 5th most common malignancy. As a multifactorial disease, *Helicobacter pylori* infection and host genetics are crucial for GC development. Autophagy, a protective cellular mechanism that is triggered upon pathogen invasion, is regulated by the Human Immunity-related GTPase M (IRGM) gene. The current study aimed to investigate the role of *IRGM*rs4958847 in *H. pylori*-induced inflammation and GC in ethnically diverse populations.

Methods: This case-control study comprised 1246 subjects from high-risk Ethnic Chinese (N=304) and Colombian (N=587) populations, and one low-risk Caucasian population (N=304). *IRGM* rs4958847 was genotyped by MADLI-TOF mass spectrometry. Using CRISPR/Cas9, a knock-in gastric epithelial cell line (AGS) was generated. Infection assays (2hr and 24hr) were conducted, and gene and protein expression levels of pro-inflammatory cytokines (IL8 and IFN-β) were analysed using qPCR and ELISA, respectively. *H. pylori* infection status as well as the presence of the virulence factors CagA and VacA were determined using the Helico Blot 2.1 kit. Statistical analyses included bivariate, multivariate and joint analyses.

Results: Multivariate analyses suggest that *IRGM* rs4958847 AA genotype increases GC risk in Chinese (A>G, OR:0.28, *p-value*:0.01) and Colombians (G>A, OR:2.63, *p-value*:0.15), but decreases risk in Caucasians (G>A, OR:0.17, *p-value*:0.09). Consistent with previous literature, male gender was found to be a risk factor in Ethnic Chinese (OR:2.01, *p-value*:0.01) and Colombians (OR:2.01, *p-value*:0.004). Joint analysis showed that Ethnic Chinese and Caucasians harbouring this polymorphism and infected with *H. pylori*, experience a significant increased GC risk of 2.5-fold. Inflammation is decreased in edited infected cells as compared with wild-type infected cells (*IL8*, Fold change:0.42, *p-value*:0.10), a result corroborated at the protein level (*p-value*:0.004).

Conclusions: *IRGM* rs4958847 is a risk factor for GC in high-risk populations; importantly, a synergistic relationship was established between *H. pylori* infection and this polymorphism. *In vitro* preliminary analysis suggests that this polymorphism plays a relevant role in *H. pylori*-related inflammation and thus, GC.

9

Remodeling of pSK1 Family Plasmids and Enhanced Chlorhexidine Tolerance in Methicillin-Resistant *Staphylococcus aureus*

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Staphylococcus aureus is an important human pathogen and a species whose evolution has been shaped in part by mobile genetic elements (MGEs). Our understanding of the evolutionary dynamics surrounding MGEs is incomplete, in particular how changes in the structure of multidrug-resistant (MDR) plasmids may influence clinically-relevant staphylococcal phenotypes. Here, we undertook a population- and functional-genomics study of 212 clinical methicillin-resistant *S. aureus* (MRSA) sequence types (ST) 239 isolates, recovered between 1980 and 2012 and representative of the two major lineages circulating in the region, to explore the evolution of the pSK1 family of MDR plasmids; illustrating how these plasmids have co-evolved with and contributed to the successful adaptation of this persistent healthcare-associated MRSA lineage. Using complete genomes and temporal phylogenomics we have reconstructed the evolution of the pSK1 plasmid family from its emergence in the late 1970s, with eight distinct structural variants having arisen within the ST239 MRSA population in Australia. Plasmid maintenance and stability was linked to IS*256*- and IS*257*-mediated structural changes, including chromosomal integration, inversion and disruption of plasmid replication machinery. Combining genomic findings with phenotypic susceptibility data for trimethoprim, gentamicin and the cationic biocide chlorhexidine, it appeared that the pSK1 family plasmids have contributed to enhanced resistance in ST239 MRSA through two mechanisms: (i) acquisition of plasmid-borne resistance mechanisms increasing rates of gentamicin resistance and reduced chlorhexidine susceptibility, and (ii) changes in plasmid configuration linked with further enhancement of chlorhexidine tolerance. While the exact mechanism of enhanced chlorhexidine tolerance in this population remains elusive, this research has uncovered a potential evolutionary response of ST239 MRSA to biocides, one which may contribute to the ongoing persistence and adaptation of this lineage within healthcare institutions.

10

Progress towards a gonococcal vaccine

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Neisseria gonorrhoeae is an obligate human pathogen and the causative agent of the sexually transmitted infection gonorrhoea. There are over 106 million reported cases of gonorrhoea each year worldwide, and if left undiagnosed or untreated, infection can lead to severe sequelae that include pelvic inflammatory disease, infertility, neonatal complications, and an increased risk of HIV. *N. gonorrhoeae* is recognised by WHO and CDC as an urgent threat to global health due to the emergence of multi-drug resistant gonococcal strains. There is currently no vaccine, and no new antibiotics or new vaccine candidates in late-stage development. However, the outer membrane vesicle (OMV) meningococcal B vaccine MeNZB, that was developed to protect against the closely related pathogen *Neisseria meningitidis,* was recently reported to be associated with reduced rates of gonorrhoea following a mass vaccination campaign in New Zealand.

Our work is focused on identifying novel gonococcal vaccine target, as well as investigating the cross reactivity to *N. gonorrhoeae* of serum raised to the meningococcal B vaccine Bexsero, which contains the MeNZB OMV component plus three recombinant protein antigens. We have characterised several highly conserved and immunogenic gonococcal candidate vaccine antigens and shown that antibodies to these proteins are bactericidal and can block gonococcal infection of cervical and urethral epithelial cells. In addition, we have found that there is a high level of sequence identity between the MeNZB/Bexsero OMV antigens, and gonococcal proteins. NHBA is the only Bexsero recombinant antigen that is conserved and surfaced exposed in *N. gonorrhoeae*. Furthermore, we have found that Bexsero induces antibodies in humans that recognise and kill *N. gonorrhoeae* in vitro. Work is ongoing to identify the full set of gonococcal targets recognized by Bexsero-induced antibodies, and their functional activity against gonorrhoea.

11

Rapid Response Pipeline for Stabilized Subunit Vaccines

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The University of Queensland has recently received $14.7M in funding from the Coalition for Epidemic Preparedness Innovations (CEPI) to establish a rapid response vaccine pipeline. This project brings together unique proprietary recombinant technology for generating stabilized subunit vaccines (the molecular clamp), a highly skilled team from some of Australia's leading scientific organizations and world-class facilities.

Molecular clamp is a broadly applicable platform technology that facilitates expression of recombinant viral glycoproteins in subunit form without loss of native antigenicity. The molecular clamp imparts superior stability over alternative trimerization domains, efficiently stabilizing soluble viral fusion proteins in their native trimeric 'pre-fusion' form. This form is equivalent to that expressed on the virion surface and the principle target for a protective neutralizing antibody response. Through stabilization of the pre-fusion form, the molecular clamp promotes the production of highly neutralizing and broadly cross-reactive antibodies. Importantly, the molecular clamp does not required prior knowledge of a proteins quaternary structure.

The goal of this project is to establish a holistic and robust pipeline to rapidly generate novel subunit vaccines purely from sequence information. Within this pipeline, pre-clinical development, including the generation of evidence for safety and immunogenicity in animal models, is to be completed within a 16 week window allowing candidate vaccines to then progress directly into Phase I clinical trials. Phase I trials, including regulatory approval, patient immunization and analysis of safety and immunogenicity will be completed within 10 weeks (week 17-26 of the pipeline). As part of the project, large-scale manufacture of >200,000 vaccine doses will be completed within a further 8 weeks. Vaccine produced through this pipeline will therefore be available for rapid deployment and provide the best possible opportunity to counter emerging viral epidemics.

12

Developing Attenuated Live Vaccines to Control Mycoplasmoses in Livestock

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Mycoplasmas are near ubiquitous causes of disease and economic loss in many livestock species, particularly in intensive agricultural systems. The chronic disease they cause affects animal welfare, increases the likelihood of more severe acute disease after infection with other respiratory pathogens, and drives a considerable proportion of the antimicrobial use in livestock. Although inactivated vaccines against most of the major mycoplasma pathogens have been marketed commercially, there is little evidence of their efficacy. However, there is very good evidence that attenuated live vaccines can be very effective, with their introduction into Australia virtually eliminating the use of macrolides in the poultry industry. The reasons for the superior efficacy of attenuated mycoplasma vaccines are complex, but are at least partially attributable to their capacity to induce effective mucosal immunity and to induce broad immunity against a range of variable cell surface antigens. Our research centre has continued to investigate methods to produce improved mycoplasma vaccines for a range of livestock species over a number of years, focussing on both rationale attenuation and on more empirical vaccine development. This has led to the identification of a number of targets for mutagenesis to develop attenuated vaccine strains, development of improved methods for mutagenesis of mycoplasmas, and the commercialisation of improved live attenuated vaccines, including the first effective vaccine against *Mycoplasma gallisepticum* for turkeys. These novel vaccines can be expected to have a global impact on the health and productivity of agricultural animals, as well as on public health.

13

Direct interaction of whole inactivated influenza A and pneumococcal vaccines enhances influenza-specific immunity

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The upper respiratory tract is continuously exposed to a vast array of potentially pathogenic viruses and bacteria. Influenza A virus (IAV) has particular synergism with the commensal bacterium *Streptococcus pneumoniae* in this niche, and co-infection exacerbates pathogenicity and causes significant mortality. However, it is not known whether this synergism is associated with a direct interaction between the two pathogens. We previously reported our approach to develop a gamma-irradiated IAV vaccine (g-Flu) capable of inducing cross-protective immunity mediated by cross-reactive cytotoxic T cell responses (1). In addition, we recently reported the development of a serotype-independent pneumococcal vaccine using genetically modified non-capsulated bacteria inactivated by gamma-irradiation (g-PN) (2). Furthermore, we reported that co-administration of g-Flu with g-PN enhances pneumococcal-specific responses (3). Our recent study shows that mucosal co-administration of g-Flu and g-PN similarly augments IAV-specific immunity, particularly Tissue Resident Memory cell (T_{RM}) responses in the lung (4). In addition, our *in vitro* analysis revealed that *S. pneumoniae* directly interacts with both g-Flu and with live IAV, facilitating increased uptake by macrophages as well as increased infection of epithelial cells by IAV. These observations provide an additional explanation for the synergistic pathogenicity of IAV and *S. pneumoniae,* as well as heralding the prospect of exploiting the phenomenon to develop better vaccine strategies for both pathogens.

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14

The *Neisseria gonorrhoeae* Methionine Sulfoxide Reductase (MsrA/B) Is a Surface Exposed, Immunogenic, Vaccine Candidate

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Control of the sexually transmitted infection gonorrhea is a major public health challenge, due to the recent emergence of multidrug resistant strains of *Neisseria gonorrhoeae*, and there is an urgent need for novel therapies or a vaccine to prevent gonococcal disease. In this study, we evaluated the methionine sulfoxide reductase (MsrA/B) of *N. gonorrhoeae* as a potential vaccine candidate, in terms of its expression, sequence conservation, localization, immunogenicity, and the functional activity of antibodies raised to it. Gonococcal MsrA/B has previously been shown to reduce methionine sulfoxide [Met(O)] to methionine (Met) in oxidized proteins and protect against oxidative stress. Here we have shown that the gene encoding MsrA/B is present, highly conserved, and expressed in all *N. gonorrhoeae* strains investigated, and we determined that MsrA/B is surface exposed on *N. gonorrhoeae*. Recombinant MsrA/B is immunogenic, and mice immunized with MsrA/B and either aluminum hydroxide gel adjuvant or Freund's adjuvant generated a humoral immune response, with predominantly IgG1 antibodies. Higher titers of IgG2a, IgG2b, and IgG3 were detected in mice immunized with MsrA/B-Freund's adjuvant compared to MsrA/B-aluminum hydroxide adjuvant, while IgM titers were similar for both adjuvants. Antibodies generated by MsrA/B-Freund's in mice mediated bacterial killing via both serum bactericidal activity and opsonophagocytic activity. Anti-MsrA/B was also able to functionally block the activity of MsrA/B by inhibiting binding to its substrate, Met(O). We propose that recombinant MsrA/B is a promising vaccine antigen for *N. gonorrhoeae*.

15

Zika Virus Non-structural Proteins as DNA Vaccine Antigens to Elicit Cell Mediated Immunity

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Introduction: The causal association of Zika virus (ZIKV) with microcephaly, congenital malformations in infants and Guillain-Barré syndrome in adults highlight the need for effective vaccines. Neutralising antibodies against ZIKV structural (pre-membrane and envelope) proteins can successfully prevent the infection [1]. However, there is a concern that anti-envelope antibodies can enhance infection of dengue virus [2], which is also endemic in regions where ZIKV circulates. Therefore, we developed novel cytolytic DNA vaccines encoding non-structural proteins (NS2/NS3 and NS4/NS5) of ZIKV. These cytolytic DNA vaccines also encode a truncated form of mouse perforin (PRF), which can more effectively prime T-cell mediated immunity (CMI) *in vivo* compared to canonical DNA vaccines not encoding PRF [3].

Method: Cytolytic DNA plasmids encoding ZIKV NS2 and NS3 (pNS2/NS3-PRF) and NS4 and NS5 (pNS4/NS4-PRF) (Brazil-ZKV2015 isolate) were used to prime/boost vaccinate female BALB/c mice. Empty DNA vector (pVAX-PRF) was used as a control. CMI to each vaccine-encoded ZIKV NS protein was evaluated *in vivo* in vaccinated mice using fluorescent target array (FTA). We have used the FTA to quantify the magnitude and avidity of CMI in vaccinated mice [4]. The assay involves challenging vaccinated mice with fluorescently bar-coded ZIKV peptide-pulsed autologous target cells, which are recognized by effector/memory $CD8^+$ cytotoxic T-lymphocyte (CTL) cells and $CD4^+$ T helper (Th) cells *in vivo*. T cell responses are assessed 18 hours later by flow cytometry analysis of FTA target cells recovered from the spleen of vaccinated mice.

Results and Conclusion: Vaccination of mice with pNS2/NS3-PRF or pNS4/NS5-PRF elicited significant $CD8^+$ CTL and $CD4^+$ Th responses *in vivo* that were mainly restricted to ZIKV NS2 and NS3, while NS4 and NS5 were poorly immunogenic. This study suggests that ZIKV NS2 and NS3 can be effective immunogens for the development of T-cell based vaccines against ZIKV. We are currently evaluating the protective efficacy of pNS2/NS3-PRF against ZIKV challenge.

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16

The "*antenatal microbiome": controversies, complications and consequences*

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The long-held belief that the fetus is sterile prior to birth has been questioned recently by a plethora of studies, using highly-sensitive molecular sequencing techniques, reporting the presence of bacterial and viral DNA within feto-placental tissues and fluids. Contradictory studies have also appeared, refuting the claims that a fetal microbiome exists and concluding that the results are confounded by the difficulties in properly accounting for external contamination. The limitations and interpretations of the conflicting evidence have elicited robust debate amongst the research community. The proponents of both sides place their findings in the context of the potential impact of microbial exposure on the development of the early-life immune system and postnatal microbiome; they argue that the sterility (or otherwise) of the fetal environment in normal, healthy pregnancies has important health implications. Yet, although there is growing evidence linking the development of the post-natal microbiome with risk of a range of disorders and diseases in later life, the health and developmental consequences of antenatal microbial exposure remain the matter of conjecture. In this presentation I will attempt to evaluate and summarise the evidence and controversies around the existence and nature of the "antenatal microbiome" and its microbiological and immunological significance for fetal, pediatric and adult health.

17

How does a long-term Paleolithic diet affect our gut health and markers of cardiovascular disease risk?

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Background: The Paleolithic diet is promoted for improved gut health in Australia. However, there is little evidence available to support these claims, with existing literature primarily examining anthropometric and cardiometabolic outcomes.

Objective : The study aimed to determine the association between dietary intake, markers of colonic health, faecal microbiota, and serum trimethylamine-n-oxide (TMAO), a gut-derived metabolite associated with cardiovascular disease (CVD).

Design: In a cross-sectional design, long-term (n=44,>1y) self-reported followers of a Paleolithic diet (PD) and controls consuming a diet typical of national recommendations (n=47) were recruited. Dietary intake was assessed via 3-day weighed diet records(3d WDR); 48-hr stool samples were assessed for short chain fatty acids (SCFA) using GC/MS and microbial composition was determined via 16S rRNA sequencing of the V4 region using Illumina MiSeq. Serum TMAO was quantified using LC-MS/MS.

Results: Participants were grouped according to adherence to the diet; namely excluding grains and dairy products. Strict Paleolithic (SP, n=22) and Pseudo-Paleolithic (PP, n=22) groups were formed. General linear modelling with age, gender, energy intake and body fat percentage as covariates assessed differences between groups. Intake of resistant starch (RS) was lower in both Paleolithic groups, compared to controls (2.62, 1.26 vs 4.48 g/day ($P<0.05$)); vegetable intake was higher in SP than controls (6.68 vs 3.83 serves/day, $P<0.01$); PERMANOVA analysis showed significant differences in microbiota composition at the genera level ($P<0.05$), with higher abundance of TMA-producer *Hungatella* in both Paleolithic groups ($P<0.001$). Serum TMAO was higher in SP compared to PP and control ($P<0.01$), and inversely associated with whole grain intake ($r=-0.34$, $P<0.01$).

Conclusions: Although the PD has been promoted for improved gut health, these results indicate long-term adherence is associated with different gut microbiota and increased TMAO concentrations. A variety of fibre components, including whole grain sources may be required to maintain gut and cardiovascular health.

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What do we mean by dysbiosis and how do we manage it?

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Homeostasis is the ability of a system to regulate its state - it is not always a good thing. Our gut microbiome profoundly influences our normal physiology through a variety of mechanisms. This is not simply a one-way effect, or something microbes 'do to us', rather our microbiome is part of us and the microbial community state is interdependent with our systemic body state. In chronic disease the concept of dysbiosis is that our body (including its microbiome) has adopted a stable state that is undesirable. Dysbiosis is different to other disease states in that it does not have a cause *per se*, but is an emergent state derived from interplay between many different factors. In managing dysbioses, two critical factors in this interplay are diet and microbial history. There is now abundant evidence that changes in our food environment and influence on early life microbial dynamics have sufficiently altered the nature of microbiome-human relationships in modern society to change patterns of public health. Chief among these are the emergence of a suite of nutrition-related diseases that include diabetes, obesity, and allergies and the prevalence of multiply antibiotic resistant bacteria in nosocomial infections. I will give an overview of the ways in which diet patterns and food components can shift the state of the human system to increase risk of dysbiotic diseases, highlighting key mechanisms we have identified in these processes.

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Cecal luminal microbial diversity profiling of laying chicks is manipulated by probiotics supplementation and *Salmonella* Typhimurium challenge

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The normal gut microbiota plays an important role in host nutrients metabolism, immunomodulation and maintenance of structural integrity of the gut mucosal barrier. Probiotics and prebiotics are used for modulation of gut microbiota in various disease conditions. *Salmonella* Typhimurium (*S*T) causes gastroenteritis in humans that often trace back to consumption of contaminated egg or egg products. Positive modulation of gut microbiota laying chickens may offer a strategy for reduction of *S*T shedding and production of safer poultry products. In the current study, the cecal luminal microbiota of laying chicks was studied using 16S rRNA amplicon sequencing on DNA obtained from the chicks that were offered supplementation with commercial probiotics and/or *S*T challenge. The load of *S*T in various organs was quantified. Irrespective of the probiotics supplementation and *S*T challenge, caecal microbiota was dominated by 22 distinct bacterial genera and 14 families that clustered into *Actinobacteria*, *Proteobacteria* and *Firmicutes* at phylum level. Taken together, probiotics supplementation increased (FDR<0.05) the abundance of *Ruminococcus*, *Trabulsiell*a, *Bifidobacterium*, *Holdemania* and *Oscillospira*, indicating their role in maintaining gut health through lowering liminal pH and digestion of complex polysaccharides. *S*T challenge decreased the abundance of *Trabulsiella*, *Oscillospira*, *Holdemania*, *Coprococcus*, *Bifidobacterium* and *Lactobacillus* and increased *Klebsiella* and *Escherichia*, indicating its role in cecal dysbiosis. Measured by linear discriminant analysis effect size method (LEfSe), group specific biomarkers were affected by probiotics supplementation and *S*T challenge. Although probiotics supplementation positively modulated the cecal microbiota, they were not effective in significantly (P>0.05) reducing *S*T load in cecal tissue and invasion into vital organs such as, liver and spleen. Probiotics supplementation positively modulated the gut microbiota, while the *S*T challenge disrupted the abundance of vital microbial communities. The early colonisation of laying chick ceca by probiotics had the potential to positively influence luminal microbiota; however, the microbial abundance and diversity were not sufficient to significantly reduce the shedding of ST in feces or invasion into internal organs during this study.

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Host nutrition manipulation for gut microbiota modulation

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The gut microbiota is intimately linked with the host in healthy and disease states, and there is great interest in diet supplementation with microbial-accessible carbohydrates (MAC) to promote microbial activity that confers host health benefits. However, MAC-supplemented diets do not consistently produce desired microbial and host responses. We propose that this variability is because the outcome of microbial competition for MACs is dependent on the availability of other required nutrients.

We categorised carbohydrates into four functional types based on host- and microbiota-accessibility: host-accessible simple (HAS), host-accessible complex (HAC), host-inaccessible microbiota-accessible (HI-MAC), and inaccessible carbohydrates (IC). To test the microbial and host response, we designed 40 defined diets that varied in the relative abundance of these carbohydrates, protein and caloric density. These diets were investigated sequentially in a mouse model, with a subset of diets tested in a host disease state using a DSS-induced colitis model.

Notably, we found that specific carbohydrates had distinct effects on the microbiota and host depending on the protein and energy intake. **Gut environment:** Using blood in stool as an indicator of intestinal epithelial health, consumption of HAC improved gut health at high carb:prot diets, but was detrimental high prot:carb diets. **Microbial composition:** *Bifidobacterium* and *Lactobacillus* are common targets of prebiotics and both taxa increased in abundance with HI-MAC intake, but *Bifidobacterium* dominated at low protein intake and *Lactobacillus* at high protein intake. **Host health:** MAC intake increased the severity of DSS-induced colitis, and symptoms were exacerbated at a high prot:carb dietary context.

We show that the outcome of MAC-supplemented diets cannot be predicted as beneficial or detrimental without knowledge of the diet context, microbial composition and gut health. We conclude that identifying specific conditions to accompany diet manipulation will increase the success in engineering desirable microbial and health outcomes.

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The Antimicrobial side of Antidepressants. A cause for Anxiety?

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The gut microbial diversity has been strongly associated with the occurrence of mood relating disorders including major depressive disorder (MDD). While the causality of this relationship is being investigated, the role of gut microbiota in the treatment of MDD has been somewhat overlooked. Currently, the dominant treatment for patients suffering from MDD is selective serotonin reuptake inhibitors (SSRIs), administered to ~10% of the global population albeit at very poor efficacy. In this presentation we provide a critical assessment of putative mechanisms by which physiologically relevant concentrations of SSRIs influence the gut microbiota and the implications that might have on mood disorders. First, an estimation of gut SSRI concentration is computed based on the pharmacokinetic properties of SSRIs and the temporal nature of their gastrointestinal transit. These concentrations are then used for an *in vitro* analysis of aerobic culturable gut microbes. The analysis reveals a significant change in microbial diversity and metabolic profile following SSRI supplementation. Analysis of individual isolates shows three types of response to the antidepressant: increased, reduced and unmodified growth rate. The antimicrobial mechanism of SSRIs is currently unknown however *in silico* analysis indicates that potential mechanisms might include inhibition of efflux pump or inhibition of amino acid

transporters. Most importantly it is also unknown whether the antimicrobial effect of SSRIs serves to enhance or decrease efficacy of treatment. This raises important issues regarding the role that gut microbiota play in the treatment of mood related behaviours, which holds substantial potential clinical outcomes for patients suffering from MDD and other mood relating disorders.

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The chitin synthase concert: orchestration of chitin synthesis at septation sites in *Candida albicans*

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During fungal cell division, cytokinesis is followed by the formation of a cross-wall, the septum, which is made of chitin and acts as a stabilising barrier between the mother and bud in yeast cells, and between compartments in hyphae. There is an absolute requirement for chitin in the synthesis of the septum for growth and viability of all fungi. However, surprisingly little is known about the mechanism of septum formation in fungi. A thorough understanding of the process of fungal septation and how it is regulated may therefore lead to the identification of tractable drug targets for future antifungal chemotherapeutic strategies.

Candida albicans is the major fungal pathogen of humans and can grow in both a yeast and hyphal form. During cell division, a primary chitinous septum is synthesised by four chitin synthase (Chs) enzymes, Chs1, Chs2, Chs3 and Chs8, all four of which are localised to sites of septation prior to cytokinesis. To understand how the four Chs enzymes work together to synthesise chitin at division sites, strains expressing pairs of fluorescently tagged Chs enzymes were constructed. These were imaged by live-cell fluorescence microscopy to elucidate the temporal and spatial distribution of the chitin synthases in relation to each other. GFP pull-downs were carried out to identify proteins which interact with the Chs enzymes and contribute to septum formation.

We demonstrate that the timing of recruitment to and the configuration of the Chs enzymes at septation sites is different in *C. albicans* yeast and hyphal cells, and that the Chs enzymes may interact with different sets of proteins in yeast and hyphal cells. Based on this information, we propose a new model for chitin synthesis during septation in *C. albicans* yeasts and hyphae which may be used to inform our understanding of septation in all fungi and allow us to exploit these essential processes in order to combat fungal infections.

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Latent *Toxoplasma* relies on effector proteins to prevent IFNg-mediated cell death

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Toxoplasma, the causative agent of toxoplasmosis, chronically infects up to a third of the world's population and can lead to acute disease reactivation in the immunocompromised and cause progressive blindness in otherwise healthy individuals. Latent forms reside in the muscle and CNS where they are able to persist for life, and how this stage is able to avoid immune clearance and cause changes in the physiology of the brain is not known. We have recently begun investigating how *Toxoplasma*, in particular, latent bradyzoite forms manipulate their host cell. We show that bradyzoite-cysts drastically alter the host transcriptional program via effector protein export. We have identified the first effector protein in bradyzoite stages that accumulates in the host cell nucleus and blocks IFNg signalling. Furthermore, we demonstrate protein export is critical for protecting bradyzoite infected host cells from undergoing IFNg-mediated programmed cell death, thus enabling persistence. This work provides the first evidence of the mechanisms used by *Toxoplasma* bradyzoites for their long-term survival and identifies host cell pathways manipulated by these latent forms.

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Dissecting the dual functionality of the Tox3 effector protein from the wheat pathogen *Parastagonospora nodorum*

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It had long been thought that necrotrophic plant pathogenic fungi use a barrage of lytic enzymes to break down host cells releasing nutrients for growth. However, in recent years it has emerged that some necrotrophic fungi facilitate disease through a strict gene-for-gene mechanism as observed in biotrophic pathogens. For the wheat pathogen *Parastagonospora nodorum*, the basis of this host specific interaction is small cysteine-rich effector proteins secreted during infection (ToxA, Tox1 and Tox3). These effectors interact with specific dominant susceptibility genes in the host leading to a programmed cell death response and disease. However, whilst we now understand the requirement of these effector proteins for disease, their modes of action remain poorly understood.

To characterise these necrotrophic effectors, a search for potential host protein binding partners for the Tox3 effector was conducted. From this work, the wheat TaPR1-1 protein was validated through three independent approaches to interact with Tox3. Subsequent analysis confirmed that Tox3 was able to interact with most acidic and basic TaPR1 proteins in wheat, but not

those harbouring a C-terminal extension. We have now generated high-resolution crystal structures of several PR-1 proteins as well Tox3 and are using these dissect the basis and function of this protein interaction.

In this talk I will present our latest findings on dissecting the dual functionality of the Tox3 effector protein. Together with its function in causing cell death through its interaction with Snn3, we demonstrate that Tox3 has an important role in mediating PR-1 defence signalling and is required for disease development. These data have not only significantly advanced our understanding of necrotrophic diseases, but also provided a rare insight into the function and mechanism of the enigmatic plant PR-1 proteins.

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Parasites of land and sea: Comparative genomics of microbial eukaryotes *Perkinsus olseni* and *Theileria orientalis*.

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Perkinsus spp. are protozoan parasites that cause enormous losses to marine mollusc populations worldwide. In Australia, *P. olseni* has been linked to severe reductions in abalone populations. Significant and devastating reductions of NSW wild abalone populations have been observed since the 1990s, which has been attributed to a *P. olseni* outbreak (Liggins and Upston, 2010). In WA, *P. olseni*has been identified in several farmed and wild mollusc species and is considered a major threat to the state's expanding abalone industry. As part of a larger project targeting this important parasite, we examined five *P. olseni* isolates and one *P. chesapeaki* isolate from local and international sources using Illumina short-read sequencing. We additionally sequenced an Australian isolate of *P. olseni* with an Oxford Nanopore MinION to produce a reference genome. Examination of these sequences has revealed differences in genome size and identity between isolates sourced from the Southern and Northern hemispheres. Notably, analysis of sequence read coverage indicates that regions commonly targeted for diagnostic quantitative PCR assays are potentially subject to substantive gene duplications that vary considerably between isolates.

Theileria orientalis Ikeda genotype is a tick-borne haemoparasite that can cause ill-thrift and anaemia in cattle. The introduction of this pathogenic genotype to naïve cattle in the early 2000s caused significant damage to cattle producers in Australia and the South Pacific (Watts et al, 2016). We previously sequenced an Australian-sourced Ikeda plus two benign genotypes (Chitose and Buffeli) and identified differences that could establish these genotypes as separate *Theileria* species (Bogema et al, 2018). However, the short-read-derived assemblies for these genotypes were severely fragmented. To further explore the genomic diversity of *T. orientalis* in Australia, we sequenced an additional 21 isolates of *T. orientalis* Ikeda variant from diverse locations and time-points. We also used nanopore sequencing technology to greatly improve the contiguity of Ikeda, Chitose and Buffeli genome sequences.

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Potential applications of vinasse as low-cost culture media for fungi

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Vinasse is a distillery effluent which is produced during sugarcane molasses-based ethanol fermentation. Although numerous researches have been conducted on potential applications of vinasse, it's still underutilized in practice. This study evaluated the potential use of vinasse in preparing growth media for ethanologenic yeast and cellulolytic fungi that are used in ethanol production from sugarcane bagasse. Vinasse agar was prepared with two vinasse concentrations (50% & 100% v/v). Two *Saccharomyces* isolates (Y4 and Ysev) were serially diluted in sterile saline (0.09%) and the 10^{-5} dilution was plated in triplicate, while yeast extract peptone dextrose (YEPD) agar was the positive control. The cultures were aerobically incubated at 37 ˚C for 24 hours. 100 µl of *Trichoderma viridae* spore suspension (1×10^{8}) was inoculated in to a series of vinasse solutions (0% to 100%, 30 ml) containing sugarcane bagasse (1.0 g) as the carbon source. After one week of incubation at 30 ˚C with 120 rpm shaking, the total cellulase activity of crude enzyme extracts were determined. Park's cellulase production medium was the positive control. In vinasse agar, the highest growth of 1.13×10^{4} CFU/ml and 1.00×10^{4} CFU/ml were observed in Ysev-cultured, 100% and 50% vinasse agar media respectively. Its growth in YEPD agar (1.05×10^{4}CFU/ ml) wasn't significantly different from values at 100% and 50% vinasse. However, Y4's growth in vinasse agar was significantly lower than Ysev. In 50 % vinasse agar, 8.33×10^{3}CFU/ml of Y4 was observed which was not significantly different from 8.6×10^{3} CFU/ml growth in YEPD agar. The lowest growth was observed in Y4 as 4.87×10^{3} CFU/ml in 100% vinasse agar. The growth in vinasse was isolate-dependent. The highest total cellulase activity of *T. viridae* was observed at 50% vinasse as 1.46 FPU/ml whereas in Park's medium it was only 0.859

FPU/ml. These results show that vinasse has facilitated the growth of yeast isolates and *T. viridae* which proves the potential application of vinasse in formulating low-cost culture media for fungi.

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Chagas disease in Australia: What are the risks?

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Introduction: Chagas disease is a zoonotic tick-transmitted parasitic disease endemic in Latin American countries. In non-endemic countries it is mainly transmitted congenitally or via blood transfusion. There is an increased risk of spread outside South America as a result of migration of asymptomatic parasitemic individuals. A current WHO initiative is to identify strategies to reduce transmission in non-endemic countries to zero. While potential vectors occur in northern Australia only two cases in immigrants from South America have been reported here. The aims of this study were to estimate the risks for congenital and for transfusion transmission of Chagas disease in Australia.

Methods: Census data (2017) on immigration to Australia from South America, births and country of birth of the mother, as well as prevalence in source countries were used to estimate the risk of congenital transmission. The risk of a parasitemic donation was estimated on the basis of reported data from Canada.

Results: Following published methodology it was estimated that 3.37% of South American immigrants were potentially parasitemic, representing 5,971 individuals in Australia, with 2,023 females of childbearing age. For those females, it was estimated that there would be 90 births annually with 4.5 exposed, potentially parasitemic newborns. Based on published data from the Canadian Blood Service, it was estimated that 199 exposed potentially parasitemic individuals are likely to present to donate blood each year, with a risk of 3.6 antibody-positive donations.

Discussion: This study suggests that the risk both of congenital transmission and transfusion transmission is very low in Australia. In addition there is universal leukodepletion of the blood supply in Australia and published data suggests that this filtration step removes the trypanosomes with the result that there is zero risk.

While in Australia identifying Chagas disease in immigrants from endemic regions of South America is an emerging challenge for general practitioners, the risks for secondary congenital or transfusion transmission here are very low.

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Inflammation and the Microbiome:A Dangerous Liaison

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The implementation of highly active antiretroviral therapy for HIV disease led to dramatic improvements in patient morbidity and mortality. That said in many HIV patients who had good CD4 T cell recovery and viral suppression there still existed persistent level of immune activation and inflammation. These changes in the immune system have been linked to persistent alterations in gastrointestinal tract permeability and microbial translocation .More recent studies have focused on the cause of these gut abnormalities and found they are linked to alterations in the gut microbiome. This presentation will provide novel data on the changes seen in the gut microbiome in HIV subjects highlighting the alterations in specific bacterial species and how the bacterial metabolic profile leads to persistent systemic inflammation especially with the loss of short chain fatty acid producing bacteria. Further data will focus on the critical changes in the tryptophan catabolic pathway and its pro inflammatory profile that contributes to non HIV co morbidities especially cardiovascular disease diabetes and neurocognitive outcomes. The additional metabolic profiles of the gut bacteria will be discussed including changes in TMAO and ceramides Data will be presented utilizing animal models to demonstrate the critical role of diet in modifying the host microbiome. The talk will conclude with a discussion of the current therapeutic paradigm for modifying the host microbiome with pre or probiotics and the prospective of fecal transplants in HIV The talk will conclude with a detailed description of the inflammatory pathways contributing to non HIV co morbidities and what novel interventional strategies are being utilized as interventional approaches. This will include studies evaluating immune based therapies targeting IL6 or IL1-beta and Jak stat inhibitors and methotrexate. In addition there will be a discussion of repurposing drugs from other therapeutic areas such as diabetes with new trials being pursued with metformin.

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Current and Future Trends in Diagnostic Microbiology

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Evolving clinical needs in infectious diseases and fluctuating healthcare trends, such as changing practice patterns and improved standard-of-care, have moved the need for rapid and more accurate diagnostics to the forefront of clinical microbiology. Laboratory consolidation, along with the shrinking technical workforce, has led to technological advances, including total laboratory automation to maximize efficiency from specimen processing through to results. The increased availability of matrix-assisted laser desorption/ionization time of flight mass spectrometry has revolutionized the clinical microbiology laboratory allowing rapid and sensitive microbial identification, as well as the potential for antimicrobial susceptibility testing and microbial typing. Syndromic molecular panels allow for the rapid detection of a large number of potential pathogens from a single specimen and have improved laboratory efficiency. Combined with antimicrobial stewardship these assays have improved patient care. The moderate complexity of these platforms also provides the potential for on-demand and near patient testing. Several point-of-care platforms are also now available which will help to improve access to healthcare in remote areas. Globally, increasing antimicrobial resistance among gram-negative pathogens challenges both clinicians and laboratorians. Rapid molecular platforms that detect ESBL and carbapenemase genes enhance surveillance. Several companies have either marketed or are in the process of developing, rapid, direct phenotypic antimicrobial susceptibility tests, allowing for targeted therapeutic decisions in less than 24 h. Single molecule technologies are being exploited to enhance enzyme immunoassays to improve *C. difficile* toxin testing and other diseases. Future technologies, including next-generation sequencing (NGS), will also provide exciting opportunities for fast and accurate species identification, resistance and virulence testing, and monitoring for the emergence and spread of a variety of pathogens. Machine learning and artificial intelligence will optimize diagnostic platforms and bioinformatics. Novel biomarkers and assays that assess host response will direct both diagnostic and therapeutic strategies. These exciting new tools will allow clinical microbiologists to view microbial infections from a new perspective, giving rise to precision medicine that will directly affect the well-being of patients.

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Exploiting the struggle for haem: a new respiratory probiotic candidate?

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Nontypeable *Haemophilus influenzae* (NTHi) is a leading causative organism of opportunistic upper and lower respiratory tract infections, including otitis media, and exacerbations of chronic obstructive pulmonary disease. The rapid development of antibiotic resistance has complicated treatment, with infections frequently proving refractory to first- and second-line antibiotics. Development of preventative vaccination strategies has also proven unsuccessful. An alternative approach may be probiotic therapy that prevents nasopharyngeal colonisation, the prerequisite step of NTHi infection.

Newly discovered strains of the closely related, *Haemophilus haemolyticus* (Hh) present a promising probiotic candidate. These strains of Hh not only share the nasopharyngeal niche, but also produce a novel inhibitory substance, hemophilin (HPL) that restricts the growth of NTHi *in vitro*. Ongoing research indicates the inhibitory effect of HPL is due to its capacity to bind haem and limit the organism's access to the essential growth factor. Disruption of haem acquisition has previously been shown to significantly dampen the pathogenic capabilities of NTHi.

This research aims to determine the therapeutic potential of HPL-producing strains of Hh (Hh-HPL) in the prevention of NTHi infections through both *in vitro* and *in vivo* investigations. Data from oropharyngeal swabs of 150 healthy adults demonstrates a potential protective benefit against NTHi colonisation in individuals carrying Hh-HPL. The incidence and density of NTHi carriage was reduced by 52% and 68% in individuals co-colonised with Hh-HPL, respectively. This potential protective benefit was further explored using short-term *in vitro* competition studies. The growth rate of NTHi was significantly reduced by 66-84% ($p<0.0001$) when co-cultured with Hh-HPL, but not Hh strains unable to produce HPL. Interestingly, exposure to NTHi increased the growth rate of Hh-HPL by 44-66% ($p<0.0001$). The competitive advantage of Hh-HPL was also maintained during long-term fitness studies. This preliminary data shows that Hh-HPL may be a potential probiotic candidate for the prevention of NTHi infections.

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A multi-component toxin from *Bacillus cereus* activates the NLRP3 inflammasome

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Recognition of microbial components by the host serves as a cornerstone in mediating an effective immune response. Innate immune sensors and the inflammasome detect both intracellular and extracellular micro-organisms. The inflammasome is an intracellular signalling complex comprising of a sensor, an adaptor protein ASC and the cysteine protease caspase-1. Inflammasomes regulate secretion of the pro-inflammatory cytokines IL-1β and IL-18 and induction of a cell death pathway known as pyroptosis. A central question in the field is how extracellular bacteria are sensed by the inflammasome in the cytoplasm? To address this question, we analysed a panel of clinically important intracellular and extracellular bacteria and identified an unknown secreted factor from the foodborne bacterium *Bacillus cereus* that uniquely activates the inflammasome without gaining cytosolic access. The tripartite enterotoxin called haemolysin BL (HBL) was identified as the novel activator of the NLRP3 inflammasome. The multi-component toxin assembled in a specific and linear order on the mammalian cell membrane to form a lytic pore, which induces potassium efflux and activates the NLRP3 inflammasome. Furthermore, HBL-producing *B. cereus* induced rapid lethality in host within 20h of infection via activation of the NLRP3 inflammasome. *B. cereus*-induced lethality was completely abrogated with administration of the NLRP3 inhibitor, MCC950. Overall, our results indicate that intracellular sensing of a toxin secreted by an extracellular bacterium is critical for the innate immune recognition of infection. Therapeutic modulation of the inflammasome can facilitate in prevention and treatment of fulminant bacterial infections.

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The not-so-sterile womb: Evidence that the human fetus is exposed to bacteria prior to birth

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Background: The human microbiome includes trillions of bacteria, many of which play a vital role in host physiology. Numerous studies have now detected bacterial DNA in first-pass meconium and amniotic fluid samples, suggesting that the human microbiome may commence *in-utero*. However, these data have remained contentious due to underlying contamination issues.

Method: Here, we have used a novel method for reducing contamination in microbiome workflows to determine if there is a fetal bacterial microbiome beyond the level of background contamination. We recruited 50 women undergoing elective caesarean section with no evidence of intra-uterine infection and collected first-pass meconium and amniotic fluid samples. Full-length 16S rRNA gene sequencing was performed using PacBio SMRT cell CCS technology, to allow high resolution profiling of the fetal gut and amniotic fluid bacterial microbiomes. Levels of inflammatory cytokines were measured in amniotic fluid, and levels of immunomodulatory short chain fatty acids (SCFAs) were quantified in meconium. Propidium monoazide (PMA) was used to test the viability of bacteria detected in meconium samples.

Results: All meconium samples and most amniotic fluid samples (84%) contained bacterial DNA. Meconium contains a low diversity and low-biomass microbiome, which was remarkably variable between patients. Importantly, PMA testing confirmed that this community consisted of viable bacterial cells. The amniotic fluid microbiome was more diverse and contained mainly reads that mapped to typical skin commensals. All meconium samples contained acetate and propionate, at ratios similar to those previously reported in infants. Neonates born from mothers with Type 2 diabetes had significantly lower levels of propionate in their meconium compared to those born from mothers with normal pancreatic function (P=0.005) or from mothers with gestational diabetes (P=0.003). These differences were not associated with alterations in the fetal microbiome, suggesting that they may be driven by the maternal microbiome.

Conclusions: Our results demonstrate that viable bacterial cells and SCFAs are present *in-utero*, and have the potential to influence the developing fetus.

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Between a rock and a hard place: *Acinetobacter baumannii* loses virulence and antibiotic resistance when escaping killing by lytic phages.

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Global health is facing the immense threat of antibiotic resistance, and new therapeutic strategies are urgently needed. In the hospital setting, *Acinetobacter baumannii* is frequently responsible for multidrug-resistant (MDR), frequently-fatal infections. The use of phages, viruses that infect and kill bacteria, is an approach gaining considerable interest due to its versatility in treating MDR infections. Although phage therapy can quickly kill bacterial pathogens, resistance to phages arises rapidly. We isolated phages from sewage samples and demonstrated their lytic activity against two clinical strains of *A. baumannii*, AB900 and A9844. Co-incubation of the phages with their hosts resulted in the emergence of phage-resistant bacterial mutants, in which we identified the presence of remarkable fitness costs, including impaired growth, reduced production of capsule polysaccharides and decreased biofilm formation. As a result, phage-resistant isolates demonstrated a diminished capacity to colonise blood, liver, kidney and spleen within a BALB/c mouse model. Most importantly, we observed that phage-resistance was associated with a 2-fold reduction in the minimum inhibitory concentration (MIC) of the antibiotics ampicillin/sulbactam, ceftazidime and minocycline. Comparative bacterial genome analyses identified SNPs in genes coding for outer membrane proteins, suggesting their association with phage infectivity and fitness costs. We propose a practical therapeutic strategy whereby phages are firstly utilised to target MDR *A. baumannii* infections, followed by the predicted emergence of phage-resistant isolates exhibiting fitness costs that can be exploited using repotentiated antibiotics and the immune response. Our findings open the door for the clinical use of phage-antibiotic combinational therapy against MDR *A. baumannii*.

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Nitroxides: a promising therapeutic strategy for the treatment of *Staphylococcus aureus* biofilm-related infections.

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Introduction: *Staphylococcus aureus* is a leading cause of nosocomial infections, particularly medical device-associated infections, causing significant morbidity and mortality among hospitalised patients.[1, 2] Bacterial attachment to medical devices and biofilm formation are major drivers of failing antibiotic therapy and the persistence of chronic infections by methicillin-resistant *S. aureus* and methicillin-sensitive *S. aureus*.[3] Thus, there is an urgent need for novel treatment and eradication strategies for *S. aureus* biofilms.

Methods: Synthetic organic chemistry was utilised to produce a new generation of nitroxide functionalised fluoroquinolone-based antibiotics with *S. aureus* biofilm eradication capabilities. Several novel biofilm-eradication agents and two commercially available compounds (CTEMPO and ciprofloxacin) were tested for biofilm eradication and/or dispersal in the MBEC^{TM} device, a reproducible high-throughput static biofilm formation system. Mature *S. aureus* biofilms were treated with serial dilutions of the specific test agent(s) and recovered cell numbers were quantified by plating for viable cell counts.

Results: Nitroxide-mediated biofilm dispersal significantly reduced *S. aureus* biofilm viable cell density at low concentrations (8 µM). Synergistic co-treatment with ciprofloxacin and CTEMPO (nitroxide) drastically improved the biofilm-eradication activity of ciprofloxacin (Minimal Biofilm Eradication Concentration (MBEC) of ≤4096 µM for ciprofloxacin versus ≤256 µM for ciprofloxacin plus CTEMPO). Synthetically incorporating a nitroxide into the base structure of ciprofloxacin improved its biofilm-eradication activity by 64-fold (MBEC ≤64 µM).

Conclusions: Fluoroquinolone-nitroxides are a new class of antibiotic which can disperse and eradicate *S. aureus* biofilms at low concentrations. Consequently, they represent a promising new therapeutic strategy which could lead to an effective treatment for *S. aureus* biofilm-related infections.

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Exploring merozoite surface protein 2 function and vaccine potential during malaria red blood cell invasion.

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Malaria parasites that invade and multiply in red blood cells (RBCs) cause >200 million cases of disease each year, with children <5 years most at risk of dying from infection. *Plasmodium falciparum* parasites cause the majority of malaria related mortality. Development of a highly efficacious vaccine is essential for long-term malaria control and eradication. Blocking the malaria parasites' merozoite from entering the RBC prevents establishment of parasite infection and growth of the disease-causing stage of the lifecycle. Vaccines raised against *P. falciparum* merozoite surface protein 2 (MSP2) have been shown to protect against infection in a clinical trial. To characterise the role of *Pf*MSP2 and its potential as a vaccine target, a transgenic line expressing *Pf*MSP2 under control of an inducible knockdown system was generated and the impact *Pf*MSP2 loss of function assessed. While substantial knockdown of *Pf*MSP2 protein expression is achieved parasite growth remains unperturbed, suggesting minimal *Pf*MSP2 is required for growth. An initial screen of sera from malaria immune Papua New Guinean adults using this *Pf*MSP2 knockdown line allowed us to explore levels of neutralising and complement-activating antibodies in the immune sera directed against *Pf*MSP2 and total merozoite antigens. The development of these tools aid our understanding of the biological function of *Pf*MSP2 and highlight the utility of transgenic parasite lines in studying antibody responses against major vaccine candidates.

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Nanostructured antibacterial surfaces

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In this invited talk, I will discuss recent advanced coming from my laboratory on the development of antibacterial technologies and coatings.

Infections are a substantial problem in healthcare. Of particular significance are infections associated with medical devices since they are notoriously difficult and costly to treat. Many of these infections begin with the attachment of an individual planktonic bacterial cell to the surface of the device. This cell(s) then divides and expresses extra cellular polymers which ultimately results in the formation of a biofilm. Once biofilm is formed, it becomes very difficult to eradicate the bacteria. The biofilm also protects the bacteria from antibiotics and the host immune system, and contributes to development of antibiotic resistance.

Thus, the goal of our work is to prevent the initial attachment of planktonic bacteria to the surface of the device. We have generated four distinct classes of antibacterial surfaces that are capable of protecting a surface from bacterial colonisation and are suitable for application on various medical devices. The surfaces are classified based on their mechanism of action as a) non-sticky, b) contact killing, c) antimicrobial compound releasing and d) stimuli responsive. As means to create antibacterial surfaces we used silver nanoparticles, conventional antibiotics, quaternary ammonium compounds, nitric oxide release, antibacterial polymers and peptides. Many of our approaches are facilitated by a technique called plasma polymerisation which allows us to place a coating on any type of medical devices regardless their shape and size or the material it is made of. Some of these coatings have intrinsic antifouling properties which allow us to prevent biofilm formation without using any toxic compounds. Other coatings can be used to attach or load antibacterial agents. In my talk I will describe how we create all these antibacterial surfaces and what are their benefits and drawbacks. An approach that we are currently applying for commercial implantable devices will also be discussed.

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Development of antimicrobial contact lenses

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Contact lenses offer an excellent alternative to spectacles to correct refractive errors. Indeed, they are superior to spectacles in many ways offering better peripheral vision and also the possibility of preventing the progression of refractive errors such as myopia. However, wearing contact lenses can cause ocular inflammation and infection. If not treated quickly and with appropriate antibiotics, the infections can progress rapidly and lead to loss of vision, even the whole eye. Contact lens-induced inflammation and infection are most commonly caused by bacteria, initially adhering to contact lens surfaces. Thus, creating antimicrobial contact lenses has the potential to reduce these adverse responses and make contact lens wear safer.

We have produced contact lens coated with a cationic antimicrobial peptide, melimine. Melimine is a membrane active antimicrobial that causes cell lysis. Importantly, growth of bacteria in sub-inhibitory concentrations does not result in bacteria becoming resistant. Laboratory experiments have shown that these lenses are active against *Pseudomonas aeruginosa(*incl. multi-drug resistant strains), *Staphylococcus aureus*(incl. MRSA), *Serratia marcescens*, *Candida albicans,* and *Acanthamoeba*sp.

After safety testing using standard *in vitro* and *in vivo* (animal) tests, the lenses were tested in 1-day trials using human subjects. There were no adverse clinical responses other than slight increase in corneal staining. Due to this, we redesigned the peptide to produce a shorter version, Mel4, that was also safe to wear, and produced no staining when attached to contact lenses. Mel4-coated lenses have finished large scale Phase II/III clinical trials with subjects wearing lenses for 3 months on a 14-day lens replacement schedule. These lenses reduced the incidence of ocular inflammation by 50%.

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The clinical relevance of anti-biofilm coated devices against *Candida* species

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Biomedical device associated infections are increasingly prevalent, and the incidence of fungal *Candida* associated infections has notably increased in recent history causing 45 % of hospital acquired infections in Australian Intensive Care Units. Prevention of this common fungal pathogen from adhering to these biomedical devices using antifungal surface coatings is paramount in minimising these events particularly in intensive care units and immunocompromised patients.

Using a novel antifungal coating, caspofungin covalently-tethered to medically-relevant biomaterials, we have demonstrated significant activity against pathogenic biofilm-producing *Candida* species including *Candida albicans, Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei* which are contributing to polymicrobial implant infections. The surface activity of a tethered antibiotic may seem unconventional and therefore we have investigated alternative hypotheses for its activity, such as whether caspofungin is released from the surface. However, we present evidence from a mounting body of experimental data from surface chemical analysis and biological studies that strongly supports a contact-killing mechanism of action which likely involves the surface interaction and disruption of the fungal cell wall.

In order to transition the surface to *in vivo* testing we have undertaken intensive *in vitro* analysis including kinetic microscopy, biofilm quantitation through the static biofilm assay and investigated the sterilisation by autoclave and the storage conditions of the surface. The coating has also proven to be effective when coated on a variety of medically-relevant biomaterials including polypropylene, polycarbonate, polyethylene terephthalate (PET), polystyrene, and glass with activity against biofilm formation of five *Candida* species. These results successfully indicate that this coating has the potential to perform well in *in vivo* and clinical trials, and good potential for commercialisation.

We conclude that the caspofungin-attached polymer coatings are an effective deterrent in biofilm formation in vitro. Therefore, these surfaces show promise as a coating for minimising Candida spp. adhesion, colonisation and subsequent biofilm formation and infections on biomedical line devices and therefore warrants further investigation *in vivo*.

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Liquid Metal Particles Used to Treat Pathogenic Mature Biofilms *via* Magneto-Physical Activation

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Bacterial biofilms related infections are a significant medical issue, responsible for both large economic costs and serious health risks to patients. Once established biofilms are difficult to treat, as the presence of extracellular polymeric substances (EPS) and complex self-organised bacterial communities provides a protective environment for the pathogens. This is particularly true at the tissue-implant interface, as the biofilm often inhibits antibiotics treatment, meaning that the complete removal often required revision surgery. Recently, stimuli-activated nanotechnology-based treatments, such as photocatalytic, photothermal and magnetic hyperthermia nanoparticles have shown promise towards disrupting mature biofilm structures. However, such studies were unable to facilitate complete biofilm disruption. In this study, the applications of biocompatible liquid metals (LM), with magneto-physical antibacterial properties are investigated as a new class of stimuli-activated biofilm treatment. Particularly, Galinstan nanoparticles magnetically functionalised with low-weight ratio magnetic iron (Fe) inclusions are exploited as magnetic responsive materials. When exposed to a rotating magnetic field these particles move at high speeds and undergo a shape transformation from spheres to high aspect ratios rods, irregular spheroids, and “nano-stars” which can physically rupture and remove pathogenic bacteria from a model surface. The magneto-physical antibacterial activity of these LM particles is tested against a range of single and co-colonised infectious biofilms of common pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*). Furthermore, the concentration of the magnetic Fe inclusion was varied in an effort to optimise the rate of antibacterial efficacy. This approach has paved the innovative way to treat the biofilm-related infections for the future applications.

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Antibiofilm activities of pentacyclic triterpenoids against methicillin-resistant and biofilm-forming *Staphylococcus aureus* (MRSA)

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has been constantly evolving and developing resistance against conventional antibiotics. One of the key features of MRSA that enables it to develop resistance to antibiotics and host immune system is its ability to form biofilm. Biofilm acts as a protective barrier and provides protection for the cells within the biofilm. Current treatment for biofilm-associated infections mainly involves surgical removal of the colonised implants which may further expose patients to hospital-associated infections. Hence, alternative therapeutics are urgently needed. Pentacyclic triterpenoids have long been used in traditional medicine and possess a wide range of pharmacological effects. In previous studies, the antimicrobial activity and mechanisms of action of three known compounds, α-amyrin, betulinic acid and betulinaldehyde against planktonic cells of MRSA were determined and elucidated. However, the antibiofilm effects of these compounds are still poorly understood. Hence, this study was carried out to evaluate the antibiofilm activities of the three compounds, as well as combinations of these compounds and oxacillin or vancomycin in pre-formed biofilms using the crystal violet and resazurin assays. The results showed α-amyrin significantly reduced the biofilm in the reference strain of biofilm-forming MRSA, while vancomycin showed significant reduction in the metabolic activity. There were no synergistic effects on the biofilm eradication in the selected combinations of the compounds and antibiotics evaluated.

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Strategies for Combatting the Formation of Fungal Biofilms on Biomaterials Surfaces

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Polymicrobial biofilms can be formed on surfaces by microbial colonisation of materials such as biomedical implant devices and are caused by bacterial or fungal species, or both kinds of microbes. In the last two decades, research has mostly been focused on bacterial infections and anti-bacterial surfaces. Although fungal infections related to biomaterials are increasing and threatening human lives, this field has received comparatively little interest. Attachment of fungal cells onto biomaterial surfaces is the first step for biofilm formation, hence, this initial attachment should be prevented.

One strategy to combat fungal biofilms is to prepare coatings with covalently bound, FDA-approved antifungal agents such as echinocandins and polyenes on the biomaterial surfaces, for long-lasting effects that are non-toxic to human cells. Using plasma polymer interlayers is a convenient strategy to functionalise surfaces with desired chemical groups allowing straightforward immobilisation of bioactive molecules on various solid surfaces. Results show echinocandins retain activity when covalently bound onto the surface which indicate they can disrupt the cell wall integrity of fungal cells. Also it has been found that these coatings can be reused several times while still maintaining efficiency against fungal cells. In contrast, polyenes do not have antifungal activity when are covalently attached onto the surface because surface attachment prevents them from reaching their cell membrane target.

A second strategy is to encapsulate and release low molecular weight antifungal molecules such as azoles. Loading fluconazole, from the approved class of azoles which are able to target cell membranes and intracellular parts of fungal cells, offers an avenue for creating coatings that can selectively deter fungal colonization while supporting mammalian cell attachment. As a carrier coating for releasing the drug, heptylamine plasma polymer is a good option for its simple deposition technique, good absorption of drug, and compatibility with innate immune cell function. Results of surface characterization techniques like XPS and TOF-SIMS and also microbiological assays will be discussed.

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Identification of estrogen receptor modulators as inhibitors of flavivirus infection

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Flaviviruses such as Zika virus (ZIKV), dengue virus (DENV) and West Nile virus (WNV) are major global pathogens for which safe and effective antiviral therapies are not currently available. To identify antiviral small molecules with well-characterized safety and bioavailability profiles we screened a library of ~2,900 approved drugs and pharmacologically active compounds for inhibitors of ZIKV infection using a high-throughput cell-based immunofluorescence assay. Interestingly, estrogen receptor modulators Quinestrol and Raloxifene hydrochloride were amongst 15 compounds that significantly inhibited ZIKV infection in repeat screens. Subsequent validation studies revealed that these drugs effectively inhibit ZIKV, DENV and WNV (Kunjin strain) infection at low micromolar concentrations with minimal cytotoxicity in Huh-7.5 hepatoma cells and HTR-8 placental trophoblast cells. Since pre-treatment of cells with these drugs was associated with more potent antiviral effects and these cells lack detectable expression of estrogen receptors, these compounds appear to inhibit early events during flavivirus infection in a manner that is independent of their known effects on estrogen receptor signaling. Taken together, Quinestrol, Raloxifene hydrochloride and structurally related analogs warrant further investigation as potential therapeutics for treatment of flavivirus infections.

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Not available

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Not available

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Structural and non-structural roles of the matrix protein of respiratory syncytial virus

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Respiratory syncytial virus (RSV) matrix protein (M) is a major virion structural protein. Similar to matrix proteins of other negative strand RNA viruses, it was predicted to have a key role in virus assembly. Interestingly, we have found that M also has a role in the nucleus of infected cells. We have shown that RSV M is localized in the nucleus of infected cells early in infection, being mostly cytoplasmic later. In the nucleus, M has functions traditionally allocated to non-structural proteins, while in the cytoplasm it is the major organizer of RSV assembly.

Using various *in vitro*, infected and transfected cell systems, we have shown that M is imported into the nucleus via the Importin-b pathway and has a CRM-1 dependent nuclear export. Regulated, timely nuclear import and export into the cytoplasm is required for optimal virus assembly, and mutations that impact on these processes result in lowered infectious virus titres. In the nucleus, M appears to inhibit cellular transcription and any mutation that impacts on this ability results in delayed RSV replication and reduced infectious titres. In the cytoplasm, M interacts with the RSV nucleocapsid-polymerase complex (vRNP), inhibiting its transcription activity. Recent work from our lab suggests that M probably facilitates the movement of the now inactive vRNPs to the site of RSV assembly at the plasma membrane. M also associates with the envelope glycoproteins at lipid rafts, the site of RSV assembly. M probably brings the vRNP and glycoproteins together via dimerization to initiate the budding process. Any changes to M's dimerization ability impact RSV budding.

The data thus far show that M protein has nuclear and cytoplasmic roles that are essential for optimal virus replication, assembly and budding.

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Discovery of novel crustacean and cephalopod flaviviruses: insights into evolution and circulation of flaviviruses between marine invertebrate and vertebrate hosts

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Most flaviviruses (family, *Flaviviridae*) are disease-causing pathogens of vertebrates maintained in zoonotic cycles between mosquitoes or ticks and vertebrate hosts. Poor sampling of flaviviruses outside vector-borne flaviviruses, such as Zika virus and dengue virus, have presented a narrow understanding of flavivirus diversity and evolution. In this study, we discovered three crustacean flaviviruses (Gammarus chevreuxi flavivirus, Gammarus pulex flavivirus and Crangon crangon flavivirus) and two cephalopod flaviviruses (Southern Pygmy squid flavivirus and Firefly squid flavivirus). Bayesian and Maximum-Likelihood phylogenetic methods demonstrate that crustacean flaviviruses form a well-supported clade and share a more closely related ancestor to terrestrial vector-borne flaviviruses than classical insect-specific flaviviruses. In addition, we identify variants of

Wenzhou shark flavivirus in multiple gazami crab (*Portunus trituberculatus*) populations with active replication supported by evidence of an active RNA interference (RNAi) response. This suggests Wenzhou shark flavivirus moves horizontally between sharks and gazami crabs in ocean ecosystems. Analyses of the mono and dinucleotide composition of marine flaviviruses compared to flaviviruses with known host status suggest some marine flaviviruses share a nucleotide bias similar to vector-borne flaviviruses. Further, we identify crustacean flavivirus endogenous viral elements that are closely related to terrestrial vector-borne flaviviruses. Taken together, these data provide evidence of flaviviruses circulating between marine vertebrates and invertebrates, expand our understanding of flavivirus host range and offer potential insights into the evolution and emergence of terrestrial vector-borne flaviviruses.

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The role of viperin in dengue virus infection

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Our laboratory and others have previously shown that the interferon (IFN) stimulated gene, viperin has anti-viral actions against dengue virus (DENV) *in vitro* (1). Here we have extended this to study DENV induction of viperin *in vivo* and DENV replication in viperin deficient (vip-/-) mice.

Intracranial DENV infection in WT mice results in infection in the brain and eye and an increase in viperin mRNA quantitated by RT-PCR in both of these tissues. Subcutaneous DENV-infection in AG129, IFN-receptor deficient mice also results in increased viperin, demonstrating IFN-independent mechanisms for induction of viperin *in vivo*. In the absence of viperin, replication of DENV was enhanced in primary murine embryonic fibroblasts (MEF) derived from vip-/- mice, consistent with previous data (1). In contrast, DENV infection and RNA levels following subcutaneous or intracranial challenge were not significantly different between wild type (WT) and vip-/- mice. Interestingly, brain IL-6 mRNA levels were significantly higher following intracranial DENV infection in vip-/- than WT mice. Further analysis is underway to assess the effect of the lack of viperin on DENV-infection and inflammatory responses in the eye.

Thus, viperin has anti-viral actions against DENV *in vitro* and can be induced by DENV infection *in vivo* including through IFN-independent mechanisms and in tissues such as the eye. DENV infection is not altered in vip-/- mice suggesting compensation *in vivo f*or the lack of viperin, potentially by other anti-viral responses such as IL-6 production, that maintains immune control of DENV-infection.

(1) Helbig et al., (2013) PNTD 7:e2178

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CRISPR/Cas9 genome-wide KO screen reveals RACK1 as a critical pan-flavivirus host factor for virus replication

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Flaviviruses, such as Dengue (DENV), West Nile (WNV) and Zika (ZIKV) are major human pathogens that inflict a significant burden on society. Cellular proteins play important roles in all facets of the flavivirus lifecycle. Therefore, understanding viral-host protein interactions essential for the flavivirius lifecycle can lead to development of effective antiviral strategies.

A CRISPR/Cas9 genome-wide KO screen was employed to isolate novel host factors critical for ZIKV replication. Briefly, cells transduced with sgRNAs targeting every gene in the genome were infected with ZIKV and cells which survived ZIKV-induced cytopathic effect were PCR amplified and sequenced using Illumina NextSeq. Bioinformatics analysis identified previously characterised host factors (EMC1/EMC6) as well as a novel candidate, RACK1. RACK1 plays multiple roles in homeostatic cellular processes as a scaffold protein and acts as an indispensable hub for signalling transduction of multiple pathways. Interestingly, RACK1 was previously identified as essential for replication of several viruses (HCV, Pox virus).

siRNA knockdown of RACK1, followed by infection with flaviviruses ZIKV, DENV, WNV, POWV, TBEV confirmed that RACK1 has a critical role in viral replication. Interestingly, YFV doesn't require RACK1 for replication. RACK1 was shown to interact with multiple non-structural (NS) viral proteins, indicating a multifaceted role in ZIKV replication. More specifically, interaction of NS1 with RACK1 was shown via confocal microscopy to localise to the ER while knockdown of RACK1 prior to infection showed that NS1 localisation is significantly altered compared to infected wildtype cells. Current work is focused on electron microscopy imaging on flavivirus NS1-RACK1 interactions. Mutational studies of RACK1 will also give further insight into the signalling pathways important for RACK1-supported flavivirus replication. Collectively, these experiments suggest RACK1 is important for replication complex formation, a critical step in establishing flavivirus replication.

Further understanding of the intricate steps in viral replication establishment will advance knowledge of the flavivirus lifecycle and may potentially aid development of broadly acting antivirals against multiple flaviviruses.

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The Center for Infectious Disease Research and Policy: Antimicrobial Stewardship Project

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The CIDRAP Antimicrobial Stewardship project was launched in July 2016 with the goal of building an online international community focused on antimicrobial stewardship. CIDRAP-ASP is led by Dr. Michael Osterholm (a member of the U.S. Department of State Science Envoy) and advised by faculty experts that encompass human and animal health around the globe. I was fortunate to be a member of the founding team as the Outreach Coordinator where I engage with antimicrobial stewardship experts from government, academia, clinical settings and industry.

Antimicrobial resistance is a critical global public health issue, and antimicrobial stewardship strategies are key to curtailing the problem. However, open access to information that is credible, and collaborative is essential. CIDRAP's Antimicrobial Stewardship Project provides current, accurate, and comprehensive information including news, resources, podcasts and webinars on the topic and works to build an online community to address leading issues. This presentation will describe the progress and components of the Antimicrobial Stewardship Project including our focus on 'One Health, Diagnostics, Clinical Tools and Strategic Partnerships' with organizations and industry partners.

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Not available

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Not available

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Capsule-switching is associated with the recent global expansion of the fluoroquinolone-resistant *Escherichia coli* sequence type (ST)1193 clone

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Introduction:

Increasing resistance to third-generation cephalosporins and fluoroquinolones amongst uropathogenic *Escherichia coli* are of critical concern to public health. Resistance is mainly driven by extended-spectrum beta-lactamase (ESBL)-producing *E. coli* – particularly the sequence type (ST)131 C2/H30Rx sub-lineage, which is mostly responsible for pandemic cases. ST1193 is globally-disseminated and is second only to ST131 in terms of clinical prevalence. We sought to characterise the emergence of this important lineage with a comprehensive genomics approach.

Methods:

Here, we used whole-genome sequencing to investigate 55 ST1193 genomes collected across Australia between 2007 and 2013. Long-read sequencing was used to assemble the complete reference genome MS10858 and determine the genomic context of genes encoding antimicrobial resistance and virulence factors in eight other isolates carrying $bla_{\text{CTX-M}}$ metallo-beta-lactamase genes. Well-characterised published genomes of strains from the same clonal complex (CC)14 (*n*=54) were used to contextualise our Australian dataset and investigate spatial clusters and lineage diversity.

Results:

Fluoroquinolone-resistant ST1193 are mediated by point mutations in *gyrA, parC,* and *parE*, distinguishing them from other lineages within CC14. Bayesian analysis predicted that fluoroquinolone-resistant ST1193 emerged in 1989. This coincides with the increase in human use of fluoroquinolones in Australia, after their inclusion in government-subsidised medications in 1988. ST1193 has two major clades with high intra-clade chromosomal similarity. Remarkably, the globally-distributed clade 1 is distinguished by recombination of a 30.4 kb region encompassing the capsular biosynthesis genes causing a switch from the K5 to K1 capsular antigen, which is associated with increased serum survivability. Assembly of MS10858 revealed an 11.5 kb composite transposon Tn*6623* containing five resistance genes on an F-type plasmid, and an *ISEcp1*-$bla_{\text{CTX-M-15}}$ element on an IncI1 plasmid. Nanopore sequencing of eight other isolates revealed a major role for IS*Ecp1* in mobilising *CTX-M-type* metallo-beta-lactamase genes, with both plasmid and chromosomal integrations observed.

Conclusion:

This work describes a comprehensive genomic characterisation of ST1193 and identifies a single recombination event in the capsule locus, associated with the global dissemination of this fluoroquinolone-resistant UPEC clone.

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Rapid respiratory pathogen testing: assessing test appropriateness and impact on antimicrobial prescribing

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Introduction

Commercial multiplex PCR testing for respiratory pathogens has increasingly become available in recent years. However, the impact of introduction of expensive testing should be evaluated. This audit evaluated the reporting times for respiratory pathogen testing by FilmArray RP2, reviewed the indications for testing, and assessed the potential impact of the assay on antimicrobial stewardship.

Materials and methods

Nose and throat swabs were tested by FilmArray RP2 on a daily basis. A subset of results with positive RP2 results was selected for clinical review by a microbiologist, and recommendations of antimicrobial therapy were made. A second review of electronic charts was performed 1 day after the final result was released to assess changes to antimicrobial usage.

Results

110 results were available for data analysis. Most testing was performed within 2 days of admission to hospital (n=95, 86.3%) and 88.2% of preliminary RP2 results were available within 3.9 hours of sample receipt in the laboratory. Frequently detected pathogens included Rhino/Enterovirus (n= 34, 30.9%), Influenza A (n=19, 17.2%) and parainfluenza virus 3 (n=15, 13.6%). The most common diagnoses for patients included pneumonia (n=21, 19.1%), asthma (n=10, 9.1%), unspecified lower respiratory tract infection (n=8, 7.3%) and chronic obstructive airways disease (n=7, 7.3%). Seventy-six (69.1%) patients were on one or more antimicrobials at the time of RP2 test reporting. A total of 50 recommendations were made on the test report (stop antibiotics n=21, 42%; review antibiotics n=12, 24%; review tetracyclines n=6, 12%, others n=11, 22%). No change was made to the antibiotic regime for 28 patients (56%).

Conclusion

Filmarray RP2 allows testing and reporting of respiratory pathogens in the early stages of hospital admission. The indications for respiratory pathogen testing appear to be varied, and appropriateness criteria should be formulated. Many patients were on antimicrobials at the time of test reporting, and half of patients continued on antimicrobials despite a recommendation to stop or review treatment.

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Induction of Erythromycin resistance in *Bordetella holmesii* confirmed by whole genome sequencing

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Bordetella pertussis, the etiological agent of whooping cough, is highly infectious and re-merging globally despite widespread vaccination. The recommended antibiotics for the treatment of *B. pertussis* infections and post-exposure prophylaxis are Macrolides, primarily Erythromycin and Azithromycin. However, mutations have occurred in the 23S rRNA gene resulting in macrolide resistance. While still uncommon, macrolide-resistant *B. pertussis* have been reported in Europe and Asia. The mechanism of resistance was identified as an A-to-G transition SNP mutation at position 2058 of the 23S rRNA gene.

The target sequence *IS481* for routine clinical diagnosis has been found in both *Bordetella holmesii* and *B. pertussis* genomes. *B. holmesii*, is most likely the main cause of false-positive results when screening symptomatic patients for pertussis with routine PCR methods. Previous studies have shown that *B. holmesii* infections range from 0-32% of nasopharygeal isolates from patients with pertussis-like illnesses. We aimed to examine the development of macrolide resistance in *Bordetella holmesii*

To generate resistance in three *B. holmesii* strains, comprising of 2 clinical isolates and a type strain, isolates were grown on HBA media with an Erthryomycin E-test or disc for 15 weeks and subcultured every 3-4 days.

B. holmesii isolates took six to twelve weeks (13-25 passages) to develop resistance (>256 µg/mL), from an MIC ranging from 0.047-0.25 µg/mL. Passaged isolates were sequenced on an Illumina NextSeq500, every month (4 weeks/8 passages), to observe any intermediate genomic changes in the isolate that may have contributed to resistance. Average coverage of the isolates ranged from 60-90x, and was able to determine mutations in the 23S rRNA gene were present in *B. holmesii.* Thus, this study was able to demonstrate a potential resistance mechanisms of *B. holmesii*.

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Weird and wacky foods and public health

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In South Australia, Local government Environmental Health Officers (EHOs) are on the front line when it comes to making sure food businesses are not going to make you sick when they make food for you. More often than not, they see the same things where ever they go…… but…..

What happens when the food technology landscape changes?

SA Health cooks up information sessions for EHOs in the moving feast that is food and public health - this is the snack size version.

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Detecting hospital transmission of superbugs using genomics

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Objectives

Antimicrobial resistant bacteria are one of the foremost threats to public health, particularly among hospital patients, who are disproportionately affected by multidrug-resistant organisms (MDROs), with corresponding high levels of morbidity and mortality. Whilst diagnostic microbiology laboratories can usually detect MDROs in patient samples, current microbiological methods have insufficient resolution to detect transmission and identify potential outbreaks, leading to delays in implementation of appropriate infection control measures. As whole genome sequencing becomes more feasible, we trialled the use of genomics for detection of MDRO transmission in hospitals in Melbourne, Australia.

Methods

A 15 month prospective multicentre study spanning four hospital networks was conducted. Hospital inpatients with MRSA, *vanA* VRE, ESBL *K. pneumoniae,* and ESBL+ciprofloxacin resistant *E. coli* from clinical or screening cultures were eligible; samples were sequenced at the central reference laboratory (Illumina NextSeq), and clinical data was collected contemporaneously. Multilocus sequence typing, antimicrobial resistance gene detection, and phylogenetic analyses were performed. Interim reporting sessions with participating hospitals were conducted to educate on genomics, communicate results, evaluate clinical utility, and identify any changes made to infection control measures arising from genomics results.

Results

Putative transmission events associated with all organisms and numerous sequence types were detected both within and between hospital networks, although relative numbers of MDROs varied. Hospitals used the detailed transmission data provided by genomics for focused outbreak investigations, to implement targeted infection prevention and control measures. Genomic evidence of transmission was useful for advocating for enhanced infection control resources.

Conclusions

Genomics can provide high-resolution discrimination between bacterial sequences and, in combination for high-quality epidemiologic data, can inform hospital infection prevention and control. This is integral to limiting the spread of MDROs in hospital. 'Ruling-out' isolates from potential outbreaks also saved infection control resources. Future challenges include improving turnaround times, streamlining transmission analyses, and developing creative ways to communicate genomic data to clinicians and hospitals.

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Collateral Damage: Biocide Use and the Co-Selection of Multidrug Resistant *Staphylococcus aureus*

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Healthcare associated infections (HCAIs) caused by *Staphylococcus aureus* are a major cause of mortality and morbidity, both locally in Australia and globally. *S. aureus* causes a variety of illnesses, ranging from skin and soft tissue infections through to pneumonia, endocarditis and bloodstream infections. Infection prevention and control (IPC) is critical for reducing the rates of *S. aureus* infection, with the use of biocidal agents such as chlorhexidine gluconate (CHG) being an important component of current IPC programs. Indeed, recent international guidelines recommend the universal use of biocides for skin decolonisation in "high-risk" hospital patients to prevent HCAIs, including those caused by *S. aureus*. This has led to concerns about possible "collateral damage" associated with the increasingly widespread and indiscriminate use of biocides such as CHG in our hospitals. Of particular concern is the possibility that CHG use might be associated with the emergence of antimicrobial resistance (AMR). Here we have used whole genome sequencing and Markov network analyses to determine the collateral antimicrobial resistances associated with biocide tolerance genes in *S. aureus*. These analyses clearly demonstrate the genetic potential for biocide-

mediated co-selection of AMR in *S. aureus.* Furthermore, using a combination of *in vitro* testing and clinically relevant skin infection models, we provide compelling experimental evidence to show that the use of biocides, including CHG, can rapidly co-select for the emergence of multidrug resistant *S. aureus* isolates.

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Laboratory-based surveillance of *Clostridium difficile* infection in the Australian healthcare and community settings in 2015

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Objectives: As part of the ongoing national *Clostridium difficile* Antimicrobial Resistance Surveillance (CDARS) study, we sought to describe the molecular epidemiology of *C. difficile* infection (CDI) in the Australian healthcare and community settings in 2015.

Methods: Ten laboratories across Australia; one private and one public laboratory from five states submitted isolates of *C. difficile* or PCR positive stool samples during two collection periods for summer/autumn (February-March; CDARS phase 4) and winter/spring (August-September/ CDARS phase 5) 2015. *C. difficile* was characterized by PCR for toxin genes and ribotyping.

Results: A total of 307 eligible samples were received, of which 45% and 47% of the total collected were submitted by private laboratories from the two collection periods, respectively. From these samples, 285 isolates of *C. difficile* [WA (n=58), VIC (n= 55), SA (n=53), QLD (n=61) and NSW (n=56)] were recovered. PCR ribotyping yielded 71 different ribotypes (RTs) and 76% of strains (218/285) were assigned to 1 of 30 internationally recognised RTs. The most prevalent RTs were 014/020 (31%, n=88), 056 (8%, n=22) and 002 (7%, n=21). Epidemic RTs 027 (n=1), 078 (n=3) and the recently emerged RT251 (n=1) were found, as well as a single isolate of RT017. The vast majority (88%) of strains were positive for the major toxin genes *tcdA/B*, of which 4% (n=12) were also positive for genes encoding binary toxin (*cdtA/B*) and 7% (n=20) were non-toxigenic strains. Similar distributions were seen for RTs between states, although phase 5 appeared to have increased numbers of RTs 056 (n= 5 vs. 17) and 002 (n=6 vs. 15).

Conclusions: A heterogeneous *C. difficile* strain population was identified in Australia. RT014/020 was the most prevalent *C. difficile* strain found in humans with CDI. This successful lineage is also the most common RT in pigs in Australia, suggesting a potential zoonotic reservoir for human CDI. Continuous surveillance of circulating *C. difficile* strains is important for tracking of prominent and emerging strain types.

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Microbiological monitoring in the production of exported meat - assessing hygiene using the Product Hygiene Indicators Program

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Australia has adopted Hazard Analysis Critical Control Point-based principles within the export meat regulatory system, requiring monitoring along the production and processing chain. An important monitoring tool within the Australian system is the Product Hygiene Indicators (PHI) Program. The PHI Program requires government and industry to undertake regular monitoring for microbiological indicators and macroscopic contaminants on carcases and boned meat. The program applies to production of meat from cattle, sheep, pigs, goats, deer, horses, camels, ratites, kangaroos and wild boar. Data from approximately 80 export meat establishments are collected on a daily basis, and data from the program in its current form are available for over ten years. Therefore the PHI program contains a very large dataset that can be used to analyse both historical and contemporary performance of the industry. The Department of Agriculture and Water Resources uses PHI data to assess industry performance and to communicate this to overseas markets. Monitoring data are also used by meat establishments to chart their relative performance within the industry and to respond accordingly. Collection of data in the PHI Program is consistent with the growing international trend for greater reliance on performance monitoring to enhance traditional inspection and audit processes.The program was recently transtioned to the Meat Export Data Collection system, the department's online performance database for

export meat establishments. The new platform allows industry and government to analyse in real time the performance of individual meat establishments and the national system. This ensures the PHI Program continues to be a valuable tool for assessing the hygiene of Australian meat exports, contributing to process improvement and demonstrating the hygiene of our exported meat to importing countries.

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The evolutionary implications of mobile genetic element acquisition in the multidrug resistant *Escherichia coli* ST101 clone.

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Extraintestinal pathogenic *Escherichia coli* (ExPEC) are a leading cause of urinary tract and bloodstream infections. Several pandemic multidrug resistant (MDR) ExPEC clones have emerged recently, with the exemplar being fluoroquinolone resistant ST131. The antimicrobial resistance (AMR) and virulence profiles of these lineages differ markedly, in concert with the mobile genetic elements (MGEs) on which AMR and virulence genes reside. The biological fitness factors determining their success are mostly undetermined, however much can be learnt from studying the emergence of new clones. *E. coli* ST101 is an emerging MDR, pathogenic lineage associated with the carriage of $bla_{\text{NDM-1,}}$ conferring resistance to carbapenems, a last-resort antibiotic. We analysed the genomes of seven ST101 isolates sequenced with Pacific Biosciences Single Molecule Real Time (SMRT) Sequencing. This technology enabled complete resolution of MGEs including plasmids, and definition of the genome-wide complement of DNA methylation. When considered together with publicly available genome data for 263 ST101 isolates, we determined how MGEs are major drivers of the evolution of the ST101 lineage. *E. coli* ST101 are monophyletic within the B1 phylogroup and cluster in two major clades. Remarkably, extensive MDR to at least 9 different antimicrobial classes was restricted to a single sub-lineage within Clade 1. This sub-lineage showed clonal expansion following the acquisition of chromosomal mutations conferring fluoroquinolone resistance, $bla_{\text{CTX-M-15}}$ and $bla_{\text{NDM-1}}$. We also found complex acquisition and dissemination pathways for $bla_{\text{NDM-1}}$ and MDR depending on the plasmid type. For IncC plasmids, acquisition was primarily due to dynamic recombination within the antibiotic resistance island (ARI-A). However, for F-type plasmids, we found evidence for acquisition of a $bla_{\text{NDM-1}}$ resistance island on one plasmid, followed by transposition to another. Lastly, we revealed how recombination events and the acquisition of a genomic island result in global methylome changes within the lineage. This work highlights the benefits of SMRT sequencing in revealing the dynamic evolutionary events and epigenetic heterogeneity that is driving the evolution of this emerging, pathogenic clone.

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Group A *Streptococcal* interactions with the host

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Group A Streptococcus (GAS, Strep A, Streptococcus pyogenes) causes mild human infections such as pharyngitis and impetigo, and serious infections such as necrotizing fasciitis and streptococcal toxic shock syndrome. Recurrent GAS infections may trigger autoimmune diseases, including acute rheumatic fever, and rheumatic heart disease. Combined, these diseases account for over half a million deaths per year globally. *GAS is a human specific pathogen, and* genomic and molecular analyses have identified a large number of GAS virulence determinants, many of which exhibit overlap and redundancy in the processes of adhesion and colonisation, innate immune resistance, and the capacity to facilitate tissue barrier degradation and spread within the human host. Our research examines the interaction of GAS with host cells and molecules at various stages of infection, with an aim to characterise mechanisms of bacterial pathogenesis.

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Immune modulation by *Helicobacter pylori* and bacterial outer membrane vesicles.

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Helicobacter pylori is a Gram negative bacterium that infects the gastric tissue of more than 3 billion people worldwide. Infection with *H. pylori* almost always persists within the host for life, and results in a spectrum of diseases ranging from gastritis in all individuals, which can progress to the formation of gastric ulcers or gastric cancer. *H. pylori* uses a range of virulence determinants and mechanisms to manipulate the hosts immune system into mounting an ineffective and chronic immune response. This ineffective immune response creates an environment in which the bacterium can survive almost indefinitely, and ultimately results in the destruction of host cells, gastric pathology and cancer in some individuals. Our research examines the cellular and molecular mechanisms whereby *H. pylori* and their products, such as outer membrane vesicles, mediate pathogenesis and chronic immune suppression in the host. Our findings ultimately aim to broaden our understanding of how *H. pylori* mediates disease in an attempt to develop novel therapies with the potential to facilitate clearance of chronic *H. pylori* infections.

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Evolution of a Clade of *Acinetobacter baumannii* Global Clone 1, Lineage 1 via Acquisition of the *oxa23* Carbapenem Resistance Gene and Dispersion of ISAba1

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Acinetobacter baumannii causes a range of hospital-acquired infections and antibiotic resistance is a critical problem, particularly when resistance genes are acquired by members of successful globally-distributed clones e.g. global clone 1 (GC1) [1-3]. Here, we investigated the evolution of an expanding sub-clade of multiply antibiotic resistant GC1 associated with carbapenem and aminoglycoside resistance.

Twenty-seven strains belonging to a specific clade of GC1 were identified, 3 in our collection and 24 in GenBank, using a range of criteria including the carriage of the Tn*6168* transposon [4], carrying the ISAba1-*ampC* structure, responsible for resistance to 3rd generation cephalosporins, in a specific chromosomal location, and a specific Outer Core oligoaccharide, OCL3. The genome sequence of the representative of Australian isolates, which was also resistant to carbapenems, was determined using Illumina HiSeq and PacBio long-read technology. A range of bioinformatics tools was used to examine the context of resistance genes, distribution of the chromosomal ISAba1 copies, and phylogeny.

Bayesian analysis showed that the Tn*6168*/OCL3 clade arose in the late 1990s, from an ancestor that had already acquired resistance to third generation cephalosporins and fluoroquinolones. Between 2000 and 2002, this clade further diverged into distinct sub-clades by insertion of AbaR4 (carrying the *oxa23* carbapenem resistance gene) at a specific chromosomal location in one group, and a phage genome in the other. Both subgroups show evidence of ongoing evolution of resistance loci and ISAba1 dispersal. Most concerning, this includes introduction of the *armA* aminoglycoside resistance gene via AbGRI3, acquired from a GC2 isolate.

Our analysis revealed the complexity of genetic events leading to resistance to multiple antibiotics in the Tn*6168*/OCL3 clade of GC1. Comparison of IS insertions sites with the dated phylogeny shows ISAba1 first entered this clade in around mid 90s with the cephalosporin resistance transposon Tn*6168* and has since expanded in both subclades. It also revealed multiple routes for the acquisition of the *oxa23* carbapenem resistance gene.

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Living on air: trace gases as hidden energy sources for the dormant bacterial majority

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Bacteria have an extraordinary capacity to persist in response to resource limitation. To achieve this, cells enter a dormant state in which they expend energy for maintenance rather than growth. Our research program has shown that the survival of environmental and pathogenic bacteria depends on previously unrecognized metabolic flexibility, including novel respiration, fermentation, and biodegradation pathways. In this presentation, I will focus on work demonstrating that many aerobic bacteria are capable of 'living on air', i.e. scavenging atmospheric hydrogen and carbon monoxide as alternative energy sources. Genetic and biochemical studies show that axenic mycobacterial cultures use these gases as respiratory electron donors when exhausted for preferred organic carbon sources. These alternative metabolic pathways are tightly regulated, critical for redox homeostasis, and necessary for long-term survival. The determinants of these processes are widespread in the genomes of aerobic bacteria and we have experimentally validated that five dominant bacterial phyla scavenge these gases. At the ecosystem level, metagenomic and biogeochemical studies show that trace gas scavengers are abundant and active in aerated soil and marine ecosystems, and are particularly important for primary production in global desert soils. I will also outline ongoing work to use these principles to understand how *Mycobacterium tuberculosis* and various enteropathogens maintain energy needs in different reservoirs. While atmospheric hydrogen and carbon monoxide in sufficiently concentrated to support growth, they are highly dependable energy sources for persistence given their ubiquity, diffusibility, and energy content. Overall, these findings redefine the minimal nutritional requirements for life and identify trace gases as the hidden energy source supporting the dormant bacterial majority in aerated ecosystems.

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The contribution of membrane transporter proteins to extensively-drug resistant phenotypes in *Acinetobacter baumannii*

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Acinetobacter baumannii is one of the most challenging nosocomial pathogens, whose infections are often associated with epidemic spread of multi- and extensively-drug resistant (M/XDR) strains. *A. baumannii* resistant to carbapenems and colistin are an emergent threat as they limit therapeutic alternatives threatening patient care and public health. We investigated the role of efflux pumps (EPs) and porins in *A. baumannii* Portuguese clinical isolates from hospital environment.

Drug susceptibility was assessed by disc diffusion. Isolates were screened for the presence of β-lactamases, metallo-β-lactamases, carbapenemases, ISAba1 and mutations in genes associated colistin resistance by PCR and DNA sequencing. The existence of active efflux was studied by checkerboard assays with efflux inhibitors (EIs), ethidium bromide real-time assays and analysis of mRNA transcriptional levels of selected efflux pump genes and porins in response to imipenem or colistin.

Of the 74 strains studied, 72 were MDR with additional resistance to carbapenems (XDR). Of these, eight evolved to TDR, presenting high-level resistance to colistin. The β-lactamases OXA-23 were detected in few strains and OXA-24 in the majority. OXA-51 were detected in all but ISAba1 was not detected upstream this oxacillinase excluding its contribution to carbapenem resistance. The results showed the existence of synergistic interactions between EIs, carbapenems, colistin and ethidium bromide extrusion. Efflux assays demonstrated a significantly increased efflux activity that can be inhibited in the presence of EIs, mainly thioridazine. The EPs *adeB*, *adeJ*, *adeG*, *craA*, *amvA*, *abeS* and *abeM* were overexpressed in response to carbapenems and colistin. An association between carbapenem resistance and expression of the porins *ompA*, *carO* or *oprD* was not found.

This study demonstrated the contribution of EPs to carbapenems and colistin resistance in M/XDR *A. baumannii* clinical strains where resistance is a combination between increased efflux activity, mutations in antibiotic target genes and β-lactamases production. Overexpression of EPs impacts the clinical outcome of *A. baumannii* infections and treatment should urgently consider alternative therapeutic combinations such as the use of EIs.

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Structural dynamics of Neisserial membrane proteins: what can we learn from in silico studies?

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Publish consent withheld

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Exploring physiological substrates of the prototypical PACE family efflux pump AceI

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Multidrug efflux pumps have gained notoriety as a major and highly promiscuous class of drug resistance determinants that contribute to the failure of antibiotic therapy and promote the persistence of pathogens in hospitals. Despite their widely-studied roles in drug resistance, for many multidrug efflux pumps drug transport is likely to be a fortuitous side reaction made possible by flexible substrate binding sites that have become beneficial to host organisms living under highly drug selective conditions in hospitals. The core functions of these pumps are likely to be linked to the physiology of the organism and the environments in which they evolved. This is almost certainly true for the AceI transport protein, the prototype for the novel PACE family of efflux pumps. The gene encoding AceI is conserved across all *A. baumannii* strains to have had their genomes sequenced, indicating an ancient origin and long term pressure for gene maintenance. Paradoxically, its only characterised substrate is chlorhexidine, which, although widely used as an antiseptic today, is purely synthetic and has been produced only since last century. This presentation will describe our progress in deciphering the core physiological functions of the AceI protein, as well as its mode of energisation.

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ZorO kills but does it save?

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Small bacterial proteins under 50 amino acids were generally overlooked in the past but now have been shown to play important roles in numerous cellular pathways throughout a variety of species. However, their characterization and functional analyses remain challenging. We are investigating the function of a 29 amino acid protein (ZorO) encoded in the chromosome of enterohemorrhagic *Escherichia coli* (EHEC), a worldwide foodborne pathogen. We previously showed ZorO overproduction results in cell growth stasis but is repressed at the post-transcriptional level via complementary base pairing by a small RNA (OrzO). Thus, the *zorO-orzO* gene pair is an example of a type I toxin antitoxin system. Nonetheless, the mechanism of ZorO toxicity, its effects and function when expressed endogenously in EHEC is not well understood. Here, using an ectopic overproduction system, we show this protein localizes to the bacterial inner membrane, causes membrane depolarization and reduces cellular ATP levels without significantly affecting cellular morphology. We also observed that specific charged amino acid residues are critical for ZorO induced toxicity. Preliminary data supports a model for ZorO oligomerizing in the membrane leading to leakage of ions. We are also assessing the conditions under which ZorO is produced to elucidate when the protein naturally functions. To address not only its own expression but also that of its antitoxin OrzO, we designed a pFA6a plasmid vector expressing two different reporter proteins under the control of the *zorO* and *orzO*promoters. These studies demonstrated that *zorO* transcription is upregulated under nutrient limiting conditions, suggesting a role for ZorO when the cells encounter adverse growth conditions. Further studies are aimed to elucidate the contribution of the gene pair in bacterial persistence and pathogenesis.

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Staphylococcal multidrug efflux pump QacA: identification of functionally-important residues in helix 12

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Staphylococcus aureus is a serious opportunistic bacterial pathogen responsible for multidrug resistant hospital- or community-acquired infections with significant morbidity and mortality worldwide. Distressingly, this 'Superbug' has developed resistance to quaternary ammonium compounds (QACs) which are widely used in medical and industrial settings as disinfectants and antiseptics. A main factor in the development of resistance to QACs is the expression of drug efflux pumps such as QacA, which is the most prevalent plasmid-encoded multidrug efflux pump found in clinical *S. aureus* isolates. QacA, is able to confer resistance to >30 structurally dissimilar monovalent and bivalent cationic antimicrobial agents. However, QacA structure-function relationships have not been fully resolved. QacA is comprised of 14 transmembrane segments (TMS) and TMS 12 has been suggested to be a component of the bivalent cation-binding region. This study aimed to delineate the functional importance of TMS 12. Toward this end, 30 amino acid residues within putative TMS 12 and its flanking region were individually substituted with cysteine and the impact of these substitutions on QacA-mediated resistance assessed. Western blotting analyses showed all QacA mutants were expressed at levels close to wild-type, implying that cysteine substitution in QacA did not affect protein expression. Minimum inhibitory concentration assays with representative antimicrobial compounds revealed G361, G379 and S387 had a significant impact (≤50% reduction) on QacA resistance capacity for at least one of the bivalent cationic substrates, indicating the importance of these residues in the interaction with these substrates. Our results confirm the functional interplay between TMS 12 of QacA and bivalent cationic substrates. Further fluorimetric transport assays and binding studies are in progress to determine as to whether TMS 12 directly involves in the substrate translocation and/or binding process. Elucidating the detailed role of TMS 12 is an imperative step towards the ultimate goal of translating the findings into development of novel antimicrobials/inhibitors that combat QacA-mediated resistance.

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Acinetobacter baumannii employs multiple pathways for zinc and cadmium export

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Acinetobacter baumannii is a Gram-negative human pathogen associated with significant morbidity and mortality. This is attributed to the bacterium's ubiquitous nature, high level of antibiotic resistance and expression of a broad arsenal of antimicrobial stress resistance mechanisms. *A. baumannii* is known to harbour an extensive repertoire of metal ion efflux systems, but none of these have been functionally characterised. Here, we study the role of membrane transport systems in *A. baumannii* zinc and cadmium resistance. Our analyses of mutant derivatives revealed a primary role for the CzcCBA heavy metal efflux system in zinc resistance. In addition to CzcCBA, CzcD, a member of the cation diffusion facilitator (CDF) superfamily, and CzcI, a putative periplasmic zinc chaperone, aid in *A. baumannii* zinc resistance. Although genetically clustered, in depth analyses reveal that *czcI*, *czcCBA* and *czcD* have distinct regulatory elements, with *czcCBA* also holding a role in resistance to the heavy metal

cadmium. Further analyses of CDF members identified *czcE* as the primary *A. baumannii* cadmium efflux system. Overall, we provide novel insight into the metal ion resistance mechanisms of *A. baumannii* and the role these systems play in enabling the bacterium to survive in diverse environments and cause significant human disease. As CDFs span multiple domains of life, these findings are of major significance across metal ion biology.

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HIV Viral Persistence and Immune Interactions

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The institution of highly active antiretroviral therapy for the treatment of HIV has led to viral suppression but HIV is not eliminated and persists in reservoirs and viral rebound almost always occurs when therapy is interrupted. It has been demonstrated in numerous published papers that inflammation is reduced on initiation of therapy but still persists in viral suppressed patients . This relationship of viral latency and chronic inflammation has not been well defined. There are numerous causes of chronic inflammation that includes microbial translocation co-infection with other viruses ie CMV ,and persistent virus. This presentation will highlight our current understanding of this complex relationship of persistent virus and inflammation by reviewing studies on this relationship in subjects on ARV and following ARV intensification. The discussion will review studies where therapeutic interruption has been used to evaluate the relation of baseline inflammation and viral rebound. The critical question in this field is what happens to viral persistence in tissue. There will be discussions on measures of the virus in critical tissue sites that include gastrointestinal tract, lymph nodes and adipose tissue and the related inflammatory responses. The talk will conclude with potential therapeutic approaches that target inflammatory responses and how they impact viral persistence.

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Disarming a killer: targeting of natural killer cells by varicella zoster virus

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Varicella zoster virus (VZV) is the causative agent of chickenpox (varicella) and shingles (herpes zoster). In the course of these diseases VZV is able to productively infect a number of specialized cell types including T cells, dendritic cells, skin cells, ganglionic cells, and others. During infection, VZV also encodes a number of functions that modulate a range of immune evasion functions, and these are likely to enable this virus to limit the efficacy of both innate and adaptive arms of the anti-viral immune response. Despite the potent anti-viral capacity of natural killer (NK) cells, this cell type has not been studied in detail in the context of VZV infection. However, clinical evidence supports a crucial role for NK cells in the control of productive VZV infection as individuals with NK cell deficiency are highly susceptible to disseminated, life threatening VZV disease. We have observed NK cells in the immune infiltrate within naturally infected human ganglia following VZV reactivation. In addition, VZV infection of epithelial cells resulted in differential modulation of ligands for NKG2D (a potent activating receptor expressed on NK cells). Recently we identified NK cells as being highly permissive to productive VZV infection. This infection results in NK cell activation but impaired cytotoxicity and cytokine responses. These studies identify a cell type previously unrecognized as being permissive to VZV infection, and also reveal a complex manipulation of NK cell anti-viral function, whereby VZV impacts on both the NK cell itself and the cells it targets.

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Cytomegalovirus latency and reactivation: new insights and clinical implications

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Not available

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Can vaccination prevent viral persistence?

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Zika and chikungunya viruses have caused major epidemics and are transmitted by *Aedes aegypti* and/or *Aedes albopictus* mosquitoes. The 'Sementis Copenhagen Vector' (SCV) system is a recently developed vaccinia-based, multiplication-defective, vaccine vector technology that allows manufacture in modified CHO cells. Herein we describe a single vector construct SCV vaccine that encodes the structural polyprotein cassettes of both Zika and chikungunya viruses from different loci. A single prophylactic vaccination of mice induces neutralizing antibodies to both viruses in wild-type and $IFNAR^{-/-}$ mice and protects against (i) chikungunya virus viremia and arthritis in wild-type mice, (ii) Zika virus viremia and fetal/placental infection in female $IFNAR^{-/-}$ mice and (iii) Zika virus viremia and testes infection and pathology in male $IFNAR^{-/-}$ mice. To our knowledge this represents the first single vector construct, multi-pathogen vaccine encoding large polyproteins, and offers both simplified manufacturing and formulation, and reduced "shot burden" for these often co-circulating arboviruses. However, Zika virus is known to persist in male testis. The current work evaluates a therapeutic vaccination approach to reduce viral load in the testis following Zika infection. In summary, we have produced a novel platform technology that can readily be accommodated in a biopharmaceutical industry-standard manufacturing process. A single vector targeting multiple diseases is an innovative approach to reducing "shot burden" for commonly co-circulating viruses.

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Exploring Mechanisms of Antiviral Resistance in Influenza with a Mass Based Phylonumerics Approach

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Understanding the pathways by which antiviral resistance mutations in influenza emerge and take hold is important for developing effective responses to the virus. Resistance mutations are typically not maintained in a population after drug treatment ceases, given their evolutionary benefit to the virus when challenged by antivirals generally carries some cost to viral fitness. However, this is not always the case where the restoration of fitness can be achieved with compensatory mutations. To help understand the origins and dynamic nature of the influenza resistance mutations, we have developed and employed a new and innovative phylonumerics approach that avoids the need for either gene or protein sequences.

Protein mass maps or fingerprints are extensively used for protein identification in proteomics applications. We have shown that they can also be used to study the evolutionary history of organisms through the construction of so-called mass trees from sets of masses (i.e numbers) that reflect a protein's sequence. Furthermore, single point amino acid mutations can be identified from mass differences alone and displayed along branches of these mass trees in this phylonumerics approach.

Frequent ancestral and descendant mutations that precede and follow the manifestation of antiviral resistance mutations in influenza neuraminidase are identified. In the case of N2 neuraminidase, the majority of mutations drive hydrophilicity changes around the active site, primarily through the incorporation or loss of hydroxyl groups, though do not impact the catalytic active site residues. These mutations either aid or restrict the entry and subsequent binding of a sialic acid or antiviral inhibitor.

Recent results from the application of the phylonumerics approach will be presented where mutations ancestral and descendant to resistance mutations will be discussed in terms of their mechanistic consequences in relation to H1N1 and H3N2 influenza strains.

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Designing peptide polymers as antibiotic adjuvants for multidrug resistant bacteria

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The continuing rise in antibiotic and multi-drug resistant bacterial infections is a major global medical health issue, which is not being met by traditional antibiotic research. Here, we show that a new class of antimicrobial agents, termed 'Structurally Nanoengineered Antimicrobial Polypeptide Particles' (SNAPPs), synthesized using ring-opening polymerization (ROP) of *α*-amino acid *N*-carboxyanhydrides (NCAs) to produce star-shaped polypeptide nanoparticles exhibit antibiotic-adjuvant properties resulting in colistin-multi-drug resistant (CMDR) bacteria becoming sensitive to antibiotics once more. Synergistic interactions were demonstrated between a model SNAPP and ampicillin, imipenem, doxycycline, gentamicin, or silver ions. We show that different peptide architectures alter the activity of the SNAPPs. We also show that the effective antibiotic dose could be decreased by 3.7 to 16 fold from the original lethal dose while retaining synergistic interactions with SNAPPs against CMDR bacteria. Further, we demonstrate that the combination treatment approach using SNAPPs is able to attenuate toxicity. Interestingly, our studies show that SNAPPs sensitise bacteria to antibiotics that were previously ineffective. Overall, this study demonstrates the potential of synergistic combinations of SNAPPs with conventional antibiotics where the SNAPP not only has antimicrobial activity in its own right but also acts as an adjuvant for the conventional antibiotic to treat CMDR bacterial infections.

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Octapeptins – Development of Novel Lipopeptides for The Treatment of MDR/XDR Gram-Negative Infections

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Infections caused by extensively-drug resistant (XDR) Gram-negative bacteria are an increasing threat to human health. The evolution of high levels of resistance is making the last-resort polymyxin lipopeptide antibiotics (colistin and Polymyxin B) obsolete, with alternate antibiotics urgently required. The octapeptins are naturally derived products, first reported in the 1970s, that are structurally similar to the polymyxins. They retain activity against polymyxin resistant isolates, but to date have been largely ignored.[1]

Intrigued by this observation, we initiated a research program to explore the potential of this class as a last resort therapy, and recently reported the first octapeptin synthesis, and confirmed its activity against polymyxin resistant clinical isolates.[2,3] The octapeptins retain activity against polymyxin-resistant Gram-negative bacteria, despite their structural similarity. Mode of action studies, employing surface plasmon resonance, membrane probe assays, and fluorescently-labelled analogues prepared for membrane localization and permeabilization studies, highlighted subtle variations in membrane interactions and permeability between the classes. An induced resistance study demostrates fundamental differences from the polymyxins, with rapid induction of high resistance by polymyxins, but not octapeptin C4, and no cross resistance between the two classes

An SAR campaign was undertaken to examine substituent effects and identify compounds with improved properties. Analogs were profiled for MIC potency, cytotoxicity and nephrotoxicity using primary human kidney cells. Advanced compounds were further assessed for PK/efficacy (mouse), and nephrotoxicity (rats). Promising compounds were advanced into *in vivo* mouse studies.

The octapeptins show promising activity against polymyxin- resistant MDR Gram-negative bacteria. Importantly, they induce resistance slowly. We have developed new analogs with improved *in vitro* activity and reduced nephrotoxicity, accompanied by efficacy in an *in vivo* mouse thigh infection model. Given the paucity of Gram-negative candidates, the octapeptins are a rare beacon of light in the fight against antimicrobial resistance.

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In the world of modern antibiotic discovery how much do we really know?

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The discovery of new antibiotics is a path well-trod, yet few of those who embark on the journey ever reach the end. For most, novel antibiotics are never more than the tight line drawn across the blue sky at the horizon, never closer, never further away. And, I am an optimist who holds discovery as a tenet of faith.

In a world where the urgency for new antibiotics increases by the day, most sensible pharmaceutical companies have withdrawn from the field or offer “me-too” analogues of known actives. Discovery is an orphan left on the world’s doorstep. There are many sound reasons that bring us to this modern-day conundrum: lack of investment, low financial returns, weak IP protection, litigation, regulatory control, a patent system that gives equal weight to the discoverer of true novelty versus me-too imitation, our belief system that finding in nature does not merit ownership ……and so the list expands.

To those crestfallen researchers who see these mountains as obstacles, I would say that never in the history of antibiotics has the world been so rosy with opportunity. Indeed, like almost every discovery over the last 80 years, it is our evolving understanding of microbes that will push innovation into the future. We have ended the era of “chase the active” bioassay-driven discovery and entered a new era where our understanding of talented microbes as a whole is the best option in town. In this realm we can see all, but as yet we know so very little. Each microbe, with up to and quite often over 50 biosynthetic gene clusters, gives rise to over 100 secondary metabolites and importantly we know the pharmacology of only a handful.

In my presentation, I intend to support this view by detailing a 25-year odyssey of discovery at Microbial Screening Technologies in Sydney. Over 500,000 microbes were isolated across Australia and screened for antibiotic activity. Along the way, there were wins, losses, draws, things we needed to develop and stuff jettisoned to approach journey’s end.

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Storage temperature influences the *in vitro* invasiveness of *Salmonella* Typhimurium and minimises disease in mice

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Foods containing raw or undercooked eggs often become contaminated with *Salmonella*. It is recommended that raw egg-based foods are acidified and stored under refrigeration, yet the incidence of foodborne cases of salmonellosis has been increasing. Previously, it was found that the culturability of *Salmonella* Typhimurium from the raw egg-based sauce, aioli, is dependent on pH and storage temperature. Here, the *in vitro* invasion of bacteria recovered from aioli preparations was tested for invasiveness into the human intestinal epithelial cell line, Caco2. Invasion was significantly reduced in low pH aioli preparations. After 48 hours, bacteria isolated from aioli samples were non-invasive. *Salmonella* in non-acidified aioli preparations exhibited reduced invasiveness compared with broth cultures but were still invasive at 72 hours post-inoculation. The ability of *Salmonella* Typhimurium in aioli (pH 3.5) to cause disease was also tested in BALB/c mice over a 21-day period. Mice were inoculated with 10^8 CFU of *Salmonella* Typhimurium stored in aioli at 5°C or 25°C for 12, 24, 48, and 72 hours. Control mice were inoculated with 10^8 CFU of *Salmonella* Typhimurium in Luria Bertani broth. Mice in infected control groups exhibited clinical signs of infection from day 1 post-infection and were all culled by day 4. Mice infected with aioli inoculated with *Salmonella* and stored at 5°C for 12 and 24 hours exhibited mild clinical symptoms from day 6. Two mice in the 5°C, 24-hour treatment group and one mouse in the 5°C, 12-hour treatment group were culled on day 7 post-infection. Faeces were monitored every three days for the presence of *Salmonella* using both culture and PCR methods. *Salmonella* was detected from infected control groups at day 3 post-infection. Faecal samples were *Salmonella* positive for 5°C, 24-hour treatment group at day 3 post-inoculation only. The 5°C, 12-hour treatment group had positive faeces at days 3 and 6 post inoculation. These results indicate that acidification of raw egg-based foods may limit *Salmonella* Typhimurium virulence.

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Engineering well-defined gold nanoparticles for the treatment of drug resistant bacteria

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Bacterial resistance to antibiotics is increasing over time. The use of nanomaterial is among the most promising strategies to overcome bacterial resistance. Therefore, nanoparticles (NPs) based treatments have attracted enormous interest because of their excellent antibacterial properties, and limited evidence for resistance. However, not all these NPs are safe in practical applications due to the intrinsic toxicity of the NPs or the released ions. The ideal NP should be highly stable, biocompatible to the mammalian cells yet effective to eradicate wide range of drug resistant bacteria. Gold (Au) is such an ideal material which does not dissociate easily into ions. However, the bulk Au or the AuNPs are not chemically active. In this talk, I shall demonstrate strategies to fabricate antimicrobial AuNPs through precise control of their size down to less than 2 nm. These ultra-small AuNPs are often known as nanoclusters, and exhibits unique molecule like properties such as HOMO-LUMO transition, chirality, magnetism, photoluminescence etc. My talk will cover the structural aspect of these AuNPs, and especially highlight how the surface structure of the ultra-small AuNPs is regulated to trigger their strong antibacterial activities against both Gram negative and Gram positive bacteria.

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Intermittent fasting effects the mouse gut microbiota and colonic mucin glycosylation in a diet-specific manner

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Intermittent fasting has been proposed to improve metabolic health, however, information on how fasting effects host-gut microbiota interactions is limited. Recent work has implied a role of the colonic mucin layer and mucin-adhered bacteria in colonic homeostasis. The colonic mucin layer is mainly composed of densely *O*-glycosylated proteins that protect the epithelium and mediate host-microbiota interactions. To examine the effect of intermittent fasting on host physiology and host-gut microbiota interactions, we performed a multiple diet feeding study in a high-fat diet-fed mouse model. Using correlation network analysis, we examined the effect of intermittent fasting on the associations between individual colonic mucin glycans and specific colonic mucin-adhered bacteria.

Fasting had a significant impact on the host physiology and gut microbiota of mice fed a high fat diet (HFD) compared to normal chow (NC). Specifically, intermittent fasting with HFD resulted in significantly lower body weight and higher glucose tolerance compared to mice fed the HFD with no fasting. Fasting had no significant impact on the NC groups. These physiological changes were reflected in the fecal and colonic mucin-adhered microbiota. The relative abundance of OTUs in the families *Lactobacillaceae, Lachnospiraceae, Muribaculaceae, Ruminococcaceae* and *Parabacteroides* significantly changed in response to fasting. Fasting affected the relative abundance of nine mucin glycans, including glycan Fucα1-2 Gal β1-3 GalNAc, which was significantly higher in the HFD group with fasting. Correlation network analysis was used to identify associations between specific mucin glycans and specific mucin-adhered bacteria, this revealed significant associations between the relative abundance of glycan Fucα1-2 Gal β1-3 GalNAc and OTUs in the family *Lactobacillaceae*. This comprehensive omics-based study is the first report on how intermittent fasting effects the associations between colonic mucin glycans and mucin-adhered microbiota.

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Inhaled bacteriophage and endolysin therapy for respiratory infections

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Emergence of multidrug resistant (MDR) bacteria is causing a global medical challenge for treatment of respiratory infections. Inhaled bacteriophage (phage) and inhaled endolysin therapies are promising alternative treatment option that is gaining a renewed and increasing amount of attention for its ability to eradicate MDR bacteria. Biologics such as phages and endolysins can be delivered as aerosols using nebulizers (liquid formulations) or dry powder inhalers (dry powder formulations). Phage PEV20 was formulated in inhalable dry powders by spray drying the phages with suitable excipients, including lactose and leucine. Our PEV20 phage powder provided efficacious anti-Pseudomonal activities against clinical MDR isolate in a mouse lung infection model. Furthermore, the safety of phage powder formulation was validated using resazurin assay and histopathological examination *in vitro* and *in vivo*, respectively. This phage powder remained physically and biologically stable after one year of storage at ambient conditions. In a separate study, we assessed the aerosol performance and *in vitro* efficacy of nebulized endolysin Cpl-1 against *Streptococcus pneumoniae*. Vibrating mesh nebulizer maintained the antimicrobial activity of Cpl-1, whereas air-jet nebulizer caused significant loss. Both nebulizers generated aerosol particles suitable for inhalation delivery. These two studies demonstrated the potential use of inhaled phages and endolysin for treatment of bacterial respiratory infections.

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Not available

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Not available

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Selection of bacteriophages for therapeutic use against multidrug resistant pathogens.

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Use of bacteriophages (phages) for the treatment of multidrug resistant (MDR) bacterial infections could be a viable alternative or adjunctive to antibiotics [1,2]. However, routine medical implementation of phages is dependent on defining standard guidelines for the preparation of therapeutic products and on the choice of treatment approach (broad application 'cocktails' *versus* tailored phage therapy) [3]. Obligately lytic phages, that specifically kill bacteria immediately upon infection, can be readily isolated from the environment, but the process of selection of candidates for therapeutic use requires a rationalised approach based on unique phage-bacteria (predator-prey) dynamics [4]. We have been investigating rational protocols for the preparation of bacteriophage therapeutics to be used against two globally disseminated pathogenic bacterial clones, *E. coli* ST131 and *K. pneumoniae* CG258 [5], along with developing curated bacteriophage libraries for therapeutic use against other important MDR pathogens.

Following a 3-step process, we have: **(1)** tested the infectivity of >60 phages (from existing collections or *de novo* isolated from diverse environmental specimens) against fully characterized sets of target bacteria (*E. coli* ST131 [n=60], *K. pneumoniae* CG258 [n=20], as well as other MDR *E. coli*, *K. pneumoniae*, *Staphylococcus* ssp. etc.). We have then **(2)** selected specific phages (n=8 against *E. coli* ST131; n=12 against K. *pneumoniae* ST258) for detailed characterisation based on specific *in vitro* lytic activity, and **(3)** tested phage combinations (cocktails) with best *in vitro* synergy *in vivo* (murine models). We have found that (**a)** using a combined genomic and molecular microbiology approach is recommended for defining best candidates for therapy; and that (**b)** phage *in vitro* activity does not readily directly translate to *in vivo* efficacy, particularly in the case of phage combinations. Our findings highlight the limitations of the 'cocktail' approach for phage applications and may facilitate the process of phage selection for personalised therapy.

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Membrane vesicles (MVs) as an outcome of bacteriophage mediated bacterial lysis

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Membrane vesicles (MVs) prevail in all the three domains of life. Bacterial MVs are mostly spherical nanostructures (20- 400 nm), which involve in diverse biological processes such as pathogenicity, horizontal gene transfer, biofilm formation, and decoys to defend bacteria from antibiotics, antimicrobial peptides and bacteriophage predation. MVs biogenesis through explosive cell lysis in Gram-negative bacteria has been reported recently, which is due to expression of endolysin, a muralytic enzymes mostly used by bacteriophage to lyse its bacterial host. Bacteriophage mediated lysis in Gram-negative bacteria has been reported as a cell bursting phenomenon similar to that of explosive cell lysis and observations of vesicular structures within phage preparations have also been reported. However, there is a lack of knowledge on the source of MVs in phage lysates and also no clear evidence

of bacteriophage mediated MVs formation. This work aimed to gain a better understanding on the processes of MVs biogenesis to determine if MVs formation occurs through phage-mediated lysis of Gram-negative bacteria, using the live-cell imaging techniques together with transmission electron microscopy (TEM). This study demonstrated explosive cell lysis of *E. coli* K-12 utilizing two distinct standard virulent phages T4 and T7. Analysis of multiple lysis events revealed that the bacteria lysed explosively either as direct blowout without any gross changes in cellular morphology or in progressive changes into spheroid morphology before rupturing. Live-cell super-resolution microscopy demonstrated that the exploding bacteria produced shattered membrane fragments that curled up inward and then rounded up forming circular MVs that persisted in the same topology thereafter. TEM revealed the presence of different forms of MVs within phage lysates, consistent with MVs formation through phage mediated bacterial lysis. To our knowledge, this is the first direct observation of MV biogenesis via phage mediated bacterial lysis. This study not only explored an unappreciated route for MVs formation but also suggested the phenomenon could be the major process responsible for the abundance of MVs in nature.

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2. Turnbull L, Toyofuku M, Hynen AL, Kurosawa M, Pessi G, Petty NK, Osvath SR, Carcamo-Oyarce G, Gloag ES, Shimoni R, Omasits U, Ito S, Yap X, Monahan LG, Cavaliere R, Ahrens CH, Charles IG, Nomura N, Eberl L, Whitchurch CB. Explosive cell lysis as a mechanism for the biogenesis of bacterial membrane vesicles and biofilms. Nat Commun. 2016; 7:11220.

84

Phage therapy in the 21st Century: AMR, Phage & Synthetic Biology

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Antimicrobial resistance (AMR) has been identified as an emerging global crisis for human health. Effective therapeutic antimicrobial alternatives are being sought to control and remove multi-drug resistant (MDR) bacterial pathogens. Phage therapy remains a much under-explored alternative to antibiotic use. Synthetic biology can enable the design, modification and synthesis of phages to help realise novel strategies to study and treat MDR pathogens that form biofilms. Our research uses synthetic biology to engineer natural phage isolates and construct synthetic phages to effectively target biofilms. Approximately 90% of urinary tract infections are caused by Uropathogenic *E. coli* (UPEC) and result in a huge global health and economic burden. Furthermore, UPEC infections are increasingly showing resistance to antibiotic treatments. We present genomic sequence data on novel phage isolates that target UPEC and demonstrate how synthetic biology can enable engineering of these phages to influence host range specificity and other characteristics. State-of-the-art tools and emerging technologies, housed within an Australia-based BioFoundry, are being utilised to achieve high-throughput phage engineering and evolution, with the view to establishing a library of customised phages that target MDR pathogens within biofilms.

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The microbial ecology of our homes

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Microorganisms are ubiquitous in our homes. Although most of these microbes are innocuous, some of these household bacteria and fungi can have important impacts on human health. Unfortunately, we have a limited understanding of how these household microbial communities vary across different geographic regions or the factors that structure their biogeographical patterns. I will highlight two recent projects that leverage the power of 'citizen science' to investigate the microbes found inside homes. In the first set of studies, we collected dust samples from ~1,500 households across the U.S. to understand the continental-scale distributions of airborne bacteria and fungi inside homes. We assessed the diversity and sources of these airborne bacteria and fungi, yielding our first insight into how airborne microbial communities are influenced by climate, home occupants, and home design. More recently, we have been focusing on those bacteria living inside showerheads. Showerheads can harbor large populations of mycobacteria, a diverse group of bacteria that includes opportunistic pathogens capable of causing nontuberculous mycobacterial (NTM) lung infections, an increasing threat to public health. To determine how the diversity and abundances of mycobacteria vary spatially and in response to changes in household water chemistry, we recruited >600 volunteer households from across the United States and Europe to sample their showerhead biofilms. We found that showerhead mycobacterial communities vary in composition depending on geographic location, water chemistry, and water source, with households receiving water treated with chlorine disinfectants having particularly high abundances of certain mycobacteria. Regions where NTM lung infections are most common were the same regions where pathogenic mycobacteria were most prevalent in showerheads, highlighting the important role of showerheads in the transmission of NTM infections. Together these results demonstrate the power of a 'citizen science'-based approach to improve our understanding of those microbes living with us in our homes and their impacts on human health.

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The global movement of fungal crop pathogens: models, predictions and perils.

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Over the past centuries crop diseases have led to the starvation of the people, the ruination of economies and the downfall of governments. Of the various challenges, the threat to plants of fungal (and oomycete) infection outstrips that posed by bacterial and viral diseases combined. Indeed, fungal and oomycete diseases have been increasing in severity and scale since the mid 20th Century and now pose a serious threat to global food security and ecosystem health (Fisher *et al*., 2012 *Nature* 484 185; Fisher *et al.,* 2016 *Philosophical Transactions Royal Society B* 1709 201; Fones *et al.,* 2017 *Microbiology Spectrum* 5 2).

We face a future blighted by known adversaries, by new variants of old foes and by new diseases. Modern agricultural intensification practices have heightened the challenge - the planting of vast swathes of genetically uniform crops, guarded by one or two inbred resistance (R) genes, and use of single target site antifungals has hastened emergence of new virulent and fungicide-resistant strains. Climate change compounds the saga as we see altered disease demographics - pathogens are moving poleward in a warming world (Bebber *et al.,* 2013 *Nature Climate Change* 3 11).

This presentation will highlight some current notable and persistent fungal diseases. It will consider the evolutionary drivers which underpin emergence of new diseases and manmade “accelerators” of spread. I will set these points in the context of a series of different disease modelling meta-analyses, which show the global distributions of crop pathogens (Bebber *et al.,* 2013 *Nature Climate Change* 3 11), their predicted movement (Bebber *et al.,* 2014 *New Phytologist* 202 901; Bebber and Gurr 2015*, Fungal Genetics and Biology* 74 62; Bebber *et al*., (2019) *Global Change Biology* (*in press*) and crop disease saturation (Bebber *et al.,* 2014 *Global Ecology and BioGeography* 23 1398). I shall present a new mechanistic model for predicting Septoria Tritici Blotch disease risk on wheat grown in temperate climes (Fones and Gurr, 2015 *Fungal Genetics and Biology,* 79 3), parametrised with experimentally-derived data for temperature and wetness-dependent germination, growth and death in *Zymoseptoria tritici* (Chaloner *et al.,* 2019 *Philosophical Transactions Royal Society B* rstb.2018.0266). I shall conclude with some thoughts on future threats and challenges, on fungal disease mitigation (and of the jeopardy of azole resistance (Fisher *et al.,* 2018 *Science* 360 739)) and of ways of enhancing global food security.

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Multivariate microbiome data analysis and omics data integration

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Our recent breakthroughs and advances in culture independent techniques, such as whole genome shotgun metagenomics and 16S rRNA amplicon sequencing have dramatically changed the way we can examine microbial communities. But are many hurdles to tackle before we are able to identify and compare bacteria driving changes in their ecosystem. In addition to the bioinformatics challenges, current statistical methods are limited to make sense of these complex data that are inherently sparse, compositional and multivariate.

I will present our latest methodological developments to identify multivariate multi-omics microbial signatures using dimension reduction methods. Our methods are implemented in our R toolkit mixOmics dedicated to biological (omics) data integration.

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Not available

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Not available

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Identifying new metabolic pathways and drug targets in microbial pathogens

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Genome-wide predictions of microbial metabolic networks are commonly used to predict the druggable space of different microbial pathogens. However, many microbial genes remain functionally undefined suggesting that these network are incomplete. Perhaps more surprisingly, recent high mass resolution mass spectrometry metabolite profiling studies have highlighted the presence of large numbers of chemically undefined metabolite peaks in microbial extracts. Undefined metabolite peaks typically account for more than half of all detected peaks, indicating that many metabolic processes and potential drug targets remain to be discovered. We have developed a new approach for defining the 'dark metabolome' of bacterial and protist pathogens that involves a labeling step (with ten ^{13}C-labeled carbon sources to specifically tag endogenous metabolites) followed by detection of labeled metabolites using complementary mass spectrometry platforms. These analyses have been used to identify a number of novel metabolites and potential pathways in different pathogens. They have also highlighted the large number of metabolites that are likely by-products of enzymes in central carbon metabolism. These metabolites can be allosterically active or toxic if they accumulate and often need to be catabolized by 'metabolite repair enzymes. These analyses suggest that characterization of the dark metabolome will reveal processes that underpin the evolution of new pathways in microbes, as well as highlighting novel drug targets.

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ARDaP: Antimicrobial Resistance Detection and Prediction from whole-genome sequence data

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Antibiotic resistance (ABR) is a major threat to human health worldwide, with increasing instances of multi-drug resistant pathogens emerging to diminish the effectiveness of antibiotics(1). Whole-genome sequencing (WGS) is rapidly changing the clinical microbiology landscape, with exciting potential for rapidly and accurately detecting ABR in diagnostic laboratories, a crucial factor in infection control and treatment. Most work to date has focussed on the development of software capable of detecting the presence of mobile genetic elements conferring ABR from WGS data(2). However, less consideration has been given to the identification of chromosomally-encoded ABR mechanisms, such as single-nucleotide polymorphisms (SNPs), insertion-deletions (indels), copy number variants (CNV), and functional gene loss.

We present an improved software for Antibiotic Resistance Detection and Prediction (ARDaP) from WGS data. ARDaP was designed with two main aims: 1) to accurately identify all characterised ABR genetic mechanisms and present the predicted ABR profile in an easy-to-interpret report; and 2) to predict enigmatic ABR mechanisms based on i) novel mutants in known ABR-conferring genes, or ii) a microbial genome-wide association approach that correlates ABR phenotypes with genetic variants to identify putative causative mutant/s.

We demonstrate the applicability of ARDaP using the Tier 1 select agent and melioidosis pathogen, *Burkholderia pseudomallei*, as a model organism due to its exclusively chromosomally-encoded ABR mechanisms and high mortality rate (3). Using an extensive, well-characterised collection of 991 *B. pseudomallei* clinical strains, we demonstrate that ARDaP can accurately detect all known ABR mechanisms in *B. pseudomallei* (>40 mutations) with high rates of precision and recall. Furthermore, ARDaP predicted four novel loss-of-function mutations that decreased meropenem susceptibility in *B. pseudomallei*; this phenotype is associated with increased treatment failure and fatality rates(3). ARDaP is a comprehensive and accurate tool for identifying and predicting ABR mechanisms from WGS data. Its clinician-friendly report(4), which summarises a given strain's AbR profile, holds great promise for informing personalised treatment regimens and treatment shifts in response to the detection of precursor or ABR-conferring mutations.

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Unveiling microbial diversity and activity in cystic fibrosis sputa using microbial metatranscriptomics

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Cystic fibrosis (CF) is the most common life-shortening inherited condition in people of European descent, affecting between ~80,000 people globally. CF pathogenesis is most prominent in the airways, where it causes the production of thick, tenacious mucus that provides ideal conditions for microbial pathogens to persist and thrive. Although metagenomics has shown promise with other clinical sample types (e.g. faeces), the CF microbiome has proven particularly challenging due to high (~99%) human DNA contamination, which overwhelms the microbial signal. Here, we used microbial metatranscriptomics (MMT) to examine the polymicrobial population of CF sputa from four Australian adult CF patients. MMT involves extracting total RNA, followed by removal of human mRNA and rRNA, leaving predominantly enriched bacterial mRNA and rRNA for sequencing. Unlike metagenomics, MMT provides an accurate snapshot of the 'active' polymicrobial population (i.e. no sequencing of residual 'dead' cells or reagent DNA contamination), it captures RNA viruses, it presents fewer ethical issues and greater human nucleic acid depletion efficiency, and it can theoretically identify gene expression differences conferring clinically relevant phenotypes for target species of interest (e.g. antimicrobial resistance caused by efflux pump upregulation). Among the four patients, MMT and 16S microbiomic sequencing yielded taxon assignments that were in broad agreement, although MMT had superior resolution; for example, MMT correctly identified miscalled *Burkholderia* sp. as *Pseudomonas aeruginosa*. Consistent with other studies, Gram-negative anaerobic bacteria were abundant, with *Prevotella* (mainly *P. melaninogenica*), and *Veillonella* spp. found in high abundance in all patients, and the pathogens *Stenotrophomonas maltophilia* and *P. aeruginosa* found in two and three patients, respectively. Taken together, we demonstrate the feasibility of MMT for unveiling the 'active' polymicrobial populations present in the CF airways. Although MMT is currently an immature method, it holds great promise for accurately characterising the composition, diversity, and function of polymicrobial infections. Future work is needed to assess the value of MMT for identifying antibiotic resistance and informing antibiotic treatment regimens in CF infections.

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Biological insights into the mechanisms that define the lipidomic landscape of *Acinetobacter baumannii*

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Acinetobacter baumannii is a Gram-negative nosocomial pathogen associated with significant morbidity and mortality in susceptible individuals. A number of persistence and resistance strategies contribute to the success of this organism, including an ability to alter the biophysical properties of the membrane in response to changing environmental conditions. This process is achieved, in part, by the fatty acid and phospholipid biosynthetic pathways. However, the molecular basis and the interplay of these lipid homeostasis mechanisms in *A. baumannii* is largely ill-defined. We have identified critical roles of two related, but functionally distinct desaturases in unsaturated fatty acid production and defined that these are co-ordinately regulated by fatty acid sensing regulators. The two major negatively-charged phospholipid species present in the *A. baumannii* membrane are phosphatidylglycerol and cardiolipin, with their biosynthesis relying upon the phosphatidylglycerolphosphatases PgpA and PgpB. We found that *pgpB* is co-transcribed with *adeIJK*, a tripartite efflux pump which has recently identified to be involved in lipid extrusion, inferring a further association with lipid homeostasis. To understand the relative contribution of the desaturases, PgpA/B and the AdeIJK efflux system in defining the *A. baumannii* lipidome, individual deletion derivatives were examined for their fatty acid and phospholipid composition and the subsequent biophysical properties of the membrane. Given the essentiality of fatty acid and phospholipid biosynthesis in bacterial viability, and their critical role in stress adaptation, investigations into the mechanisms of lipid homeostasis are crucial for understanding how this pathogen of global significance adapts to host-induced and environmental stress.

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Howard Walter Florey: Baron of Adelaide and MarstonHoward Walter Florey: Baron of Adelaide and Marston

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Not available

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History of the Institute of Medical and Veterinary Science

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The history of IMVS is a fascinating story of far-sighted idealism, service, extraordinary personalities and political difficulties. From the early 1900's, the SA Government Laboratory for Bacteriology and Pathology (SAGLBP) provided a comprehensive State-wide service that included diagnostic testing, public health, preparation of vaccines and sera, veterinary and agricultural work. With leadership from Dr (later Sir) Trent de Crespigny, this was consolidated into the Institute of Medical and Veterinary Science under its individual Act of Parliament in 1938, including the brief to pursue research into problems with a direct bearing on the diagnosis and treatment of disease of humans and animals.

Continuing expansion occurred. Many innovations followed, including the very early introduction of computer facilities for data processing and autoanalysers; responsibility for public health and food hygiene work; an extensive state-wide network of regional laboratories and patient collection centres; a bulk-billing arrangement with the Commonwealth for private pathology tests, and the successful winning of the majority of the State's private pathology; many successful research programmes, including the formation of the Hanson Centre for Cancer Research. Staff included many individuals who played a recognised role internationally in microbiology, and many who went on to be leaders in the profession in Australia. With a staff of over 1,000, IMVS was one of the largest diagnostic/research institutions in the country.

Over recent years the IMVS underwent a number of reviews, partly prompted by its somewhat anomalous position in the accepted structure of State health services, partly due to financial difficulties across the whole of the health sector. Research came to be not always seen as a valid activity for the IMVS, and privatisation was raised as a possible solution to the "problem" on occasions. Finally, in 2008 the IMVS Act was repealed, the Council dissolved, and a new entity SA Pathology was created as a part of the Central Northern Health Service within the SA Department of Health.

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CJ: Charles James Martin, an unstoppable plugger

Cheryl Power[1]

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Howard Walter (1898-1968) was born in Malvern, Adelaide.
After graduating MB BS 1921 from the University of Adelaide he sailed for England as a Rhodes scholar.
He studied under Sir Charles Sherrington at the University of Oxford then transferred to Cambridge to complete his doctoral thesis.
In 1935 he was appointed professor at the Sir William Dunn school of pathology at Oxford . It was here he made his greatest contribution to science : the development of penicillin.
He was assisted in this work by Ernst Chain and Norman Heatley.Florey was knighted in 1944, won (with Chain and Fleming) the Nobel prize for physiology and medicine in 1945 and received many other awards.
In 1965 he was created Baron Florey of Adelaide and Marston. When he died in 1968 his estate was sworn for probate at thirty thousand pounds. He had made no fortune from his work for in his time ethical medical principles forbade the patenting of penicillin.

When told of the immense value of his work to relieve the suffering of mankind, work that saw the inauguration of the antibiotic era, Florey said that the project was originally driven by scientific interests, and that the medicinal discovery was a bonus.

1. L.Bickel. Rise up to life (Syd.1972)

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Student-Professional-Academic Co-creation of collaborative and active learning approaches in the new UTS PC2 Superlab

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Laboratory skills are considered a core competency in microbiology graduates (and biomedical sciences in general). However, it is challenging to ensure active, inquiry based learning can occur in laboratory settings within the typical bachelor degree curriculum along with sufficient learning and practise in core-competencies. UTS has designed a new PC2 superlab (HIVE Superlab) that is focused on biomedical and biotechnological discipline areas. The design approach has been based on a 'flipped laboratory model' where the classes are expected to operate as collaborative, student lead inquiry based learning projects. The challenge is to design these learning approaches for success. Using a literature review, and consultative process we set out to design learning 'blue prints' for the HIVE Superlab. The blue prints have been informed from the perspectives of academics, professional, and students and existing literature. This talk will present our findings from our literature analysis, consultations and focus groups and our resulting proposed learning design blueprints.

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A 'Students as Partners' Approach to Co-creation of Assessment and Feedback to improve Engagement

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Cook-Sather et al. (2014) outline the benefits of a students as partners paradigm; demonstrating increased engagement for learners and development of self-awareness and agency for student peer leaders. This presentation showcases the application of a multi-disciplinary pedagogical partnership project founded on principles of co-creation, peer mentorship and curriculum change agents to transform didactic, underperforming anatomy courses in undergraduate medical sciences. In addressing problematic first-to-second year transitions, we propose a cohort-based learning community model that stands in contrast to 'students-as-consumers' and departs from the 'sage-on-the-stage' model of teaching. We aim to evaluate the effectiveness of co-created health science assessment in adaptive learning platforms to improve deep learning and confidence levels in the Adelaide Medical School.

Strategies to embrace co-construction of knowledge within the classroom and personalised design of content and feedback by peer leaders, will be provided. In-class activities included co-design of a Anatomical Dialogue Assignment and short-answer response questions for the final examination by 2nd year students; advocating utility of Blooms taxonomy and clinical stem writing. Exam performance demonstrated a minimum grade of 72 ± 11% for student-generated submissions; with a significant improvement in mean examination score ($p<0.05$). Informed by technology preferences, 3rd year peer leaders developed flexible social media revisions channel for interactive, authentic revision content and deployed co-created quizzes in Canvas. Analytical reports provided by Facebook inc. and Canvas informed study habits and engagement. 69% of the student cohort followed the Instagram channel, with impression data demonstrating regular out-of-class interaction with stories.

Course transformation is quantified through a reduction in fail rates from 36% to 12% from 2015 to 2018; retention rates increased (91%) and students saw the benefits to *showing up* to learning sessions (53% attendance increase). Enlisting a Students-as-Partners model has the potential to reignite the spark of interest, inquiry and curiosity in students to not only engage, but excel, without compromising academic rigour. Further, transferability of this approach within the Neuroscience major at Swinburne University of Technology will be showcased.

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Not available

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Not available

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Phosphohydrolase genes in soil are locally responsive but globally conserved

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Phosphorus is an essential macro-nutrient supporting biological productivity in natural and agricultural ecosystems alike. Phosphorus chemical pathways in soil are complex, but organo-phosphorus compounds typically constitute a major component. This organic pool is turned over by a suite of microbial phosphohydrolase enzymes (alkaline and non-specific acid phosphatases and phytases) which cleave phosphoester (P-O) bonds, releasing assimilable orthophosphate for both the soil microbiome and plant communities. Understanding how edaphic factors and soil management can influence phosphohydrolase distribution is important to understanding both environmental biogeochemical processes and managing agricultural fertility using more benign approaches. Shotgun metagenomic methods indicate that alkaline phosphatase *phoD* and *phoX*, class A and C non-specific acid phosphatases and β-propeller phytase genes are the most abundant and responsive genes in soil, but that the relative abundance of each group within communities fluctuates. These shifts do not appear to be dependent upon localized phosphorus bioavailability: in a long-term fertilization experiment under wheat in the UK, genes respond to edaphic factors, particularly exchangeable calcium which acts as a co-factor for several alkaline phosphatase and phytase enzymes. In a second long-term phosphorus fertilization study under Maize and Sorghum in Brazil, gene abundance responds to plant species, being more abundant under Sorghum than Maize. These same genes are present in metagenomes generated from geographically- and ecologically-distinct soils and marine sediments from around the globe. The phylogenetic diversity of these genes is highly conserved and the sequences present are consistent with horizontal genetic transfer event predictions based upon bipartition dissimilarities between PHO gene and 16S rRNA phylogenies. Currently available data suggests the following hypothesis: phosphohydrolase genes are maintained within microbiomes by horizontal genetic transfer processes, but are selected for within particular environments based upon, as yet, unidentified plant associations or edaphic factors.

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The prevalence and distribution of microbial communities that mediate the nitrogen cycle in Australian soils

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The soil biological nitrogen cycle is mediated by physiologically diverse microbial communities that have evolved in accordance with heterogeneous soil microhabitats. It is likely that the distribution of microbial taxa and functions associated with the transformation of the many forms of soil nitrogen is characteristic of the diverse microhabitats of Australian soils. Assisted by various genetic sequencing technologies and databases such as BASE (Biome of Australian Soil Environments; Bissett *et al* 2016) and other industry supported databases, over 1000 samples are utilised to describe the mainly bacterial and archaeal taxa that are typically associated with nitrification in the major climatic zones of Australia, incorporating Christmas and King Island and northern Antarctica. It compares the impacts of disturbance associated with agriculture and non-agricultural across a range of soil orders on the distribution and abundance of prominent autotrophic taxa implicated in ammonium and nitrite oxidation. It also presents an examination of the distribution and abundance of genes typically associated with the N cycle such as amoA, amoB and nifH and the key physical and chemical correlates that explain geographical occurrence patterns. The implications of describing the abundance and distribution of taxa and N cycle genes for measuring, monitoring and managing soils in food and fibre production systems in Australia are explored.

1. Bissett et al. GigaScience (2016). Introducing BASE: the Biomes of Australian Soil Environments soil microbial diversity database 5:21 DOI 10.1186/s13742-016-0126-5

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Whole genome sequence analysis of Clostridium difficile RT017 strains in hospital patients in Cape Town, South Africa

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Clostridioides difficile (*Clostridium difficile*) infection (CDI) is a potentially significant cause of diarrhoeal illness in South Africa, particularly in TB patients who receive long-term intensive antimicrobial therapy. Previously *C. difficile* ribotype (RT) 017 was identified as the most commonly isolated RT in Cape Town hospitals. To investigate potential strain transmission, a selection of RT017 isolates was analysed by whole-genome shotgun sequencing (WGS) using the Illumina MiSeq platform. The analysis included 48 strains from patients attending nine Cape Town hospitals, including two specialist TB institutions, between September 2014 and September 2015, along with 10 strains isolated in 2012 and sequenced as part of an earlier study. Three different approaches were used to investigate strain relatedness – core genome single nucleotide variant analysis (cgSNV) based on a comparison of high-quality candidate SNVs, core genome multi-locus sequence typing (cgMLST), based on 2270 core genome loci and multi-locus variable-number tandem-repeat analysis (MLVA) based on 7 tandem repeat loci. The overall results of the different approaches were largely congruent with all three methods identifying clusters of related isolates according to previously published cut-offs. However, there were differences in the total number and size of clusters with five clusters of 7-18 isolates identified by cgMLST, eight clusters of 2-14 isolates identified by cgSNV and eleven clusters of 2-12 isolates identified by MLVA. Evidence of within-ward and inter-hospital transmission of clonally-related strains was observed, suggesting that patient

movement and/or community sources played a role in strain transmission. In support of *in vitro* antimicrobial susceptibility testing, WGS predicted several antimicrobial resistance determinants, including mutations in the *gyrA* and *gyrB* (fluoroquinolone resistance) and *rpoB* (rifamycin resistance) genes. Transposons carrying *tetM* (Tn916, tetracycline resistance) and *ermB* (Tn6194, MLSB resistance) homologues were identified, with truncated forms of the latter (lacking the *ermB* gene) present in some strains. Taken together, the results suggest that multidrug resistant, clonally-related RT017 strains are circulating within patients attending public hospitals in the greater Cape Town area.

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The Burden of contamination of restaurant non-food surfaces in Qassim region, Saudi Arabia

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Introduction: Restaurants are one of the most common cause of food poisoning worldwide by the contamination in the source of food, the way of food processing or staff. Microbial contamination of non-food contact surface can occur at various times of food processing. It can be transmitted through table, dishes, menus and other surfaces. Many resistant bacteria and their mode of resistance can come from natural environments including soils and water. Therefore, this study aimed to isolate and characterise bacterial species that contaminate non-food contact surfaces in different restaurants through Qassim region, Saudi Arabia.

Methods: This was an experimental study design performed on selected restaurants including high-ways, fast food and five-stars rated restaurants in Qassim region, Saudi Arabia. Forty-two (N=42) samples were collected from tables, dishes and menus. Collected samples were then cultured and each isolated bacterium was identified using standard bacteriological methods. Isolates were further analysed using Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF technology) and Beckman Coulter MicroScan.

Results: Thirteen different species of microorganisms were successfully isolated and identified. The most isolated bacteria were *Panatoea* spp. (27%) followed by *Staphylococcus* spp.(25%), *Micrococcus* spp. (20%), *Enterococcus faecalis* (9%), *Bacillus* spp. (8%), *Acinetobacter* spp.(3%), *Paenibacillus faecis* (2%), *Ewingella americana* (1%), *Proteus spp.*(1%), *Psycobacter spp.* (1%), *Pasteurella spp.* (1%), *Massilia timonae* (1%) and *Aspergillus fumigatus* (2%). A number of isolated bacteria were also found to be resistant to amoxicillin, ampicillin, azithromycin and vancomycin.

Conclusion: This study elucidates the restaurants as potential areas of bacterial accumulation. Isolates had not been reported before like *Ewingella americana* and *Paenibacillus faecis* were successfully found in this study. The results of current study may help to guide the cleaning protocols on microbial contamination of non-food contact surfaces in restaurants and improves the food safety for the public. Further studies are required to elaborate biofilm existence which might act as a long-term source of contamination.

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Antimicrobial susceptibility of bovine respiratory disease isolates obtained from Australian feedlots (2014-2018)

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Introduction:

Bovine respiratory disease (BRD) causes high morbidity and mortality in beef cattle worldwide. Antimicrobial resistance (AMR) monitoring of BRD pathogens is critical to promote antimicrobial stewardship in veterinary medicine for treatment and control of BRD. In this study, the antimicrobial susceptibility of BRD pathogens - *Pasteurella multicoda (Pm), Mannheimia haemolytica (Mh)* and *Histophilus somni (Hs)* - was determined to 18 antimicrobials including those commonly used in the treatment of cases in Australian feedlots including macrolides, tetracyclines and ceftiofur.

Methods:

BRD pathogens were obtained from Veterinary Diagnostic Laboratories and direct submissions from large feedlots in two states from 2014-2018. A total of 55 *Mh,* 75 *Pm* and 35 *Hs*, were identified using MALDI-TOF MS. The BRD pathogens underwent minimum inhibitory concentration (MIC) testing to screen for antimicrobial resistance using the Sensititre system and MICs were

interpreted using CLSI VET08 (CLSI, 2018). Resistant isolates were subtyped using Random Amplified Polymorphic DNA (RAPD-PCR) assay using arbitrary primers to resolve clonality.

Results:

Antimicrobial susceptibility testing confirmed that all *Mh* and *Hs* isolates from 2014-2018 were susceptible to all antimicrobials tested. All *Pm* isolates from 2014-2015 were susceptible to all tested antimicrobials. Resistance to at least one antimicrobial (ampicillin, oxytetracycline, penicillin, tilmicosin and tulathromycin) increased to 12.5% and 17.4% for the 2016-2017 and 2018 isolates, respectively. The nine resistant *Pm* isolates belonged to three different RAPD patterns.

Conclusions:

An increased proportion of resistance was observed over the sampling period and tulathromycin resistance was observed for the first time in *Pm* from cattle in Australia. The two isolates that were resistant to tulathromycin belonged to the same clonal lineage, but were from QLD and SA, respectively. Tulathromycin is commonly used for treatment of BRD in Australia, hence the use of a regular antimicrobial resistance surveillance program for beef feedlots is necessary to detect changes in the susceptibility patterns for detection of emerging types of resistance and effective treatment of BRD.

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Bacterial Contamination with the use of Automated Teller Machines (ATMs) in Buraydah city, Saudi Arabia

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Introduction: Automated teller machines (ATMs) are widely used by many people, and they tend to harbor a numerous number of microorganisms on their surfaces. The hand borne transmission through ATMs is one of the most important routes for the spread of infectious agents in the community. The aim of this study is to investigate whether ATMs can act as potential vectors for bacterial contamination.

Methods: This is an observational study using cross-sectional study design. The study included collection of 30 surface swab from the ATMs of 5 different bank in Buraydah city, Saudi Arabia. The samples were collected using sterile cotton swab soaked with sterile water. The samples were then cultured immediately in to Blood agar and MacConkey agar. Each isolated Bacteria was identified using standard bacteriological methods after 24 hours of incubation. Selected isolates were further analysed using Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF technology).

Results: Nine different microorganisms were isolated & identified. The majority (53.97%) were *Staphylococci* spp., being 25.40% *S. aureus* and 28.57% *S. epidermitis*. Other isolated bacteria were 11.11% *Acinetobacter junii*, 1.59% *Acinetobacter baumannii,* 1.59% *Neisseria macacae*, 3.18% *Escherichia coli*, 6.35% *Pseudomonas aeruginosa*, 7.93% *Klebsiella* species and 14.28% *Micrococci*species.

Conclusion: Isolates not reported before like *Acinetobacter junii, Acinetobacter baumannii*, and *Neisseria macacae* were successfully found in this study. This study reveals that a high level of bacterial contamination was detected on ATMs. The study also gives a public health concern as ATMs are strongly suggested to be potential areas for pathogen accumulation. It is highly suggested that the hands should be sterilized by hand sanitizer after ATM usage and ATMs should be well cleaned to reduce microbial contamination. Further studies are required on the bacteria isolated in this study to investigate their biofilm and antimicrobial susceptibility.

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Calibrated Dichotomous Susceptibility Disc Testing of Polymyxin B: A Review

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In response the emergence of plasmid-mediated resistance due to *mcr-1* and increasing evidence against agar based testing of polymyxins, the CDS reference laboratory undertook a review of its disc testing method. A combination of intrinsically resistant and naturally susceptible Enterbacteriaceae and pseudomonads were tested in parallel by agar dilution, disc diffusion and broth microdilution.

With the increasing worldwide prevalence of multi-drug resistant (MDR) Gram negative bacteria, polymyxin has re-emerged as a last resort treatment. Polymyxins have *in vitro* activity against *Pseudomonas aeruginosa, Klebsiella* spp., *Enterobacter* spp., *Escherichia coli, Salmonella* spp., *Shigella* spp., *Citrobacter* spp, *Yersinia pseudotuberculosis* and *Haemophilus influenzae*. It is not active against *Burkholderia* spp., *Proteus* spp., *Providencia* spp., *Morganella morganii, Serratia* spp., *Brucella* spp., *Neisseria* spp., *Chromobacterium* spp., and Gram-positive bacteria.

The minimal inhibitory concentration (MIC) determined by agar and broth dilution were compared to the annular radius obtained by disc diffusion for categorical agreement. The results are presented along with a commentary on the future of polymyxin B 300 u disc testing for CDS users.

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Does banknote act as a reservoir and environmental vehicles for the spread of pathogenic microorganisms?

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Contaminated paper currencies could be a potential source of multi-drug resistance organisms (MDROs). Therefore, there is a likelihood that banknote acts as a reservoir and environmental vehicles for the spread of MDROs, representing a risk to individuals handling paper currencies and posing a public health hazard.

The aim of this study was to evaluate the knowledge of Saudi population on contaminated currency notes and to assess the load of microbial pathogens of paper currency.

This was an observational study using the cross-sectional study design and experimental study. The data was obtainedfrom an online survey using a structured questionnaire. The data wasthen analyzed using EPI INFO V7.Currency notes used for microbial isolation were collected from all Saudi currency denominations from different places including slaughter houses, gas stations, hospital cafeteria. They were assessed through microbiological culture and biochemical techniques. The non-identified isolates were further analyzed using Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF).

The total participants in this survey was 1415 Saudi adult citizens. Approximately 60% of respondents did not know the level of contamination on the currency notes. Among the respondents, 57.1% reported not to wash their hands after handling contaminated currency, the majority of which was male, being 78%. Fifteen types of bacteria and one type of fungi were found on the surface of currency notes, including *Enterococcus faecalis, Staphylococcus aureus, Acinetobacter baumannii, Rhizopus, Bacillus* spp*., Klebsiella* spp*., Escherichia coli, Pantoea septica* and*Paenibacillus faecis.*

The Saudi adult awareness about contaminated currency notes was inadequate. To our knowledge, some isolated bacteria have never been previously isolated, including *Enterococcus faecalis*and *Paenibacillus faecis*. The outcomes of this study showed that currency notes could play a role of microorganisms' transmission.

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Examination of Microbial Colonisation and Antibiotics Susceptibility in Pharmacies Door Handles in Qassim Region, Saudi Arabia

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Introduction: The transfer efficiency of microbes to the hands is greater from nonporous surfaces such as acrylic, glass, ceramic tile, laminate, stainless steel and granite when compared with porous surfaces. The community pharmacies door handles are made of nonporous surfaces which raise the concern in possible transfer of potentially pathogenic and multi-drug resistant (MDR) bacteria in the community. This study aimed to isolate, identify the bacterial species and evaluate the antibacterial susceptibility pattern of bacterial contaminants from door handles of community pharmacies in Qassim region, Saudi Arabia.

Methods: This was an observational study primarily conducted in the Qassim region, Saudi Arabia. One hundred samples were collected during the period between February and April 2019, from the door handles of community pharmacies among different cities and countryside. Identification and characterisation were performed by culturing the samples on different agar media and following the standard bacteriological methods. Further analysis was also done using Siemens MicroScan.

Results: All pharmacies door handles were found to be colonised with various types of bacteria. Sixteen types of bacteria were identified, including *Staphylococcus* spp. (41.79%), *Bacillus* spp. (16.45%), *Micrococcus* spp. (13.78%), *Panatoea* spp. (9.35%), *Acinetobacter* spp. (8%), *Enterococcus faecalis* (3.12%), *Enterobacter* spp. (1.77%), *Shigella sonnei* (1.33%), *Serratia plymuthica* (1.33%), *Weeksella virosa* (0.88%), *Klebsiella rhinoscleromatis* (0.88%), *Rhizobium radiobacter* (0.88%), and *Salmonella paratyphi A* (0.44%). Isolates were also evaluated for antibacterial susceptibility testing that revealed several MDR bacteria.

Conclusions: This study shows that pharmacies door handles are contaminated with a diverse range of bacterial species and can act as a potential source of direct bacterial transmission to the community. Therefore, community awareness on hand hygiene should be raised and the use of automatic doors is highly recommended to reduce the chances of the transmission of pathogenic and MDR bacteria in the community. The efficacy of standard cleaning of the door handles should be revised. Further studies on the biofilm existence are also required.

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Women Who Develop Preeclampsia Have Lower Abundance of The Butyrate-Producer *Coprococcus* in Their Gut Microbiota

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BACKGROUND: The gut microbiota plays a role in maintaining human health and in the development or prevention of illness partially through the release of metabolites. Throughout pregnancy, physiological changes to hormonal, immunological and cardiovascular systems and metabolic regulation are required to sustain fetal growth. In early pregnancy, the capacity of the gut microbiota to produce the short chain fatty acid (SCFA) butyrate is inversely correlated with systolic blood pressure. However, it is unclear if the gut microbiota composition is altered in women developing hypertension or preeclampsia later in pregnancy. In the present study, we investigate gut microbiota composition at 28 weeks gestation between women who go on to develop preeclampsia and non-hypertensive pregnant controls.

METHODS: The composition of gut microbiota was investigated by 16S rRNA sequencing of faecal samples obtained from pregnant women in the SPRING cohort (Study of Probiotics IN gestational diabetes) at 28 weeks gestation. Gut microbiota composition was compared between pregnant women who developed preeclampsia (n=11) and controls (n=200). Quantitative real-time PCR was used to assess the density of butyrate-producing genes.

RESULTS: Women who develop preeclampsia had significantly decreased abundance of the genera *Coprococcus*, *Parabacteroides*, *Roseburia*, Unclassified *Christensenellaceae* and Unclassified *Clostridiales*. Abundance of the *Coprococcus* genus, which is known to express butyrate-producing genes, is significantly positively correlated with the gene density of the butyrate genes *BUT* and *BUK*and total butyrate-production.

DISCUSSION: The results suggest that reduced abundance of butyrate-producing bacteria contributes to increased risk of preeclampsia in pregnant women.

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Comparison of the Elution Buffer and Concentration Methods for Detecting Norovirus from Various Foods

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Norovirus (NoV) is a leading cause of gastroenteritis outbreaks in human. Common sources of NoV outbreaks include contaminated foods such as fruits, produce and ready-to-eat foods. The detection methods for NoV in various foods are important for preventing and investigating foodborne outbreaks through foods contaminated with NoV. This study aimed to compare the elution buffers and concentration methods for detecting NoV from various foods. Twenty-five gram food samples were artificially inoculated with murine NoV (MNV). Spiking experiment with MNV-1 was conducted to determine the efficiency of viral elution-concentration methods. The inoculated virus on each food was washed using 0.25M threonine-0.3M NaCl (pH 9.5), TGBE (100mM Tris-HCl, 50mM glycine, 3% beef extract (pH 9.5)), 0.25M glycine-0.3M NaCl, Phosphate buffered saline (PBS) and TRIzol reagent. As the next procedure, viral concentration was conducted using polyethylene glycol (PEG) and ultrafiltration (UF). Real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed to detect MNV-1 RNA. The result showed that the recovery rate of the most efficient methods for the elution and concentration was 52.7% for lettuce by TGBE-PEG, 38.3% for cabbage by TGBE-ultrafiltration, 32.3 % for onion by TRIzol, 66.4% for cucumber by TGBE-PEG, 62.1% for tomato by TGBE-PEG, 59.1% for apple by TGBE-ultrafiltration, 71.6% for grape by TGBE-PEG, 24.1% for strawberry by TGBE-ultrafiltration, 68.9% for sesame leaf pickle by TRIzol, 70.0% for pickled radish by TRIzol, 71.8% for salmon by PBS, 72.8% for laver by PBS-PEG, 75.9% for Gimbab by TGBE-ultrafiltration, 37.0% for sandwich by Threonine-ultrafiltration, 38.3% for salad by TGBE-ultrafiltration, 54.8% for a boiled egg by TGBE-ultrafiltration, and 57.3% for raw beef by Glycine. Based on our results, it is concluded that these methods could be practically exploited for detection of NoV in various foods.

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Optimisation of a microbiological method for the re-isolation of a *Salmonella* Typhimurium *aroA* deletion mutant from mixed samples

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Vaxsafe® ST (strain STM1) is the only live attenuated vaccine registered in Australia for use in the control of *Salmonella* in poultry. Attenuation was achieved through the inactivation of *aro*A in the parent strain, *Salmonella enterica* subsp. enterica serovar Typhimurium strain 82/6915. The attenuation that makes STM1 an ideal vaccine candidate also renders it difficult to reisolate from mixed samples such as chicken faecal or cloacal swab samples. The microbiological protocols, used for *Salmonella* detection, described in the ISO 6579-1 and EP Monograph 2.6.13 guidelines result in low and inconsistent re-isolation of STM1. Further development and optimisation of these methods was carried out to improve the recovery rate of Vaxsafe® ST. The optimised method was evaluated using mixed samples where faecal material was present to mimic field

conditions. Multiple aspects to the ST reisolation method were re-evaluated and optimised to increase Vaxsafe® STM1 recovery from mixed samples with complex and diverse competing flora. Evaluation of several commercially available plating media indicated that the Oxoid™ OSCM media was the best and most specific STM1 reisolation media. The addition of an antifungal-antimicrobial cocktail during pre-enrichment and aromatic supplement combination during selective enrichment led to the improved STM1 reisolation frommixed backgrounds. The optimised method improved growth of pure STM1 and reisolation of the parent strain, but the reisolation rate was reduced in the presence of competing flora, e.g., in chicken faecal material. This reduction in recovery rate is due to the *aro*A attenuation of STM1, rendering it less able to compete with other faecal flora compared to the wild type parent ST 82/6915.

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Co-culturing *E. coli* with a *Salmonella* Typhimurium *aroA* deletion mutant can restore H_2S production in the mutant strain

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Salmonella enterica serovar Typhimurium strain 82/6915 was attenuated by transposon mutagenesis (Tn10) generating the vaccine strain STM-1, which resulted in functional inactivation of *aroA* (Alderton *et al*., 1991). Inactivation of *aroA* renders the strain auxotrophic for aromatic amino acids (tryptophan, tyrosine and phenylalanine) and is associated with the loss of detectable H_2S production on XLD media. A *Salmonella* spp. with functional AroA results in cream coloured colonies with black centres (H_2S), which is characteristic of *Salmonella* spp. on XLD media. The parent strain can produce H_2S on XLD media. Observations in the field and in *in vivo* chicken trials when STM-1 was recovered from mixed samples containing common chicken intestinal microflora, e.g., *E. coli*, identified colonies of STM-1 that appeared to produce H_2S on XLD agar. The role of co-culturing an *Escherichia* spp. with STM-1 in compensating for the *aroA* deletion and restoring the H_2S producing phenotype was investigated by cross-streaking and co-culturing in broth then co-plating on XLD agar.

H_2S production is observed in *Salmonella* Typhimurium D*aroA* strains when co-cultured with *E. coli* and to a lesser extent, *E. fergusonii*. H_2S production is restored only when STM-1 is in physical contact with an *Escherichia* colony. Growing STM-1 in the presence of filter sterilised supernatant from an *Escherichia* culture does not lead to H_2S production in STM-1. It is likely that the compound responsible for phenotypic reversion to H_2S production is not in sufficiently high concentrations in the supernatant or is not stable in the environment, which is why colony to colony contact is required. Continual cell turnover is the likely source of compounds produced downstream of *aroA* in the aromatic amino acid biosynthesis pathway, e.g., the branch chain intermediate chorismate.

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Chlamydial infection and on-farm risk factors in dairy cattle herds in South East Queensland

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Chlamydia spp. in dairy herds have been associated with reduced performance and occasional, but severe, disease manifestations. While chlamydial infections are well described in sheep, very little is known about these infections and the associated risk factors in cattle, including in Australia. This study sought to understand chlamydial infection and on-farm risks in dairy cattle herds from Southeast Queensland (SE Qld) region of Australia.

In total, 228 paired vaginal and rectal swabs were collected from 114 outwardly healthy dairy cows from four farms in SE Qld. Risk factors were rated by observational study and included: hygiene of cows, walkway and parlour, perinatal mortality, external replacements, mode of breeding, calving pen management, heat reduction strategies and feed ration usage. Screening for chlamydial pathogens (*Chlamydia pecorum*, *Chlamydia psittaci* and *Chlamydia abortus*) was done using species-specific quantitative polymerase chain reaction (qPCR) assays. Detected levels of chlamydial infection were evaluated against the on-farm risk factors known to correlate with increased chlamydial infection loads. *C. pecorum* was found to be present at high levels in all four farms, with an overall prevalence of 56.1% (64/114). *C. abortus* and *C. psittaci* were not detected in any animals with *C. abortus* believed to be exotic to Australia. No statistically significant relationship was found to exist with risk factors and *C. pecorum* infection levels in these dairy farms.

A retrospective screening of healthy and diseased beef cattle from Central NSW also revealed high prevalence for *C. pecorum*, but that other chlamydial infections such as *C. psittaci* are common. In beef cattle, *C. pecorum* infections could range from sub-clinical shedding through to Sporadic Bovine Encephalomyelitis associated with high mortality.

This study suggests that *C. pecorum* is the endemic species infecting cattle in Australia, and that these infections are likely to cause economical loses to the producers as well the overall industry.

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Vancomycin Resistant *Enterococci* (VRE) screening quality assurance program: a five-year review of methods and performance

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Background:

VRE are strains of *Enterococci* that have developed resistance to vancomycin, a glycopeptide antibiotic used to treat serious enterococcal infections.

Vancomycin-resistant *Enterococcus* was first described in England in 1988. Since then, it has increasingly become a major nosocomial pathogen worldwide.

Given the importance of accurately reporting VRE, the RCPAQAP Microbiology introduced the program "*Enterococci* for identification, antimicrobial susceptibility and *van* gene detection" in 2011. It was renamed in 2013 to become the "Vancomycin Resistant *Enterococcus* (VRE) Screening" program to be more aligned with the relevant guidelines. Participants enrolled in this program are currently from Australia, New Zealand, Asia and Europe. Enrolments grew from 52 participants in 2011 to 96 in 2018.

Material/methods:

Four simulated samples representing rectal swabs are sent twice a year. Once reconstituted, samples are suitable for enterococcal culture and/or molecular testing. Participants are asked to perform "VRE screen" testing as per their laboratory protocol.

The RCPAQAP direct data entry is used to capture methods, results and overall comments. From 2014 to 2018, methods and algorithms used by participating laboratories to test for VRE were analysed and participant performance was assessed.

Results:

The majority of participants use culture-based methods. However, overtime we have seen a minor increase from around 25% in 2014 up to 31% in 2018 of participants employing molecular methods alone or in combination with culture and other methods

Overall, participants delivered an acceptable level of performance, with greater than 90% reporting correct responses.

Conclusion:

Over the course of five years, whilst false positive and false negative results remain an issue for some participants, overall there was a high level of concordance.

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Rapid Differentiation of Staphylococcus from Positive Blood Cultures Using The Xpert® MRSA/SA BC Assay

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Sepsis is a life-threatening condition and one of the leading causes of death in hospital patients worldwide. Timely recognition and management of patients is of paramount importance as delays are associated with high morbidity and mortality. This study evaluated the utility of the Cepheid® Xpert® MRSA/SA BC Assay to rapidly differentiate between *Staphylococcus aureus*, Methicillin-resistant *S. aureus* (MRSA) from other Gram positive cocci (including Coagulase negative *Staphylococcus* (CoNS)) from positive blood cultures where Gram positive cocci in clusters were seen in Gram stain.

Positive Blood Cultures in which Gram positive cocci in clusters were seen in Gram stain from March 2016 - July 2018 were included in the study (n=100). Samples were tested using the Xpert® MRSA/SA BC Assay for the *spa*, *mecA* and SCC*mec* genes according to the manufacturer's instructions and compared to results obtained from in-house *mecA/nuc* PCR, Vitek 2 Susceptibility testing (bioMérieux) and conventional bacterial culture. Isolates were identified by MALDI-TOF MS (Bruker).

The correct call of *S. aureus* (23%), MRSA (15%) or *S. aureus* negative (58%) was obtained from 96% of specimens tested. Three (3) false negative (3%) and one (1) false positive MRSA result (1%) was obtained.

The Xpert® MRSA/SA BC Assay was able to rapidly differentiate between *S. aureus* and MRSA from other Gram positive cocci in a reliable manner with the potential to significantly decrease morbidity and mortality in septic patients with *S. aureus* bacteraemia.

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Genetic characterisation of phage receptor sites in *Bacillus cereus* and *Serratia marcescens*

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The rise of antibiotic resistance in bacteria represents a large threat to human health. Consequently, new therapies are urgently needed. Bacteriophages (phages) are viruses that infect and kill bacteria. The use of phage therapy, either as a complement or alternative to antibiotics, may provide an attractive means to treat infection and curb the rise of antibiotic resistance. In this study, we have focused our efforts on the isolation and investigation of phages targeting clinically relevant pathogens, *Bacillus cereus* and *Serratia marcescens*. Genomic and ultrastructural investigation of JWP1, a novel siphoviridae phage targeting *B. cereus*, a gram positive spore-forming bacterium, revealed a distinctive genome organisation and highly elaborate and unique tail structure. We designed and implemented a CRISPR-Cas9 system in *B. cereus* to demonstrate that JWP1 is in fact a flagellotropic phage, that is, it utilises the host flagellum as the site of attachment. Specific deletion of the host flagellin genes was shown to be protective against phage infection. *Serratia marcescens* is an opportunistic, gram negative, nosocomial pathogen. We have isolated four novel siphoviridae phages that infect clinical isolates of *S. marcescens* and described their genomes. In an effort to understand how phage-host interactions and spontaneous resistance of these *S. marcescens* hosts to phages may occur in a clinical setting, we are currently employing mutagenesis studies followed by whole-

genome sequencing. This will allow us to determine host to factors essential for phage infectivity and efficacy. Ultimately, the isolation of phages to clinically relevant pathogens and understanding their infectivity will lead to effective treatment options in an era of rising antimicrobial resistance.

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Evaluation of Nucleic Acid Extraction Methods for Detection of DNA Viruses in Urine Specimens

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Urine specimens may contain inhibitory elements that interfere with real-time polymerase chain reaction (qPCR). Our laboratory has been extracting viral nucleic acids from urine specimens via a manual spin column protocol using the QIAamp Viral RNA Mini Kit. Buffer AVL in this kit can remove PCR inhibitors in urine. Automated nucleic acid extraction is performed using EZ1 Virus Mini Kit, which does not contain any reagents that removes PCR inhibition. Thus, an evaluation was performed on automated extraction and manual extraction, in terms of performance and processing time, and whether automated extraction is able to replace manual extraction.

Twenty-eight PCR-positive urine specimens were selected, 24 positive for cytomegalovirus and 4 adenovirus-positive. All specimens were retested in parallel, using automated extraction method as well as manual extraction in the same test setup, to ensure comparability of results and integrity of specimens. The specimens were retested using in-house real-time PCR assays, each with an additional reaction to which 400 copies of virus DNA was added (spiked controls) in order to monitor PCR inhibition.

All 28 samples were positive using real-time PCR after both extraction protocols. Spiked controls showed no inhibition in any of the samples. The Ct values did not differ significantly from each other ($p=0.25$). Automated extraction is able to process up to 14 specimens and requires approximately 45 minutes. This is advantageous for high numbers of specimens. Manual extraction of 1 specimen requires approximately 20 minutes, and is beneficial for small numbers of specimens.

The results suggest that automated extraction for urine specimen is acceptable, and has the potential of replacing manual extraction. However, due to the relatively small number of cases evaluated, further evaluation is necessary.

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Investigating the use of Nanopore sequencing for pathogen surveillance in aquatic birds

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Victoria has diverse avifauna and supports a range of sites where large numbers congregate to breed, migrate, or escape drought. Two important sites for Victorian birds are Melbourne Water's Western Treatment Plant (WTP), which supports tens of thousands of waterfowl, and Phillip Island Nature Parks (PINP), which supports the largest little penguin colony in the world. Due to the ecological and economic importance of these populations, pathogen surveillance is a desirable management tool to identify potential threats and the risk of disease in advance of, and to reduce the incidence of, mass mortality events. With the advent of third-generation sequencing technology, such as Nanopore sequencing through the MinION device, it has become possible for in-field (or near-field) sequencing of samples for rapid simultaneous detection of organisms of interest, and previously unknown potential pathogens. We collected field faecal samples from 46 penguins (PINP) and 121 waterfowl (WTP) to determine optimal extraction methodologies for Nanopore sequencing and investigate the potential of this tool for pathogen surveillance. We obtained the best results for quality and concentration of nucleic acids using traditional phenol-chloroform extraction methods, followed by a kit-based post-extraction 'clean up'. We found that kit-based extraction could be used on samples after concentration by ultracentrifugation. Sequencing was undertaken on a subset of samples to determine which library preparation kit provided the best results. Optimal results were obtained by using Nanopore's low input sequencing kits. The viruses most commonly identified by k-mer matching algorithms were bacteriophage, and invertebrate and plant viruses. DNA from *Clostridium botulinum* and *Clostridium perfringens* was detected in 6/18 and 8/18 samples, respectively, of the sequenced waterfowl species. These organisms are known to cause significant morbidity and mortality to avian specieswhen infections result in disease outbreaks. Further advances in automation of extraction of nucleic acids and subsequent library preparation, Nanopore sequencing technology itself, and rapid dataanalysis, may allow in-field pathogen surveillance of important wildlife populations in the near future.

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Multilevel genome typing and molecular epidemiology of the seventh pandemic clone of *Vibrio cholerae*

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Cholera, caused by *Vibrio cholerae,* in modern history (1817 - present) has spread across the world in seven pandemics. The seventh pandemic started in 1961 and continues today. The global epidemiology and population structure of the seventh pandemic clone have recently been analysed using single nucleotide polymorphisms (SNPs) from genomic data. However, the relatedness of strains between studies is poorly communicated through comparing SNPs. In this study, a multilevel genome typing (MGT) scheme capable of classifying the seventh pandemic was established. Fundamentally, the MGT is based on the multi-locus sequence typing (MLST) concept and assigns sequence types (STs) based on a strain's combination of alleles. However, the concept is expanded to include a series of MLST schemes, capable of comparing population structure at multiple resolutions.

The *V. cholerae* MGT scheme consisted of 3760 loci which were shared across all analysed seventh pandemic strains. The core loci were then organised into nine MGT schemes, with the lowest, MGT1, composed of 17 loci and the highest, MGT9, consisting of 3760 loci (the seventh pandemic core genome). The genetic relationships calculated by smaller schemes of the MGT recapitulated previous findings reporting the large-scale transmission of the seventh pandemic across the globe. Conversely, the larger MGT schemes provided an increased discriminatory power and were able to examine smaller scale trends such as the Nepalese source of the 2010 Haiti outbreak. Additionally, classification of over 5000 seventh pandemic strains showed the MGT is appropriate for analysing large datasets. The MGT identifiers describing the seventh pandemic relationships are not affected by subsequent analysis (are stable) and can be directly compared between various studies (are transferable). The seventh pandemic MGT will allow tracking of new and existing clones and will be useful for controlling future outbreaks and pandemic spread of cholera.

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Evaluation of automated reading of Sensititre™ SGP1MBD panel

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Introduction

Commercial microbroth dilution (MBD) panels are commonly used for determining minimum inhibitory concentrations (MIC). Manual reading of these panels is tedious, time consuming and potentially error-prone. This study compared the accuracy of automated versus manual reading of Sensititre™ SGP1MBD panels (Sensititre) when tested on multi-drug resistant organisms (MDRO). A secondary objective was to compare susceptibility results obtained for a subset of antibiotics between MBD and Vitek® 2 Compact (Vitek).

Methods

MIC testing was performed by Vitek and Sensititre on 42 unique clinical MDRO isolates. Following incubation, Sensititre were read manually and using two automated commercial readers namely Sensititre™ Vizion (Vizion) and BIOMIC® digital reader. Categorical interpretation was determined using CLSI guidelines, with categorical agreement defined as concordant, minor errors, major errors or very major errors, using manual reading as the gold standard. In addition, Sensititre results for Meropenem, Imipenem, Piperacillin-tazobactam, Levofloxacin, Amikacin and Aztreonam were compared to Vitek results.

Results

Isolates included *Acinetobacter baumanii* (n=8) and Enterobactericeae (n=34) strains positive for ESBL (n=12), AmpC (n=13), carbapenemases (n=16) and *mcr-1* (n=2). A total of 714 drug-organism combinations were tested. 99.7% and 98.5% categorical agreements were achieved for BIOMIC® and Vizion results respectively when compared to manual reading. All errors observed were minor and there was no significant result discordance for any antibiotic. MICs obtained by all three methods were within one-fold dilution difference. There was 83.3% categorical agreement for Sensititre results when compared to Vitek. Of the 16.7% errors observed, 60% (n=25) were minor and 40% (n=17) were major errors. No very major error was observed. Carbapenems demonstrated the most number of discordant findings, with 46.2% (12/26) minor errors and 50% (8/16) major errors.

Conclusion

Automated reading of Sensititre by both systems is reliable as both showed no significant result discordance when compared with manual reading. Discordant findings between Vitek and Sensititre were most notable in Carbapenems amongst the six tested antibiotics.

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Antimicrobial susceptibility of *Clostridium difficile* from the Asia-Pacific region

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Background

Clostridium difficile, a spore-forming anaerobe, causes toxin-mediated diarrhoea and pseudomembranous colitis, primarily among hospital inpatients. Some of the most severe outbreaks of *C. difficile* infection (CDI) have been caused by strains with enhanced antimicrobial resistance, particularly fluoroquinolone resistance. These strains include *C. difficile* ribotype (RT) 027, which originated in North America, and RT 017, which is most widespread in Asia. Despite being the most common cause of

hospital-acquired infection in the developed world, and frequent misuse of antimicrobials in Asia, little is known about *C. difficile* in Asian countries. We aimed to determine the resistance profiles of a large collection of *C. difficile* isolates from Asia to an array of antimicrobials.

Methods

C. difficile isolates (*n*=414) from a 2014 study of 13 Asia-Pacific countries were tested for susceptibility to moxifloxacin, amoxicillin-clavulanate, erythromycin, clindamycin, rifaximin, metronidazole, vancomycin and fidaxomicin by the agar dilution method according to the Clinical & Laboratory Standards Institute and EUCAST guidelines. The most common strains among the collection were RTs 017 (*n*=68), 014/020 (*n*=45), 018 (*n*=41), 002 (*n*=38), 012 (*n*=20) and newly emerging strains QX 239 (*n*=15) and RT 369 (*n*=17).

Results

All isolates were susceptible to metronidazole, vancomycin, amoxicillin-clavulanate and fidaxomicin. Moxifloxacin resistance was detected in all countries except Australia, in all RT 369 and QX 239 (MIC_{50}=16mg/L and 32mg/L respectively) strains, 92.7% of RT 018 (MIC_{50}=32mg/L) and 70.6% of RT 017 (MIC_{50}=32mg/L) strains. All RT 012, 369 and QX 239 strains were also resistant to erythromycin (MIC_{50}= >256mg/L) and clindamycin (MIC_{50}= >32mg/L). Rifaximin resistance was common in RT 017 strains only (63.2%, MIC50=>32mg/L) and was not detected in Australian, Japanese or Singaporean isolates.

Conclusions

Antimicrobial susceptibility of *C. difficile* varies highly by strain type and by country across Asia. Emerging RTs 369 and QX 239 show high rates of resistance and high MICs, and the most common strain in Asia, RT 017, is highly resistant to many antimicrobials. Ongoing surveillance is clearly warranted.

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Identification and characterisation of spontaneous teixobactin-tolerant mutants in *Enterococcus faecalis*

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Teixobactin is a new antimicrobial active against a number of multidrug-resistant pathogens, including *Staphylococcus aureus* and *Enterococcus faecalis*, with no reported mechanisms of teixobactin resistance. Understanding how resistance could develop will be crucial to the success and longevity of teixobactin as a new potent antimicrobial. Antimicrobial tolerance has been shown to facilitate the development of resistance and we show *E. faecalis* is intrinsically tolerant to teixobactin at high concentrations. We subsequently chose *E. faecalis* as a model to elucidate the molecular mechanism underpinning teixobactin tolerance and to understand how this may contribute to the development of teixobactin resistance. We attempted to isolate teixobactin resistant and/or tolerant *E. faecalis* mutants using transposon mutagenesis and spontaneous mutagenesis via long-term exposure and serial passaging. No mutants displaying altered susceptibility to teixobactin were isolated using transposon mutagenesis and long-term exposure. Six mutants were isolated from three strains of *E. faecalis* by spontaneous mutagenesis using serial passaging resulting in both higher minimum inhibitory concentration (MIC) values of 4 mg/L (2-fold increase over wild-type) and minimum bactericidal concentration (MBC) values of 64 - >128 mg/L (2-8-fold increase over wild-type). Whole-genome sequencing of these mutants identified mutations in cell wall biosynthesis, with isoprenoid biosynthesis (*mvaD/E*) and exopolysaccharide transport (*epaM*) appearing to play important roles. Further analysis showed that mutants displayed a reduced cell wall thickness with significant changes in gene expression in the absence of teixobactin compared to the parental strain exposed to teixobactin-induced antimicrobial stress. We propose that mutations in *mvaD/E* and *epaM* results in a decrease of cell wall biosynthesis and activation of an “antimicrobial stressed” state.

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Two distinct uptake hydrogenases differentially interact with the mycobacterial respiratory chain to energize cells during persistence

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Soil microorganisms metabolize the trace quantities of atmospheric hydrogen to persist in environments when nutrient sources are limited. High-affinity hydrogenases are the key enzymes that drive the oxidation of atmospheric H_2. Although previous studies have indicated the importance of these hydrogenases in microbial persistence, the mechanism on how they function during carbon limitation is still unclear. In this work, we show how mycobacterial uptake hydrogenases Hup and Hhy are utilized and how they interact with the respiratory chain to generate energy during persistence. Gene expression and biochemical analyses revealed that the hydrogenases have differential expression profiles and activities. Although both hydrogenases are highly expressed and active upon carbon depletion, Hup is most upregulated and active in early-stationary while Hhy in mid- to late-stationary phase. In addition, both hydrogenases are membrane associated and solubilization experiments revealed that Hup forms a weak physical membrane association while Hhy is tightly bound to the membrane. This suggests that both hydrogenases interact with the electron transport chain during carbon limitation. This interaction is confirmed when respiratory chain uncouplers significantly decreased the H_2 oxidation activities of Hup and Hhy. Respiratory measurements also revealed that electrons derived from the activities of both hydrogenases are fed into the quinone pool and are ultimately transferred to cytochrome bc_1-aa_3 complex. Interestingly, Hhy is also capable of terminally feeding the electrons to the cytochrome *bd* oxidase. From these findings, we present a model of how the mycobacterial hydrogenases are functionally linked to the respiratory chain during carbon starvation. The electrons derived from atmospheric H_2 oxidation are used to maintain a membrane potential and support ATP

generation via aerobic respiration. This has implications for how energy is conserved in oligotrophic environments, where microorganisms scavenge atmospheric trace gases to support microbial persistence.

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Evaluation of APAS® Independence for routine urine culture reading within a private clinical setting.

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Background: As culture reading can be highly subjective and time-demanding the use of artificial intelligence such as the APAS® Independence offers to introduce a higher level of consistency and reliability between results. This evaluation was performed to determine if the APAS® Independence was able to be integrated into routine workflow while still maintaining its accuracy, consistency and throughput.

Materials/Methods: Routine samples were set up in duplicate onto HBA/Brilliance media (Thermo Fisher Scientific) and incubated separately. One group of plates was incubated strictly for 18 hours based on APAS® manufacturer guidelines, while the other was incubated overnight to coincide with routine plate reading workflow. Both sets were processed through the APAS® Independence and the growth was evaluated and compared by experienced microbiologists.

Results: The APAS® Independence showed a high level of agreement for both the 18 hour incubation and the routine incubation (>97%). In terms of determining significant vs non-significant growth the APAS showed a sensitivity of 99.2%

Conclusions: The APAS® Independence showed a high level of agreement, reliability and consistency when evaluating plates that have been incubated and processed in a routine clinical setting. This coupled with a high throughput rate may provide benefits when integrated into an appropriate workflow.

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Treatment of *Salmonella enterica* serovar Typhimurium with DsbA inhibitors in physiologically relevant *in vitro* conditions does not result in resistance development

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In Gram-negative bacteria, inhibition of the disulfide bond (DSB) oxidative protein folding machinery -a major facilitator of bacterial virulence- is considered an attractive antivirulence strategy. We have previously developed small molecule inhibitors of the prototypic DsbA enzyme from *Escherichia coli* K-12 (EcDsbA) and showed that they can block DsbA homologues found in pathogens. EcDsbA inhibitors were active against uropathogenic *E. coli* (UPEC) and *Salmonella enterica*, both of which contain more than one copy of DsbA. Our inhibitors attenuated UPEC and *S.* Typhimurium virulence without affecting their growth in rich medium. Here we test the evolutionary robustness of DsbA inhibitors as antivirulence antimicrobials by looking for resistance development in *S.* Typhimurium in physiologically relevant *in vitro* conditions. We demonstrated that DsbA inhibitors had a fitness cost on *S.* Typhimurium, cultured in physiologically relevant conditions, a phenotype that was DsbA-specific, as a *dsbA* null mutant cultured under the same conditions had a similar growth defect. Despite the fitness cost of DsbA inhibitors on *S.* Typhimurium growth, no inhibitor resistance was detected following treatment at sub-IC50 concentrations. No mutations were identified in the target gene and all inhibitor-treated *S.* Typhimurium culture displayed DsbA-mediated virulence phenotypes similar to the untreated pathogen. Importantly, these phenotypes remained sensitive to inhibition by DsbA inhibitors to the same extent as bacteria not previously exposed to inhibitor. Our work provides *in vitro*evidence that DsbA inhibitors do not select for resistant mutants in physiologically relevant conditions, suggesting that they could offer evolutionary robust new antimicrobials to be used instead or in combination with antibiotics.

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Comparison of two Schistosoma IgG Enzyme immunoassays

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Although not endemic to Australia, Schistosomiasis serology is the mainstay for diagnosis of this chronic parasitic infection in a variety of settings including refugees, migrants and travellers to endemic countries. These groups will all have varying prevalence of infection.

The aim of this study was to compare the Euroimmun Schistosoma IgG ELISA kit to the Novalisa anti-Schistosoma IgG ELISA as feedback from clinicians had queried the specificity of the Novalisa ELISA and its potential high rate of false positive results in low risk populations.

In 2018 the seroprevalence of Schistosoma from 2480 specimens tested at Sullivan Nicoloaides Pathology was 19.2% (477) tested positive, 3.3% (81) tested equivocal, and 77.5% (1922) tested negative, with a total positive/equivocal rate of 22.5%. Positive and negative predictive values have been calculated based on a seroprevalence of 20%.

70 samples in total were tested; a sensitivity panel that included 15 high positive (Novalisa >1.5) and 15 low positive equivocal patients (Novalisa 0.90 -1.5) samples and 40 specificity samples that included 20 challenge sample from patients with strongyloides, malaria, filariasis, and amebiasis. All specimens that had not previously been tested using the Novalisa assay (cross-reactors) were tested using both assays. For the analysis equivocal results were treated as positive.

62 episodes (88.5%) were concordant, and 8 discordant (11.5%). Based on the Novalisa results the sensitivity of the Euroimmun assay was 79.4% (CI: 62.1-91.3), specificity 97.2% (CI: 85.5-99.9); PPV 79.4% (CI: 49.2-95.3) and NPV 97.2% (87.7-99.6). Discordant analysis based on clinical history and follow up samples suggested a number of false positive calls with the Novalisa. The adjusted sensitivity of the Euroimmun assay was 96.4% (CI: 79.8-99.8), specificity 97.6% (CI: 85.5-99.9); PPV 92.9% (CI: 64.2-99.6) and NPV 99.1% (89.2-99.9). Cross reactions with Strongyloides positive specimens and a malaria specimen for both assays. The Euroimmun assay was less likely to detect false positive results in the HIV insurance specificity panel.

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Inconsistencies in reporting Anti-Streptolysin O: A Five Year Review

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Anti-streptolysin O (ASO) is an antibody produced in response to the streptolysin O toxin produced by Group A streptococcus (GAS) bacteria. GAS is responsible for most strep throat infections and if left untreated may result in post-streptococcal complications such as rheumatic fever and glomerulonephritis. Diagnostic reporting of ASO results is based on clinical cut-offs with reference to population studies. The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) has a Streptococcus serology module which includes reporting ASO both quantitatively (IU/mL) and qualitatively (significant / non-significant) linked to an associated clinical scenario. RCPAQAP undertook a 5 year retrospective review of the program data to identify any trends in performance and reporting of ASO.

Data from 6 survey specimens with varying levels of ASO (258 sets of data from 43 laboratories) from 2013 to 2017 was reviewed. Statistical analysis only included data sets where participants returned results for all six surveys. Clinical notes for the selected specimens all stated, “a symptomatic 15-year-old patient”. Quantitative and qualitative results were analysed against the cut-off values provided by each laboratory. Assessment of assigned results was determined by a consensus of ≥ 80%.

Three specimens had a consensus result of “non-significant”, two were “significant”, and no consensus was achieved for one specimen. Inconsistencies were identified in the interpretation of the quantitative results against the nominated cut-off. In addition, significant variation was noted in the cut-off values provided both between (1IU/mL to >480IU/mL) and within different method groups (e.g. 116-300IU/mL).

In conclusion, participants provided a variety of cut-off values for the same clinical notes over the 5 year study period. This poses the question on how clinical cut-off values are being determined and reviewed to ensure consistent results are being provided to clinicians. The variation in cut-off values (even within the same method group) highlights a lack of standardisation in this area and the need for the pathology profession to act on harmonising reporting practices.

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RCPAQAP pilot program for Strongyloides detection

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Background: Strongyloidiasis is an infectious disease caused by the parasitic roundworm *Strongyloides stercoralis.* Strongyloidiasis is endemic in tropical and subtropical regions and has been shown to have high prevalence in some remote communities in Australia [1,2]. In immunocompromised individuals, Strongyloides infection can be fatal. In 2018, RCPAQAP Serology issued a Strongyloides Pilot Program to assess the suitability of introducing an external quality assurance (EQA) program.

Methods: The Strongyloides pilot survey was distributed to Australian laboratories for Strongyloides IgG testing as per routine laboratory protocols. The survey consisted of one negative specimen and three positive serum specimens distributed in one survey. The majority of participants used a commercial assay, with only one participant using an In-house assay. Qualitative (pos/neg) and quantitative (OD, Index and S/CO) data was analysed.

Results: Qualitative results showed 100% consensus for all specimens. Inconsistencies were identified in the reporting of cut-off values and units.

Conclusion: Results reflect a high concordance in qualitative reporting of Strongyloides IgG. The introduction of an EQA program may be beneficial for reviewing the quality of results when reporting low level positive specimens with result values close to the clinical cut-off value and assist laboratories in the accurate reporting of Strongyloidiasis.

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Spore forming bacterial populations in New Zealand dairy feeds

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Since the early 2000s forages and supplementary feeds utilised on New Zealand dairy farms have changed but the potential consequences of those changes on the spore populations in the raw milk are unknown. To investigate the potential effect that the bacterial spore populations within the feeds can have on the bacterial spore populations in raw milk, samples of raw milk and the feeds consumed were analysed for their spore populations.

Bacterial spore selection and detection was based on standard microbiological methods, with a heat treatment at 80°C for 12 min to select for spores, followed by detection using two agars and three different incubation temperatures, to capture a wide spectrum of psychrotrophic, mesophilic and thermophilic spores.

From this analysis, the highest counts of bacterial spores were found in tuber-feeds (fodder beet (*Beta vulgaris*) and turnips (*Brassica rapa*)) and palm kernel expeller (made from *Elaeis guineensis)* followed by pasture silage (*Lolium Perenne + Trifolium Repens*) and feed concentrates. Low counts of spores were present in raw milk samples.

The bacterial spores found were identified using a MALDI-TOF-MS Biotyper (Bruker). These data showed that ~ 70 % of the bacterial spore species identified in milk, could also be identified in feeds from the same farm. The results also showed variations in the diversity of the spore populations between different feed samples, milk samples and different farms.

This study indicates that the bacterial spore populations within cow feeds could influence the spore populations present in the raw milk.

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Insights into the dynamic wheat microbiome – responses to challenges by pathogens and beneficial microorganisms.

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As we move into the era of sustainable food production we are developing microbial inputs that are applied to broad acre crop production at a scale which is disrupting traditional chemical usage in modern agriculture. These inputs are derived from, but also affect the microbiomes of the crop plants. The microbiomes within the endosphere and the rhizosphere of wheat seed and plants are dynamic systems which change with the age of crop, the soil, climate and the presence of phytopathogens in the soils.

In this study, we challenged these communities by adding *Streptomyces* and *Paenibacillus* biocontrol strains, separately, to wheat seeds growing with either severe *Rhizoctonia solani* disease or *Pythium* rootrot disease. Wheat plants were grown in the field and compared to plants grown in the same soil in a glasshouse. The bacterial and fungal microbiomes of wheat roots (endosphere) and rhizosphere soils were monitored for 20 weeks, from seed to mature plant stage. A total of 233 samples from a Rhizoctonia infested system and 254 samples from a Pythium infested system were analysed. The results showed highly dynamic and diverse microbial communities over the weeks, with crop age being the main differentiator followed by the presence of biocontrol agents. Bacteria played a stronger role in the early stages of wheat growth while fungi gained importance later on. The initial wheat microbiome was enriched was enriched by the rhizosphere soil microbiome with distinct fungi and bacteria admitted into the core microbiome at different time points.

Streptomyces biocontrol strains promoted plant growth and maturation and the root microbiome stressed such differences at OTU Level with specific disease-responsive OTUs revealed among fungi and bacteria and unclassified Micrococcaceae. Some differences in particular taxa were evident between field and glasshouse trials, but there was a consistency in microbial populations at each time point.

The application of biologically sustainable approaches in agriculture may limit the damaging effects of serious fungal diseases and preserve high levels of microbial biodiversity in soils.

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Group B Streptococcus prevalence, serotype distribution and colonisation dynamics in Western Australian pregnant women

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Streptococcus agalactiae, or Group B Streptococcus (GBS), is a leading neonatal pathogen that causes sepsis, meningitis and pneumonia. Globally, strategies have been implemented to address vertical transmission, and in Western Australia (WA) culture-based screening at 35-37 weeks gestation is part of routine care to guide antibiotic administration. In this study we aimed to describe antenatal GBS colonisation within a large population in WA; this is a population that has received little attention in previous research. A cohort of 814 pregnant women attending antenatal clinics (2015-2017) provided self-collected vaginal and rectal swabs at ≤22 weeks (n = 814) and ≥33 weeks (n = 567) gestation. These were assessed for GBS presence using culture and PCR and serotyping was conducted using molecular methods. Lifestyle questionnaires and medical data were collected. We observed an overall GBS colonisation rate of 24%, with 10.6% of positive participants transiently colonised. Ethnicity (Aboriginal, Torres Strait Islander and African), maternal age ≥25 years, vitamin use, frequent sexual intercourse (≥5 times/week) and use of sex toys were associated with GBS colonisation following logistic regression analysis. The dominant serotypes identified were Ia (27.9%), III (20.9%), II (16.3%), V (15.8%), Ib (8.4%), VI (5.1%), IV (2.8%), NT (1.9), VIII (0.5%) and IX (0.5%) at visit one, with V (18.9%) preceding serotype II (18.2%) at visit two. Serotype VII was not detected. This is the first cohort study to assess GBS colonisation in WA pregnant women and will be highly beneficial for guiding future clinical practice and therapeutic options, in particular, selection of suitable vaccine candidates.

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Automated urine particle analysis: A performance evaluation of three analysers in the investigation of urinary tract infection

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Objective:

Whilst considered the gold standard, manual microscopy has become increasingly replaced with automation in the investigation of urinary tract infection. We evaluated the performance of cobas u 701 (Roche Diagnostics) and Atellica UAS 800 (Siemens Healthineers) against our incumbent IRIS iQ200 (Beckton Dickinson). Detection of particles was compared to the reference standard of microscopy, and a correlation of analyser organism detection was made to semiquantitative culture counts and significant bacterial growth.

Methods:

608 urine samples were run on all analysers and compared to manual microscopy. Data was recorded quantitatively for white blood (WBC) and red blood cells (RBC), semi-quantitatively for bacteria and squamous epithelial cells (SEC), and qualitatively for yeast. WBC and RBC data were divided into categories of 0-10, 11-100, 101-500, and >500 cells/uL. Assessment of bacteria detection was also performed by comparison with total bacterial counts on culture, and with the presence of significant growth of bacteria requiring release of susceptibility results.

Results:

Within critical ranges of WBC counts of 0-10 and >500 cells/uL, all platforms had concordance of ≥85% with microscopy. In the non-critical cell types of RBC and SEC, concordance was lower with no clearly superior performance. Concordance for detection of yeast ranged from 72.9-81.7%. The NPV of nil organisms detected by each platform ranged from 74.9-77.9% across all analysers compared to microscopy. When compared to growth of a predominant organism, and to total bacterial counts on culture, the NPV remained below 80%.

Conclusion:

Both the u 701 and UAS 800 were considered acceptable alternatives to the iQ200. All performed similarly in correctly designating WBC, RBC, and SEC categories. However, performance for detection of RBC and SEC was poor. Additionally, the NPV for detection of bacteria using any method as the gold standard, was considered too low to permit screening out samples not requiring culture.

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Genetic and biological analysis of *Legionella pneumophila* isolates from clinical and environmental water in Korea

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Legionnaires' diseases (LD) is a bacterial infectious disease which is transmitted into the respiratory tracts through aerosol droplet generated in a water system contaminated with *Legionella*. *Legionella* is known to more than 60 sub-species and the most representative strain is *Legionella pneumophila*. LD occurred 210 cases during the last 3 years in Korea, but there are few studies on the characteristics of Korea isolates. To identify the genetic characteristics of Korea isolates, two strains from clinical (KP14) and environmental isolates (KJ08261) were selected from the 100 Korea isolates by phylogenetic studies, and the analyzed by

whole genome sequencing. Biological characteristics including growth kinetics, host cell invasions, and intracellular survival, were compared with reference strain (Philadelphia-1). As a results, the genome size of the two isolates was analyzed to 3,559,584 bp and 3,698,611 bp, respectively, which were larger than those of reference strain (3,397,754 bp). The number of ORFs for two isolates and reference strain was 3,320, 3,178, and 3,003, respectively. The similarity of total genome between three *L. pneumophila* strains is 96%, which is somewhat lower than the similarity of 97% between the clinical and environmental isolates. Common 2,677 genes were shared in all three strains, and the number of specific genes were 117, 233, and 390, respectively. Growth rates in BYE broth were not significantly different from the three strains, but the infectivity and survival abilities in macrophage cells were observed to be the best in the environmental isolate. This study provided the differential genetic and biological data between the clinical and environmental isolates in Korea and our results will be useful as a basic data for the further study of LD in Korea. This research was supported by a fund (2018-NI001) by Research of Korea Centers for Disease Control and Prevention.

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Transcriptomic analysis of growth and host infection related genes for *Legionella pneumophila* isolated from Korea

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Legionella in waterborne environments is biofilm-forming, growing in an inhibited state, or growing and surviving in protozoa such as *amoeba*. It is necessary to sense environmental changes and response by controlling the expression of appropriate genes for successful infection in human. To study the pathogenicity in *Legionella pneumophila* isolated from Korea, we analyzed the expression profiles of transcriptome in the clinical isolate (KP14), environmental isolate (KJ08261), and reference strain (Philadelphia-1). In order to investigate the changes of growth phase dependent gene expression in *L. pneumophila*, we measured the growth rate and compared the differential expressed genes at early exponential phase and early stationary phase. In addition, to study whether the gene necessary for efficient host infection was pre-expressed when *Legionella* as exposed to the host environment, it was compared with BYE broth and DMEM media. Interestingly, the results showed that the profiles of the genes that changed when exposed to DMEM media, were very similar as *Legionella* reached the early stationary phase. Expression of genes for type II and IV secretion system, flagella, and alpha-amylase related genes were up-regulated and genes related to host infection were also increased. However, down-regulation genes for expression of RNA-polymerase and ribosome machinery components are expected to result in a significant decrease in transcription and translation, which may be related to a negative effect in growth rate. Our results will be used for further studies on pathogenicity of Korea isolates. This research was supported by a fund (2018-NI001) by Research of Korea Centers for Disease Control and Prevention.

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Continued evolution of the versatile multi-drug resistant *Escherichia coli* ST131

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The rise of multidrug-resistant pathogens has become a global health concern. One notable pathogen is Escherichia coli ST131, a lineage that is responsible for million cases of urinary tract infections (UTIs) annually (1). UTIs occur via the ascending route through the urethra, mostly by residential gut *E. coli* crossing the physical barrier. Before the pandemic ST131 lineage emerged, UTIs were mostly treatable with antibiotics. However, with resistance to almost all frontline antibiotics, ST131-associated UTIs have become difficult to treat and life-threatening, imposing a huge burden to resource-drained public healthcare (2). Worryingly, we present recent data that certain strains within the ST131 lineage have evolved mechanisms for enhanced transmission by acquiring genes that allow them to be pathogenic also inside of the gut, using both cell line and mouse infection models. These strains represent a significant public health threat and further research on their pathogenesis is warranted.

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Contribution of usage to endoscope working channel damage and bacterial contamination

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The number of endoscopy related infections and outbreaks has increased rapidly over the last few years. Previous study demonstrated biofilm formation associated with damaged areas of endoscope channels (1). Protection of bacterial biofilms within pits and scratches of endoscope channels makes cleaning difficult, contributes to endoscope contamination and pathogen transmission.

We hypothesis that the passage of instruments and brushes through endoscope channels during procedures and cleaning contribute to the channel damage, bacterial attachment and biofilm formation. In this study, we compared the roughness and bacterial attachment in used endoscope channel compared to new endoscope channel in vivo and in vitro.

The surface profiles of ten clinically used retired and seven new colonoscope biopsy channels were analysed by contact profilometer.

For in vitro study, a flexible endoscope biopsy forceps with 2.8 mm of diameter was repeatedly passed through a curved 3.0 mm diameter Teflon tube 100, 200, 500 times. Atomic force microscopy was used to determine the degree of inner surface damage.

10^8/ml *Escherichia coli* or *Enterococcus faecium* culture was circulated in an enclosed flow system containing new Teflon tube and tube with 500 times biopsy forceps passes for one hour at 37°C. Bacteria attached to the inner surface of Teflon tube was determined by colony forming units.

Average surface roughness of clinically used colonoscope biopsy channels was 3.7-fold of the new colonoscope biopsy channels (P=0.03). Surface roughness of Teflon tubes with 100, 200 and 500 times biopsy forceps passes was 1, 1 and 3.2-fold (P=0.025) of the new Teflon tubes respectively.

The bacterial number of *E. coli* and *E. faecium* attached to Teflon tubes with 500 times biopsy forceps passes was 2.9-fold (P=0.021) and 4.3-fold (P=0.018) of the bacterial number attached to the new Teflon tubes respectively.

Our study confirmed the association of endoscope usage with biopsy channel damage and increased bacterial attachment. The endoscope channel after 500 times of usage posed a great risk of bacterial contamination, pathogen transmission and should retire.

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Investigation of pneumococcal gene expression associated with pneumonia pathogenesis

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Streptococcus pneumoniae (the pneumococcus) is the most common cause of community-acquired pneumonia. However, it is also commonly found as an asymptomatic coloniser of the upper respiratory tract. It is not clear how this pathogen may change from the colonisation state to causing disease. This study investigated nasopharyngeal swabs and lung aspirates collected from pneumonia patients as part of ongoing surveillance in The Gambia, along with pneumococcal isolates obtained from these clinical samples. Our aim was to elucidate the molecular processes by which the pneumococcus can transition from the carriage to infection state, and identify potential genes involved in the pathogenesis of pneumococcal pneumonia. Genomic analysis revealed three non-silent genomic variations: two substitutions (202G>A in *rpoC* and -18G>T in *psaB*) and one insertion (469insC in *glnA*). Transcriptomic changes were examined using RNA extracted directly from clinical samples by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and the 2^{-DDCt} method. We found that *ply* (pneumolysin), *nanA* (neuraminidase A), *yeeN* (putative virulence regulator), and *psaB* (manganese transporter) were upregulated (> 4-fold) in lung aspirates compared with nasopharyngeal swabs; whereas *spxB* (pyruvate oxidase) and *eno* (enolase) were downregulated (2- to 168-fold). For *cpsA* (capsule), *luxS* (quorum sensing), *endA* (DNA-entry nuclease), and *lytA* (autolysin), disparate expression patterns were observed across serotypes, ranging from 23-fold downregulation to 57-fold upregulation. Upregulations or downregulations of the ten genes were also seen in vitro when comparing expression in artificial sputum with nose-like media. However, direction and/or magnitude from the in vitro results were not always consistent with those in the clinical samples. In addition, limited upregulations or downregulations (both <4-fold) were seen in the same ten genes when pneumonia patients were compared with healthy controls using nasopharyngeal swabs. This is the first study investigating pneumococcal gene expression using samples collected directly from patients. These findings indicate the critical role that the host plays during pneumococcal pneumonia, and provide new insights into the complex process of pneumonia pathogenesis.

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Detection of *Helicobacter pylori* and its virulence genes (*cag*A, *dup*A, and *vac*A) among patients with gastroduodenal diseases in Chris Hani Baragwanath Academic Hospital, South Africa

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ABSTRACT

Background: The global prevalence of *H. pylori* approaches 50%, with prevalence rates between 20 and 40% in developed countries and up to 90% in Africa and other developing nations of the world. Development of *H. pylori*-associated diseases is determined by a number of virulence factors. This study aimed at determining the prevalence of *H. pylori* infections and virulence genes (*cag*A, *dup*A, *and vac*A); the relationship between virulence factors and gastroduodenal diseases among patients.

Methods: Gastric biopsies were obtained from patients and cultured, DNA was extracted from biopsies / cultured isolates for PCR assay after which samples were investigated for pathogen and virulence factors using standard laboratory procedures. Data of associated risk factors were obtained with the aid of questionnaires.

Results: Of the 444 participants, *H. pylori* was detected in 115 (25.9%) from culture analysis and 217 (48.9%) by direct PCR method. Ninety-eight (85.2%) of the culture-positive patients were also detected by PCR giving an overall prevalence of 52.7% (234/444). The highest number of *H. pylori* isolates 76.9% (180/234) was obtained from patients suffering from pangastritis. The *Cag*A virulence gene was found in 62% (145/234), *dup*A in 53.4% (125/234) and *vac*A in 90.6% (212/234). *Vac*A genotype s1m1 was the most prevalent [56.4% (132)] followed by s2m2 [11.5% (27)], s2m1 [10.3% (24)] and [s1m2 9.4% (22)]. There was a significant association observed in *vac*A s1 and peptic ulcer disease, as well as *vac*A s1/m2 and gastric erosion ($P<0.05$).

Conclusion: The study revealed a significant association between virulence genes and the development of certain forms of gastric infections while the variations in *H. pylori* detection and the associated risk factors investigated in the study were not significantly related.

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High diversity of TcdA-negative, TcdB-positive and non-toxigenic *Clostridium difficile* in Thailand

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It has been previously reported that there is a high prevalence of TcdA-negative, TcdB-positive (A-B+) *Clostridium difficile*, all of which belong to a single ribotype (RT) 017, as well as non-toxigenic *C. difficile* in Thailand, many of which are resistant to multiple antimicrobials. However, these findings were limited to a single tertiary hospital in Bangkok. In this study, 145 *C. difficile* strains isolated from clinical specimens from 13 provinces of Thailand during 2006 – 2018 were ribotyped and toxin profiled. In addition, minimal inhibitory concentrations (MIC) for eight antimicrobials were determined for 100 *C. difficile* strains isolated during 2006 – 2015. Forty-nine per cent of the strains (71/145) were non-toxigenic. Among the toxigenic strains, the most common toxin profile was A-B+ (46/145; 32%) and, of A-B+ *C. difficile*, the most common RT was RT 017 (28/145; 19 %). In contrast to previous studies, 18 A-B+ *C. difficile* strains (12 %) belonged to 12 new RTs. All *C. difficile* strains remained susceptible to vancomycin and metronidazole, however, a slight increase in MICs for metronidazole was noticed ($MIC_{50/90}$: 0.25/0.25 mg/l during 2006 – 2010 compared to $MIC_{50/90}$: 1.0/2.0 mg/l during 2011 – 2015). Resistance to moxifloxacin occurred in 73 % of RT 017 strains, consistent with previous reports on this RT. Given that the use of fluoroquinolones (FQs) is poorly controlled in Thailand, it is possible that FQ resistance in RT 017 will drive its spread, as it had happened in many countries during recent decades. In contrast, only 22 % of other A-B+ RTs were resistant to moxifloxacin. Non-toxigenic strains in this study had low rates of antimicrobial resistance in contrast to previous reports. This study suggests that there is a great diversity of A-B+ and non-toxigenic *C. difficile* in Thailand, both of which may have played a role in the pathogenesis of CDI in this country, resulting in the unique clinical characteristics often seen.

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Emergence of *Clostridium difficile* ribotype 106 in Western Australia

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Recently, there has been a shift in the epidemiology of *Clostridium difficile* infection in the United States, with *C. difficile* ribotype (RT) 106 becoming the most common strain of *C. difficile* isolated from adults and children with both community- and hospital-associated *C. difficile* infection, surpassing RT027. In Western Australia (WA), the first *C. difficile* RT106 strain was detected in September 2015 (1 case). Since then there has been an increase in the prevalence: 2016 (9 cases), 2017 (12 cases) and 2018 (19 cases). As part of an investigation into this rise in RT106 in WA, we performed whole-genome sequencing (WGS) and core genome typing on 12 selected RT106 strains from 5 hospitals in WA isolated during 2016 – 2018. For comparative analysis, we included genomes of RT106 circulating in the United Kingdom (UK) and the United States (US). Interestingly, the 12 RT106 strains could be further divided into 3 different, but closely related multilocus sequence types (ST): ST28 (n=6), ST42 (n=5) and ST 8 (n=1), the latter the first report of this ST within RT106. All ST 28 strains from WA were closely related, and two came from the same ward (separated by 55 days) and had no SNP differences, suggesting the spread within the hospital. Three ST 42 strains isolated from different patients (over 163 days) in different hospitals were also identical, suggesting, possibly, a common source of *C. difficile*. The remaining ST42 strains were distinct from one another. All RT106 strains from WA were different from the strains from the UK and US. These findings suggest that there may be at least two closely-related *C. difficile* lineages of RT106 in WA instead of a single strain. They also suggest that PCR ribotyping should only be used as a screening tool for *C. difficile* outbreaks and that WGS is needed for confirmation.

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Comparison of Calibrated Dichotomous Susceptibility disc test and Thermo Fisher Sensititre broth microdilution methods with ISO broth dilution MIC among Enterobacteriaceae

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Comparison of CDS disc diffusion and Thermo Fisher Sensititre™ broth microdilution methods using Enterobacteriaceae strains with established ISO broth dilution MICs

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Antibiotic resistance in Enterobacteriaeceae family is on the rise and accurate reporting of the antimicrobial susceptibility is important as it has a direct impact on patient response to treatment. A study was undertaken using seventeen members of Enterobacteriaceae family against eleven antibiotics belonging to six classes. The Calibrated Dichotomous Susceptibility (CDS) disc diffusion method and the Thermo Fisher Sensititre™ micro broth dilution methods were compared with the MIC results of the gold standard, ISO broth dilution (performed by two independent laboratories attached to the United Kingdom National External Quality Assessment Scheme). Sensititre™ GNX2F plates were used for the study and the antibiotics which were common to all the three methods were cefotaxime, ceftazidime, piperacillin- tazobactam, amikacin, gentamicin, tobramycin, ciprofloxacin, ertapenem, imipenem, meropenem and tigecycline. Categorical agreement (susceptible or resistant) was used to calculate the concordance with CDS testing. Essential agreement was used for broth dilutions, with consensus defined as MICs that were within ± 1 doubling dilution of the MIC determined by the ISO broth dilution. A discordance was categorised as a minor error when a susceptible isolate was reported as intermediate/resistant and a major error when an intermediate/resistant isolate was reported as susceptible. The CDS test correlated well with the reference standard with no major errors. The Sensititre™ test correlated well for all antibiotics except with cefotaxime, where there was a major error.

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A comparative study for the detection of Salmonella species in faeces by culture and Seegene multiplex PCR

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Infectious gastroenteritis is caused by a wide range of enteric pathogens of which Salmonella spp is an important pathogen with public health importance. The conventional detection is by the culture isolation, biochemical identification and serotyping which are labour intensive, time consuming and costly. Multiplex PCR is expected to overcome all these drawbacks of culture along with having a good sensitivity and specificity. We evaluated the performance of culture and Seegene multiplex PCR over a 14 month and tested 4897 faecal specimens. The Salmonella isolates belonged to 28 serotypes. There was a good concordance between culture and multiplex PCR.

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Development of Surface Plasmon Resonance in Combination with Loop-Mediated Isothermal Amplification for Detection of *Clostridium perfringens*

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Clostridium perfringens is one of the most common causes of food poisoning. The standard culture method of detection is time-consuming and labor-intensive. In this work, we developed a platform for surface plasmon resonance (SPR) as DNA biosensor for high sensitivity and label-free detection of *C. perfringens*. The biotinylated single-stranded oligonucleotide probe targeting a specific sequence in the *cpa* gene of *C. perfringens* was immobilized onto carboxydextran surface, which was functionalized with streptavidin for the detection of the DNA sample of *cpa*-specific *C. perfringens*, which was amplified by loop-mediated isothermal amplification (LAMP) technique. The sensitivity of our method was 10 CFU/ml and 10^3 CFU/ml, respectively in pure culture and spiked food sample using boiling method for genomic DNA extraction. In addition, the sensor surface could be regenerated at least 10 cycles with high specificity. This technique is a simple and powerful tool for the detection of *C. perfringens* in food samples. It can be extended into an array sensor, which has a potential in development into a screening test for foodborne pathogens.

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Examining relapse versus re-infection in recurrent NTHi infections of COPD patients through whole-genome analyses

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Recurrence is a characteristic feature of nontypeable *Haemophilus influenzae* (NTHi) infection, particularly during COPD exacerbations. This makes the management of COPD more challenging. Recurrent infections are caused either through persistence and relapse of earlier infection or by re-infection with a new strain. For a number of bacterial pathogens, whole genome sequencing has been shown to provide a level of resolution that can differentiate between strains from relapsed and new infections. This presented an opportunity to develop in this study a simple workflow for genome-wide comparison of paired isolates of NTHi. In this pilot study, we examined four pairs of longitudinal isolates of NTHi from the sputum of COPD patients sampled at baseline and recurrence. The isolates were sequenced on an Illumina MiSeq platform and low-quality bases and adaptors were removed from fastq reads using TRIMMOMATIC. Based on *in silico* multilocus sequence typing (MLST) profile, longitudinal isolates from the same patient were found to be non-unique. The paired isolates were further analysed in respect of the number and location of single nucleotide polymorphisms (SNPs). The genome of the baseline isolate was assembled *de novo* and was annotated automatically using SPADES and PROKKA, respectively. The sequence reads of re-infection isolates were then mapped to the annotated reference baseline genome, and variants were then called using SNIPPY. For each of the longitudinal isolates, the analysis found that there was little genetic distance (<5 SNPs) between members of each pair of longitudinal isolates *i.e.* 2, 0, 0, and 0 SNPs. Based on this pilot set of paired NTHi isolates, the data to date suggest that relapse of an earlier infection may be more common than re-infection with a new strain in COPD exacerbations. Further analysis of a larger number of longitudinal isolates is required, but should this trend hold, it would have implications for the effectiveness of antimicrobial therapy in the treatment of COPD exacerbations, particularly in relation to NTHi.

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Analysis of fungal concentration and species from indoor air of diverse buildings in Korea

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Fungi present in indoor environment are one of contaminant sources that affect on indoor air quality. When they become air-borne particles, fungi could serve as pathogens and/or irritants that cause infection, allergy, asthma, and dermatitis and trigger itching and coughing. To get scientific information on fungal bioaerosol, we investigated fungal concentration and diversity in indoor air of diverse buildings in Korea. Culture-based measuring method of ISO 160000 was used for fungal sampling. The level of fungal concentration ranged from 100 to 1,000 cfu/m^3. Among the investigated buildings, the highest level of fungal concentration was found in sauna bath facility buildings. In general, the level of fungal concentration was higher in underground buildings than above ground buildings. About 4,000 fungal isolates were obtained from indoor air samplings which were performed from 2006 to 2016. Morphological and molecular methods were used for species identification. 99 genera and 233 species were identified. Ascomycota (83.2%) was dominant group followed by basidiomycota (15.88%), zygomycota(0.02%), and oomycote(0.004%) groups. Among ascomycota, eurotiomycetes containing *Penicillium* and *Aspergillus* species was 40.2%, indicating it is dominant taxon group in indoor air of diverse buildings. Incase of basidiomycota, agaricomycetes containing *Trametes* species was dominant taxon (91.89%). Among the identified 233 species, *Alternaria tenuissima* was dominant species, followed by *P. funiculosum*, and *Aspergillus niger*. When dominant *Penicillium* and *Aspergillus* species were grown at different conditions, they produced good quality conidiospores only at certain growth conditions. However, they could produce spores at diverse ranges of pH and temperature. These data imply that they are present as bioaerosol in indoor air in diverse buildings.

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Comparative Genomic Analysis of *Lactococcus garvieae* Isolated from Fish and Humans

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Lactococcus garvieae is a well-known fish pathogen in aquaculture, but is also found in other animals like cattle, poultry, pigs, dogs and cats. In recent years, it has emerged as an occasional human pathogen possibly as a result of zoonotic infection. The aim of this study was to use whole genome sequencing to characterise *L. garviae* causing human infection in Singapore to determine their likely source.

The genomic DNA of 11 local *L. garvieae* isolates (Fish = 6, Human = 5) were sequenced using Illumina MiSeq and assembled with Galaxy 18.05. An additional 17 whole genome sequences of *L. garviae* isolated overseas were included for comparative genome analysis. Resistance genes were identified using ABRICATE. Virulence genes identified in previously published literature were sought by BLAST search. Multilocus sequence typing was performed with SRST2 and the concatenated loci were aligned using MEGA 7.0 and a neighbour joining tree constructed. Genetic clusters (GCs) were inferred from the tree. Clonal complexes (CCs) were also identified by eBURST v3.

Two local isolates belonged to CC3. The rest were singletons, with 6 novel STs. Similarities were observed between the STs of local fish and human isolates with those of fish, food and human clinical isolates overseas. There were 3 GCs with local isolates distributed across all of them. Virulence genes (putative internalin and putative mucus adhesin) were found to be specific to GC2 and GC3. Antimicrobial resistance genes were more common in local isolates compared to those overseas.

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A Small RNA Transforms the Multidrug Resistance of *Pseudomonas aeruginosa* to Drug Susceptibility.

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Bacteria with multiple drug resistance (MDR) have become a global issue worldwide, and hundreds of thousands of people's lives are threatened every year. The emergence of novel MDR strains and insufficient development of new antimicrobial agents are the major reasons that limit the choice of antibiotics for the treatment of bacterial infection. Thus, preserving the clinical value of current antibiotics could be one of the effective approaches to resolve this problem. Here we identified numerous novel small RNAs that were downregulated in the MDR clinical isolates of Pseudomonas aeruginosa (*P. aeru*), and we demonstrated that overexpression of one of these small RNAs (sRNAs), AS1974, was able to transform the MDR clinical strain to drug hypersusceptibility. AS1974 is the master regulator to moderate the expression of several drug resistance pathways, including membrane transporters and biofilm-associated antibiotic-resistant genes, and its expression is regulated by the methylation sites located at the 50 UTR of the gene. Our findings unravel the sRNA that regulates the MDR pathways in clinical isolates of *P. aeru*. Moreover, transforming bacterial drug resistance to hypersusceptibility using sRNA could be the potential approach for tackling MDR bacteria in the future.

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Development of a Real-time PCR Assay for Simultaneous Detection of Helicobacter pylori and Screening Mutants Associated with Clarithromycin Resistance

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Introduction:

It is estimated that more than half of the world's population is infected with *Helicobacter pylori* (HP). Increasing HP resistance to clarithromycin also increases the risk of treatment failure. The aim of this study was to develop a Taqman probe based asymmetric real-time PCR assay for simultaneous detection of HP infection and identification of point mutations in the 23S rRNA gene responsible for clarithromycin resistance.

Materials and methods

Primers for the HP 23S rRNA gene were selected from a region close to commonly reported mutations, while the Taqman probe was selected from a region across the mutations. During PCR amplification, annealing of the Taqman probe on the HP target sequence (if present) is indicated by increasing fluorescence. During the high-resolution (HRM) melt stage, the melting of Taqman probe occurs at different temperature for the normal (clarithromycin susceptible) and the mutant (clarithromycin non-susceptible) targets. For identification of HP, the PCR was evaluated for analytical sensitivity & specificity, limit-of-detection (LOD), linearity and precision against known HP and other bacterial isolates. For identification of clarithromycin resistance, the PCR was tested against HP isolates with known clarithromycin susceptibilities, as previously determined by Etest.

Results:

This real-time PCR gave a performance of 100% sensitivity (78/78) and 100% specificity (25/25). For mutation detection, 62 phenotypically sensitive isolates showed an average Tm of 75.8°C (SD 0.3°C), compared to a Tm ranging from 71.5°C to 73.8°C (depending on mutation type) for 16 clarithromycin-resistant isolates. PCR reaction efficiency was 93% over range of 4.7E+07 to 4.7E+04 bacteria per reaction, while the LOD was 15 bacteria per reaction. For the inter-assay & intra-assay reproducibility, one ATCC sensitive strain and two clarithromycin resistant strains (A2142G & A2143G) were tested in 5 individual PCR runs. Both Ct value and Tm were consistent.

Conclusion:

This PCR assay demonstrates the capability to both detect HP, and to differentiate wild-type and clarithromycin resistant mutations in the 23s rRNA gene.

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Function of novel WzzB:WzzE chimeric proteins in O antigen and ECA biosynthesis

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The ability of bacteria to synthesise complex polysaccharide chains at a controlled number of repeating units has wide biological functions which include: symbiosis, biofilm formation and immune system avoidance. Complex polysaccharide chains such as the O antigen component of lipopolysaccharide and the enterobacterial common antigen (ECA) are synthesised by the most common polysaccharide synthesis pathway in bacteria, known as the Wzy-dependent pathway. The O antigen (B) and ECA (E) are polymerized into chains via the inner membrane proteins WzyB and WzyE, while the co-polymerases WzzB and WzzE, modulate the number of repeat units in the chains or “the modal length” of the polysaccharide via a hypothesised interaction. The data generated in this project is the first to show the phenomenon that WzzE is able to partially regulate O antigen modal length via a potential interaction with WzyB. To investigate this, one or both of the transmembrane regions (TM1 and TM2) of the co-polymerases (WzzE and WzzB) were swapped to create six chimera proteins. Several chimeric proteins were found to significantly increase O antigen modal length control, while others reduced modal length control, as detected via colicin E2 sensitivity assays, LPS analysis by SDS PAGE and silver-staining and ECA Western immunoblots. These results suggest that the TM2 region of Wzz proteins plays a critical role in O antigen and ECA modal length control presumably via the interaction with Wzy proteins.

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An update on serotype distribution and antibiotic resistance on invasive Streptococcus agalactiae infection among pregnant women and neonates of Hong Kong from 2006-2018

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Background

Streptococcus agalactiae (GBS) infection in pregnant women and neonates has been a worldwide concern due to its lethal threat in neonates causing early- and late-onset disease (EOD and LOD). Universal screening of GBS in pregnant mothers for intrapartum prophylaxis (IAP) was launched in 2012 across Hong Kong hospitals and has greatly reduced the incidence of invasive neonatal and pregnant women infections. We report the prevalence of serotypes and antibiotic resistance among pregnant women and neonates with invasive GBS infection in the period 2006-2018 in one of the hospital clusters of Hong Kong.

Methods

Non-duplicate patient isolates of archived GBS strains isolated from neonates (n=73) and pregnant women (n=73) from 2006-2018 in one of Hong Kong’s hospital clusters were serotyped. Antibiotic resistance data by disc diffusion test according to CLSI was obtained where six antibiotics were tested: Ampicillin, Clindamycin, Erythromycin, Levofloxacin, Penicillin, and Vancomycin.

Results

EOD and LOD cases dropped from 8 and 7 cases in 2011 to 1 and 4 cases in the subsequent year respectively. The prevalent serotypes were III (n=44) followed by Ia (n=11) and Ib (n=11). Most of the Ia isolates were sensitive to antibiotics (64%), while 82% and 84% of Ib and III isolates were resistant to erythromycin and clindamycin. Incidence of invasive GBS in pregnant women reduced from 10 cases in 2011 to 5 cases in 2012. The most prevalent serotype was Ia (n=19) followed by III-2 (n=13), Ib and II (n=10 for both serotypes). Serotype Ia strains were mostly sensitive to antibiotics (74%), while 60% of Ib and 54% of III-2 strains showed resistance to clindamycin and erythromycin.

Conclusion

Although IAP reduced incidences of invasive infection in neonates and pregnant women drastically, multidrug resistance persisted in over 70% of the strains in our study. Active surveillance on the change in antibiotic resistance is required to prepare the healthcare sector for improving treatment regimens to this high risk population.

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Environmental sources of *Clostridioides (Clostridium) difficile* in the hospital

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Introduction

The notion that *Clostridioides difficile* infection (CDI) is a hospital-acquired infection transmitted between symptomatic patients is questionable following the isolation of clinically important *C. difficile* strains in production animals, food and the environment, and the lack of evidence of transmission based on whole genome sequencing studies. Driven by the use of antimicrobials in production animals, and subsequent use of contaminated animal manure in composted gardening products, the environment outside of hospitals is contaminated with *C. difficile* spores. We suspect environmental *C. difficile* is being carried into the hospital on the soles of contaminated shoes and subsequently contributes to CDI in the hospital.

Objective

The initial stage of this study was to determine the prevalence and circulating strains of *C. difficile* on the hospital floor and the shoes of visitors, patients and hospital personnel.

Methods

Selective direct and enrichment cultures were performed on shoe and floor samples collected using MWE Polywipe sponges between January and March 2019. Toxin profiling and PCR ribotyping were used to characterize all isolated *C. difficile* strains.

Results

C. difficile was isolated from 77.3% (58/75) of floor samples and 76.7% (46/60) of shoe samples. The floor samples (58/58) tested positive by enrichment culture only. For the shoe samples, 43/46 (93.5%) were positive by enrichment only. Of the 3 shoe samples that were positive by direct culture, 20 spores/shoe were present on all 3. Non-toxigenic ribotype (RT) 010 predominated in both the floor (98.0%) and shoe (96.3%) samples. Toxigenic *C. difficile* RT043 was isolated from the shoe of 1 staff member.

Conclusions

In Australia, *C. difficile* RT010 is one of the most common RTs found in compost, lawn and asymptomatically-colonized people. While non-toxigenic, its presence implies it is being transported into the hospital. On-going surveillance of *C. difficile* on hospital floors and shoes is continuing to look for any seasonality. Later, comparative genomic analysis will be performed on environmental *C. difficile* and clinical cases of CDI.

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Evaluation of peracetic acid sporicidal wipes in removing *Clostridioides* (*Clostridium*) *difficile* spores from rubber surfaces

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Introduction

Clostridioides (*Clostridium*) *difficile* is the most important cause of infectious diarrhoea in hospitalized patients. *C. difficile* spores are highly resistant to disinfectants and frequently recovered from hospital surfaces. This study aimed to examine the effectiveness of a peracetic acid/hydrogen peroxide-based sporicidal wipe (Clinell Sporicidal; GAMA Healthcare Ltd, London, UK) in removing *C. difficile* spores from rubber surfaces, as a proxy for shoe soles or tires on hospital equipment.

Methods

Rubber stoppers (65 mm in diameter) were inoculated with 0.1 ml of suspension containing various concentrations of non-toxigenic *C. difficile* spores (ranging from 10^3 to 10^6 cfu/ml). After drying in a vacuum chamber, the stoppers were then pressed onto an activated sporicidal wipe (according to manufacturer's instruction) for 1, 3, 5, 10, 15 and 20 sec. The number of residual spores on the stoppers was determined using contact plating onto selective ChromID agar (bioMerieux) which was incubated anaerobically for 48 h in a Don Whitley Scientific A35 anaerobic chamber. The assay was repeated three times. Average $\log_{10}$ reductions in spores were calculated for each inoculum size and contact time.

Results

The average $\log_{10}$ reductions of *C. difficile* spores were 3.61, 3.63, 3.76, 3.73, 4.00 and 3.93 for 1, 3, 5, 10, 15 and 20 sec of contact with the sporicidal wipe, respectively. The mean $\log_{10}$ reductions for inocula of roughly 10^3, 10^4, 10^5 and 10^6 cfu/ml were 3.69, 4.21, 3.80 and 3.41, respectively. No significant differences in spore reduction were observed between different contact time or inoculum size.

Conclusions

In conclusion, Clinell's sporicidal wipe was effective in significantly reducing spores on rubber within seconds of contact. This finding suggests that routine use of sporicidal wipes on hospital's products could be beneficial in reducing the risk of *C. difficile* transmission from contaminated surfaces.

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Preventing the fish 'cold'; Tasmanian Atlantic salmon reovirus vaccine development in farmed Atlantic salmon

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In Tasmania, Atlantic salmon aquaculture is the largest primary production industry in the state and is now worth one billion dollars annually to the local economy. The isolation of Tasmania from salmonid populations in Europe, North and South America helps maintain biosecurity and protects the industry from numerous devastating exotic diseases. While Tasmania may be free of the highly virulent microbes found elsewhere in major salmon-producing countries, it has its own unique pathogens and our research group has developed and commercialised a number of bespoke vaccines. Currently, we are developing three new vaccines, one of which is for the Tasmanian Atlantic salmon reovirus (TSRV).

TSRV, a member of the reoviridae family, was first isolated from Australian brood-stock in 1990 and in recent years, it has become more prevalent in farmed Atlantic salmon as production and water temperatures increase. The virus, known to cause direct multifocal hepatocellular necrosis in salmon is also thought to predispose infected fish to other coinfections. With a ubiquitous distribution across all marine farming sites and a high carriage level when water temperatures peak, the salmon industry has invested in the development of vaccine.

In this presentation, we will show the development of a novel TSRV challenge model, which has provided a platform to test vaccine candidates. To date, the challenge model has produced robust correlating datasets (including virus titration, feed intake and antibody responses) that reflect a promising level of vaccine performance.

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Suggestion for a Consensus MLST Scheme Applicable for all Pneumocystis Species

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Multilocus sequence typing (MLST) is considered the preferred approach for the analysis of genetic diversity of *P. jirovecii*, but no consensus scheme is currently established. Previous research compared all MLST schemes currently used worldwide, to determine a MLST scheme that would combined loci with the highest amplification efficiency and discriminatory power. Based on the analysis of available sequences *CYB, SOD, mt26s* were identified to produce the most discriminatory MLST scheme for *P. jirovecii* genotyping. The herein presented study looked at the efficiency of the scheme in a real-life setting and assessed whether the scheme could be used for all PCP infected animal isolates, as well as a world-wide standardized MLST scheme for all *Pneumocystis* species. The newly identified MLST loci (CYB, SOD and *mt26s)* were amplified and sequenced from 30 clinical PCP isolates and 2 canine samples. In addition the DNA concentration needed for a successful amplification was assessed. The CYB, SOD and *mt26s* scheme successfully generated MLST data from 28/30 PCP isolates, even with minimal DNA concentrations. It was noted that no MLST sequence data were obtained if the DNA concentration was diluted below 20 ug/ml. Further optimization to the protocol is currently underway to ensure higher amplification rates. More mammal isolates over a larger range of animal species is required to confidently recommend a universal consensus scheme for all *Pneumocystis.*

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Diversity of ACC Deaminase-positive Bacteria – plant and soil factors

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Plant-microbe interactions play a key role in alleviating the impacts of abiotic and biotic stresses on plant health and production. Gene specific NGS techniques can be employed to enable a better understanding of the factors influencing the diversity and dynamics of specific microbial functional groups. Climate change induced fluctuations in the amount and pattern of rainfall, across Australia and worldwide, exposing crops to multiple water stress conditions pose most difficult challenges facing agricultural sustainability and food security. Soil bacteria that produce 1-Aminocyclopropane-1-Carboxylic Acid (ACC) Deaminase have been shown to promote plant growth under abiotic stresses such as water stress by lowering stress ethylene levels through deamination of ACC. Using gene specific primers for the bacterial gene encoding ACC deaminase (*acd*S), we examined the diversity (NGS sequencing) and abundance (qPCR) of ACC deaminase positive bacteria in soils and plants from cropping fields in South and Western Australia and Central Himalaya Kumaun region, India.

A total of 25 genera mostly belonging to the phyla Proteobacteria and Actinobacteria accounted for the majority of *acd*S gene harbouring microbes in Australian and Indian soils and wheat roots. Bacteria belonging to the Comamonadaceae and Bradyrhizobiaceae families were the most abundant groups. However, significant differences were observed in the *acd*S community composition at the genus level e.g. *Acidovorax, Variovorax, Bradyrhizobium, Ensifer and Methylibium*. Also, significant differences were observed between wheat cultivars and water-stress conditions in both countries. Beta diversity analysis indicated significant variation in *acd*S-community composition between locations (South Australia, Western Australia and North India), plant type (wheat vs. perennial grasses) and varieties. *Panicum coloratum* L. cv. *Bambatsi,* a summer active perennial c4 grass, supported greater diversity (chao1 and Shannon indices) of *acd*S gene harbouring microbiome in roots. These results indicate that although edaphic and environmental factors are the major drivers of *acd*S community, a cultivar-based selection of specific genera may be occurring suggesting a variety-based response in terms of plant-*acd*S community interactions.

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Using genome-wide screening to identify genes important for bacterial attachment to plant surfaces

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One of the major factors limiting global food and fibre production is the loss of crop yields due to plant disease. Pesticides are used to control many plant diseases, but pathogens are becoming increasingly resistant, and new disease control methods are needed. One such approach is the use of biological control agents. *Pseudomonas protegens* Pf-5 is one of the best characterised biocontrol bacteria and in lab studies has the ability to control crop diseases. However, field trials of biocontrol bacteria often show unreliable colonisation and persistence on plant surfaces, hindering their efficacy.

We used Transposon Directed Insertion Site Sequencing (TraDIS) to identify genes important for surface attachment, a crucial step in bacterial colonisation of plant surfaces and biocontrol activity. TraDIS combines a dense library of randomly generated loss-of-function mutants with massive parallel sequencing to allow the simultaneous study of all non-essential genes in the genome and en masse identification of important genes. We identified 55 genes important for Pf-5 seed surface attachment, with many involved in cell wall and cell membrane biogenesis. Other important functions included cell cycle control, transcription, energy production, and inorganic ion and nucleotide metabolism and transport. Targeted knockouts and mutant phenotype characterisation of specific genes within this set will provide information on their roles in attachment. A greater understanding of the genes important for attachment to plant surfaces may improve our ability to effectively use biocontrol bacteria in Australian agriculture.

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Bacterial consortia reduce severity of Fusarium wilt in cotton

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Soil bacteria colonise plant roots and interact intimately with their plant host. Some colonisers promote plant growth and may also exhibit antagonistic activities against pathogenic microorganisms. There have been a number of studies using biocontrol agents to control crop pathogens with mixed results. It has been proposed that inconsistent outcomes of biological approach are linked to use of single species biocontrol products. Only few studies have attempted to use a consortium which can arguably have better ability to survive in new environment and hence improved efficacy.

Our aim was to isolate multiple bacterial species and to develop a bacterial consortium which demonstrated antagonistic activities against Cotton Fusarium Wilt disease caused by *Fusarium oxysporum* f.sp. *vasinfectum* (Fov). The cotton wilt is a major constraint of cotton farm productivity in Australia. We tested if applying the bacterial consortium improve control of Fusarium wilt compared to single isolates and no treatment.

In this study, two glasshouse experiments were conducted to (1) test the efficacy of three single isolates in reducing impact of Fusarium wilt on germination and disease severity at early stage of plant development, and (2) test whether a consortium could provide a better control of pathogen and if the efficacy of biocontrol agents is modified by cotton cultivars. A number of antagonistic bacteria was isolated from the roots of healthy cotton plants and a bacterial consortium was developed based on their ability to grow together with synergistic relation. Our results showed that single isolate reduces disease incidence by up to 97%. Bacterial consortia also improved seed germination by 50% but impact of disease incidence was modified by crop cultivars.

Overall, our results demonstrated that biocontrol agents are effective in reducing disease incidence under glasshouse conditions with two different soils. Currently we are investigating whether soil and plant microbiomes have responded either to disease incidence or to use of biocontrol agents and this result will also be presented.

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Two long stories from the Northern Territory

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The population of the remote Australian Northern Territory (NT) experiences a distinctive and challenging infectious disease burden. This presentation encompasses two narratives of NT-focussed work that have occupied over 30 years. Consistent with the rest of the world, *C. trachomatis* is a major cause of sexually transmitted infections (STIs). However, in the NT, it also causes the distinctive ocular disease trachoma, which is transmitted amongst young children in facial secretions, and is not regarded as an STI. In the 1980s and 1990s, ocular isolates were obtained from children in the remote NT, associated with clinical patterns consistent with trachoma. Whole genome analysis revealed three lineages all unrelated to 'ocular" strains elsewhere in the world, apart from at the *ompA*, and*pmpEFGH* genes, for which "ocular" variants appear to have been acquired by recombination. Subsequent work has shown that one lineage is globally distributed, and is weakly tropic, so often also associated with STIs. There is no evidence that the other two exist outside Australia, and they have strong ocular tropism. These data have assisted the development of a *C. trachomatis* genotyping method, *Ct*GEM, that identifies ocular lineages known to exist globally, and also discriminates the major phylogenetic groups of the entire species. There is a high burden of skin infections in the remote NT,

and *Streptococcus pyogenes* is largely responsible. It is likely that this contributes to the high prevalence and incidence of rheumatic heart disease in the NT. An effective *S. pyogenes* vaccine would be of considerable benefit. It is unclear with *emm* type-specific or non-specific vaccine have the greatest potential. Analysis of *emm* types from 1810 isolates collected in the NT since 1987 indicates that a 30-valent *emm* type-specific prototype vaccine developed in the United States will have poor efficacy in the NT, unless there is cross protection between *emm* types, and a strategy for eliciting immunity against *emm* cluster D4 is developed. Possible priorities for research will be discussed.

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Not available

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Not available

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Not available

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Not available

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Immune regulatory role of flagellin from uropathogenic *Escherichia coli* during acute urinary tract infection

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Introduction: Urinary Tract Infections (UTIs) are a huge public health problem affecting an estimated 300 million people each year worldwide [1]. UTIs are predominantly caused by uropathogenic *Escherichia coli* (UPEC). A protective role for the regulatory cytokine IL-10 in the control of acute UTI has previously been demonstrated [2], however, UPEC factor(s) associated with IL-10 induction have yet to be identified. We hypothesized that UPEC flagellin (FliC), the major flagellar filament protein contributes to the induction of IL-10 during acute UTI.

Methods: UPEC reference strain CFT073 and their *fliC* mutant were used [3]. Extraction and purification of FliC to homogeneity was performed using a protocol based on sequential steps of mechanical shearing, ultracentrifugation, and chromatography. Human U937 monocytes and J774 mouse macrophages were used to define the biological activity of native FliC. RNA sequencing was applied to map the transcriptomic response of the bladder in response to FliC in female C57Bl/6 and TLR5$^{-/-}$ mice.

Results: UPEC CFT073 derivatives and purified FliC induces significant amount of IL-10 within 5h of infection in both *in-vitro* and *in-vivo* studies. Transcriptomic data demonstrate significant IL-10 upregulation in the bladder in response to FliC among other innate responses consistent with prior literature. Additionally, it revealed multiple host factors not previously reported to be associated with host responses to FliC.

Conclusion: We propose that flagellin of UPEC contributes to early immune regulatory cascades via IL-10 induction. These findings may be useful to identify potential targets for manipulating the infectious process and disease pathogenesis.

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Investigation of the IcsA-mediated *Shigella* adherence

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Publish consent withheld

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Comparison of the role of extracellular vesicles (EVs) from methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from ampicillin stressed and unstressed state on β-lactam antibiotics

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EVs produced by Gram-positive bacteria contain several bacterial compounds and play important roles in interbacterial communication to cope with the antibiotic condition. Roles of EVs isolated from MRSA grown on ampicillin stressed condition and normal condition on several β-lactam antibiotics were compared. The EVs of methicillin-resistant *Staphylococcus aureus* (MRSA) ST692 were isolated in an environment with no stressed and with stressed by adding ampicillin at a lower concentration than minimum inhibitory concentration (MIC). The isolated EVs were incubated with sensitive *S. aureus* in the presence of several β-lactam antibiotics. The several β-lactam antibiotics treated with EVs from the stressed condition and normal condition was measured with LC-QQQ. Each of EVs was analyzed using LC-ESI-MS/MS to compare their respective protein compositions.

The level of EVs production from MRSA under stressed conditions was increased to about 8.8 fold compared with that of EVs under unstressed condition. The growth kinetics and CFU counting results revealed that EVs from MRSA under stressed condition could protect susceptible *S. aureus* in the presence of several β-lactam antibiotics (ampicillin, cefoperazone, cefazolin, amoxicillin, cephalexin, and cloxacillin) better than EVs from MRSA under unstressed conditions. The result of LC-QQQ showed that several β-lactam antibiotics can be degraded by EVs. In protein analysis, several proteins related to β-lactam antibiotics were found in EVs. Especially, EVs isolated from stressed condition showed 5.4 times more β-lactamase than EVs isolated under normal condition.

In this study, the presence of ampicillin could increase the production of EVs from MRSA. The EVs from MRSA under stressed conditions have a potent ability in protecting susceptible *S. aureus* in the presence of β-lactam antibiotics compared with unstressed conditions. The difference in these roles seems to be due to different protein compositions of EVs. Our observation can serve as a caution on the abuse of β-lactam antibiotics in real life because this could lead to escalating antibiotics resistance, which is currently becoming a serious social problem.

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Taking aim at the flavivirus NS1 protein

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Emerging flaviviral pathogens represent a significant current and projected global health crisis. Dengue virus (DENV) is the leading disease-causing arbovirus in the world, with over 390 million infections annually, resulting in more than 500,000 severe cases requiring hospitalization. Adding to this burden, the recent global spread of the related Zika virus (ZIKV) has caught governments and aid agencies by surprise and resulted in the WHO declaration of a global health emergency. The co-circulation of related flaviviruses poses significant complications for accurate diagnosis as well as vaccine design. However, similarities between flaviviruses also offers the potential for the development of future treatments and vaccines that could offer broad protection against these important human pathogens. All flavivirus genomes encode a protein known as non-structural protein 1 (NS1). We recently revealed that DENV NS1 acts like a viral toxin, driving the induction of inflammatory cytokines and directly damaging the endothelial barrier. In this talk I describe progress we have made towards new therapies that target NS1, and the potential for NS1 as a broad spectrum antiviral target.

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Cysteine proteinase propeptides exhibit secondary functions as bacteriocins

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Porphyromonas gingivalis is an important pathogen in the development of chronic periodontitis, a subgingival plaque biofilm-associated disease characterised by progressive loss of alveolar bone and soft tissue attachment to the tooth. Increase in subgingival plaque numbers of *P. gingivalis* in conjunction with *Treponema denticola* is a predictor of disease progression. In the mouse periodontitis model *P. gingivalis* at low numbers (<0.01% of the biofilm) can induce plaque dysbiosis, with increased plaque biomass and shifting the species composition to a pathogenicity-associated profile. This has been attributed to the hydrolytic activities of *P. gingivalis* proteinases RgpA, RgpB and Kgp, collectively called gingipains, and associated dysregulation of immune responses. The gingipain zymogens have unusually large, 25 kDa, N-terminal propeptides (PP) that are removed during extracellular activation. The fate *in vivo* of the cleaved PP fragments has not been determined. We show that the Arg-gingipain propeptides produced in *Escherichia coli* (rRgpA-PP and rRgpB-PP) inhibit RgpA and RgpB with nM Ki thus are promising therapeutics. Furthermore, application of rRgpA-PP and rRgpB-PP prevents the establishment of mono- and multispecies biofilm and importantly disrupts established mono- and multi-species biofilms *in vitro*. The anti-biofilm effect was found unexpectedly to be due to antimicrobial functions of rRgpA-PP and rRgpB-PP, including against *P. gingivalis*, via a mechanism independent from gingipain activities. However, rRgpA-PP and rRgpB-PP do not harm all species, including *T. denticola*, demonstrating selectivity of action. Gingipain propeptides may represent the first identified members of a novel class of bacteriocin, ones that derive from a larger precursor protein where *in situ* they have an alternative function. We propose that *in vivo,* the released gingipain propeptides via selective killing or growth inhibition could promote the observed pathogenic dysbiosis of the subgingival plaque biofilm observed in periodontal disease.

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Not available

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Not available

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Autoinducer 2 and Galactose: How Quorum Sensing Dictates Sugar Metabolism in *Streptococcus pneumoniae*

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Through an unknown mechanism, *Streptococcus pneumoniae* (the pneumococcus) is able to switch from asymptomatic coloniser to invasive pathogen whilst colonising the nasopharynx. In this niche, cell-to-cell communication is achieved through the coordinated release and sensing of signalling molecules known as autoinducers in a process known as Quorum Sensing (QS). We have focused on characterising the Autoinducer-2 (AI-2)/LuxS QS system and observed that AI-2 QS signalling is dependent upon the membrane bound protein FruA for the import of AI-2, which enables the pneumococcus to utilise galactose as a carbon source by upregulation of the Leloir pathway. We have been investigating whether AI-2 phosphorylates the Leloir pathway regulator GalR after FruA-mediated uptake. GalR is known to possess three potential phosphorylation sites: S317, T319 and T323. Substitution mutants have been generated to either abrogate (A) or constitutively mimic (D or E) phosphorylation at these sites, either singly or in combination. Subsequent growth assays and transcriptional analyses revealed complex phenotypes. Interestingly, $galR_{AAA}$, $galR_{DDD}$ and $galR_{EEE}$ strains fail to grow in Chemically Defined Media with galactose as the sole carbon source, indicating that these sites are essential for galactose utilisation. However, the presence of endogenous intracellular AI-2 may be having an impact on the Leloir pathway, thereby complicating interpretation of mutant phenotypes. This is being addressed by the introduction of a *luxS* deletion mutation into each of the *galR*substitution mutants, rendering these strains unable to produce AI-2. Additionally, the membrane-bound protein FruA, the putative AI-2 receptor, has been successfully over-expressed and purified, enabling direct biochemical characterisation of its interaction with AI-2. The outcomes of this study will provide greater understanding of the relationship between intracellular sugar metabolism and cell-to-cell communication within the pneumococcus, parameters that are known to profoundly impact virulence phenotype. Additionally, greater translational outcomes will be delivered by bettering our understanding of how to control bacterial colonisation and interfere with the community-like behaviours of biofilms.

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Surfaceome analysis of Australian epidemic *Bordetella pertussis*

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Background: *Bordetella pertussis* causes whooping cough, the least controlled vaccine-preventable disease in Australia. Since the switch to the acellular vaccine (ACV), Australian epidemic strains changed to single nucleotide polymorphism (SNP) Cluster I from SNP Cluster II. Our previous proteomic analysis identified upregulation of transport proteins and downregulation of immunogenic proteins in the whole cell and secretome of Cluster I which was associated with higher fitness in the mouse model. Additionally, current ACVs have been shown to be less efficacious against current epidemic strains in mice models and there is an ongoing need to discover new antigens to improve the effectiveness of ACVs. One important source of novel antigens is the surfaceome. Therefore, the aim of this study was to establish surface shaving in *B. pertussis* to compare the surfaceome of SNP Cluster I and II and identify novel surface antigens for vaccine development.

Methods: *B. pertussis* strains L1423 (SNP Cluster I) and L1191 (SNP Cluster II) were grown in THIJS media. Surface shaving with 1 µg of trypsin for 5 min and LC-MS/MS were used to identify surface proteins. Flow cytometry and CFU were used to assess cell lysis after shaving.

Results and conclusion: Surface shaving identified 666 peptides from 126 proteins with the most abundant being virulence-associated and known outer membrane proteins. The proportion of previously identified immunogenic proteins was significantly higher in the surfaceome than in the whole cell and secretome. Of the 126 proteins, 6 were unique to L1423 including two transport proteins. There were 2 proteins unique to L1191, both of which are immunogenic. Two immunogenic type III secretion system proteins were downregulated in L1423, providing further evidence of proteomic adaptation in Cluster I. This study established the surface saving method for the analysis of *B. pertussis* surfaceome and identified unknown surface proteins which may be potential vaccine antigens.

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Strategies to treat recalcitrant biofilm infections.

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The inexorable increase in multidrug resistant infections combined with a decrease in new antibiotic discovery and the lack of compounds to treat recalcitrant infections, such as those associated with sepsis and chronic biofilm infections, is creating a potential crisis in human medicine. Thus it is imperative to consider alternatives to conventional antibiotics for treating infections.

Cationic host defence (antimicrobial) peptides are produced by virtually all organisms, ranging from plants and insects to humans, as a major part of their innate defences against infection. They are a key component of innate immunity and have multiple mechanisms that enable them to deal with infections and inflammation including an ability to favourably modulate the innate immune system, and distinct antibiotic and anti-biofilm activities.

We have defined a class of peptides that act against biofilms formed by multiple species of bacteria in a manner that is independent of activity vs. planktonic bacteria. We have now developed novel anti-biofilm peptides that (i) kill multiple species of bacteria in biofilms (MBEC <1 mg/ml), including the ESKAPE pathogens and other major clinically relevant Gram negative and Gram positive bacteria, including, (ii) work synergistically with antibiotics in multiple species and in animal model infections, and (iii) are effective in human skin organoid and animal models of biofilm and abscess infections. Structure activity relationships studies showed no major overlap between anti-biofilm and antimicrobial (vs. planktonic bacteria) activities, and indeed organisms completely resistant to antibiotic peptides can still be treated with anti-biofilm peptides. The action of such peptides is dependent on their ability to trigger the degradation of the nucleotide stress signal ppGpp.

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Cross-talk between host and bacterial metabolisms influences the susceptibility of biofilms to aminoglycoside antibiotics

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Antibiotic susceptibility of bacterial pathogens is typically evaluated using *in vitro* assays that do not consider the complex host microenvironment. This may help explaining a significant discrepancy between antibiotic efficacy *in vitro* and *in vivo* in chronic lung infections, with some antibiotics being effective *in vitro* but not *in vivo* or vice versa. Nevertheless, it is well-known that antibiotic susceptibility of bacteria is driven by environmental factors. We previously demonstrated that lung epithelial cells enhance the activity of aminoglycoside antibiotics against biofilms of the opportunistic pathogen *Pseudomonas aeruginosa*, yet the mechanism behind was unknown. The present study provides mechanistic understanding on how lung epithelial cells stimulate aminoglycoside activity. To investigate the influence of the local host microenvironment on antibiotic activity, an *in vivo*-like three-dimensional (3-D) lung epithelial cell model was used. We report that conditioned medium of 3-D lung cells, containing secreted but not cellular components, potentiated the bactericidal activity of aminoglycosides against *P. aeruginosa* biofilms, including resistant clinical isolates, and several other pathogens. We found that 3-D lung cells secreted endogenous metabolites (including succinate and glutamate) that enhanced aminoglycoside activity, and provide evidence that bacterial pyruvate metabolism is linked to the observed potentiation of antimicrobial activity. Biochemical and phenotypic assays indicated that 3-D cell conditioned medium stimulated the proton motive force (PMF), resulting in increased bacterial intracellular pH. The latter stimulated antibiotic uptake, as determined using fluorescently labelled tobramycin in combination with flow cytometry analysis. Our findings reveal a cross-talk between host and bacterial metabolic pathways, that influence downstream activity of antibiotics. Understanding the underlying basis of the discrepancy between the activity of antibiotics *in vitro* and *in vivo*may lead to improved diagnostic approaches and pave the way towards novel means to stimulate antibiotic activity.

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Associational resistance to predation by protists in a mixed species biofilm

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Mixed species biofilms exhibit increased tolerance to a number of stresses compared to single species biofilms. The aim of this study was to examine the effect of grazing by the heterotrophic protist, *Tetrahymena pyriformis*, on a mixed species biofilm consisting of *Pseudomonas aeruginosa*, *Pseudomonas protegens* and *Klebsiella pneumoniae*. The spatial organization and quantification of fluorescently tagged strains within the biofilm was determined by confocal microscopy. Protozoan grazing significantly reduced the single species *K. pneumoniae* biofilm, and while the single species *P. protegens* biofilm also displayed sensitivity to grazing, the biofilm biovolume was not significantly affected. Unlike *K. pneumoniae* and *P. protegens* biofilms, *P. aeruginosa* biofilms exhibited grazing resistance to predation by *T. pyriformis*. Additionally, mixed species biofilms containing *P. aeruginosa* also displayed grazing resistance, indicating that the sensitive strains gained associational resistance to predation by *T. pyriformis*. Rhamnolipids produced by *P. aeruginosa* were shown to be toxic for the protist. However, a rhamnolipid-defective mutant strain of *P. aeruginosa* maintained grazing resistance, which suggests that predation protection is a multifactorial phenotype. A single species biofilm of a mutant of *P. aeruginosa* lacking rhamnolipids and the type III secretion system was grazing resistant. However, the overall grazing tolerance of the biofilm was reduced when the single and double knockout mutants

of *P. aeruginosa* were introduced to the mixed species biofilm, suggesting that both play partial roles in predation resistance. This study demonstrates that residing in a mixed species biofilm can be an advantageous strategy for grazing sensitive bacterial species, as *P. aeruginosa* protects the whole community from protozoan grazing.

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Repurposing the metal chelator diethyldithiocarbamate to inhibit bacterial growth of staphylococci

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Background: *Staphylococcus aureus* and *Staphylococcus epidermidis* are major causes of infectious disease, including infections associated with hernia mesh and surgical sites. Current medical care fails to effectively control these infections, particularly when bacteria establish antibiotic-resistance. In addition, the formation of biofilms (i.e. bacterial clusters embedded in a protective matrix) on hernia mesh and surgical sites frequently leads to clinical complications and increased healthcare costs. The lack of effective treatment strategies to combat antibiotic-resistant bacteria and biofilms is a major unmet need.

Objective: Investigating the antibacterial activity of an innovative treatment comprising diethyldithiocarbamate (DDC) combined with copper(II) (Cu) against staphylococci.

Methods: The minimal inhibitory concentration (MIC) of DDC and Cu was determined in 2 methicillin-resistant *S. aureus* (MRSA 1 and MRSA 2) and 2 *S. epidermidis* (SE 1 and SE 2) strains. To assess the potential synergy between the two compounds, checkerboard assays were performed with planktonic and biofilm bacteria. The fractional inhibitory concentration index was calculated and used to define synergism, near synergism or additive effects of DDC and Cu.

Results: The MIC of DDC was 64 for MRSA 1 and 2, and 32 µg/ml for SE 1 and 2. The MIC of Cu was >256 µg/ml in all strains tested. Synergistic effects of DDC and Cu (DDC-Cu) were observed in the 2 planktonic MRSA strains. However, DDC-Cu reached near synergism in MRSA 1 biofilms and additive effects in MRSA 2 biofilms. In all *S. epidermidis* strains DDC-Cu showed additive effects in both planktonic cells and biofilms.

Conclusion: Synergistic and additive effects against MRSA and *S. epidermidis* make DDC-Cu a potential new treatment strategy against staphylococci. Future research will expand on current antibacterial and antibiofilm studies, and will focus on determining the mode of action of DDC-Cu. In addition, studies will focus on the development of drug-delivery approaches for applications in hernia mesh infections and surgical site infections.

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Staphylococcus aureus biofilm exoprotiens impairs the nasal epithelial barrier

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Publish consent withheld

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Large Scale-Free Network Organization is Likely Key for Biofilm Collective Behaviour

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Living organisms are complex, dynamical and dissipative systems, often considered to be in a far from equilibrium state. That is, for their survival, living systems exchange matter dynamically and are able to evolve spontaneously under certain environmental perturbation towards a critical point for a phase transition. This happens without fine-tuning their system parameters. The formation of biofilm by certain microorganisms, such as *Escherichia coli*and *Saccharomyces cerevisiae*, is a common example. Such phase transformation is known to break the symmetry of the system leaving it invariant, or in a collective mode. At the critical point, the system achieves universality, that is, all differences between individuals will be reduced to follow common "universal" properties. The fascinating self-organizing behavior of biology has triggered scientists across diverse disciplines to study the underlying *sync*mechanisms. Non-linear Kuramoto model has been used to study synchronized or *sync*behavior in numerous fields, however, its application in biology is scare. Here, I introduce the non-linear model and show that large scale "small-world" or "scale-free" networks are crucial for spontaneous *sync*even for low coupling strength. To verify this prediction, we performed multi-dimensional transcriptome-wide analysis of *Saccharomyces cerevisiae*biofilm in wildtype and 6 biofilm regulating overexpression strains (*DIG1*, *SAN1*, *TOS8*, *ROF1*, *SFL1*, *HEK2*). The overall results show that low to middle expressed genes, not the highly expressed, are key for scale invariance in biology. Together, our data indicate that scale-free network connectivity structure with low coupling, or expression levels, is sufficient for *sync* behavior of living cells.

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What's New in the Detection of Bloodstream Infections?

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Globally, bloodstream infections are among the most serious infections resulting in significant morbidity and mortality. While blood cultures remain the standard of care, they are slow and insensitive. Syndromic panels that allow for rapid identification of a broad range of pathogens and resistance markers from positive blood cultures have been a major advance and have enhanced antimicrobial stewardship interventions. Existing platforms are being expanded and a novel platform that uses ferrocene labeled probes and electrochemical analysis and voltammetry to detect targets has just been released. Matrix assisted laser desorption ionization time of flight mass spectrometry has also been used successfully to identify organisms in positive blood culture bottles. The first rapid phenotypic susceptibility testing platform from positive blood cultures became available two years ago. The use of these rapid methods, in combination with antimicrobial stewardship, has improved antibiotic utilization, both in terms of de-escalation and earlier time to appropriate therapy. However, while studies show decrease in length of hospital stay and reduced hospital costs, very few studies demonstrate a reduction in mortality. Direct from whole blood testing has the ability to decrease poor patient outcome and has been available in Europe and other areas for more than a decade. However, the available assays are either limited in the pathogens detected, are less sensitive than blood cultures, or have been commercial failures because of cumbersome and prohibitively expensive instrumentation. A variety of new technologies are in development, appear promising, and will also be discussed in this presentation. At the same time, novel biomarkers are being tested and exploited to better assess the patient who presents with sepsis.

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Not available

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Not available

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Clinical metagenomics of fungal infections as basis for precision-based medicine

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Fungal infections in humans affect 25% (~1.7 billion) of the world population, with invasive fungal disease (IFD) alone causing 1.6 million deaths/year. They are treatable and result in good outcomes if diagnosed in a timely manner. However, current mycological diagnostics take days to weeks, lack specificity and sensitivity, leading to vital delays in treatment, inappropriate therapy, increasing morbidity and mortality and high healthcare costs. Therefore, early identification of the causative pathogen is vital to improve disease outcome. New technologies, such as long-read sequencing-based metagenomics, having the potential to radically transform clinical diagnosis, as they permit real-time detection of multiple pathogens directly from clinical specimens (metabarcoding). The utilization of these technologies is currently hampered due to the lack of standardized protocols, low pathogen:host DNA ratio, sub-optimal DNA quality, limited reference data and inadequate bioinformatics algorithms. To evaluate and compare the resolution of amplicon based short-read Illumina and metagenomics based long-read MinIONsequencing, first a pilot study was conducted with a defined "mock" community of 12 fungal species. Illumina correctly identified 7/12 and MinION6/12 species. Second, metagenomics based long-read MinION sequencing was applied to detect *Pneumocystis jirovecii*, the non-culturable agent of *Pneumocystis*pneumonia, directly from BAL and sputum specimens. Of the total reads obtained 70 - 95% were assigned a human origin, while most microbial reads were classified as bacteria and only ~10% were fungi.Comparison of all fungal and bacterial reads obtained by WIMP and BLAST for species concordance showed a good correlation for bacterial reads (70.2-97.4%) but not for fungal reads (0.19-10.2%). Whilst *P. jirovecii*was identified, it did not produce the strongest signal, indicating that patient fungal loads, false-positives and sequencing errors need to be taken into account in analysis algorithms. Long-read based metagenomics as diagnostic tool for fungal infections will result in a drastic reduction in turn-around time to 24-48h and significant improvement of accuracy, leading to a more cost-effective and highly improved patient care.

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A Genotyping strategy for *M. intracellulare* isolates in QLD

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Mycobacterium avium Complex (MAC) is a group of opportunistic non-tuberculous mycobacterial species (NTM) which includes *M. avium* and *M. intracellulare*. MAC organisms, particularly *M. intracellulare,* are a significant cause of pulmonary infections in older patients, often in the presence of underlying lung disease. When compared with *M. avium, M. intracellulare* is more often isolated from immuno-competent patients. The disease may be an infection with the same organism or chronic disease with multiple strains. Re-infection following treatment can occur. As the source of the infections is environmental, there is the

potential for ongoing infections with multiple genotypes as well as colonisation without disease. Treatment of NTM disease is problematic because of the resistant nature of the organisms.

Does repeated isolation of the organism indicate a chronic infection with the same genotype or re-infection with a different genotype? Is the strain associated with significant disease or does it represent colonisation? This information would help to target antibiotic therapy or avoid further costly investigations and treatment. By comparing environmental and patient isolates there would also be the possibility of determining the potential source of the infection. Do some strains have characteristics which predispose patients to chronic infection and disease? Are there preventative measures the patient could take to prevent infection? A genotyping strategy would enable clinicians to understand the nature of infection of *M. intracellulare* in a patient *M. intracellulare* is the most common NTM isolated in respiratory isolates in QLD. The incidence of infections due to *M. intracellulare* is increasing both in QLD and worldwide. This preliminary study investigated the use of a 7-loci MIRU-VNTR genotyping method using a random selection of clinical isolates of *M. intracellulare*. The results showed a discriminatory method suitable for further investigation of both clinical and environmental isolates of *M. intracellulare.*

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Development of a novel real-time PCR assay for detecting the bacterial pathogen *Stenotrophomonas maltophilia*

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Cystic fibrosis (CF) is a commonly inherited fatal disease in European descendants that is caused by defects in the *CFTR* gene. The excessive production of mucus produced by this defect, coupled with a typically exaggerated and ineffective immune response, provides optimal growth conditions for airway infections of various opportunistic pathogens with high antibiotic tolerance including *Pseudomonas aeruginosa*, *Burkholderia cepacia* species, *Staphylococcus aureus*, *Achromobacter xylosoxidans*, and *Stenotrophomonas maltophilia*. In addition to its contributory role in CF pathogenesis, *S. maltophilia* is increasing in prevalence in other clinical settings, including bloodstream, wound and catheter-associated infections. Currently, there is no rapid and highly-accurate method for detecting this naturally multi-drug resistant and potentially life-threatening opportunistic pathogen, suggesting that its true prevalence is likely being underestimated. This study used large-scale comparative genomics of *S. maltophilia* and near-neighbour species to identify a specific genetic target for this emerging pathogen, with subsequent development and validation of a newly designed real-time Black Hole Quencher-based PCR assay for its detection. Comparative genomic analysis of publicly available *Stenotrophomonas* spp. genomes identified a single 4kb region that was specific to *S. maltophilia*. Microbes BLAST analysis of non-*S. maltophilia* matches to the PCR amplicon revealed several isolates submitted to GenBank that we confirmed as *S. maltophilia* using phylogenomic analysis, thereby representing species assignment errors in the NCBI database. Upon PCR assay optimisation, we incorporated our assay with a previously published universal 16S rDNA target to enable the simultaneous identification of *S. maltophilia* and confirmation of DNA integrity. Our assay successfully detected 89 clinical *S. maltophilia* samples derived from both CF sputa and acute non-CF infections with 100% accuracy. Our novel assay surpasses existing phenotypic and genotypic methods for the identification of this organism, and will improve the diagnosis and subsequent treatment of this under-recognised pathogen by enabling its accurate detection from polymicrobial clinical and environmental samples.

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Application of MALDI-TOF MS in foodomics

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Foodomics is a relatively new discipline that applies advanced omics approaches in the areas of food safety, food quality and nutrition. Although some research has been done in food safety using some of the more recent omics approaches, such as proteomics and metabolomics, few studies have explored their use in analysing real food samples. In addition, the limitations around these approaches have not been highlighted. The current work focusses on different applications of a proteomics in assessing microbial food safety using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). This platform was used for two different applications: identification of foodborne pathogens and spoilage bacteria from different culture media and artificially spiked foods; and strain level typing. In the case of pathogen identification from different culture media, the identification rates were found to be influenced by culture conditions (media and incubation time). Limitations in the commercial database were hypothesized as the reason for the variable identifications rates from the different media. Post-customisation of the database led to an overall improvement in the identification rates. In the case of artificially spiked foods, detection of various foodborne pathogens from various complex foods (ground beef, chicken pâte, cantaloupe and Camembert cheese) was evaluated. Detection to the species level could be achieved for spiking loads of 10cfu/mL in most foods in 18-30h. Interfering peaks from protein rich matrices, such as meat, and other inherent microbiota, such as those found in cheese, could pose a challenge for achieving a lower detection threshold using this platform. However, contrary to the currently available pathogen detection systems, MALDI-TOF MS provides a universal platform for detection of multiple pathogens from food. In the case of strain level typing for *Listeria monocytogenes*, good congruence was observed between MALDI typing and the gold standard PFGE suggesting that it has a potential to provide a more rapid and cost-effective solution.

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The planetary health imperative to eliminate nuclear weapons: progress and prospects

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The World Health Organisation has concluded that nuclear weapons pose the greatest immediate threat to human health and welfare. The planetary health imperative to prohibit and eradicate the only weapons posing an existential threat is urgent. The danger of nuclear war is growing. The hard-won agreements that have constrained nuclear proliferation since the Cold War are being progressively dismantled, and a new arms race between NATO/US and Russia is escalating. Rather than disarming, all 9 nuclear-armed states are massively investing in retaining and modernising their arsenals with new capacities and lowering the threshold for their use.

Successful approaches to the control of other indiscriminate and inhumane weapons have stigmatised, prohibited and are advancing the progressive elimination of biological and chemical weapons, landmines and cluster munitions. Evidence-based advocacy by scientists and health professionals on the catastrophic consequences of any use of nuclear weapons, together with compelling testimony of survivors of nuclear weapons use and testing, have underpinned a Humanitarian Initiative.

On 7 July 2017, the historic United Nations Treaty on the Prohibition of Nuclear Weapons was adopted. The Treaty is a planetary health good of the highest order. Expected to enter into force during 2020, it provides the first comprehensive and categorical prohibition of nuclear weapons, and is the only currently defined path for all states to achieve the eradication of nuclear weapons, the stated aspiration and legal obligation of all governments. Regrettably, the Australian government provides assistance for possible use of US nuclear weapons and opposes the Treaty.

The 2017 Nobel Peace Prize, the first to an Australian-born entity, was awarded to the International Campaign to Abolish Nuclear Weapons (ICAN) *"for its work to draw attention to the catastrophic humanitarian consequences of any use of nuclear weapons and for its ground-breaking efforts to achieve a treaty-based prohibition of such weapons"*.

The Treaty and health evidence-based advocacy are key to ensuring nuclear weapons are ended before they end us.

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The Osteocyte as a Target Cell and Disease Nidus in Human Periprosthetic Joint Infection

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Purpose

Periprosthetic joint infection (PJI) is a devastating complication of joint replacement surgery. Osteocytes, comprising 90-95% of all cells in hard bone tissue, are increasingly recognised as a critical cell type in bone physiology. We examined the potential role of osteocytes in PJI, with the rationale that their involvement could contribute to the difficulty in detecting and clearing PJI.

Methods

S. aureus, the most common pathogen in PJI, was chosen to test for their ability to infect human primary osteocyte-like cells *in vitro* and human bone samples *ex vivo*. Bone biopsies were retrieved from patients undergoing revision total hip arthroplasty for either aseptic loosening or PJI. Retrieved bacterial colony number and morphology from cell lysates were determined. Gene expression was measured by microarray and/or qPCR.

Results

Susceptibility of osteocytes to *S. aureus* was all confirmed in *in vitro* cell culture, *ex vivo* organ culture and PJI human bone samples. 24h post-invasion, transcriptome analysis of osteocyte-like cells revealed a strong host immune response. Consistent patterns of host gene expression were observed in both *ex vivo* infected human bone and in PJI patient bone samples. Internalised bacteria switched to the quasi-dormant small colony variant (SCV) form over 5d and later to a viable but non-culturable (VBNC) form. At a late stage, levels of acute immune response markers were attenuated compared to 5d but remained upregulated.

Conclusion

We have provided evidence for the involvement of human osteocytes in PJI [1]. The multiple events of phenotypic switching of *S. aureus* suggest that infection of osteocytes may contribute to a chronic disease state. The osteocyte may serve as a reservoir of bacteria for reinfection, perhaps explaining the high prevalence of infections that only become apparent after long periods of time or recur following surgical/medical treatment. Our findings also provide a biological rationale for the recognised need for aggressive bone debridement in the surgical management of PJI.

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The Mathematical and Scientific Basis for Developing and Setting Epidemiological Cut-offs Values

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Minimum inhibitory concentrations are an in vitro measure of antimicrobial activity. They are measured on an interval and not a continuous scale, and therefore have special mathematical and statistical properties. For instance, the MIC value is at the upper end on the interval, but because of standard plotting techniques this property is often incorrectly overlooked when analysing data. Conventionally, MICs are measured on a 2-fold dilution scale. This accident of history turns out to be correct because wild type MICs are distributed on a lognormal scale. Wild types, namely isolates without phenotypically expressed resistance mechanisms, are important to recognise and characterise. They serve as the foundation for establishing susceptibility testing breakpoints and the detection of emerging resistance. The epidemiological cutoff value (ECOFF) is the MIC that identifies the upper end of the wild type population and is the lowest possible breakpoint for a species. The bell-shaped curve of MICs in the wild-type population is a result on variation in two broad parameters: biological (strain-to-strain) variation and assay variation. Assay variation is in turn a composite of variation in reagent/material, intra-laboratory and inter-laboratory variation. Assay variation can be observed in repeated testing of the same strain, such as that which happens with quality control isolates. In setting ECOFFs, all these sources of variation ideally need to be included. The European Committee on Antimicrobial Susceptibility Testing has developed standard procedures ECOFF-setting that incorporate as many of these types of variation as possible. There are several methods for estimating ECOFFs. Visual interpretation has led the way, but more recently some of mathematical and statistical techniques have been developed. The most widely favoured at thpresent is the iterative statistical method which has been implemented in Excel as 'ECOFFinder', which is available on the EUCAST website as a free download.

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High throughput robotic antimicrobial resistance surveillance system for diverse applications

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Antimicrobial resistance (AMR) is one of the most prominent biosecurity issues affecting animals and humans in modern society. AMR in animals is a major global issue in both disease-causing zoonotic pathogens and commensals in the microbiota of healthy livestock. Globally, antimicrobial resistance surveillance and monitoring is widely acknowledged as a critical response to AMR and is one of the priorities of the WHO Global action plan. There are a number of barriers in effective AMR surveillance in food producing animals. These include high labour costs associated with conventional culture and antimicrobial susceptibility testing, lack of epidemiologically determined representative sample collection and sample size, poor study power and the inability to rapidly genotype resistant isolates. In addition, large national surveys undertaken to date are not truly representative of herd level data and do not provide insights useful for veterinarians and farmers implementing farm control measures via antimicrobial stewardship and infection control. Addressing these barriers requires an inexpensive and accurate means for objectively defining AMR risks at the herd and national level.

With the recent development of automated robotic systems and high throughput next generation sequencing platforms, it is now feasible to develop cost effective tools to monitor AMR on large numbers of representative samples obtained from livestock. Application of this tool to individual herds would deliver an accurate description of their AMR status. This presentation will focus on advancing AMR surveillance through robotic multi-platform integration and identify balancing the role of phenotype and genotype from a One-Health perspective.

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Aerobic CO respiration by carbon monoxide dehydrogenases is a widespread mechanism of bacterial persistence

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Carbon monoxide (CO) is a ubiquitous atmospheric trace gas produced by natural and anthropogenic sources. Some aerobic bacteria can oxidize atmospheric CO and, collectively, they account for the net loss of ~250 million tonnes of CO from the atmosphere each year. However, the physiological role, genetic basis, and ecological distribution of this process remain incompletely resolved. In this work, we addressed these knowledge gaps using the genetically tractable aerobic actinobacterium *Mycobacterium smegmatis*. Shotgun proteomic and transcriptional analyses revealed this bacterium upregulates the catalytic subunit of a form I carbon monoxide dehydrogenase by 50-fold when exhausted for organic carbon substrates. Whole-cell biochemical assays in wild-type and mutant backgrounds confirmed that the enzyme oxidizes CO, including at sub-atmospheric concentrations, and supports aerobic respiration. Contrary to current paradigms on CO oxidation, the enzyme did not support chemolithoautotrophic growth and was dispensable for CO detoxification. However, it significantly enhanced long-term survival, suggesting that atmospheric CO serves a supplemental energy source during organic carbon starvation. Phylogenetic analysis indicated that atmospheric CO oxidation is a widespread and ancestral trait of CO dehydrogenases. Homologous enzymes are encoded by 685 sequenced species of bacteria and archaea, and we confirmed genes encoding this enzyme are abundant and expressed in terrestrial and marine environments. CO dehydrogenases are also conserved and expressed in the pathogen *Mycobacterium tuberculosis*. On this basis, we propose a new survival-centric model for the evolution of CO oxidation and conclude that, like atmospheric H_2, atmospheric CO is a major energy source supporting persistence of aerobic heterotrophic bacteria in different environments.

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Multi-level Genome Typing: A stable genomics-based strain typing system for short and long-term epidemiology.

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Both long and short-term epidemiology are fundamental to disease control and require accurate typing of bacterial isolates. The implementation of whole genome sequencing in many public health laboratories has led to an explosion of genomic data that has the potential to provide highly sensitive and accurate descriptions of strain relatedness. Previous efforts to implement typing regimes using this data have mainly focussed on outbreak detection or used clustering methods to identify larger groups of isolates.

We have developed multilevel genome typing (MGT), using multiple, hierarchical multilocus sequence typing (MLST) schemes of increasing size that allow examination of genetic relatedness at resolutions from 7 gene MLST to core genome MLST. This system avoids clustering methods which can provide unstable naming schemes and derives each identifier directly from the sequence. Once assigned, the string of sequence types from each scheme, known as a genome type (GT) will not change. We implemented this system for *Salmonella enterica* serovar Typhimurium and typed 9799 isolates with publicly available data. Previously described *S.* Typhimurium populations can be identified and named, such as the DT104 multidrug resistance lineage (GT 19-2-11) and two invasive lineages of African isolates (GT 313-2-3 and 313-2-752). Further we show that outbreak detection clusters, derived from the MGT, are capable of accurately distinguishing 54 outbreak isolates from 5 background isolates in five outbreaks.

MGT provides a universal and stable nomenclature at multiple resolutions for bacterial strains and could be implemented as an internationally standardisable strain identification system that accommodates both long-term and short-term epidemiological needs. It will allow better temporal and spatial tracking of bacterial clones that are associated with clinically relevant phenotypes such as antimicrobial resistance and disease severity, facilitating better diagnosis, clinical management, disease control and prevention.

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Comparative genomics of Australian *Elizabethkingia* clinical isolates resolves incorrect speciation and reveals additional population diversity

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Members of the genus *Elizabethkingia* are environmental bacteria that can cause opportunistic hospital-acquired infections with the potential for life-threatening complications. Although clinical outbreaks have been documented in the United States, Taiwan, Mauritius and Hong Kong over the last 10 years, very little is known about the species diversity or evolutionary relationships between strains and species. Further, epidemiological factors such as transmission routes and pathogenesis potential of many *Elizabethkingia* species remain undefined.

To resolve the relationships among *Elizabethkingia* species, 22 clinical isolates collected from throughout Queensland, Australia, over a 16-year period were subjected to speciation using both the VITEK MS system and whole-genome sequencing. These 22 genomes were combined with 100 publicly available *Elizabethkingia* genomes. Using the comparative genome pipeline SPANDx, 127,130 core-genome biallelic single-nucleotide polymorphisms (SNPs) were identified among these genomes. Phylogenomic reconstruction using these SNPs robustly identified 14 *E. anophelis*, 3 *E. miricola*, 2 *E. meningoseptica* and 1 *E. brunniana* strains in the Queensland isolates. In addition, two pairs of *E. anophelis* Queensland strains were near identical, suggesting patient to patient transmission.

Consistent with previous studies, *E. anophelis* was the dominant pathogenic species. Phylogenomic analysis resolved eight VITEK MS speciation errors within the *E. miricola*, *E. brunniana* and *E. meningoseptica* clades. Furthermore, 60% of Australian clinical isolates resided on novel branches within the global phylogeny, with no overlap found between Australian cases and those of international origin. Representing almost 15% of available public data, these Australian genomes add valuable reference diversity to the *Elizabethkingia* genus from a previously unsampled geographic region. Taken together, these findings highlight the need for phylogenomic-based approaches for correct assignment of *Elizabethkingia* species; and reveal untapped diversity present in this genus.

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Ecological and genomic attributes of novel bacterial taxa that thrive in subsurface soil horizons

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While most bacterial and archaeal taxa living in surface soil horizons remain undescribed, this problem is exacerbated in deeper soils owing to the highly oligotrophic conditions found in the subsurface. Additionally, previous studies of soil microbiomes have focused almost exclusively on surface soils, even though the microbes living in deeper soils also play critical roles in a wide range of biogeochemical processes. We examined soils collected from 20 distinct profiles across the U.S. to characterize the bacterial and archaeal communities that live in subsurface soils and to determine whether there are consistent changes in soil microbial communities with depth across a wide range of soil and environmental conditions. We found that, irrespective of location, bacterial and archaeal diversity decreased with depth, as did similarity of microbial communities to those found in surface horizons. We observed five phyla that consistently increased in relative abundance with depth across our soil profiles: Chloroflexi, Nitrospirae, Euryarchaeota, and candidate phyla GAL15 and Dormibacteraeota. Leveraging the unusually high abundance of Dormibacteraeota at depth, we assembled genomes representative of this candidate phylum and identified traits that are likely to be beneficial in low nutrient environments, including the synthesis and storage of carbohydrates, the potential to use carbon monoxide (CO) as a supplemental energy source, and the ability to form spores. Together these attributes likely allow members of the candidate phylum to flourish in deeper soils and provide insight into the survival and growth strategies employed by the microbial taxa that thrive in oligotrophic soils. I will finish by highlighting microbial adaptations to oligotrophy more generally and the strategies that can be used to study those microbes with long generation times and low rates of activity that are typically difficult to study using more traditional cultivation-based approaches.

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Microbial dynamics in a thawing world

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High northern latitudes are at the leading edge of global climate change with the effects of warming already evident in degrading permafrost. Increased thawing of permafrost, a significant global carbon pool, makes previously sequestered carbon available for microbial degradation. Increased ambient temperature results in a transition from pristine (frozen) permafrost, through an intermediate (thawing) state, to fully degraded and flooded end state (thawed). Transition to a thawed state has been associated with dramatic increases in biogenic production of methane and a change in overall greenhouse gas balance. Our research aims to investigate microbial communities associated with this shift with a view to predicting post-permafrost community composition and function, and global warming feedbacks. Microbial communities along such a thaw gradient in Stordalen Mire, northern Sweden were characterised using meta-omic approaches. Our results reveal distinct differences in the microbial communities at each site along the thaw gradient, which can be directly associated with thaw state and increasing methane emission.

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Bacteria living on thin air - strategies for survival in the harsh Antarctic environment

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Antarctica is the coldest, driest and windiest continent on the planet. Yet the desert soils of east Antarctica host a great diversity of microbial communities that have evolved unique strategies to survive under the extremely harsh conditions. Microbes dominant terrestrial Antarctica and in doing so must endure frequent freeze-thaw cycles, complete winter darkness, limited nutrient and water availability and intense UV radiation. We have found that in the dry desert surface soils of the Windmill Islands and Vestfold Hills regions, very few phototrophs such as cyanobacteria or algae exist. Instead, novel bacterial phyla with new functional capacities are thriving under the stressful conditions. By combining metagenomics with differential coverage binning and functional assays we proposed that trace gas chemosynthesis, a new form of primary production was supporting microbial communities living in these nutrient poor environments. This aerobic energy-capture process relies on the oxidation of atmospheric levels of hydrogen and carbon monoxide gas to provide the fuel required to fix CO_2 via the Calvin-Benson-Bassham cycle, and provides new understanding of the nutritional limits for life. Today I will focus on the significance of this alternative form of primary production, while providing new information on the physiology and environmental drivers of *Candidatus Eremiobacteraeota* (WPS-2) and *Candidatus Dormibactereaota* (AD3), two yet to be cultured bacterial phyla whose members have the genetic capacity to live on thin air.

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Transfer of Environmental Microbes to Humans in Urban Green Space Environments

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Urbanisation has significantly changed human lifestyles with increases in population density, pollution exposure, and incidences of immune diseases. In parallel, there has been a decrease in green space environments and their microbial diversity. Decreased human microbial diversity is linked to immune diseases, which have increased worldwide in the past 50 years within urban areas. There are several hypotheses on microbial exposure (e.g. Hygiene, Old Friends and Biodiversity hypotheses) that describe urban

populations as ‘too clean’ and that these populations are no longer exposed to diverse environmental microbiota and microbial stimulants. These exposure factors likely alter and disrupt immune priming, and therefore contribute to the development of immune diseases. The Microbiome Rewilding Hypothesis proposes to decrease the prevalence of immune diseases observed today by increasing human-associated microbial diversity through urban biodiversity restoration. However, the microbial interaction between humans and their surrounding environment is not well understood. Here, we present the diversity and taxonomic profiles of microbes transferred from urban green space environments to the skin and respiratory tract of two individuals. Using a 16S rRNA metabarcoding approach, we analysed skin (n=90) and nasal (n=90) swabs collected from urban green spaces within Australia, the United Kingdom, and India, as well as air (n=30), soil (n=15), and leaf (n=15) samples from Australia. Morning controls of both skin (n=16) and nasal (n=16) swabs were collected allowing for before- and after-exposure comparisons. We found increased microbial richness and phylogenetic diversity after green space exposure in skin and nasal samples for all three countries. Beta-diversity showed that skin swabs became more similar to the microbiota of soil and leaf samples, while nasal swabs became more similar to microbes found in air samples. Interestingly, the nasal swabs from India were distinctly different from all other samples and contained less overall microbial diversity. Overall, this study improves our understanding of human-environmental microbial interactions and suggests that exposure to these environments may lead to positive outcomes for immune diseases.

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The Australian Microbiome – insights into Australia’s terrestrial microbial diversity

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The Australian Microbiome (AM) initiative is a collaborative network of researchers providing publically available data characterizing Australia’s environmental microbial diversity. The AM produces both amplicon sequences (bacteria, archaea, eukaryote and fungal targets) and shotgun metagenomes, primarily from soil and marine environments. Currently, terrestrially derived data in the AM comprises several thousand samples covering a large part of the observable environmental gradient. Every sample is accompanied by rich sample specific metadata describing its physical and chemical composition, as well as its geolocation and regional descriptors. Methods used to collect samples, perform wet lab analyses and downstream bio-informatic analyses are standardised. Sequence and sample specific metadata is available via the AM data portal (https://data.bioplatforms.com/organization/about/australian-microbiome), as both raw sequences data and curated sequence abundance tables, sequence data is also submitted to international nucleotide archives. Data produced by the AM is also available for direct export to and analysis by the Galaxy Australia server.

AM data has been able to provide insights into the drivers of soil microbial community composition and turnover, the role of climate in shaping Australia’s microbial diversity, the effects of various land-uses and perturbation levels on microbial communities, the role of microbial communities in successful land regeneration and the description of novel diversity within Australian soils.

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Polymicrobial Nature of Chronic Oral Disease

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Polymicrobial biofilms are complex, dynamic microbial communities that are important for the persistence and proliferation of participating microbes in the environment. Some chronic human diseases are caused by dysbioses in these communities that usually result in reduced diversity and a community dominated by a group of specific pathobionts. In the human oral cavity dysbiosis in subgingival plaque communities can lead to an increased abundance of specific anaerobic pathobionts, including the highly proteolytic, sessile *Porphyromonas gingivalis* and the motile spirochaete, *Treponema denticola*. *In vivo*, increases in *T. denticola* and *P. gingivalis* abundance are predictive of chronic periodontal disease progression. *P. gingivalis* and *T. denticola* co-exist in deep periodontal pockets and have been co-localized to the superficial layers of subgingival plaque as microcolony blooms adjacent to the epithelial cell layer, where they benefit from a dysregulated host inflammatory response. *P. gingivalis* and *T. denticola* specifically adhere to each other and decorate each other with outer membrane vesicles. *P. gingivalis* and *T. denticola* exhibit synergistic pathogenicity in animal models of disease, synergistic biofilm formation and development, and display a symbiotic relationship in nutrient utilization and growth promotion. Co-culture causes significant changes in global gene expression in both species, with *T. denticola* genes encoding virulence factors and glycine catabolic pathways being significantly up-regulated. *T. denticola* utilises glycine as a major energy and carbon source whilst in monoculture *P. gingivalis* produces excess free glycine. Free glycine release by *P. gingivalis* is stimulated by *T. denticola* which is due to the collaborative hydrolysis of peptides by proteases of both species. These processes enable these species to partition their environment and avoid competition for nutrients. *T. denticola* motility although not usually considered as a classic virulence factor, is involved in synergistic biofilm development between *P. gingivalis* and *T. denticola*. Collectively these data suggest an intimate relationship between these two species that has evolved to enhance their survival and virulence.

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Not available

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Not available

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Prevention of nontypeable *Haemophilus influenzae* colonisation and otitis media in mice by microbial interference with a closely-related commensal species

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Nontypeable *Haemophilus influenzae* (NTHi) is the major pathogen in otitis media (OM, middle ear infection). Colonisation of the nasopharynx with NTHi is a prerequisite to developing OM. Therefore therapies that prevent NTHi colonisation may prevent OM. Microbial interference involves the use of commensals to compete with pathogens to beneficially alter the host microflora. We previously demonstrated that pre-treatment of human respiratory epithelium with *Haemophilus haemolyticus*, a closely related commensal of NTHi, can prevent NTHi colonisation and infection of the epithelial cells. We have now assessed whether a rodent commensal *Muribacter muris* (from the same family as *H. haemolyticus* and NTHi) can prevent NTHi OM *in vivo*.

Methods: Using the murine NTHi OM ascension model, BALB/c mice were intranasally pre-treated with either *M. muris* ($5x10^7$ colony-forming units, CFU) (n=12) or saline (n=15) on Day 0. On Day 1, mice were challenged with $1x10^{4.5}$ plaque-forming units of Influenza A virus (strain MEM, H3N2) followed by a Day 3 intranasal challenge with $5x10^7$ CFU of NTHi (strain R2866Spec^r). Mice were monitored daily and scored clinically. Nasal washes and middle ear bullae were collected on Day 6. Homogenised ear tissue and nasal washes were plated onto selective media.

Results: Intranasal treatment of mice with *M. muris* reduced NTHi colonisation from 6×10^5 CFU/mL to 9×10^3 CFU/mL, p=0.0004. *M. muris* pre-treatment also prevented development of NTHi OM, with 8% (1/12) of pre-treated mice developing OM compared with 67% (8/12) of untreated controls, p=0.0682. The inflammatory response to NTHi was reduced in pre-treated mice, with lower KC (IL-8 homolog) levels in the middle ear compared with controls (p=0.0058). Weight loss was reduced (10.4% versus 13.8%; p=0.0006) and clinical scores improved (5.5 versus 7.2; p<0.00001) in pre-treated mice.

Conclusion: We have demonstrated that microbial interference can prevent NTHi OM in a murine model. Human challenge studies using microbial interference of NTHi are warranted to reduce the global burden of OM.

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Deep Sequencing of Microbial Communities in Cystic Fibrosis Airways

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Introduction: Cystic fibrosis (CF) is the most common life-shortening genetic disorder in Australians. CF leads to several clinical outcomes, particularly chronic lung disease, which causes the highest rate of mortality in CF patients. It remains unclear how microbes can deeply infect the CF airways and why the immune response is ineffective in eradicating them [1]. Traditional culture methods for pathogen identification are laborious and insensitive to microbial community diversity [2].

Methods: This study performed deep ("metagenomic") Illumina and Oxford Nanopore Technologies sequencing of the microbial communities in CF sputa to understand their microbial composition, and to identify potential links between persistence and antimicrobial resistance (AMR). Due to high human DNA contamination (~99%) in CF sputa, methods for enriching microbial DNA using saponin were investigated [3].

Results: Our metagenomic approach identified *Pseudomonas aeruginosa* as the most abundant pathogen based on either total DNA (99% human contamination) or microbial enriched DNA, consistent with culture results. In addition, AMR genes and strain mixtures within species were identified using both sequencing platforms. Using the saponin method, the human DNA was depleted by ~50-80%, enabling greater detection of microbial reads.

Conclusions: Our results provide exciting preliminary data showing that metagenomic sequencing is a powerful method for characterising microbial populations in the CF airway microbiome. Future studies will use our depletion methods prior to metagenomic sequencing to examine microbial changes over time and in response to treatment, with the aim of better understanding microbial populations, persistence and evolution, and ultimately, informing patient treatment.

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Novel antimicrobial strategy by arming the essential nutrient for the targeted elimination of *Porphyromonas gingivalis*

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The Gram-negative anaerobe *Porphyromonas gingivalis* is a “keystone” pathogen in chronic periodontitis whereby the capacity of the organism to dysregulate local host defence mechanisms drives a shift in the dental plaque microbiota towards dysbiosis. Subsequent host response against the dysbiotic microflora results in progressive destruction of supporting structures of the teeth and eventual tooth loss. Experimental models and epidemiological data show that *P. gingivalis* infection may also contribute to systemic pathology such as atheroma formation and rheumatoid arthritis. Critically, one strategy *P. gingivalis* employs to avoid immune surveillance is by invading oral epithelial cells. High levels of intracellular *P. gingivalis* can be recovered from the oral cavity of subjects with periodontitis, and this provides a reservoir for recurrent infection after therapy. Effective targeting of intracellular *P. gingivalis* is therefore an important aspect of enhanced antimicrobial therapy. Characteristically, *P. gingivalis* is unable to synthesise the porphyrin macrocycle and relies on exogenous porphyrin, most apparently as haem from host sources. Here we show that under iron-limited conditions prevailing in tissue environments, *P. gingivalis* expresses a haemophore-like protein, HusA, to mediate uptake of essential porphyrin as well as survival within epithelial cells. The solution structure of HusA presents the haem binding site as a shallow hydrophobic pocket on the a-helical structure but without evidence for coordination of prosthetic iron. This unusual binding feature also accommodates a variety of porphyrins including abiotic deuteroporphyrin IX, with higher binding affinity than for haem. We further show that an amino acid linked deuteroporphyrin-metronidazole conjugate with restricted antimicrobial specificity could function as a “Trojan horse” linked to HusA binding, for effective killing of intracellular *P. gingivalis*.

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Veterinary mycology and updates on agents of aspergillosis

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Veterinary mycology encompasses a range of naturally acquired infections in a variety of animal species, including birds, reptiles, fish and mammals. Many fungi of veterinary importance also cause disease in humans, prompting recent researchers to adopt a one-health investigative approach.

Yeast and mould infections in animals include aspergillosis, cryptococcosis, candidiasis, dermatophytosis, scedosporiosis, penicilliosis and many other diseases caused by pigmented and non-pigmented fungi. Infection can be localised to a particular body system or organ, or disseminated. Many antifungal therapies have been adapted from human medicine, with animal only products also employed, including azoles, echinocandins, polyenes, allylamines, griseofulvin and flucytosine. Additionally, some infections warrant the use of surgical management, including debriding and debulking procedures. Prognosis varies depending on the agent of disease and type of infection, host immuno-competency, and therapies used.

The past decade has seen an increase in cases of sino-nasal and sino-orbital aspergillosis in domestic cats. The majority of cases have been reported from pet cats living in Australia. Further investigation of these and other clinical (human and other animal) and environmental isolates has discovered novel cryptic species within *Aspergillus* section *Fumigati,* belonging to the *Aspergillus viridinutans* species complex. A number of fungi within this complex cause aggressive infection that is non-responsive to triazole drugs, resulting in poor clinical outcomes for humans and other animals. This has prompted investigations into antifungal susceptibility patterns, assessment of mechanisms of resistance and extrolite production for this species complex. The findings are of importance to both veterinary and human medicine.

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Fungal IP_7-protein interaction and invasive fungal disease: a dangerous liaison

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Invasive fungal diseases (IFDs) affect 300 million people and cause >1.5 million deaths annually, matching deaths from tuberculosis and exceeding those from malaria. A major factor contributing to the high morbidity and mortality is that only a few antifungal drug classes have been developed, the therapeutic effect of which is compromised by poor bioavailability, poor efficacy and/or toxicity. We have discovered a novel strategy for antifungal drug therapy by demonstrating that the inositol polyphosphate signalling molecule, IP_7, is critical for IFD. Using the model pathogen *Cryptococcus neoformans*, we showed that IP_7 is produced by the phospholipase C/inositol polyphosphate kinase (IPK) pathway. Blocking IP_7 production by inhibiting IPKs is feasible, as IPK-selective inhibitors have already been generated. To develop IPK inhibitors into antifungal drugs, a better understanding of how IP_7 promotes IFD is essential. I will discuss how we are addressing this key gap in knowledge, opening the door to developing a novel class of antifungal agent.

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The role of mutators in microevolution and the emergence of antifungal drug resistance in *Cryptococcus neoformans*

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The relative success of a species is determined by their ability to adapt rapidly to changes in environmental conditions. This adaptation occurs by the process of microevolution, in which a small proportion of the microbial population possessing phenotypes which facilitate growth are selected for and these cells become predominant in the population in a short time frame. Passaging of microbes *in vitro* can also lead to the selection of microevolved derivatives with differing properties to their original parent strains. The phenotypic traits are generated by mutations in the microbe's DNA sequence arising from unrepaired errors occurring during DNA replication or from environmental damage to DNA. Mutations can be pre-existing in the microbe's genome, or can be rapidly acquired in response to the host environment in a process termed adaptive evolution. Adaptive evolution is greatly enhanced by an increased mutation rate, which provides higher genetic diversity within a population on which selection can act. Strains which exhibit an elevated mutation rate, often 100-200-fold that of wildtype, are termed mutators. A mutator phenotype is advantageous in rapidly changing environmental or stressful conditions. The objective of this study was to ascertain if mutators existed within environmental, laboratory and clinical populations of *Cryptococcus neoformans* and to investigate the role mutation rate plays in microevolution and the emergence of resistance to antifungal drugs. The findings provide support for the hypothesis that this pathogenic fungus can take advantage of a mutator phenotype in order to cause disease, but only in specific pathways that lead to such a trait without a significant trade off in fitness.

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Molecular analysis of two strains of Barmah Forest virus isolated by Australian Defence Force

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Epidemic polyarthritis (EPA) caused by Barmah Forest virus (BFV) infection is the second most frequently notified arboviral diseases after Ross River virus (RRV) in Australia with approximately 1,200 cases reported annually over the last decade. The prototype strain of BFV (BH2193) was isolated from *Cx. annulirostris* trapped near the Barmah Forest area of Northern Victoria in 1974. Since then, outbreaks have been recorded in every Australian state as well as Papua New Guinea. Two BFV strains were isolated from Australian Defence Force (ADF) training areas (TA). MIDITully.2017 strain was isolated from a homogenized pool of *Verrallina spp.* mosquitoes captured in ADF Tully TA (17.9° S, 145.9° E), Queensland 2017 while MIDIWB78.2018 strain was isolated from a homogenized pool of *Cx. annulirostris* mosquitoes captured in ADF Wide Bay TA (25.3° S, 152.8° E), Queensland in 2018. The complete nucleotide sequences comparison with the only available whole genome sequence in GenBank, the prototype BFV BH2193 strain, revealed remarkable conservation between 1974 and 2018 with a maximum divergence of only 2.7% genome wide, but both ADF BFV strains contain multiple nucleotide insertions in the 3' untranslated region (UTR). At the amino acid level, MIDITully.2017 and MIDIWB78.2018 have a total of 32 and 33 amino acid substitutions evenly spread in non-structural and structural proteins, respectively. These mutations/deviations from the prototypic sequence and the insertions in the 3'UTR might have a dramatic impact on viral fitness and disease transmission capacity of the virus warranting further investigation. Phylogenetic analysis of all available BFV E2 nucleotide sequences deposited in GenBank between 1974 and 2019 segregated the viruses obtained by ADF into one of two BFV clades.

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Causal factors contributing to host immune system change in koalas immunized against chlamydial infection.

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The koala (*Phascolarctos cinereus*) is an iconic Australian marsupial currently experiencing localized extinctions in areas of Queensland and New South Wales. Disease is one factor contributing to this decline of this species, and arguably the most prevalent diseases in koalas are caused by infection with the intracellular pathogen, *Chlamydia*. Antibiotics can clear chlamydial infection, but their use is complicated by potentially eliminating beneficial bacteria in the koala gut. A chlamydial vaccine is one promising alternative to antimicrobial treatment for koalas that does not yet exist. Recent studies show that wild koalas infected with *Chlamydia* have a reduced chlamydial load and a lower prevalence of disease after vaccination with a multi-subunit vaccine as compared to unvaccinated koalas. Measurements of cytokine and antibody (IFNγ, IgA, IgG, IL-10, IL-17A and TNFα) abundance are available from these studies, however the effects of vaccination on these immune parameters are less understood. In this study we first created *a priori* hypotheses to make biologically relevant structural equation models to determine the causal factors in chlamydial load change in immunized koalas. We tested whether the data fit each *a priori* model using three fit indices: a Bollen-Stine bootstrap, a comparative fit index, and a root mean square of error of approximation. If multiple models fit, we chose the best fitting model based on Akaike's information criterion relative to sample size (AICc). We found that the best model at time 0 (pre-immunization) fit IFNγ, chlamydial load, and chlamydial disease. Our model at 6 months post vaccination also supports these variables, and indicates vaccination had a strong negative effect on chlamydial load. It was not surprising that IFNγ was a core component of our models, as previous studies found IFNγ inhibits chlamydial growth *in vivo* and mechanisms

for this have been described. Determining the effect of vaccination on IFNγ and chlamydial load will be useful to evaluate the effectiveness of future koala vaccine trials.

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Multilevel genome typing and global epidemiology of *Salmonella* Enteritidis

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Salmonella enterica serovar Enteritidis is a common foodborne pathogen causing both invasive and non-invasive infections. Multilevel genome typing (MGT) is a recently developed novel hierarchical genome typing approach. In this study, a MGT framework for *S.* Enteritidis was established, including 9 multi-locus sequence typing (MLST) schemes of different resolutions, composed of increasing numbers of genes (from seven genes for MGT1 to 4986 genes for MGT9). MGT1 refers to the seven house-keeping genes MLST of *Salmonella* which is of the lowest resolution. A total of 26,670 publicly available genomes from 89 countries were analyzed with MGT. As the MGT levels grow, numbers of recognised sequence types (STs) increase in size from 252 STs by MGT2 to 20,153 STs by MGT9. STs at each MGT level were further grouped into clonal complexes (CCs) when STs differed by atmost one allele. Using CCs at the middle-resolution level of MGT4, CCs prevalent in all continents as well as CCs restricted to a single continent were found. Two CCs were common in all continents representing global epidemic lineages, with one CC more prevalent in North America and Europe. These two CCs, which included 87.5% of all *S.* Enteritidis isolates, were found to have different potential to cause outbreaks according to the number of highly similar clusters defined by MGT9. We also identified two CCs at MGT4 that contained exclusively the invasive *S.* Enteritidis strains prevalent in Africa. By analyzing the presence of virulence and multi-drug resistance genes, MGT of various resolution levels were able to identify unique clades of *S.* Enteritidis strains that harbored different virulence associated loci, such as the *Yersinia* high pathogenicity island (HPI), type VI secretion system, and multi-drug resistance genes. In conclusion, MGT of *S.* Enteritidis is a hierarchical, simple and stable method for the epidemiological surveillance of *S.* Enteritidis, which enables additional insights into the diversity and convergence of the virulence and multi-drug resistance of different clones.

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Case report: whole genome sequencing based investigation of maternal-neonatal listeriosis

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Background

Neonatal listeriosis is a rare but severe disease manifesting as septicemia and central nervous system (CNS) infections with a high fatality rate of around 20% to 30%. Whole genome sequencing (WGS) is a promising technique for pathogen identification and infection source tracing with its high resolution.

Case presentation

A case of neonatal sepsis with listeriosis was reported with positive blood culture for *Listeria monocytogenes*. The case was investigated to confirm the vertical transmission of the infection and identify the potential food source of the maternal *L. monocytogenes* infection using WGS. *L. monocytogenes* was isolated from the neonate's blood sample the day after caesarean delivery and from the mother's genital and pudenda swab samples 5 days and 13 days after caesarean delivery. WGS showed that the isolate from the neonate was identical to the main genome type of the isolates from the mother, with only one of the 4 isolates from the mother differing by one single nucleotide polymorphism (SNP). By WGS, one *L. monocytogenes* isolate from a ready-to-eat (RTE) meat sample in the patients' community market shared the same sequence type but was ruled out as the cause of infection, with 57 SNP differences to the strain causing the maternal-neonatal infection. The food isolate also carried a novel plasmid pLM1686 that harbored heavy metal resistance genes. After caesarean section, the mother was treated with cephalosporin, which *L. monocytogenes* is naturally resistant to, which may explain why genital and pudenda swabs were still culture-positive for *L. monocytogenes* 13 days after delivery.

Conclusion

Genital swab culture for *L. monocytogenes* had been informative in the diagnosis of maternal listeriosis in this case. The high resolution of WGS confirmed the maternal-neonatal transmission of *L. monocytogenes* infection and improved source tracing.

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Comparative proteomics of Australian epidemic *Bordetella pertussis*

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Sulfate is an important modulator for virulence factor expression in *Bordetella pertussis*, the causative organism for whooping cough. During infection, sulfate is released when respiratory epithelial cells are damaged which can affect gene expression. The current predominant strains in Australia are found in single nucleotide polymorphism (SNP) cluster I (*ptxP3*/*prn2*). It has been reported that *ptxP3*strains have higher mRNA expression of virulence genes than *ptxP1* strains under intermediate sulfate-modulating conditions (5 mM $MgSO_4$). Our previous proteomic study compared L1423 (cluster I, *ptxP3*) and L1191 (cluster II, *ptxP1*) in Thalen–IJssel (THIJS) media without sulfate modulation and identified an upregulation of transport proteins and a downregulation of immunogenic proteins. To determine whether proteomic differences exist between cluster I and cluster II strains in intermediate modulating conditions, this study compared the whole cell proteome and secretome between L1423 and L1191 grown in THIJS media with 5 mM MgSO4 using iTRAQ and high-resolution multiple reaction monitoring (MRM-hr). Two proteins (BP0200 and BP1175) in the whole cell were upregulated in L1423 [fold change (FC) >1.2, false discovery rate (FDR) <0.05]. In the secretome, four proteins from the type III secretion system (T3SS) effectors were downregulated (FC < 0.8, FDR < 0.05) while six proteins, including two adhesins, pertactin (Prn) and tracheal colonization factor A (TcfA), were upregulated which were consistent with our previous proteomic study. The upregulation of Prn and TcfA in SNP cluster I may result in improved adhesion while the downregulation of the T3SS and other immunogenic proteins may reduce immune recognition, which may contribute to the increased fitness of cluster I *B. pertussis* strains.

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Pertactin-Negative and Filamentous Hemagglutinin-Negative *Bordetella pertussis*, Australia, 2013–2017

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Background: Pertussis (whooping cough) is a vaccine preventable disease caused by the bacterium *Bordetella pertussis.* Despite high vaccine coverage, pertussis has re-emerged to cause epidemic level disease. Our prior studies have shown a rapid increase in the proportion of *B. pertussis* isolates not expressing Pertactin (PRN) during the 2008-2012 epidemic. As a new pertussis epidemic occurred in 2015 and to further our understanding of pertussis epidemiology, we genotypically and phenotypically characterised 78 Australian *B. pertussis* isolates from 2013-2017, and compared their molecular characteristics with isolates from the 2008-2012 epidemic.

Methods: Whole-genome sequencing was performed on 78 clinical isolates collected from 2013-2017 to detect single-nucleotide polymorphisms (SNPs) in virulence genes and to determine their SNP profiles (SP). Together with 27 previously sequenced Australian *B. pertussis* isolates, a total of 105 isolates were analysed to determine their phylogenetic relationships. Western immunoblotting was performed to detect the expression of the acellular vaccine antigens; pertussis toxin (PTX), PRN and filamentous haemagglutinin (FHA) proteins.

Results: The 78 isolates were typed into two SPs: SP13 (SNP cluster I, *ptxP3*, 96.15% [75/78]) and SP18 (non-cluster I, *ptxP1*, 3.85% [3/78]). The majority (75/78, 96.15%) of the SP13 isolates had the *prn2* and *fim3A* allele. Three non-cluster I SP18 isolates were genotyped as *ptxP1-fim3A*-prn1*. The frequency of *ptxP* and *fim3* alleles were higher than those observed during the last epidemic. For PRN, 89.74% (70/78) of the isolates were found to be PRN-negative. By contrast, the percentage of PRN-negative isolates increased from 5.13% in 2008 to 77.78% in 2012. One novel PRN inactivation mechanism and a novel *fim2-3* allele were also found in this study. Importantly, we detected the first FHA-negative *B. pertussis* isolate in Australia.

Conclusion: The Australian 2013-2017 pertussis epidemic was predominantly caused by PRN-negative isolates, with local and interstate expansion. Our results suggest that *B. pertussis* continues to evolve under vaccine-induced selective pressure.

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SpeeDx PlexPCR RespiVirus Assay Detect a Broad Range of Contemporary Human Influenza Strains

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Background: Human influenza virus infections have a world-wide distribution. Seasonal influenza epidemics occur regularly both in the Northern and the Southern hemispheres each winter. These influenza epidemics are estimated to cause approximately 500,000 deaths per year world-wide. The most outstanding characteristic of influenza viruses is their rapid evolution which leads to great variability. This can impact the performance of laboratory diagnostic tests, including nucleic acid amplification tests (NAATs). We investigated the inclusivity of SpeeDx® ***Plex****PCR*® RespiVirus assay against contemporary human strains of influenza A and B viruses.

Methods: We conducted *in silico* analysis of ***Plex****PCR* components, namely the target specific ***Plex****Zyme* and Primer sets, using the international database Global Initiative on Sharing All Influenza Data (GISAID) (https://www.gisaid.org). We analysed influenza strains from 2015-2019 in Europe and Oceania region. The ***Plex****Zyme* and Primer sets were analysed against the matrix protein (MP) gene of Influenza A and B viruses. We also performed *in silico* analysis of the same ***Plex****Zyme* and Primer set against the 2018/2019 vaccine strains. Finally, we performed *in vitro* studies of the quality assurance samples (QCMD and QAP).

Results: Over 10,000 sequences for human strains of influenza A and B were downloaded and analysed. The ***Plex****Zyme* and Primer sets were placed in a very conserved region which would not result in false negatives. All strains from 2018/2019 vaccine strains were detected or are expected to be detected based on sequence conservations. Synthetic gene block sequences similar to 2018/2019 vaccine strains were used for *in vitro* detection. All quality assurance samples from QCMD and QAP were detected using the SpeeDx ***Plex****PCR* RespiVirus assay.

Conclusion: The SpeeDx ***Plex****PCR* RespiVirus assay detects a wide range of contemporary human influenza viruses. The incorporation of primer sets in a conserved region and inclusion of target specific PlexZymes negates potential false results.

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Structure and function of the Enterobacterial Common Antigen polysaccharide polymerase WzyE

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The outer membranes of Enterobacteriaceae are covered in a heterogeneous population of surface polysaccharides which include lipopolysaccharide (LPS) O-antigen and Enterobacterial Common Antigen (ECA). Both are biosynthesised by Wzy-dependent biosynthetic pathways, the most common polysaccharide biosynthetic pathway in bacteria. The polysaccharides repeat units are polymerized into linear chains within the periplasm by the glycosyltransferase Wzy, whereby, the degree of polymerization is controlled by the co-polymerase Wzz prior to export to the outer membrane. Wzy proteins, which are members of the Shape, Elongation, Division, Sporulation (SEDS) protein family, are poorly characterized both functionally and structurally due to low sequence homology and their intrinsic polytopic integral membrane protein nature. The vast majority of research has been orientated towards WzyB from the O-antigen biosynthetic pathway. While WzyE, the Wzy polymerase from the ECA biosynthetic pathway has yet to be investigated, it is known that the polysaccharide it assembles, ECA, plays crucial roles in maintaining outer membrane integrity as well as providing resistance to bile-salts and other detergents. Initial alignments of a large number of WzyE protein sequences enabled identification of conserved residues throughout the polypeptide. Through the use of site-directed mutagenesis, conserved residues were altered to determine their importance in the polymerase function as determined by Western immunoblotting of ECA. Topology mapping via C-terminal PhoA-LacZ_{α} reporter fusions enabled the topology of WzyE to be investigated. This will allow for a greater understanding of how Wzy proteins function, and provide insight into Wzy-dependent polysaccharide polymerization.

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Prevalence of fecal coliforms and *Escherichia coli* in drinking water from source and point-of-use in Rohingya camps, Bangladesh

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Background: An estimated 1.16 million Rohingya people originally displaced from Myanmar have been living in 32 camps in Cox's Bazar district of Bangladesh. Newly arrived Rohingya peoples are living in spontaneous settlements, and there is an increasing need for humanitarian assistance; including shelter, clean water, and sanitation. Collectively, a total of 6057 water points and 50087 emergency latrines have been built to support the needs of inhabitants of the camps. Safe drinking water is essential for life. Water is unsafe for human consumption if contaminated with pathogenic microorganisms. A quality water supply (adequate, safe and accessible) must be ensured to all human beings for healthy life. Herein we report microbial contamination of drinking water from a large habitation of Rohingya population.

Methods: We have collected a total of 12650 drinking water samples from source and point-of-use and tested for fecal coliforms and *E. coli* contamination.

Results: It has been found that 28.14% (n=896) water samples derived from tube wells were contaminated with fecal coliforms and 10.6% (n=337) were contaminated with *E. coli*; also, 73.96%, (n=4644) samples from stored household sources (at point of use – POU) were found contaminated with fecal coliforms while 34.67% (n=2177) were contaminated with *E. coli.* It was also observed that higher percentage of POU samples fall in the highest risk category (based on number of *E. coli* contamination per 100 mL sample) than that of their sources.

Conclusions: Our findings indicate secondary contamination during collection, transportation and storage of water due to lack of knowledge of personal and domestic hygiene. Hence, awareness buildup is necessary and the contaminated sources should be replaced as well as the POU water should be treated by a suitable method to provide safe water in the camps.

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A Case of *Nocardia brasiliensis* in a Gardening Wound

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A Case of *Nocardia brasiliensis* in a Gardening Wound

Robekah McDougall – Launceston General Hospital

A 65-year-old male presented to the Emergency Department at the Launceston General Hospital on the 29th of November 2018 with suspected cellulitis on his right arm and wrist. He was also systemically unwell with fever, rigors and was hypotensive. He had a history of rheumatoid arthritis and was on medication that is known to be an immunosuppressant. It was noted that he had initially injured his right wrist gardening 4 weeks prior. Routine pathology tests were ordered. His total WCC was 15.0 10^9/L, neutrophil count 12.4 10^9/L and CRP 193 mg/L. These results all pointed towards an infection. At this point the patient was sent to theatre to operate on his infected wrist. A right wrist tissue and right wrist swab was collected and sent to the microbiology department for routine microscopy, culture and sensitivity testing. The direct gram stain of the tissue and the swab showed numerous leukocytes however, no organisms were observed. The culture plates had no growth after 24 hours incubation and were re-incubated for a further 24 hours. After 48 hours incubation, a light growth of dry, white colonies was observed on the chocolate and blood agar CO_2 plates. A Gram stain of the colonies was performed, and branching Gram positive bacilli were seen. Following suspicion based upon these results, a Ziehl-Neelsen and Modified Ziehl-Neelsen stain was performed. The Ziehl-Neelsen stain was negative and the Modified Ziehl-Neelsen stain demonstrated partially acid-fast bacilli. These results were pointing towards the isolate being a *Nocardia* species. As our laboratory is unable to formally identify Nocardia or perform susceptibility testing, this isolate was forwarded to a reference laboratory for confirmation of identification and susceptibility testing. The isolate was later confirmed to be *Nocardia brasiliensis* by polymerase chain reaction (PCR). The patient is expected to make a full recovery.

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Proteome analysis during fruit body development in the edible mushroom *Lentinula edodes*

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Lentinula edodes, popularly called *Shiitake* or black mushroom, is one of the most widely cultivated edible mushrooms in the world. During fruit body development essential for sexual reproduction, *L. edodes* displays a dramatically morphological differentiation as well as other mushroom-forming basidiomycetous fungi. To aid in effective breeding, constructing a genetic map and gene expression studies have been performed in *L. edodes*. Although discriminatively expressed genes in morphological changes were identified, those molecular mechanisms are not well known. Compared with the analysis of transcriptomes, protein analyses are still limited. The proteomic analysis techniques have progressed within recent years. In this study, we analyzed intracellular protein changes during fruit body development in *L. edodes* using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and applied expressed sequence tags (ESTs) data to tandem mass spectrometry (MS/MS) data analysis.

The fruiting body formation stage was divided into three developmental stages: vegetatively growing mycelia, primordia and fruiting bodies. Nonetheless, primordia contained slightly various proteins, quite similar patterns of protein spots were obtained in primordia and fruiting bodies. In contrast, there found many unique protein spots in vegetatively growing mycelia. Next, specifically and abundantly expressed protein spots during fruiting body formation were subjected to the subsequent MS/MS analysis. As a result of computational searches against fungal EST databases, approximately90% of spots were found to be identical to the amino acid sequences deduced from ESTs in *L. edodes*. In addition, several protein modifications such as phosphorylation and glycosylation were successfully detected. The obtained data helps to understand the molecular mechanism of fruiting body development in *L. edodes* and may provide us a new insight into the improvement of yield efficiencies for cultivations of edible mushrooms.

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Candidatus Dormibacteraeota, a trace gas scavenging phyla of soil bacteria.

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Candidatus Dormibacteraeota, previously known as candidate division AD3, is a phylum of yet to be cultured soil bacteria that are found ubiquitously across the globe. Usually representing less than 1% of the microbial population, they have been detected in relatively high abundances (up to 15%) within Antarctic desert surface soils. This enigmatic phylum has also been reported to be present in high abundances in other cold environments, predominantly permafrost. Recently, we used shotgun sequencing and differential coverage binning on metagenomes obtained from two arid desert sites, Robinson Ridge and Mitchell Peninsula, in Eastern Antarctica. Six microbial assembled genomes (MAGs) were retrieved that were > 90% complete and contained

between 0-4.6% contamination. Analysis of all six MAGs showed that Dormibacteraeota is a distinct phylum, with four of the genomes matching the typestrain Dormibacter sp., while the others matched a group commonly referred to as JG37-AG-4. Genomic analysis revealed that all six of the genomes have the capacity for aerobic respiration of glycerol and xylose, while the JG37-AG-4 genomes were also capable of anaerobic respiration. All six MAGs also contained type 1h hydrogenases, indicating their capacity for scavenging atmospheric hydrogen. While four also contained one or more carbon monoxide dehydrogenase genes, suggesting the atmospheric CO oxidation potential. Three MAGs also contained a type 1E RuBisCO. This data indicates the phylum are metabolically diverse, being genetically capable of trace gas chemosynthesis. We next aimed to visualise the Dormibacteraeota. FISH probes were designed targeting the two main groups which were subsequently validated using Clone-FISH. Isolation of cells from Antarctic soil showed that members of both groups within the Dormibacteraeota are cocci in shape, ranging from approximately 1 µm in length. We propose that the Dormibacteraeota are a phyla perfectly adapted to life in the cold and nutrient devoid environment of Antarctic soils.

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LIFE PROCESSES IN OUR GROUNDWATER

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Aquifers are oligotrophic environments that harbour diverse and novel microorganisms. These microorganisms form networks of microbial food webs which govern biogeochemical processes and cycle nutrients. Currently, little is known about the functional capacities of these subsurface ecosystems and how globally problematic anthropogenic pollutants, such as nitrate, can effect ecosystem health. This project aims to study how these communities respond to and mitigate inputs of pollution. We hypothesize that aquifer metabolism will strongly reflect non-agricultural and agricultural influences on groundwater chemistry, and that groundwater microorganisms play an important role in mitigating nutrient pollution. A series of wells in a globally common aquifer type – alluvial sandy gravel - were selected along a nutrient gradient in Canterbury, New Zealand. Biological groundwater samples were recovered using filtration. Microbial cells were captured using a 0.22 µm pore size filter. In order to sample both groundwater communities and communities attached to the aquifer substrate, we used a low-frequency *in situ* sonication to induce biofilm detachment. Chemical parameters of the groundwater such as nitrogen species, sulfur species, organic carbon and metals were measured. The nitrate gradient across the well series ranges from 0.45 to 12.6 g/m^3. Results from 16S amplicon data shows changes in community composition across the gradient. In particular, higher abundances of N_2-producing anaerobic ammonium-oxidizing Planctomycetes (Brocadiales) were found at anoxic sites. Consistent with this observation, excess N_2 was detected in groundwater at these anaerobic sites, indicating removal of N from the system. Whole genome and transcriptome sequencing is in process in order to gain insights into active gene pathways across the nitrate gradient. To expand the scope of these findings a survey of aquifers of similar lithology across New Zealand is also underway. This will allow key steps in the nitrogen cycle to be quantified across different nutrient conditions using Droplet Digital PCR. Information from this survey will determine the dominant N-cycling mechanisms in nutrient polluted and pristine aquifers.

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Bacteriophages For The Control Of 'Rattles' In Foals

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Rhodococcus equi causes a suppurative bronchopneumonia, colloquially termed 'rattles' in foals. This globally distributed bacteria, is endemic to many Australian farms and has a prevalence of 1-10%. Diagnosis and treatment of rattles is expensive and is a multi-million dollar burden on the Australian horse industry.

Without a vaccine, current control strategies are primarily aimed at reducing the environmental bacterial proliferation and aerosol transmission. However, as a saprophyte the organism easily survives and propagates in soil and the gastrointestinal tract of foals, and is shed to the environment via faeces. Treatment for *R. equi* infections are limited to long term and expensive combination antibiotic therapy of rifampicin and a macrolide, with mounting evidence of resistance worldwide.

Bacteriophages affect microbial populations via lysis of bacterial cells or by integration of the phage into the bacterial genome, which can influence virulence. Using a screen panel of *R. equi* isolates, bacteriophage have been isolated from mare and foal faeces and paddock soil samples collected from NSW horse farms with varying incidence of 'rattles'. Isolated phage are characterised by host range and lytic activity and a selection of these are to undergo electron microscopy and whole genome sequencing. By comparing findings between these paired samples and correlating this with disease prevalence and bacterial burden (quantitated by qPCR), we are unique in our aim to determine the impact of bacteriophage populations on the ecology of virulent *R. equi*, specifically within the gut of foals and in the foal's environment.

Preliminary findings show that phages were more commonly isolated from soil than from mare and foal faeces. Soil crude lysates generally display a larger *R. equi* host range than those from foal faeces sampled in the same area. Thus, soil samples will be targeted in future for isolation of phage candidates that could potentially be used for environmental biocontrol of *R. equi.*

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Virulence genes *rib* and *bca* in serotypes of Group B streptococcus (GBS) isolated from symptomatic pregnant women in tertiary care hospital in East Coast Malaysia

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Group B streptococcus (GBS) is a leading cause of maternally-acquired invasive infections in neonates. Maternal immunization with GBS vaccine is of utmost demand for prevention of these infections. Required knowledge concerning vaccine candidates includes serotype-specific polysaccharides and GBS virulence proteins. This study aimed to undertake capsular serotyping and virulence factor genes identification for the local GBS isolates as a pilot study to identify vaccine candidates. Standard microbiological methods, according to the Centers for Disease Control and Prevention (CDC) recommendations, were used to identify GBS serotypes. A total of 62 GBS isolates from high vaginal swabs of symptomatic pregnant women were collected from the 1st of March 2018 to 30th of July 2018. The isolates identity was reconfirmed by molecular methods. Latex agglutination test was performed to determine the GBS serotypes according to the specificity of the capsular polysaccharide. Of the 62 examined GBS isolates, 48 were serologically typeable, representing (77.4%), and 14 were serologically non-typeable representing (22.6%) of the samples. Serotype Ia and Ib (16.1% each) was the most common capsular types, followed by II, V, and VII (9.7% each), III (8.1%), VI (6.5%), and VIII (1.6 %). Among all ten GBS serotypes, serotypes IV and IX were not detected in the present study. Real-time PCR revealed that 42 (67.7%) isolates harbored the *rib* gene while 61 (98.4%) isolates harbored the *bca* gene. Our findings showed that the five widely known prevalent serotypes in other regions in the world which are considered as candidates for pentavalent CPS-conjugate vaccine do not match the CPS distribution in symptomatic pregnant women in Kuantan. On the other hand, the frequency of virulence genes *rib* and *bca* is higher in our isolates, which tentatively makes the proteinaceous vaccine, N-terminal domains of Rib and AlpC a more suitable choice for GBS prevention in this geographical area. However, further wider study recruiting larger number of isolates from various Malaysian states is required to confirm this conclusion.

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FUNCTIONAL METABOLIC INTERMEDIATE (2-PYRON-4,6-DICARBOXYLIC ACID) FROM LIGNIN

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Most of the chemical industry is dependent on fossil resources as raw materials for both energy and chemicals. Fossil resources are becoming increasingly expensive as the supply decreases and they affect the climate and environment. The utilization of biomass resources in valuable fuels and industrial products is becoming important aspect for conversion from nonrenewable resources to renewable bioresources. Lignin is one of the most important carbon resources, existing in woody plants at 15-30% by dry weight. The effective utilization of lignin waste from pulp production and bio-ethanol fermentation has been main concern of the society. PDC, 2-pyrone-4,6-dicarboxylic acid is the terminal chemical substance of lignin bio-degradation before streaming into the TCA cycle in *Sphingobium* sp. SYK-6 and has never appeared in chemical synthesis. The metabolic conversion of lignin into stable and functional intermediates has been extensively investigated, and we recently reported the massive production of 2-pyrone-4,6-dicarboxylic acid (PDC) from lignin via protocatechuic acid by the action of transformed bacterium. PDC consists of the polar pseudo-aromatic ring system and two carboxylic acids. By using it character as bi-functional monomer for stepwise polymerization, our group has synthesized the biomass-based polymer consisted of PDC. In the presented study, the production of PDC from vanillic acid, one of the major component of monomeric lignin, by the combination of genes encoding demethylase, protocatechuate 4,5-dioxygenase, 4-carboxy-2-hydroxymuconate-6-semialdehyde dehydrogenase in transgenic bacterium *Pseudomonas putida* PpY1100 and PDC polyester which has the tenacious adhesive property to metals and glasses was described.Polyester consisted of PDC was synthesized. Tenacious adhesion properties of bio-based polyesters as high as about 30 ~ 60 MPa against several metals were reported. Generally, the biomass-based materials are poor in terms of usefulness. The biomass-based material presented here is prospect for not only an alternative to petroleum one but also the useful material with higher quality compared to the ordinary one.

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Trans-generational effects of commensal microbiota on pupal production and body weight of a polyphagous fly

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There is increasing evidence showing the important role of commensal microbiota on the fitness of the animals, from affecting nutrient metabolism and energy regulation, to supporting immune system and modulating behavior. While direct effects of commensal microbiota on host physiology are progressively understood, little is known about their trans-generational effects on animal performance. We addressed the gap by manipulating the commensals of parental fruit fly pest (the Queensland fruit fly-*Bactrocera tryoni*) using the germ-free model, then investigating the effects of commensal microbiota on the development of flies in both parental and offspring generations. We found that the developmental time and adult emergence rate were not affected by commensal manipulation, but the pupal production was significantly different between treatments whereby fewer pupae were observed from the germ-free treatment. Flies of control and reinfection treatments were heavier than flies of germ-free treatment at all life stages (larva, pupa, adult) in both generations, suggesting that the presence of commensal microbiota consistently support the body weight gain. Together, our results indicate that the commensal microbiota has the long lasting effect on the development of the animals.

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Identification of common Apergillus species using Matrix-Assisted Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS)

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Background:

The launched of MALDI microbial ID system in 2006 has revolutionized the way clinical diagnostic Microbiology laboratories operated by enabling Laboratory to rapidly identify Bacteria and now the common molds.

Lau A et al have offered a rigorous tube protein extraction method for fungus which does not only render the organism nonviable but it also allows the intracellular proteins to be freely available for analysis hence producing good-quality spectrum which lead to successful identifications obtained.

Sue S et al have also validated the MALDI-TOF for the identification of fungus focusing on the Australian demographics and were also successful with obtaining identification using the method outlined by Lau.

Both research groups concluded that the successful identifications of any fungus using MALDI-TOF MS is dependent on the fungal coverage in the existing database and to date this is insufficient unless complimented with an in-house database.

Summary:

Extraction method described by *Lau A, Steven D et al* have proven to be robust and feasible to adopt as part of Bacteriology routine workflow. Using this method, reliable and reproducible identification of Aspergillus fumigatus complex and Aspergillus niger complex was obtained.

Prospective studies (*Lau A, Steven D et al & Sue S, Catriona H et al*) have shown that reliable identifications to species level could be obtained from the primary RUO library when the cut-off score is lowered to ^{3}1.70 to ^{3}1.99 instead of the recommended ^{3}2.00 by the manufacture.

The data obtained from our validation reflected this, as we were able to achieve 100% correlation with 30 isolates (A. fumigatus and A.niger complex) when compared against phenotypic method.

At Royal Prince Alfred Hospital (RPAH), we have improved the utility of our existing Brucker MALD-TOF MS and TAT for the identification of A. fumigatus complex and A.niger complex without the added cost to the department's current resources hence also improving patient care.

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Effective host depletion and microbial enrichment method for bovine genital tract microbiome research

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In 2016-2017, the Australian beef industry gross value was $A16.85 billion, which is 20% of the total farm production in Australia ($62b). Overall beef production is dependent on the lifetime reproductive efficiency of the cow, as the relative cost of raising a breeding female decreases with increased calving. It has been estimated that neonatal calf mortalities, pestivirus and bovine genital campylobacteriosis (vibriosis) collectively contribute to ~$236m in production losses in Australian cattle industries each year. Genital tract microbiome has a direct impact on bovine reproductive health and susceptibility to infectious diseases which may be associated with infertility and pregnancy losses.

16S ribosomal DNA amplicon sequencing has been utilized to characterize both human and bovine vaginal microbiomes and has generated confident characterization of representative microorganisms for different gynaecological and obstetric conditions.

However, it is accepted that there is an underlying bias in 16S metagenomic sequencing. Hence, shotgun whole-metagenome sequencing (WMS) is proposed for this study to overcome this bias. Additionally, shotgun WGS is able to generate more valuable insights into the phylogenetic composition, metabolic capacity and functional diversity. The major challenge in using WGS from clinical samples is the large amount of host genetic material present in these samples.

We conducted a preliminary study to examine the effects of four host depletion and microbial enrichment methods on cattle genital tract samples. Soft-spinning and benzonase extraction were shown to be the most efficient host elimination methods, yielding more comprehensive taxonomic and functional profiles for the microbiome in bovine genital tracts than the other depletion methods evaluated.

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Screening of bacteriophage library displaying scFv antibody fragments from schistosome-infected buffalo for diagnostic reagents for schistosomiasis

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Schistosomiasis is an acute and chronic parasitic disease caused by blood flukes of the genus *Schistosoma.* The Kato-Katz method is the standard recommended by World Health Organization for qualitative and quantitative diagnosis of intestinal schistosomiasis caused by *S. japonicum* and *S. mansoni*, because it is highly specific, relatively simple and inexpensive. However, Kato-Katz is less useful in light infections and other available detection methods are neither practical nor sensitive enough for routine or large-scale screening. Thus, improved tests that are also more cost effective are demanded for assessment of infection.

The water buffalo (*Bubalus bubalis*) is a natural host and reservoir for propagation of *Schistosoma*. Although it can be infected, the water buffalo does not succumb to schistosomiasis, suggesting a robust immune repertoire. In addition, *B. bubalis* belongs to Bovidae family like cows (*Bos taurus*), which have been reported to possess a diverse complementary determining region (CDR) 3 antigen receptor.

In this study, a phage display single-chain Fragment of variation (scFv) library derived from a schistosome-infected water buffalo in China was screened against schistosome antigens, to select antibody that could be used as diagnostic reagent for schistosomiasis. The schistosome antigens, which were highly expressed at various stages of life cycle of the parasite, were initially cloned and over-expressed in vector with maltose-binding protein as solubility tag, purified by immobilized metal affinity chromatography, and subsequently used in biopanning the library. The scFv fragments that bound to the antigens were isolated and characterized. However, when tested against bovine serum albumin, lactoferrin and hemoglobin, the scFvs were also enriched, suggesting non-specificity to the schistosome-antigens.

Our next step is to construct a larger phage display scFv library from the Philippine carabao. Since the Philippine carabao is more exposed to harsher conditions and more parasites, it should offer a diverse immune response and produce potent antibodies. The isolated schistosome-specific scFv will be fused to alkaline phosphatase, which will be used in developing a direct enzyme-linked immune-sorbent assay (ELISA) format diagnosis.

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Genomic epidemiology of erythromycin-resistant *Bordetella pertussis* in China

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Erythromycin is the empirical treatment of *Bordetella pertussis* infections. China has experienced an increase in erythromycin-resistant *B. pertussis* isolates since they were first reported in 2013. Here, we undertook a genomic study on Chinese *B. pertussis* isolates from 2012 to 2015 to elucidate the origins and phylogenetic relationships of erythromycin-resistant *B. pertussis* isolates in China. A total of 167 Chinese *B. pertussis* isolates were used for antibiotic sensitivity testing and multiple locus variable-number tandem repeat (VNTR) analysis (MLVA). All except four isolates were erythromycin-resistant and of the four erythromycin-sensitive isolates, three were *non-ptxP1*. MLVA types (MT), MT55, MT104 and MT195 were the predominant types. Fifty of those isolates were used for whole genome sequencing and phylogenetic analysis. Genome sequencing and phylogenetic analysis revealed three independent erythromycin-resistant lineages and all resistant isolates carried a mutation in the 23S rRNA gene. A novel *fhaB3* allele was found uniquely in Chinese *ptxP1* isolates and these Chinese *ptxP1-ptxA1-fhaB3* had a 5-fold higher mutation rate than the global *ptxP1-ptxA1 B. pertussis* population. Our results suggest that the evolution of Chinese *B. pertussis* is likely to be driven by selection pressure from both vaccination and antibiotics. The emergence of the new non-vaccine *fhaB3* allele in Chinese *B. pertussis* population may be a result of selection from vaccination, whereas the expansion of *ptxP1-fhaB3* lineages was most likely to be the result of selection pressure from antibiotics. Further monitoring of *B. pertussis* in China is required to better understand the evolution of the pathogen.

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Isolation of *Clostridium difficile* from paediatric patients in Western Australia

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Introduction

Clostridium difficile is an anaerobic, Gram positive, spore forming rod, the most common cause of hospital-acquired diarrhoea in adults in the developed world. Research on *C. difficile* infection (CDI) among paediatric patients is limited. Epidemiological typing of *C. difficile* isolates conducted in Western Australia (WA), between 2011 and 2012 revealed an increasing number of CDI cases in children with malignancy.

Objective

The aim of this study was to analyse the molecular epidemiology of CDI in paediatric patients in WA over a period of a year, a period of time when patients were at Princess Margaret Hospital (PMH) in the first half of the year, and were then moved to Perth Children's Hospital (PCH) for the second half of the year.

Methods

This was a retrospective study, examining stool PCR positive *C. difficile* and ribotyping data for the positive stool samples recorded during 2018 (1st January to 28th December). For 1st January to 9th June, data came from PMH and for 13th June to 28th December, the newly opened PCH.

Results

A total of 31 stool samples (from 25 patients) were PCR positive for *C. difficile* at PMH, for patients aged between 1 to 17 years, from 11 different wards (with the highest prevalence recorded from the oncology ward, followed by the emergency ward). A total of 53 stool samples (from 31 patients) were PCR positive for *C. difficile* at PCH, for patients aged 6 months to 16 years, from 13 different wards (with the highest prevalence recorded from the haematology/oncology ward). The most common ribotypes (RTs) isolated were toxigenic RTs 020, 014, 106 and 056.

Conclusions

An increase in prevalence was observed in patients at PCH, when compared to PMH. Understanding the importance of isolating *C. difficile* (particularly toxigenic strains) in young children and the implications this has on their treatment is significant in aiding the development of effective laboratory testing strategies for CDI in children.

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Unravelling the three "-omes" of *Pandoraea fibrosis*

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Introduction

Pandoraea species are emerging pathogens in cystic fibrosis (CF) patients, however, the contribution to disease progression remains elusive[1]. Current treatments comprise of imipenem and trimethoprim-sulfamethoxazole (co-trimoxazole). A new species has recently been identified, *Pandoraea fibrosis*, but the conventional treatment regime was unsuccessful clinically[2]. This study aimed to assemble the genome, investigate the transcriptome, expand the panel of antimicrobial susceptibility testing and evaluate the progression of resistance.

Methods

The sequential *P. fibrosis* clinical isolates 6399 and 7641 were obtained from a CF patient (Royal Hobart Hospital, Tasmania, Australia)[3]. DNA was extracted using the DNeasy Blood and Tissue kit, RNA via the RNeasy Mini Kit with an additional rRNA depletion and poly(A) tailing for direct RNA sequencing. Samples were sequenced on a MinION (Oxford Nanopore Technologies) sequencer. Combined with prior Illumina sequencing[4], genomes were assembled using Unicycler, variants determined via GATK (impact using snpEff) and resistance genes detected with ResFinder3.1. As no breakpoints (CLSI, EUCAST guidelines) exist for *Pandoraea*, resistance was determined using closely related species. Evaluating the progression of resistance was performed by passaging isolates in increasing concentrations of either imipenem or co-trimoxazole for 10 days followed by 3 antibiotic-free passages (n=4).

Results

The completed genome assembly for each isolate was a singular 5.59 Mb circular contig. Genome length differed by one nucleotide and three missense mutations. ResFinder detected one gene conferring resistance to beta-lactams, *blaOXA-154* (82% identity). The top differentially expressed genes were associated with efflux pumps (≥1.4-fold increase in 7641). Of the 40 antibiotics assayed, *in vitro*susceptibility was identified for imipenem, co-trimoxazole, doxycycline and minocycline. Selection of resistance revealed one 7641 replicate developing resistance against imipenem at day 7 and MICs were similar to MBCs. Co-trimoxazole non-susceptibility was observed temporarily at day 9 for one 7641 replicate, however, was unstable and MBCs were commonly ≥4-fold higher than MICs.

Conclusion

Overall, this study has provided further insight into the genome, resistome and transcriptome of *P. fibrosis*.

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Molecular epidemiology of *Clostridium difficile* isolated from piglets in Thailand and Malaysia

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Data regarding the epidemiology of *Clostridium difficile* infection (CDI) in South East Asian countries is limited, as is information on possible animal reservoirs of *C. difficile* in the region. This study aimed to investigate the prevalence of *C. difficile* in piglets and the piggery environment in Thailand and Malaysia, and determine the molecular epidemiology of *C. difficile* isolated.

Piglet rectal swabs (*n*=224) and piggery environmental specimens (*n*=23) were collected between 2015 and 2016 from 11 farms located in Thailand and Malaysia. All specimens were tested for the presence of *C. difficile* with toxigenic culture. PCR assays were performed on isolates to determine the ribotype (RT), and the presence of toxins A, B and binary toxin genes. Whole genome sequencing was used to determine the evolutionary relatedness of isolates of RT QX083 (the most prevalent RT identified) common to pigs and humans from Thailand and Indonesia.

C. *difficile* was recovered from 35% (58/165) and 92% (54/59) of the piglets, and 89% (8/9) and 93% (13/14) of the environmental specimens from Thailand and Malaysia, respectively. PCR assays performed on all strains from Thailand, and 30 strains from Malaysia (23 piglet and 7 environmental isolates) revealed all strains to be non-toxigenic. The most common strain belonged to a novel RT QX083, which accounted for 88% (51/58) of the piglet and 78% (7/9) of the environmental isolates from Thailand, and all of the isolates from Malaysia. Core-genome single nucleotide variant (SNV) analysis showed that piglet RT QX083 isolates from Thailand and Malaysia differed by only 18 SNVs. Moreover, these strains were, on average, 30 SNVs different from the human strains from Thailand and Indonesia, indicating that piglet and human *C. difficile* RT QX083 likely shared a common ancestor in the last two decades.

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Dual DNA barcoding for the molecular identification of the agents of invasive fungal infections

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Fungal diseases have an increasing global impact on human and animal health. Invasive fungal diseases alone cause over 1.6 million deaths/year. To reduce this burden advancements in diagnoses are essential. DNA barcoding has recently been established as the gold-standard identification technique for pathogenic fungi. This method relies on the selection of short, standardized DNA regions - DNA barcodes - that are divergent at the species level. For pathogenic fungi, the primary barcode (internal transcribed spacer region) and secondary barcode (*translational elongation factor 1α*) have been established, and together form the dual barcoding scheme. Clinical implementation of these barcodes relies on databases of quality-controlled reference sequences, e.g. the “ISHAM Barcoding Database”, aiming to provide reference sequences for both fungal barcodes. The current study aimed to generate secondary fungal barcode reference sequences to complement the primary fungal barcode, and to evaluate the discriminatory power of the dual barcoding scheme. As a result, 270 reference sequences were generated from 90 pathogenic fungal species. To evaluate the dual barcoding scheme, barcoding gap analysis of each barcode and in combination was performed for select fungal taxa. Barcoding gap analysis revealed barcoding gaps when using the secondary barcode for all taxa where the primary barcode was unable to do so. For all taxa analysed the combination of the two barcodes revealed barcoding gaps. These results indicate that either the secondary barcode alone or in combination with the primary barcode enables accurate identification of the studied fungal species. As such, the dual barcoding scheme in combination with the extension of the “ISHAM Barcoding Database” establishes a highly accurate and rapid identification system for fungal pathogens for routine diagnostics of mycoses.

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Calibration of Ceftolozane-Tazobactam for the CDS Method

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The Calibrated Dichotomous Sensitivity (CDS) test is a disc diffusion method calibrated using agar dilution. The method is maintained by the CDS Reference Laboratory at St George Hospital, Australia.

The CDS reference laboratory is responsible for supporting users as well as upkeep of the method. Before any new antimicrobial agent can be included in the manual it is necessary to confirm that there is a correlation between zone size and minimal inhibitory concentration (MIC).

Ceftolozane-tazobactam is a novel antimicrobial agent with activity against *Pseudomonas aeruginosa* (including drug-resistant strains) and other common Gram-negative pathogens (including most extended-spectrum-β lactamase [ESBL]-producing *Enterobacteriaceae* strains).

A selection of current and historical strains of Enterobacteriaceae and Pseudomonas species were tested against ceftolozane in the presence of 4 mg/L tazobactam to establish the breakpoint and determine whether a uniform zone size could be applied for use with the CDS disc diffusion test.

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Multiplex PCR-Next Generation Sequencing for Culture-Independent Typing of *Bordetella pertussis*

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Background: *Bordetella pertussis* is the causative agent of pertussis (whooping cough), an acute respiratory disease. Despite high immunization coverage in Australia, pertussis has re-emerged, with current strains belonging to SNP cluster I carrying non-vaccine alleles (*ptxP3/prn2*), which can be further typed into 5 epidemic lineages (EL1-EL5), reflecting microevolutionary events. Increased use of PCR-based diagnostic techniques is gradually phasing out culture in many countries including Australia, creating challenges for ongoing disease surveillance. This study aimed to establish culture-independent typing in *B. pertussis* for continuous epidemiological surveillance of how *B. pertussis* is evolving against vaccine selection pressure.

Materials/methods: A multiplex PCR protocol was designed for eight targets distinguishing SNP cluster I and EL1-EL5 based on SNP in current Australian epidemic strains. Custom amplicons were amplified with Illumina adapter-linked primers and sequenced using Miseq next generation sequencing (NGS). Ten-fold serial dilutions of DNA templates were used starting from the lowest 2 ng/µl to the highest 0.2 pg/µl (DNA copy numbers 4.63×10^6 to 46.3 respectively).

Results: Amplification of DNA was evident in all dilutions from agarose gel electrophoresis. Bioinformatic analysis of sequenced reads showed that all reads were successfully mapped to the reference genome and the corresponding bases in the SNP targets were correctly called. All SNP targets could be detected up to 2 pg/µl and in case of the highest dilution (0.2 pg/µl), six out of eight targets were detected. At individual target level, total reads and their distribution by percentage showed *ptxP3* and *ampD* had the best amplification. *ptxP3* also had the highest coverage across all dilutions.

Conclusion: This study tested NGS of multiplex PCR products for culture-independent typing of *B. pertussis*. Our results found the method to be highly specific, sensitive and accurate, allowing for correct assignment of SNP cluster and epidemic lineages.

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Unravelling the transposition mechanism of *res* hunter transposon Tn*502*

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The emergence of multiple antibiotic resistant bacteria is a major global health problem. A key contributor to the spread of resistance is mobile genetic elements, including transposons. The *res* hunter family of transposons is of particular interest as one subgroup of its members contains an integron (*int*) module which can serve as a gene capture and expression system and can facilitate accumulation and expression of drug-resistance cassettes. The other subgroup contains a Hg(II)-resistance module in place of the integron. Transposons that belong to this family exhibit an insertional and orientational specificity for a single target in plasmid RP1 located within the resolution (*res*) site of the *par* operon in the presence of its cognate resolvase (ParA). Tn*502* a member of the Hg(II)-resistance *res* hunter family, contains a distinctive transposition module (*tniABQ-res-tniR* & *tniM*) and differs from other members as it is able to transpose in the absence of the preferred site at a reduced frequency.

The transposition mechanism of these transposons is not well understood. In this study, we constructed deletion mutants of each *tni* gene revealing that deletions of *tniA*, *tniB* or *tniQ* completely abolished transposition whilst deletion of *tniM* reduced transposition frequency. To further elucidate the transpositional mechanism of Tn*502,* we expressed the following proteins, TniA, TniB, TniQ, TniM and ParA and performed protein-protein interaction assays. Our results reveal a complex array of interactions among the Tni proteins and that all Tni proteins and the external ParA resolvase are involved in the transposition mechanism. This study suggests that TniQ is likely to be a scaffold protein in the transposition complex and an external resolvase (ParA) has a pivotal role in transposition to the preferred site while TniM enhances the transpositional activity. Elucidating the molecular mechanisms of *res* hunter family will inform efforts to control the spread of antibiotic resistance.

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Quality Characteristics of Mango Flavoured Fermented Dairy Drink

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Foods are fermented for many different reasons such as to extend the product shelf-life, improve the sensory profile and enhance nutritional composition. Fermented foods are considered an ideal method of delivering beneficial probiotic bacteria to the gut. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (1). To confer a health benefit, a minimum therapeutic level (10^6-10^7 cfu/g or ml of carrier food product) must be maintained throughout the shelf-life of the product. Currently, the greatest difficulty for manufacturers is maintaining probiotic viability during production, storage and digestion (2). Dairy based probiotic food and beverages are currently highly popular and recently there appears to be an increased interest by food manufacturers in the production of functional fruit and milk beverages (3). Mangos are a good source of vitamins, dietary fibre and the mineral magnesium and potassium. They also contain the phytochemicals which could provide additional health benefits. As mango are a seasonal fruit, when the supply is in surplus the excess fruits are often processed into purees or juices to avoid wastage. This study aimed to determine whether mango juice can improve the viability of probiotics in a fermented dairy-based beverage whilst maintaining its quality characteristics. Formulations containing a *Lactobacillus acidophilus* La-5 culture, whole cow's milk and varying concentrations of mango juice (0%, 10%, 20%, 30% and 40% (w/w)) were produced and stored for five weeks at 4°C. Results showed that probiotic viability was enhanced with the addition of 10% mango juice throughout the storage. Additionally, this formulation significantly improved probiotics tolerance when exposed to *in-vitro* gastrointestinal digestion ($p<0.05$). The sensory analysis indicated that mango juice at 10% addition had minimal influence on the beverage sensory scores but increased the sensory scores as levels increased from 20% to 40%. Results suggest that commercialisation of such a product must find a balance between consumer liking and maintaining therapeutic amounts of probiotics in these types of products.

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Disrupting Bacterial Water Channels to Prevent Growth of *Staphylococcus aureus*

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Background: Persistent and recurrent *Staphylococcus aureus* infections can be associated with small colony variants (SCVs). SCVs are slow growing bacteria that show elevated antibiotic-resistance and capacity to form biofilms. As a result, standard medical care fails to eradicate SCVs, implicating quality of life and increasing health care costs. Alternative strategies to combat SCV's related infections are required; one of which includes targeting aquaporin channels (AQP). Bacterial aquaporin, AQPZ, shares homology to mammalian AQPs 1 and 4 and plays a role in osmoregulation in *Escherichia coli*. No AQPZ blockers have been discovered, but bumetanide derived agents effective in blocking mammalian AQPs 1 and 4 have shown to inhibit AQPZ in preliminary data. We hypothesise that if *S. aureus* SCV expresses AQPZ, novel AQPZ modulators would block this channel, disrupting the biofilm matrix, subsequently improving antibiotic delivery and efficacy.

Methods: The minimal inhibitory concentration (MIC) of the AQPZ modulator AqB013 was determined on planktonic SCVs. Bacterial growth in the presence of AqB013 was measured over 24 h. The AlamarBlue viability assay determined anti-biofilm efficacy following 24 h treatment exposure to different concentrations of AqB013 (0.078 mM to 2 mM). To assess biofilm removal, the crystal violet assay was performed. *E. coli* ATCC 25922 was used as a control strain. Triplicates were normalised to vehicle control (DMSO) and plotted after statistical analysis by one-way ANOVA.

Results: The MIC of AqB013 was 1 mM. Bacterial growth of planktonic SCV was inhibited by AqB013 in a dose dependant manner. AlamarBlue assays showed a significant decrease in viability of SCV biofilms at AqB013 concentrations of 0.125 mM (58% decrease) and 0.06 mM (71% decrease) ($P<0.0001$). AqB013 did not remove the SCV biofilm matrix.

Conclusion: The effect of AqB013 on SCV suggest the possible presence of an aquaporin. Confirming these findings may lead to a new treatment for persistent and recurrent *S. aureus* SCV infections.

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Host diversity driven phage evolution which leads to host range expansion

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Microbiome sequencing has shown that phage and bacteria coexist and co-evolve in complex communities (Stern *et al.*,2012, Weitz *et al.*,2013). Since, microbial communities support all life forms on the earth and bacteriophages are potential antimicrobial agents, it is important to understand the molecular details of phage evolution. A major challenge of phage biology and evolution is to determine the dynamics and driving forces of host range expansion.

A phage can target one specific host (specialist), or many hosts (generalist) (Koskella & Meaden, 2013). Generalists and specialists are both found in natural and clinical microbial communities (de Jonge *et al.*, 2019). However, microbiome studies

typically take a “snapshot” of phage diversity at one or a few time points, so it’s difficult to know dynamics of generalism and specialism over time. Experimental evolution is a useful tool to explore the genetic and phenotypic changes in phage and their hosts during evolution in a controlled laboratory environment (Buckling & Brockhurst, 2012). Here we test whether generalism is an intermediate state during the evolution of a specialist by evolving a novel T7-like phage (JB) in presence of diverse strains of *Escherichia coli*. We found that JB quickly adapted to new host in the presence of original host by diversifying into at least two subpopulations: one that specialized on the original host and new type that could infect the new host. Repeating this experiment across a range of host *E. coli* strains, we found that the new types were sometimes specialists on the new host, and sometimes generalists. This data suggests that generalism may be a transient state during the evolution of specialists, but that could be stable under certain conditions.

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Multidrug-resistant *Salmonella* isolated in Australia harbour a conjugative plasmid similar to that from a *Salmonella* strain isolated in Canada

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Red meat has been implicated as a source of foodborne bacterial pathogens such as *Salmonella enterica.* Antimicrobial resistance to antibiotics is a major concern to both human and red meat species. The aim of this study was to determine the presence of antibiotic resistance genes and occurrence of conjugative plasmids in antibiotic resistant *Salmonella* strains isolated from different red meat species in Australia. Whole genome sequencing of *S.* Heidelberg (n = 3) and *S.* Typhimurium (n = 1) was performed using the Illumina Miseq Sequencer (V3 2 x 300 bp reads). Genomes were assembled using SPAdes and annotated using Rapid Annotation using Subsystems Technology (RAST). Bioinformatic tools were used to determine antimicrobial resistance genes, plasmid replicons and orthologous average nucleotide identity. Aminoglycoside, beta lactam, macrolide, sulphonamide, tetracycline and trimethoprim resistance genes were detected in *S.* Heidelberg 329 and *S.* Typhimurium 2470. Three plasmids were detected in *S.* Heidelberg 329 and two plasmids in *S.* Typhimurium 2470. *S.* Heidelberg 329 and *S.* Typhimurium 2470 harboured a similar plasmid to a multidrug resistant *S.* Heidelberg strain N13-01290 plasmid pN13-01290_23 isolated in Canada. *S.* Heidelberg 329 and *S.* Typhimurium 2470 have plasmids belonging to the IncHI2 group similar to plasmid pN13-01290_23. *S.* Heidelberg 632 and *S.* Heidelberg 2581 had only aminoglycoside and fosfomycin resistance genes. *S.* Heidelberg 632 has two plasmids belonging to the IncII group while no plasmids were detected in *S.* Heidelberg 2581. This study suggests the IncH12 group plasmid is present in strains from different countries and may infer that these plasmids are involved in the transmission of multiple antibiotic resistance genes on a conjugative plasmid. This may confer an advantage towards the persistence and survival of *Salmonella*.

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Combination of duplex loop-mediated isothermal amplification with lateral flow biosensor assay for detection of *Campylobacter jejuni* and *Salmonella spp*.

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Publish consent withheld

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Fishing for *Neisseria gonorrhoeae* –a custom amplicon panel for detection from clinical metagenome samples

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Gonorrhoea, caused by *Neisseria gonorrhoeae* (*Ng*), *i*s the second most common sexually transmitted disease worldwide. Natural competence combined with a high rate of recombination gives *Ng* an increased capability to evolve antimicrobial resistance (AMR). *Ng* has evolved resistance to every class of antibiotic used to treat it in the past. Currently the recommended dual first-line treatment regime is azithromycin coupled with ceftriaxone. However, isolated cases of superbugs, which are resistant to both antibiotics have been reported recently, highlighting the threat of the spread of superbugs.

Tracking of AMR is essential to provide an effective treatment regime and to counteract the spread of antimicrobial resistant pathogens. Sufficient surveillance is lacking in many countries, including New Zealand. Since the move to nucleic acid amplification diagnostic tests (NAAT) for detecting gonorrhoea in New Zealand, information on AMR is limited to a national survey every few years.

We develop a cost-effective custom amplicon panel for metagenomics sequencing to directly type *Ng* from clinical samples and track AMR. We first tested the species specificity of currently used typing schemes (NG-MAST and NG-STAR), since clinical samples contain diverse microbial profiles. Our findings suggest that currently used typing genes are *Neisseria* specific, with one exception (23S gene). The creation of synthetic metagenomes allows the simulation of a range of clinical samples with varying pathogen load, representative of the swabbing quality and infection status of clinical samples. Amplicon sets will be tested in synthetic metagenomes, prior to clinical samples.

Usage of the amplicon panel for routine diagnosis of gonorrhoea will improve our ability to understand the evolution, emergence and spread of AMR in *Ng*. Ongoing surveillance provides key information to make informed decisions for effective treatment of common pathogens.

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Defining the mechanism of Kidney infection due to Group B streptococcus

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Group B streptococcus (GBS) causes pyelonephritis in adults but the mechanisms of infection by which GBS colonizes the kidneys *in vivo* are unknown. We investigated GBS colonization of the kidneys in mice following experimental challenge via the hematogenous route (transient bacteremia model) or transurethral route (bladder infection and cystitis model). Adult female mice were examined for bacterial dissemination to the kidneys and other organ systems at 24-72 h and tissue samples were assessed for histopathological changes. Comparisons included analysis of different challenge inoculum doses ranging between 10^7-10^9CFU and investigation of several GBS serotypes, including representative strains of serotype V (NEM316), III (BM110, 874391) and Ia (807). Mice with transient, low-level GBS bacteremia routinely developed acute pyelonephritis secondary to high-level kidney colonization. Kidney infection progressed with high GBS burdens that were sustained in the tissue for days in contrast to bacterial clearance in other organs, including spleen, liver and heart. The histopathological changes of acute pyelonephritis due to GBS were characterized using stains for hematoxylin and eosin, bacteria, neutrophils, macrophages, mast cells and T lymphocytes; this revealed recruitment of a mixed inflammatory cell population that infiltrated the renal medulla of infected mice in focal areas of discrete micro-abscesses. In contrast, bladder infection leading to cystitis in mice did not result in ascending spread of GBS to the kidneys. We conclude that transient bacteremia, rather than preceding infection of the lower urinary tract, is the predominant condition that leads to GBS kidney infection and subsequent development of acute pyelonephritis.

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Proteomic comparison of Australian epidemic *Bordetella pertussis* biofilm cells

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Background: Whooping cough is an acute respiratory disease caused by *Bordetella pertussis*. Despite developed vaccination programs, there has been a rise in pertussis notifications in Australia with an epidemic occurring between 2008 and 2012. The rise in incidence has been associated with pathogen adaption and the use of an acellular vaccine (ACV) that only targets 3-5 antigens. Recent studies have shown that *B. pertussis* readily forms biofilms, and this may lead to greater persistence in the respiratory tract. However, the proteins involved with biofilm formation and persistence in *B. pertussis* are not fully understood. The aim of this study was to use proteomic analysis to determine differences in protein expression between biofilm and planktonic cells of a recent Australian epidemic strain.

Method: L1423, a clinical isolate from the 2008-2012 Australian *B. pertussis* epidemic, was grown in THIJS media to 96 h in biofilm and 12 h in planktonic conditions. The proteins were extracted and label free liquid chromatography tandem mass spectrometry (LC-MS/MS) with spectral counts was used to identify and quantify the expressed proteins. Fold change < 0.8 and > 1.2 were considered downregulated and upregulated, respectively. Significance was assigned as $p < 0.05$ and $q < 0.05$.

Results and conclusion: There were 969 proteins identified with 590 proteins differentially expressed between biofilm and planktonic cells. There was a downregulation of proteins associated with *de novo* synthesis and an upregulation in central metabolism and nutrient transport in biofilms cells which may allow *B. pertussis* to persist under nutrient limiting conditions. Upregulation of toxins (pertussis toxin, dermonecrotic toxin and adenylate cyclase toxin) associated with immune modulation and downregulation of pertactin and type 3 secretion system proteins may increase immune evasion. Together, these proteomic changes in metabolism and virulence could increase the persistence of biofilm cells in the host.

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Automated Resistance Detection: Comparison of BD Phoenix to bioMérieux Vitek2 for Susceptibility Testing of Multi-drug Resistant Isolates

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Objective: There is a lack of literature comparing the performance of the BD Phoenix M50 (PHX) with the Vitek 2XL (V2) system in susceptibility testing of multidrug resistant organisms. We evaluated the accuracy, reliability and reproducibility of these systems using a collection of organisms with confirmed resistances (molecular +/- phenotypic).

Methods: The PHX NMIC-404 and PMIC-84 panels and the V2 AST-N246 and AST-P612 cards were tested for analytical reproducibility across different organism categories. 10 ATCC organisms, 200 Gram negative bacilli (GNB) and 100 Gram positive cocci (GPC) were used in this study. Isolates were tested simultaneously on both platforms and minimum inhibitory concentration (MIC) values of antibiotics common to both systems compared. Organisms with >1-fold difference in MIC had broth microdilution (BMD), (Thermo Fisher Sensititre panels GN3FG- and GPALL1FG+) performed as a reference standard.

MIC data was compared across the instruments and analysed by MIC and categorical interpretation (CSLI standards). MIC measurements were classified as concordant (EA – essential agreement) if MIC results were within 1 dilution of each instrument, while categorical interpretation was classified (CA – category agreement) using standard definitions of susceptibility errors (minor (mD), major (MD) and very major discrepancies (VMD).

Results: 10 ATCC strains each tested 5 times found 100% EA on both instruments.

For the 200 GNB tested, overall EA and CA were 95.8% and 90.7%, respectively. 50.5% of isolates required BMD to resolve discrepancies. The VMD, MD and mD rates were 0.16%, 0.48%, 1.18% and 0.29%, 0.61%, 1.09% for PHX and V2, respectively. The antibiotic with highest percentage of discrepant MICs overall was cefepime with an EA and CA of 79% and 65.5%.

Relative discrepancies were low for GPC, the overall EA and CA was 95.0% and 94.7%.

Conclusion: The performance of both systems was comparable with a low level of VMD and MD. Evaluation of a resistant population revealed generally acceptable results similar to more susceptible populations.

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Automated Resistance Detection: Comparison of the expert systems of BD Phoenix and bioMérieux Vitek2 for Susceptibility Testing of Multi-drug Resistant Isolates

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Objective:

The rising incidence of multidrug resistant organisms has made interpretation of antibiograms more challenging for Microbiologists. Assistance through use of interpretive algorithms in automated platforms such as the Vitek® 2XL has been valuable. The comparative performance of these systems has not been well documented. The expert systems (ES) of the BD Phoenix M50 (PHX) and the Vitek 2XL (V2) was tested against a collection of organisms with confirmed resistances (molecular +/- phenotypic).

Methods:

Isolates were tested simultaneously on both platforms. The instruments interpretation of resistance mechanisms was evaluated based on the ability to accurately classify a number of key resistance mechanisms including extended spectrum β-lactamases (ESBL), acquired AmpC β-lactamases (AmpC), carbapenem resistant Enterobacteriaceae (CRE), vancomycin resistant Enterococci (VRE), glycopeptide non-susceptible Staphylococci and MRSA (Methicillin resistant *Staphylococcus aureus).*

Results:

From 200 Gram negative bacilli tested, there were 158 isolates which harbored 178 designated acquired resistances. For specificity, 42 isolates were included that had no acquired resistance mechanisms detected either phenotypically or genotypically. The sensitivity for the ES corresponding to the reference genotype/phenotype was (PHX 90%, V2 77%), although the error rate was higher when analysed as a proportion of total tests (PHX 91%, V2 78%).

100 Gram positive cocci were tested. For 30 VRE, the sensitivity of the ES to correctly classify vanA & vanB was 100% for both systems. For low MIC vanB, the sensitivity was 30% for PHX, 10% for V2, however the limitation of the PHX system was its inability to differentiate between vanA & vanB. Both ES correctly classified methicillin resistance across the variety of Staphylococci tested. The sensitivity of the V2 ES to alert to possible hVISA/VISA was 53.3% compared to 0% on the PHX.

Conclusion: The performance of the ES was difficult to compare due to different levels of sophistication of the reporting algorithm, however, the V2 had superior performance, with a greater specificity. Both systems were designed to maximise sensitivity and should be considered screening algorithms only.

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Characterization of Phase Variable Genes of *Moraxella catarrhalis*

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Moraxella catarrhalis is a human host-adapted, opportunistic bacterial pathogen of the respiratory mucosa. Although asymptomatic colonization of the nasopharynx is common, *M. catarrhalis* can ascend into the middle ear where it is a prevalent causative agent of otitis media in children, or enter the lower respiratory tract where it is associated with acute exacerbations of chronic obstructive pulmonary disease in adults. Phase variation is the high frequency, random, reversible switching of gene expression that allows bacteria to adapt to different host microenvironments and evade host defences, and is most commonly mediated by repetitive tracts of DNA. Bioinformatic analysis identified 17 unique DNA sequence repeat tracts that appeared to vary in size between five closed *M. catarrhalis* genomes. We investigated the six top candidates by enriching populations with single repeat units and show phase variation of *uspA1*, *uspA2*, *mid/hag*, a conserved hypothetical gene and*modO*. We demonstrate that phase variation of *uspA1* (from high to low expression) occurs during repeat exposure to human serum, while phase variation of *uspA2* to a low expression variant similarly occurs over 72 hours of biofilm growth. We also identify and confirm the variable expression of two novel phase variable genes encoding a Type III DNA methyltransferase (ModO) and a conserved hypothetical gene (MC25239_RS00020). These data reveal the repertoire of phase variable genes mediated by simple sequence repeats in *M. catarrhalis* and indicate that phase variation occurs and correlates with altered gene expression under conditions mimicking human infection.

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Role of *Shigella flexneri* 2a serotype determinants in pathogenesis and as bacteriophage Sf6 receptors

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Shigella flexneri serotype $2a_2$ bacteria are the most prevalent serotype that cause shigellosis in developing countries. Chemical modifications (eg. glucosylation and O-acetylation) of lipopolysaccharide (LPS) O antigen (O polysaccharides) contribute to a wide diversity of serotypes. The increase in new serotype strains due to serotype conversion and the rise of multi-antibiotic resistant *Shigella* have led to the re-emergence of interest in phage therapy as a therapeutic strategy. Bacteriophage Sf6 is a *Shigella*-specific phage that infects serotype Y and X strains by targeting and hydrolysing the LPS O antigen via its tailspike protein (TSP). This study determined the impact of the various O antigen acetylations and glucosylation on *S. flexneri* 2457T ($2a_2$) LPS and their effect on pathogenesis and bacteriophage Sf6 sensitivity. We created a collection of isogenic mutants using *S. flexneri* 2457T as the parent strain and deleted O antigen modification genes in different combinations. Deletion of these genes converted serotype $2a_2$ to various 2a and Y sub-serotypes. These isogenic mutants were compared with respect to their LPS profiles, adherence and invasion efficiency on different cell lines, and sensitivity to Sf6 infection. We identified a novel serotype variant that was unexpectedly sensitive to Sf6 infection, suggesting that *Shigella* has acquired a specific O antigen modification gene that provides protection against this phage. In addition, we also provide the first molecular evidence that shows Sf6TSP is able to hydrolyse the LPS O antigen of serotype $2a_2$ and its sub-serotype in a dose-dependent manner.

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Investigation of a complex resistance module in the IncM plasmid pVS20

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Plasmids are extrachromosomal, self-replicating genetic elements which naturally exist in bacterial cells. They are the platforms on which various transposable elements are assembled and recombined. Additionally, plasmids are considered the key agents in the dissemination of various antimicrobial resistant genes through horizontal gene transfer. Hence, this contributes to the evolution of prokaryotes which enable them to survive in niches which were previously lethal. This was the case with members of the *Enterobacteriaceae* which were identified to be resistant to different classes of β-lactam antibiotics reported around the Mediterranean countries. Numerous studies have demonstrated that the origin of the worldwide spread of genes giving rise to carbapenems resistance were associated with the IncL and IncM plasmids.

Plasmid pVS20 (91 kb) was isolated from a clinical *Serratia marcescens* strain from a local Melbourne hospital. The plasmid is conjugative, can only replicate in members of the *Enterobacteriaceae* and confers multiple antimicrobial resistance. The complete DNA sequence of pVS20 and laboratory derived mutants was determined. Bioinformatic analysis of the sequence revealed that pVS20 is an IncM plasmid which features a backbone that is interrupted by a large (31.3 kb) nested transposable element located in the backbone gene *mucB*.

The nested structure denoted Tn*6607* is built on a Tn*1721* related platform and contains remnants transposon of Tn*1000-like*, Tn*5393c*, Tn*1*, IS*1,* Tn*1696* (incorporating the integron In4), and three copies of IS*26*. The loss of resistance traits observed with the pVS20 mutant appears to be due to the activity of the IS*26* elements, which render Tn*6607* inheritably unstable. Tn*6607* in pVS20 is closely related to a similar element in another IncM plasmid isolated from *Serratia marcescens*, R1215. We investigate the possibility of dissemination of the complex resistance region to other plasmids and to the chromosomes of strains unable to maintain pVS20.

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Study for Monitoring Antimicrobial Resistance Trends (SMART) in Australia and New Zealand (ANZ) in a Regional and Global Context

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Background: SMART has monitored global *in vitro* antimicrobial susceptibility patterns of clinical Gram-negative bacilli isolates since 2002. This poster presents recent ANZ susceptibility data for UTI and IAI, and ANZ resistance mechanisms within the Asia-Pacific (AP) and global context.

Methods: Sites collected up to 250 consecutive Gram-negative isolates. Susceptibilities were determined by broth microdilution and MICs interpreted by EUCAST criteria. Multiplex PCR screening to detect beta-lactamase genes.

Results: Species distribution for 2017 ANZ UTI and IAI isolates largely reflected AP species distribution. Locally, the top three pathogens in IAI (n=604) were *Escherichia coli* (48%), *Pseudomonas aeruginosa* (13%) and *Klebsiella pneumoniae* (11%) and in UTI (n=666) were *E. coli* (50%), *K. pneumoniae* (14%) and *P. aeruginosa* (11%).

Susceptibilities for ANZ UTI and IAI isolates (2016 - 2017 combined) were as follows: amongst 1180 *E. coli*, rank-order susceptibility was carbapenems and colistin (100%), amikacin (99%), ceftolozane/tazobactam (97%), piperacillin/tazobactam (92%); for 329 *K. pneumoniae*: amikacin, carbapenems and colistin (99%), ceftolozane/tazobactam (91%), cefepime and aztreonam (80%), and ceftazidime, ceftriaxone and piperacillin/tazobactam (79%); for 296 *P. aeruginosa*: colistin (100%), ceftolozane/tazobactam (99%), amikacin (98%), cefepime (93%), carbapenems (92%) and ceftazidime and piperacillin/tazobactam (90%).

Rates of ESBLs in ANZ *Enterobacterales* (2017, all infection sources) were among the lowest in the AP region being 11.5% in ANZ *E. coli* (n=729) vs 25.6% for AP *E. coli* (n=2516), and 15.2% in ANZ *K. pneumoniae* (n=257) vs 24.5% for AP *K. pneumoniae* (n=1696).

In 2017, ANZ carbapenemase rates (0.7%, n=1564) were second lowest in AP (2.5%, n=5667). The carbapenemases identified in ANZ *Enterobacterales* (n=13) isolates were IMP (62%), NDM (23%) and OXA-48 like (15%); NDM was the most frequently detected across AP (52%). IMP carbapenemases were most frequently detected in AP *Enterobacter cloacae*. Globally, KPC is the dominant carbapenemase detected in *Enterobacterales* in the Americas while OXA-48 like is dominant in Europe, Africa and Middle East.

Discussion: ESBL and carbapenemase rates in ANZ are among the lowest in Asia Pacific.

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A study of antimicrobial susceptibility pattern of *Bacteroides* species to amoxicillin-clavulanate, clindamycin and metronidazole from clinical isolates between 2015-2019 at Austin hospital

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OBJECTIVE:

Susceptibility testing is not routinely performed on anaerobes due to perceptions of predictable susceptibility, and lack access to reference methods. In recent years however, emerging resistance of anaerobes to antimicrobial agents have been noted, hence periodical monitoring of antimicrobial resistance patterns is important.

The study aimed to analyse the resistance patterns of *Bacteroides* spp from clinical specimens to amoxicillin-clavulanate, clindamycin and metronidazole.

METHOD:

A total of 61 clinical isolates were screened from sterile sites from 2015-2019. 93% of isolates analysed were from Blood cultures and 7% were from intra-abdominal and soft tissue infections.

An E-test was performed on all isolates of *Bacteroides* spp isolated from these specimens. The final MICs were read at 48 hours and were categorised as R, S or I using the CLSI breakpoints.

RESULTS:

57% of the isolates tested were *B. fragilis* sp, and the remainder 43% were other *Bacteroides* spp. Amoxicillin-clavulanate and metronidazole non susceptibility was noted in only 3 and 4 isolates respectively. Clindamycin non-susceptibility was most frequently detected, being present in 37% of isolates.

DISCUSSION:

This survey found an increased non-susceptibility to clindamycin when compared to previous published Australian data in 1992 and 2018 by Chen *et al.* and Hughes *et al*. In these surveys Clindamycin Susceptibility was noted at higher rate of 86-89% compared to 63% in this study. The overall non-susceptibility data of *Bacteroides* spp to amoxicillin-clavulanate and metronidazole was in concordance to previous studies. However direct comparison with the studies is difficult given the differences in methodology and clinical breakpoints used.

Based on this study, clindamycin should not be used for empiric therapy of anaerobic infection given the high rate of non-susceptibility. Amoxicillin-clavulanate and metronidazole remain suitable choices for empirical treatment, but routine testing should be considered for sterile sites to reduce treatment failure and decrease the morbidity rate of these infections.

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Rapid Screen System (RSS) for Mycobacteria via Digital Pathology

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Microscopic examination of clinical specimens is a critical initial screening tool for the laboratory detection of mycobacterial infections worldwide.

Microscopy using an auramine-rhodamine stained smear which may be followed by a Ziehl-Neelsen stain to confirm positives is a standard algorithm to screen for acid fast bacilli in specimens from patients suspected of infection with mycobacterium species.

This process can be a laborious task. Our aim was to automate this and increase the sensitivity of microscopy by developing a Rapid Screening System (RSS) which digitises a stained smear on a glass slide and performs automatic ranking on the smear regions.

Based on the ranking, the regions are sorted and displayed to the microscopist in descending order from the most likely regions with auramine-rhodamine stained bacilli present, to the least likely.

The RSS consists of three components: the scanning system; the analytic system that provides automatic region ranking and the smart viewer displaying the images captured by the scanning system that are ranked based on information from the analytic system.

In a preliminary study of 107 samples, this RSS had good negative predictive value (NPV) and significantly increased sensitivity, which are critically important for a primary screening test particularly when testing a low prevalence patient population. The developed RSS also reduces screening time for positives to only seconds. The RSS is designed to be an open system as it can be used to scan and process other stained slides such as Gram stained specimen preparations. The RSS system increases screening sensitivity and reduces labour thereby allowing better utilisation of laboratory staff.

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Performance Qualification of Bruker MALDI Biotyper® 4.0 Microbial Identification System within a Regulatory Compliance Testing Laboratory

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The Microbiology Section of the Therapeutic Goods Administration conducts risk-based compliance and investigative testing of therapeutic goods to verify microbiological quality with regulatory requirements. Identification of microorganisms presents challenges due to the variety of environmental organisms encountered, the slow-growing nature of some organisms and the potential for organisms to be sub-lethally damaged by exposure to sterilising or antimicrobial agents. To determine if an organism poses a risk to patient safety or product spoilage, it is vital that identification is correct with a high degree of confidence.

Matrix Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry (MALDI-TOF MS) systems are established as rapid and reliable identification systems in many fields of microbiology. Performance qualification (PQ) of a Bruker Maldi Biotyper® 4.0 system was conducted to investigate its suitability to identify with a high degree of confidence, bacteria likely to be encountered during testing of therapeutic goods.

PQ comprised four separate phases: specificity, reproducibility, robustness and accuracy. A total of 1474 data point identifications were analysed: 94.2% were correct to species level, 2.4% were correct to genus level only, 2.1% resulted in no identification, 1.1% resulted in a not reliable identification and 0.3% were identified incorrectly.

Results indicate reliable capability of the system to rapidly identify many of the PQ microorganisms to genus or species level, with known limitations evident for some organisms, e.g. some species of Bacillus and Streptococcus. Extension of PQ studies will further investigate suitability of the system for identification of other relevant organisms e.g. yeasts and probiotic bacteria.

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Preserving proteins during microbial dormancy speeds return to growth state

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All living organisms require nutrients to grow and reproduce. When nutrient quantity or quality is low, organisms reduce their growth rate and enter a dormant state characterized by arrested physiological activity and critical for cell survival. We now report that preserving proteins during dormancy speeds the return to a growth state. We establish that the bacterium *Salmonella enterica*reduces proteolysis by adenosine triphosphate (ATP)-dependent proteases by decreasing ATP amounts when starved for magnesium, carbon or nitrogen. In contrast, ATP reduction allows degradation of non-functional proteins to continue unimpeded thereby avoiding their potential toxic effects. The yeast *Saccharomyces cerevisiae* also reduces ATP amounts and ATP-dependent proteolysis when starved for nutrients. Drugs that increase ATP amounts delay entry into the growth state by promoting ATP-dependent proteolysis. Thus, the better the ability to preserve proteins during dormancy, the faster prokaryotes and eukaryotes exit the dormant state as soon as nutrients become available. Starvation-promoted protein longevity likely also plays a role in the germination of bacterial spores and in antibiotic persistence.

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Rapid detection and identification of common *Salmonella* serovars using target genes selected from comparative genomic analysis

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Salmonella enterica is a highly diverse species with more than 2600 serovars. The ability to distinguish serovars within the species is important for surveillance. With the uptake of whole-genome sequencing technology, serovar determination by traditional serotyping is being to be replaced by inference of serotypes from genome sequences. In this study, we aimed to identify new serovar-specific gene markers for the common *Salmonella* serovars and to validate the genomic signatures for accurate detection of five most common serovars in Australia.

A total of 2258 publicly available *Salmonella* genomes were selected to include serovars with at least 5 genomes available. The genomes were annotated using PROKKA. Pan-genome and core-genome were analyzed by Roary using an 80% sequence identity threshold. The genes specific to each serovar were identified from the pan-genome's accessory genes using an in-house python script with a cutoff of 20% false negatives and 10% false positives. Multiple Cross Displacement Amplification (MCDA) was employed to develop highly sensitive assay to detect the common serovars.

Comparative genomics of 2258 *Salmonella* accessory genomes identified a minimum of 131 serovar-specific gene markers to allow *in silico* typing of the 106 serovars with 95.1% accuracy. To evaluate the usefulness of gene markers for assay development, we selected seven genes which were specific to the five most common *Salmonella* serovars in Australia: Typhimurium, Enteritidis, Virchow, Saintpaul, and Infantis. Seven MCDA assays targeting these seven genes for rapid identification of the five *Salmonella* serovars were developed and evaluated. They demonstrated a sensitivity of 50 fg/ul (10 copies) on the pure DNA and were specific to the target serovars. The assays were also rapid with a result detectable within 8 minutes.

A new in silico serotyping method based on genomic data of the common Salmonella serovars is described. The gene markers identified in this study have also been used to develop a rapid, accurate and sensitive serotyping MCDA assay which opens the way for culture independent serotyping directly from clinical samples.

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Establishing mouse infection models with clinically relevant bacterial strains for examining Mucosal-associated invariant T (MAIT) cell immunity

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Bacterial infections are a global threat to human health and are particularly important in immuno-compromised individuals. Extensive research has been conducted to understand anti-bacterial immunity, often using mouse models of bacterial infections. However, clinical bacteria are often not well suited to infect laboratory animals, which makes it difficult to optimize an *in vivo* infection model.

MAIT cells are an abundant population of innate-like T cells. MAIT cells are activated by recognizing 5-(2-oxo-propylideneamino)-6-D-ribitylaminouracil (5-OP-RU), a small molecule antigen derived from vitamin B2 (riboflavin) biosynthesis, a shared and essential metabolic pathway for many microorganisms. The role of MAIT cells in control of bacterial infection is only beginning to be elucidated. Recent studies suggest they play a pivotal role in the early immune, either by killing infected cells directly or secreting cytokines. This has been demonstrated in mouse infection models of several bacteria, but most of these bacterial strains were not those seen in human patients.

In this study, we infected laboratory strains of mice with three clinically-important bacteria and investigated the MAIT cell response upon infection. The three bacteria, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*, are all clinically important pathogens bearing a riboflavin metabolic pathway and the strains used were isolated from hospitalized patients. Using a MAIT cell reporter assay we found that each of these bacteria could produce antigen to activate MAIT cells when cultured *in*

vitro. In the following *in vivo* study, we infected C57BL/6 mice with a sub-lethal dose of *E. coli* and observed an accumulation of MAIT cells at the site of infection. Moreover, MAIT cells were educated to specific functional phenotypes upon infection, as reflected by their secretion of cytokines. These results confirmed the possibility of establishing clinically-relevant bacterial infection models in mice. It is hoped that our study will contribute to filling the gap between bench and bedside.

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Discovery & preclinical development of tetrapeptide ETC-670 for treating MRSA skin infections and nasal decolonization.

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S. aureus is the primary pathogen responsible for skin infections. They also reside in nasal passages and can be transferred to surgical wounds by self-inoculation. Mupirocin is the first-line topical antibacterial agent used for treating skin infections and nasal decolonization. Its introduction since 1985 has led to the emergence of mupirocin-resistant *S. aureus*. Indeed, a survey by seven public-sector hospitals in Singapore revealed 31% of clinical isolates were mupirocin-resistant, highlighting the need for a new antibacterial agent. I shall present ETC-670, a tetrapeptide bacterial membrane disruptor currently in pre-clinical development in Singapore as a replacement for mupirocin.

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An evaluation study on Illumina's iSeq 100 sequencing platform using the two commonly performed bacterial analysis.

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Over the last decade, advances in next-generation sequencing (NGS) technology have resulted in the development of a variety of sequencing platforms to meet up the different user's needs. Among these platforms, Illumina's iSeq 100 has become an ideal choice for individual labs where only a certain number of throughput power is needed. In this study, we have evaluated the performance of the iSeq 100 on two most commonly sequenced bacterial analysis methods; namely, 16S rRNA based microbial taxonomic profiling (MTP) and whole genome sequencing (WGS). For MTP analysis, we have incorporated Illumina's newest 2 x 250 library kit for the iSeq 100 platform and applied it to four different samples (Soil, Stool, Fermented beans and Microbiome standards). Through the use of ChunLab's EzBioCloud database and rigorous bioinformatic pipelines, the comparison study with MiSeq data has shown high correlations in composition, alpha, and beta diversity. Likewise, the performance of bacterial WGS was measured using three different iSeq 100's library kits (1 x 150, 1 x 300 and 2 x 150) with *Escherichia Coli* K12. The generated sequence data were examined in TrueBac^{TM} ID, a fully automated genome identification system. All kits produced sufficient quality genomes that contain all the up-to-date bacterial core genes (UBCG), high N50 values, and high ANI value towards *E. coli* genome. The evaluation conducted here shows promising results in the two most commonly performed microbial analysis, MTP and WGS, using the iSeq 100 platform.

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The Australian Microbiome – a collaborative network characterizing Australia's microbial diversity

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NOTE: Author IS Australian Microbiome Consortium

The Australian Microbiome (AM) initiative is a collaborative network of researchers that aims to provide publically available data characterizing Australia's environmental microbial diversity. The initiative is primarily administered and supported by CSIRO, Director of National Parks through Bush Blitz, Bioplatforms Australia and the Integrated Marine Observing System (IMOS). The AM produces both amplicon sequences (bacteria, archaea, eukaryote and fungal targets) and shotgun metagenomes, primarily from soil and marine environments. The AM currently comprises >2.5x10^{9}amplicon sequences, from 7723samples and >42x10^{9} metagenomic sequences from 962 samples. Every sample is accompanied by rich sample specific metadata describing its physical and chemical composition, as well as its geolocation and regional descriptors. Methods used to collect samples, perform wet lab analyses and downstream bio-informatic analyses are standardised. Sequence and sample specific metadata is available via the AM data portal (https://data.bioplatforms.com/organization/about/australian-microbiome), as both raw sequences data and curated sequence abundance tables, sequence data is also submitted to international nucleotide archives. Data produced by the AM is also available for direct export to and analysis by the Galaxy Australia server.

AM was formed by aligning the previous Marine Metagenome (MM) and Biomes of Australian Soil Environments (BASE) projects into a single Australian microbial diversity resource. Soil data comprise samples from two depths per site, covering the breadth of Australian soil heterogeneity, as determined by soil chemical and physical maps and overlying plant community data. Soil samples also include limited samples from Antarctica. Marine data also covers depth profiles and includes relatively high temporal resolution time series data from IMOS national reference stations, as well as both pelagic and near shore samples from around the Australian coast line. Near shore samples also include both sediments and symbiont microbiomes.

The AM is currently expanding to include greater temporal and spatial coverage with an expected 3500 amplicon samples sequenced and 400 metagenomes in the next 3 years. AM will also continue to improve secondary data types and accessibility.

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Panning for Gold in Mould: Genomics-guided discovery of bioactive molecules from fungi

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Fungi are a rich source of bioactive small molecules. However, the large number of secondary metabolite (SM) biosynthetic gene clusters (BGCs) encoding these molecules in their genomes suggests their biosynthetic potential is far greater than we previously appreciated. The mining of fungal genomes therefore holds great promise for the discovery of new chemical entities for pharmaceutical and agricultural applications. As more and more fungal genomes become available, the accompanying number of BGCs is quickly becoming unmanageable. Along with improving molecular genetic tools to accelerate the translation of BGCs to small molecules, we must devise strategies to prioritise BGCs most likely to encode the biosynthesis of novel small molecules and molecules with new or improved bioactivities or functions. Here, we will discuss our strategies and successful examples of using genomics and synthetic biology to facilitate the discovery of bioactive compounds. This includes the discovery of phytotoxic compounds from plant pathogens and antimicrobial compounds.

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Including microbial eukaryotes in metagenomic surveys with CCMetagen

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Microbial eukaryotes are diverse, ubiquitous, and play important functional roles in environmental and host-associated microbial communities. However, micro-eukaryotes are far less studied than their bacterial counterparts, due in part to methodological challenges. Several methods are available to identify taxa directly from metagenome data, but their species-level identification tends to be highly inaccurate or relies heavily on reference databases of complete genomes, which are particularly scarce for microbial eukaryotes. We used a novel concept in read mapping to develop CCMetagen – a metagenome classifier that is highly accurate and fast enough to use the entire NCBI nucleotide collection as reference, facilitating the inclusion of microbial eukaryotes in metagenomic studies. High accuracy is achieved by assessing all read-mapping possibilities, rather than attempting to classify individual reads. Using simulated fungal and bacterial metagenomes, we found that species-level identifications obtained with CCMetagen are between 17x and 1580x more precise than other commonly used metagenome classifiers. We applied CCMetagen to characterize the gut microbiome of wild birds using RNA-based metagenomic data (metatranscriptomics). Besides prokaryotes, we found an abundant and diverse community of micro-eukaryotes, with fungal taxa composing 50% of the family-level diversity of the bird microbiome. Our work opens possibilities to confidently include microbial eukaryotes in studies seeking ecological and evolutionary insights from metagenomes.

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Geomicrobiology and mining

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In Australia, the search for new ore is focused under cover. In these deeply weathered terrains, geochemical and mineralogical dispersion halos surrounding the emplaced ore should contain biological targets for exploration, even with 'inert' materials, e.g., the biogeochemical cycling of gold that can produce a variety of materials (colloidal and octahedral gold). Regarding metal extraction, microbiology has important environmental consequences in the generation of acid mine (rock) drainage, damaging 10's of thousands of km of streams globally and is important in bioleaching, producing 15% of the world's copper. The role of geomicrobiology should be further exploited in bioleaching of low-grade ore or mine tailings, further reducing the environmental impacts of mining. These soluble metals can be subsequently recovered via the application of acidophilic sulfate reducing bacterial bioreactors precipitating metal sulphides, integrating mine water remediation with selective biomineralisation. With respect to remediation, we are looking at iron ore waste stabilisation and mineral carbonation. Supergene enriched iron ore deposits are typically protected by a goethite-cemented ferruginous duricrust layer (canga). In these deposits, the canga forms extensive deposits blanketing ancient erosion surfaces, is tough, moderately hard, well consolidated, permeable and very resistant to erosion and chemical weathering, protecting the relatively soft enriched iron ore below. This protective canga horizon is therefore, essential to supergene iron ore enrichment and formation of high-grade iron ore. Active, biogeochemical iron cycling is essential for the 'self' healing cementation/re-cementation occurring in canga, which should be exploited for the remediation of iron mine sites, post-mining. Adding value to mine waste, biogenic magnesium carbonate mineral precipitation from fine-grained Mg-rich tailings generated by mining operations could potentially offset net mining greenhouse gas emissions. As a proof of concept, cyanobacteria in a wetland bioreactor enabled the precipitation of magnesite ($MgCO_3$), hydromagnesite [$Mg_5(CO_3)_4(OH)_2 \cdot 4H_2O$], and dypingite [$Mg_5(CO_3)_4(OH)_2 \cdot 5H_2O$] from acid leached ultramafic mine tailings.

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Microbial interactions with petroleum-based plastics and bioplastic pollutants in aquatic environments

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Not available

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Using Antarctic soil bacterial communities to monitor, remediate and develop guidelines for diesel fuel contamination in polar soils.

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Publish consent withheld

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Effect of bio-stimulation on the distribution and composition of the microbial community of a polycyclic aromatic hydrocarbon-contaminated landfill soil during bioremediation

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Dumping wastes generated from oil industry to the local waste disposal facilities remains the most common means of oil waste management. In industrial landfills, the most serious ecological problem is the contamination of soil and local groundwater by landfill leachate. Polycyclic aromatic hydrocarbons represent hazardous group of harmful chemical compounds that threaten human and ecosystem health. This study focuses on a PAH-contaminated soil from a landfill site in New South Wales, Australia. Soil was exposed to two different bioremediation treatments, natural attenuation and biostimulation using pea straw. The bacterial community composition and diversity of the PAH-contaminated soil were also examined using high throughput Illumina MiSeq sequencing. The results revealed that PAHs were degraded naturally by indigenous microorganisms; however, the addition of plant residues led to enhanced degradation (66.6%) at the beginning of the treatment, although in all treatments a degradation plateau occurred between 43 and 102 days of incubation during bioremediation. Quantitative PCR analysis showed an increase in the number of 16S rRNA and ITS gene copies as well as genes associated with Gram-positive PAH-degrading bacteria in the soil amended with pea straw. Next generation sequencing results and diversity indices revealed that a high proportion of the sequences from all soil samples belonged to the *Proteobacteria* phyla; gammaprotobacteria was the dominant class in all the investigated samples. *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Cyanobacteria* were the most dominant in the natural attenuation and pea straw treated soil bacterial communities. The largest shift in bacterial communities was in the pea straw amended soils with increased abundances by Day 42 of *Pseudomonas*, *Pseudoxanthomonas*, *Lewinella and Parvibaculum; these organisms are all known for their abilities to degrade petroleum hydrocarbons. However, by Day 102, these organisms were decreased or not detected in both NA and PS treated soil samples at the end of incubation which may explain the degradation plateau. These findings suggest that Gram-negative PAH-degrading bacteria, mainly Pseudomonas sp., may be the key contributor to PAH-degradation in the landfill-polluted soil.*

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Approaches to quantifying antimicrobial use in Australian dogs and cats

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Antimicrobial use drives the development of antimicrobial resistance. With resistant bacteria able to transfer between humans and animals, veterinary antimicrobial use is coming under increased scrutiny. Much of the focus so far on veterinary antimicrobial use has been in food animals, but attention is shifting to use in other species, particularly those that have close contact with humans, such as dogs and cats. Methods of measuring antimicrobial usage in small animal veterinary practice have been largely reliant on surveys or analysis of clinical records. Surveys provide information on self-reporting of prescribing intentions in hypothetical clinical scenarios, but may not accurately reflect actual usage. In contrast, analysis of veterinary medical records provides valuable data on actual usage. However, with relevant details largely in free text fields, analysis of electronic records has been performed manually, thus limiting the number of records that can be assessed. Additionally, assessing antimicrobial usage in cohorts restricted to those attending veterinary practices, does not assess the rate of antimicrobial prescribing in a population of animals independent of their need for veterinary attention. This may overestimate usage per animal if extrapolated to the wider population.

To overcome these limitations, analysis of pet insurance claim data provided an opportunity to investigate the exposure of companion animals to antimicrobials at a population level, independent of their need for veterinary intervention. This demonstrated exposure to antimicrobials in this cohort of animals was much lower than community antimicrobial use in humans in Australia.

Additionally, access to big data via VetCompass (181 Australian veterinary clinics contributing medical records) and the use of natural language processing to analyse unstructured free text in over 3 million records is enabling characterisation of veterinary antimicrobial usage patterns on a larger scale.

These data sets provide a unique opportunity to establish baseline antimicrobial usage data in dogs and cats, identify targets for antimicrobial stewardship interventions and monitor the impact of these interventions on veterinary antimicrobial prescribing in Australia.

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Not available

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Not available

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Use of antimicrobials in a veterinary teaching hospital

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Veterinary training for antimicrobial stewardship is critical to promote responsible use of antimicrobials and to prevent the development of antimicrobial resistance. Veterinary students receive this training in the preclinical and clinical curriculum. The aim of this presentation was to investigate antimicrobial stewardship in the clinical curriculum. Clinical data was investigated to determine the antimicrobials prescribed for various conditions. Antimicrobials prescribed for the treatment of canine allergic dermatitis and dog-to-dog bite wounds were further explored.

In 2018, there were 349 prescriptions for cats, 435 for avian, exotic or wildlife species, 610 for cattle, 1,465 for horses and 2,146 for dogs. In cats, the most commonly prescribed antimicrobials were amoxicillin clavulanic acid (AMC) (39%), ceftiofur (15%) and first generation cephalosporins (8%). Ceftiofur was prescribed most commonly to treat abscesses or wounds. In cattle, tetracycline (41%) was the most common antimicrobial prescribed, followed by ceftiofur (14%), macrolides (8%) and penicillin (6%). Ceftiofur was most commonly prescribed to treat septic calves and respiratory disease. In horses, penicillin (34%), aminoglycosides (35%) and trimethoprim sulphonamides (11%) were most frequently prescribed. Imipenem was used once for septic arthritis. Ceftiofur (3%) was most commonly used to treat sepsis and colic. Fluoroquinolones (3%) for orthopedics, colic and respiratory disease. In dogs, the most commonly prescribed drugs included AMC (36%), first generation cephalosporins (21%), metronidazole (5%) and fluoroquinolones (5%). Ceftiofur (0.5%) was prescribed most commonly for wound infections. Meropenem was prescribed twice to one dog that had a cholecystoduodenostomy. Few cultures were performed with the diagnostic laboratory only receiving 629 samples.

The antimicrobials used in dogs and cats at the veterinary teaching hospital were similar to other Australian studies. The use of critically important drugs was low, though a high use of ceftiofur was noted in cattle and cats. Veterinary teaching hospitals often receive referral cases and this may account for the use of critically important antimicrobials. In the majority of cases antimicrobials were prescribed in accordance with best practice.

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Transmission of critically important antimicrobial resistant E. coli between Silver Gulls, Feral Pigeons and Little Penguins occupying different ecological niches within an urban environment

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Carriage of critically important antimicrobial (CIAs) resistant pathogenic bacteria by wild animals is of concern. High-level FQ and ESC-resistant and low frequency of carbapenem and colistin resistant *E. coli* carriage by Australian Silver Gulls was reported in our previous study. We hypothesise that carriage of CIA-resistant *E. coli* among Silver Gulls leads to transfer of resistant bacteria to other bird species sharing the same environment. Three bird species foraging from a central place (Penguin Island, Perth, WA) occupying different foraging niches were included in this study to characterize and assess the potential source and determine the possible pathways of transmission of CIA-resistant *E. coli*.

Methods: Cloacal swabs from three bird species were cultured on selective media and presumptive *E.* coli isolate were subjected to identification by MALDI-TOF MS, antimicrobial susceptibility testing and a subset to next generation sequencing (n=55) on Illumina NextSeq 500 platform.

Results: CIA resistant *E. coli* carriage were 53%, 10% and 11% among Silver Gulls,

feral pigeons and Little Penguins respectively. Predominant STs were ST131, ST 69, ST 10, ST450 and ST695 amongst *E. coli* from all three species. *E. coli* ST1598 and ST 95 were detected in Penguins. The resistance genes detected were $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}14}$ in *E. coli* from all three species. The other resistance genes detected only amongst isolates from seagulls included $bla_{CMY\text{-}2}$ (3%) and one isolate each carrying $bla_{CTX\text{-}M\text{-}3}$ and $bla_{CTX\text{-}M\text{-}45}$.

Conclusion: This study reports the carriage of CIA resistant *E. coli* by all three bird species. The presence of CIA resistant *E. coli* in the cloacal swabs and the sharing of identical human associated pathogenic *E. coli* clones like ST131, ST69, ST95 and ST10 between the bird species indicates inter-species transmission. Penguin Island is a popular tourist destination and has high visitor numbers up to 80 000 engaged in coastal recreational activities. Contamination of such an environment with clinically significant CIA resistant *E. coli* warrants further investigations to determine the source of faecal contamination.

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Emerging and well-known chlamydial infections in Australian domesticated and wildlife animals: On the heels of infection spill-over

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In Australia, the most successful chlamydial species are the infamous koala (and sheep) pathogen *Chlamydia pecorum*, and *Chlamydia psittaci,* an emerging pathogen with zoonotic potential. Little is known about the exact host range for these pathogens, nor about infections caused by other chlamydial organisms in Australian livestock or wildlife. Considering that chlamydial organisms can be encountered by humans at the animal/human interface, or native wildlife at the domesticated animal/wildlife interface, we investigated molecular epidemiology of chlamydial organisms infecting Australian domesticated and wild animals.

We screened a catalogue of > 400 samples taken from domesticated sheep, cattle, pigs, and horses, and wild deer and range of wild psittacine and non-psittacine birds using pan-*Chlamydiales* as well as species-specific assays. Whole genome sequencing was performed on a subset of *C. pecorum* and *C. psittaci* positive samples from several livestock and bird hosts to evaluate genetic diversity of infecting strains.

Overall, chlamydial DNA was detected in >60% samples, including all domesticated and wildlife hosts. Sequence analyses revealed that genetically diverse novel, as well as traditional, chlamydial organisms infect an expanded range of Australian animals. Not surprisingly, we have detected new hosts for the infamous *C. pecorum* and *C. psittaci*, raising new questions regarding reservoirs for these pathogens, in addition to concerns about risks of spill-over between livestock, humans, and native wildlife[1].

Whole genome molecular typing revealed that the *C. psittaci* strains from Australian livestock and birds are diverse, however mainly cluster in two clades: the globally distributed, highly virulent parrot ST24 clade; and pigeon-associated ST35 clade, again indicative of spill-over of *C. psittaci* infections from birds. In contrast, livestock *C. pecorum* strains clustered into a clonal ST23 clade, a genotype globally associated with sheep polyarthritis and cattle encephalomyelitis, but genetically distinct from koala strains.

Future research must expand on these molecular findings to understand the epidemiology as well as the zoonotic potential posed in association with these new reservoirs and new chlamydial infections.

1. Jelocnik, M, Taylor-Brown, A, O'Dea, C, et al. Detection of a range of genetically diverse chlamydiae in Australian domesticated and wild ungulates. Transbound Emerg Dis. 2019; 00: 1– 6. https://doi.org/10.1111/tbed.13171

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Characterising novel metabolic pathways in *Coxiella burnetii*, the causative agent of the zoonotic disease Q fever

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The zoonotic pathogen *Coxiella burnetii* poses a serious threat to global public health. *C. burnetii* replicates intracellularly within a unique vacuole derived from the phagolysosome, known as the *Coxiella*-containing vacuole (CCV). To cause disease, *C. burnetii* must not only survive the bactericidal environment within this vacuole, but also obtain energy and nutrients to replicate. Investigating the metabolic pathways required by *C. burnetii* to survive inside host cells may identify novel therapeutic targets. Recent stable isotope labelling studies revealed that *C. burnetii* is capable of synthesising lactate, despite the apparent absence of a genetic pathway for lactate production. In this study we are investigating two potential lactate-producing pathways. Malolactic enzymes, found in lactic acid bacteria, convert malate to lactate. Our bioinformatic analysis revealed that Cbu823, currently annotated as a NAD-dependent malic enzyme, possesses 43% identity with the malolactic enzyme of the lactic acid

bacterium *Oenococcus oeni*. As *C. burnetii* already possesses a putative malate dehydrogenase, MDH (Cbu1241), Cbu0823 may function as a malolactic enzyme. Alternatively, Cbu1241 may perform a dual function and possess MDH and LDH activity. We have successfully expressed and purified recombinant Cbu0823 and Cbu1241 as 6xHis N-terminal fusion and GST N-terminal fusion proteins respectively. In vitro enzyme assays demonstrated that Cbu1241 possesses MDH activity but lacks LDH activity, at least in vitro, and that Cbu0823 has malic enzyme activity. Current work is examining the malolactic enzyme activity of Cbu0823. Future work will examine the role of this pathway in *C. burnetii* intracellular replication.

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Metabolic control of host-pathogen interactions in infection

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It has recently become clear that metabolic shifts underpin immune response to infection, giving birth to the field of "immunometabolism". To date, the focus has largely been on host metabolic pathways. However, microbes also adjust their metabolism, to survive and thrive during infection. We are studying the nexus of host and pathogen metabolism, to understand how it impacts on host responses and immune evasion by pathogens to ultimately control infections. Our experimental system is *Candida albicans*, a yeast that can cause systemic infections with high mortality. *Candida* mounts a counter-attack against innate immune phagocytes through sophisticated pathways that involve a developmental switch from yeast to hyphal morphology, and metabolic competition for an essential nutrient. Our recent work has illuminated how the interaction of host and pathogen glucose metabolism controls macrophage viability and immune responses to *C. albicans*. We have further shown that mdivi-1, a putative inhibitor of mitochondrial division, represses hyphal development of *C. albicans* through a novel mechanism. Treatment with mdivi-1 delays macrophage cell death in response to *C. albicans*challenge, and reduces inflammatory responses.

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Title not available

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Not available

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Heterogenous cell types correlate with disease manifestation in the yeast pathogen *Cryptococcus*

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Cryptococcus is normally described as a spherical encapsulated yeast of ~7 µm diameter, however under certain conditions cells can display considerable heterogeneity in size and shape. Giant cells >15 µm, micro cells < 2 µm, irregularly shaped cells, and cells with enlarged or shed capsule have been described *in vivo* and *in vitro*. There is conflicting data on whether these phenotypes enhance or reduce virulence and disease progression. This study examined the presence of morphological variants in two *Cryptococcus* collections: clinical isolates obtained from from HIV-AIDS patients in Botswana, and a set of strains derived over many years from the *C. neoformans* reference strain H99. Using conditions designed to simulate host infection, we assessed strains for the presence of cell morphotypes and correlated their presence with clinical and virulence data. Giant cells were on a spectrum of increasingly larger cells, while micro cells appeared to be an independent cell type and were strongly associated with the presence of extracellular capsule. In the Botswana clinical collection, the presence of larger cells and capsules was positively correlated with higher $CD4^+$ T-cell count and negatively correlated with indicators of intracranial pressure, while micro cells and shed capsule had opposite associations, and in addition were negatively associated with indicators of cerebral inflammation. This suggests that larger cell types are more likely to occur in early stage infection when $CD4^+$ count is still high and intracranial pressure is relatively low, with a transitions to smaller cell phenotypes as disease progresses, when micro cells and shed capsule may also act to dampen the host immune response. These findings were corroborated by the H99 derivative set, where strains that have been characterised as more virulent were the most abundant producers of micro cells and shed capsule. Taken together our analyses suggest that cell plasticity is important for virulence, can alter over the course of infection and is likely to be epigenetically determined.

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Beyond *Diptera*: exploring *Wolbachia*-pathogen interactions in two *Lepidoptera* cell lines

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The endosymbiotic bacteria *Wolbachia pipientis* manipulates the reproductive success in infected arthropods through cytoplasmic-incompatibility and also has been demonstrated to induce RNA-virus refractoriness in the dipteran models; *Drosophila melanogaster* and the yellow fever mosquito *Aedes aegypti*. While the molecular mechanism of the cytoplasmic incompatibility has been solved questions still remain about the *Wolbachia* mediated virus-restriction phenotype and how extensive this phenomenon may be within other arthropods. To explore this we set out to examine broad patterns of *Wolbachia*-mediated virus interference in *Lepidoptera* cells. To achieve this we generated four lepidopteran cell lines stably transinfected with two *Wolbachia* supergroup A and B strains for the diamondback moth *Plutella xylostella* (Px.wAlbB and Px.wMelPop-CLA) and *Spodoptera frugiperda* cells (Sf9.wAlbB and Sf9.wMelPop-CLA). Production of the cell lines has been successful with stable *Wolbachia* infection now for over forty passages and with the next stage to challenge cell lines with a diverse range of viral species: a dsDNA baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV) a negative sense ssRNA virus Spodoptera frugiperda rhabdovirus (Sf-RV) (Family: *Rhabdoviridae*) and also Flock House Virus (FHV) (family: *Nodaviridae*). Preliminary virus inoculation trials with Sf9 cells suggests that *Wolbachia* infection has no effect on AcMNPV and Sf-RV while FHV trials are ongoing. This work improves our understanding of *Wolbachia*-mediated pathogen interference and may provide potential bio-control strategies for virus infections of agriculturally beneficial arthropods

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Life in the recycling bin: understanding nutrient acquisition and utilisation in *Coxiella burnetii*

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Coxiella burnetii is a Gram-negative bacterium which causes Q fever, a complex and life-threatening infection with both acute and chronic presentations. *C. burnetii* replicates within a unique vacuole derived from the host cell lysosome and little is known about the utilisation of carbon sources by *C. burnetii* within this compartment. Here we used ^{13}C-stable isotope labelling to investigate central carbon metabolism of axenically and intracellularly cultivated *C. burnetii* bacterium, at both log and stationary phases of growth. Important differences in utilisation of the ^{13}C-glucose and ^{13}C-glutamate substrates across the two growth conditions, and at the two different growth stages, were observed. Both bacterial populations were capable of using ^{13}C-glucose and ^{13}C-glutamate as carbon sources, with observed labelling of intermediates in glycolysis and gluconeogenesis, respectively, and in the TCA cycle. This demonstrates that *C. burnetii* maintains metabolic flexibility even within the unique intracellular replicative niche. Characterisation of the putative *C. burnetii* glucose transporter, CBU0265, led to a significant decrease in ^{13}C-glucose utilisation, but did not abolish glucose usage, suggesting that *C. burnetii* express additional hexose transporters. Intracellular infection of human cells and *in vivo* studies in the insect model showed that loss of CBU0265 had no impact on intracellular replication or virulence. These analyses demonstrate that *C. burnetii* are able to use multiple carbon sources *in vivo* and contribute important understanding of how this unique pathogen replicates within the harsh lysosome-derived vacuole.

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Host adaptation and convergent evolution increases antibiotic resistance without loss of virulence in a major human pathogen

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As human population density and antibiotic exposure increase, specialised bacterial subtypes have begun to emerge. Arising among species that are common commensals and infrequent pathogens, antibiotic-resistant 'high-risk clones' have evolved to better survive in the modern human. *Klebsiella pneumoniae* is a Gram-negative enteric bacterium and a significant cause of human disease. It is a frequent agent of pneumonia, and systemic infections can have high mortality rates (60%). OmpK35 and OmpK36 are the major co-regulated outer membrane porins of *K. pneumoniae*. OmpK36 absence has been related to antibiotic resistance but also decreased bacterial fitness and diminished virulence. A mutation that constricts the porin channel (Gly134Asp135 duplication in loop 3 of the porin, OmpK36GD) has been previously observed and suggested as a solution to the fitness cost imposed by loss of OmpK36. In the present study we constructed isogenic mutants to verify this and test the impact of these porin changes on antimicrobial resistance, fitness and virulence. Here, we show that the major matrix porin (OmpK35) of *K. pneumoniae* is not required in the mammalian host for colonisation, pathogenesis, nor for antibiotic resistance, and that it is commonly absent in pathogenic isolates. This is found in association with, but apparently independent of, a highly specific change (OmpK36GD) in the co-regulated partner porin, the osmoporin (OmpK36), which provides enhanced antibiotic resistance without significant loss of fitness in the mammalian host. These features are common in well-described 'high-risk clones' of *K. pneumoniae*, as well as in unrelated members of this species and similar adaptations are found in other members of the Enterobacteriaceae that share this lifestyle. Available sequence data indicate evolutionary convergence, with implications for the spread of lethal antibiotic-resistant pathogens in humans. Our data provide evidence of specific variations in OmpK36 and the absence of OmpK35 in *K. pneumoniae* clinical isolates that are examples of successful adaptation to human

colonization/infection and antibiotic pressure, and are features of a fundamental evolutionary shift in this important human pathogen. (PlosPathogens, 2019)

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Palaeomicrobiology: prospects and pitfalls

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Palaeomicrobiology—the study of past microorganisms—is a rapidly emerging field of research. The discovery that ancient microbial DNA can be preserved within archaeological dental calculus has opened the door to investigating changes to dental microbiota (and individual microbial genomes) throughout human history. In this talk I will introduce this nascent field, highlighting the prospects, pitfalls, and challenges remaining. I will share the latest methodological advances, and present new findings relating to changes of dental microbiota and genomes through time.

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Microbiomes associated with oral cancer: Passengers turned drivers.

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Activity is rapidly increasing in this field. Critical considerations/caveats include: association does not prove cause and effect; composition is significantly different by sample type [saliva, surface swab, tissue within the neoplasm]; nature of control sample [contralateral mucosa, matched subject with benign mucosal lesion; matched healthy subject – with/without tobacco/other risk factors]; fungi, bacteria, archaea, viruses are all relevant; stage is important [do oral potentially malignant disorders harbour an oncogenic flora?, are associated organisms of primary importance or only secondary invaders?]; laboratory techniques are critical; 16s or shotgun sequencing; contamination is a significant confounder. The functional metabolome of the microbiota is what matters.

Candida species are heavily involved, as well as bacteria capable of metabolizing alcohol to acetaldehyde, which adducts DNA. High-risk Human Papillomaviruses drive the majority of squamous cell carcinomas of the oropharynx and a minority of oral cavity SCC. No consistent small group of bacteria emerge: phyla Firmicutes, Bacteroides and Spirochaetes are enriched in our Indian cases, and at species level Fusobacterium nucleatum and Porphyromonas gingivalis are abundant in several studies: these are described in other gastrointestinal cancers and are oncogenic *in vitro*. These are keystone pathogens of destructive periodontitis, and there is strong epidemiological evidence of association between severity of periodontal disease and risk of oral cancer.

What is consistent is the function of the microbiota: most consortia described are pro-inflammatory. We propose < https://link.springer.com/article/10.1007/s40496-019-0215-5> a “passenger turned driver” scenario whereby organisms initially colonise cancer tissues, including the deep invading front, and consortia are selected by the anerobic and acid environment of the tissues. As they flourish, their metabolic products are strongly pro-inflammatory, favouring tumour progression.

Maintaining a health-associated oral microbiome [by sensible diet and reasonable oral hygiene], and treatment of periodontal disease, should contribute to risk mitigation. Might probiotics have a role? Might antibiotics be a treatment component? As always, avoidance of tobacco and areca nut, mimimal alcohol intake, and diets rich in antioxidant macro- and micro-nutrients are the mainstay of prevention.

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Development of an *in vitro* biofilm model of the supragingival plaque microbiome

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Despite the emergence of various monoculture and multispecies *in vitro* systems, current protocols inadequately mimic the bacterial diversity of oral biofilms which may contain up to 700 bacterial taxa, with many species yet to be identified.

The primary aim of this study was to establish an *in vitro* biofilm model of the supra-gingival oral microbiome which reflects biofilm diversity. The establishment of a successful model will allow the development of anti-microbial/biofilm agents that selectively target bacteria associated with oral diseases including caries and periodontal disease.

Supra-gingival plaque samples (n=6) were individually inoculated into polypropylene 3D printed flow cells and cultured on hydroxyapatite (HA) discs for 14 days in Artificial Saliva Medium (ASM) and a new-modified medium (SHI). New generation sequencing (NGS) was used to analyse the microbiome at plaque collection and after 14 days growth.

Confocal microscope and scanning electron microscope imaging demonstrated the successful development of plaque biofilms over 14 days in ASM and SHI. Biofilm thickness, bio-volume and viability showed no significant difference at 14 days for either culture media ($p>0.05$). NGS demonstrated a significant decrease in bacterial composition and diversity between the original inoculum and 14 day biofilms when using ASM ($p<0.001$). However, this alteration was limited to a common phylum, *Actinobacteria* (PERMANOVA test: 25.34; $p<0.01$). Five domain phyla and over 26 genera were maintained in 14-day biofilms grown in ASM, representing 65% of the original inoculum. LIVE/DEAD™ BacLight™ staining revealed that the established biofilm consisted of an average of 73% live and 27% dead bacteria across experiments regardless of which growth media was used.

The study demonstrated the capability to grow oral biofilms from supragingival plaque with a highly complex and diverse range of bacterial species in 3D-printed flow cells over 14 days using ASM or SHI.

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Identification and removal of contaminating microbial DNA from PCR reagents

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Background: Over the past decade microbiome studies have increased in popularity, due to the increasingly user-friendly workflows and affordability of 16S rRNA gene sequencing. However, in recent years, it has become apparent that many studies are plagued by entrenched methodological errors, resulting in the production of large amounts of erroneous data which have been spuriously interpreted. One of the major problems facing microbiome research is reagent-derived contamination, which can compromise the integrity of microbiome data, particularly in low-biomass samples.

Method: Using a commercially available dsDNase treatment protocol we have decontaminated our PCR master mix to assess the extent to which our DNA extraction kit and PCR master mix introduce contamination to bacterial DNA profiles generated by 16S rRNA gene sequencing. Four blank extraction controls (ECs; extracted in separate batches) and two blank PCR controls (NTCs) were processed with and without decontaminated PCR master mix. The resultant bacterial DNA levels/profiles were assessed by qPCR, endpoint PCR, and 16S rRNA gene sequencing.

Results: The vast majority of contamination in our workflow was derived from our PCR master mix. Decontaminated NTCs appeared to be true negatives upon qPCR and endpoint PCR screening. ECs amplified using decontaminated master mix appeared an average of 4.4 cycles later than those amplified without decontaminated master mix by qPCR, and were undetectable by endpoint PCR. Importantly, dsDNase treatment resulted in a 99% reduction in the number of contaminating bacterial sequence reads following Ion Torrent 16S rRNA gene sequencing.

Conclusions: We have identified the PCR master mix as the primary source of contamination in our workflow, and shown that enzymatic removal of this contamination drastically reduced the blank signal and improved precision. Decontamination of PCR master mixes may have the potential to improve the sensitivity and accuracy of low-biomass microbiome studies.

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Multidrug resistance in tuberculosis and nontuberculous mycobacteria: a slow selective process in progress in One-Health/One-World

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Worldwide, tuberculosis (TB) epidemic is characterized by a global decrease of incidence of antibiotic susceptible forms and an alarming onset of multidrug resistant (MDR) *Mycobacterium tuberculosis* strains, resistant to isoniazid and rifampicin. In these cases, and especially in cases of extensively drug resistant tuberculosis (XDR-TB), where the bacillus is also resistant to second-line antibiotics, fluoroquinolones and second-line injectables, there is an urgent need to stop the rise of resistance to the small therapeutic armamentarium available to fight tuberculosis. Nowadays, molecular tools are crucial to control and prevent tuberculosis and in particular of its resistant variants. Taking advantage of worldwide association genomic studies was possible to demonstrate the global evolution and dissemination of the different *M. tuberculosis* lineages, their association with latency, disease severity and ability to become drug resistant: old lineages are associated with latent tuberculosis and modern lineages are associated with active disease, fast transmission and drug resistance. Furthermore, the modern lineages are genetically better equipped to counteract the host response to the infection and become XDR through the acquisition of mutational resistance in antibiotic target genes associated with the overexpression of efflux pumps that overall, promote high-levels of resistance. The more we combat these strains with the standard diagnostic and therapeutic protocols, without knowing their genetic background and physiological response, the more they acquire resistance and limit patient clinical outcome. Drug-sensitive tuberculosis is easily curable...drug-resistant is much more complicated!!! The same evolutionary process of selection of resistance is now seen in nontuberculous mycobacteria (NTM), such as *M. avium* and M. *abscessus* that are becoming even more virulent, drug resistant and life threatening.

The inclusion of molecular tools in the mycobacteriological classic diagnosis algorithm allows early detection with clinical relevance, essential for a timely response and effective treatment of TB and NTM. Only through a complete interconnection between molecular laboratory data and clinical action can one avoid the development and transmission of resistant strains caused by inadequate therapeutic options.

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Viral infections in the ageing: a major cause of morbidity and a challenge for vaccine development

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Ageing results in increased susceptibility to several bacterial and viral pathogens, eg Influenza, Respiratory Syncytial Virus (RSV), Cytomegalovirus (CMV) and Herpes zoster, owing to Immuno-senescence. This is an ageing related decline in all immune

response modalities leading to increased incidence and severity of these infections. Vaccine responses to these important viral diseases are also impaired. Although immunization is one of the most effective measures for disease control, until recently we lacked vaccines for influenza and Herpes zoster with the same efficacy in this age group as paediatric vaccines (>90%) and indeed any for RSV and CMV. However there have been recent major advances in vaccine development include identification, design and production of antigens, adjuvants and mode of delivery. The new recombinant Herpes zoster vaccine is an excellent example. The combination of the most appropriate and immunodominant recombinant varicella zoster virus antigen, glycoprotein, E combined with the adjuvant system AS01B, consisting of MPL (from bacterial cell wall) and QS21 (from tree bark), resulted in 90% efficacy, even in those over 80 years. This remarkable efficacy is highly adjuvant dependent. In mice the adjuvant stimulates a cascade of innate immune responses in lymph nodes draining the intramuscular injection sites. This is followed by stimulation of CD4 and (weak) CD8 T cell immunity as well as boosting anti-gE antibody. Currently we are examining such adjuvant induced responses in human lymph node explants to compare with mouse models and how HSV naturally induces immunity after initial infection of genital mucosa. Such knowledge may help further improve vaccines by more appropriate adjuvant selection for other viral diseases in the ageing and for the closely related Herpes simplex virus (HSV).

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Phage Therapy in the Postantibiotic Era

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Antibiotic resistance is arguably the biggest current threat to global health. An increasing number of infections are becoming harder or almost impossible to treat, carrying high morbidity, mortality, and financial cost. The therapeutic use of bacteriophages, viruses that infect and kill bacteria, is well suited to be part of the multidimensional strategies to combat antibiotic resistance. Although phage therapy was first implemented almost a century ago, it was brought to a standstill after the successful introduction of antibiotics. Now, with the rise of antibiotic resistance, phage therapy is experiencing a well-deserved rebirth. Phage therapy is versatile, from conventional approaches such as the use of phage cocktails, to novel strategies, including the use of phage-antibiotic combinations, phage-derived enzymes, exploitation of phage resistance mechanisms, and phage bioengineering. However, in order to take advantage of phages as a therapeutic resource, we must understand the biological and evolutionary bases of their interaction with their bacterial and human hosts. This talk will illustrate these principles, using the development of a novel strategy of phage therapy against multidrug-resistant *Acinetobacter baumannii* as an example. Finally, the benefits of phage therapy beyond the clinical perspective, including opportunities for scientific outreach and effective education, interdisciplinary collaboration, cultural and economic growth, and even innovative use of social media, will be discussed. Phage therapy is more than just an alternative to antibiotics.

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Anti-biofouling: Selective copper uptake and release in seawater

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The use of copper based coatings to prevent biological growth has been widely practiced in the past. However, leaching of the copper has increased the concentrations in harbours/marinas and affecting marine life and the environment. It is therefore timely to develop a sustainable method in which no copper is added to the sea. Herein, we are proposing the use of cross linked polyethylene mine (PEI) coated on conducting carbon cloth as a copper adsorbing material and electrochemically triggered release of copper. Our approach is to use copper-binding polymers to capture the copper naturally present in the sea, and release it upon an electric stimulus, creating a biocidal copper flux across the coating–water interface. This would form a never-ending cycle of copper uptake and release.

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The Emergence of *Microsporum audouinii* in Australia

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Microsporum audouinii is an anthropophilic dermatophyte causing non-inflammatory infections of the scalp and skin. Once the cause of tinea capitis epidemics in Europe and North America, it is now endemic to regions of Africa and Eastern Europe. As a result of this, and with an increase in immigration from African countries, there has been a significant increase in *Microsporum audouinii* infections in Australia.

In tinea capitis infections, invaded hairs show an ectothrix invasion and fluoresce a bright greenish-yellow under Wood's ultra-violet light. Identification of this isolate however is difficult due to poor growth and lack of sporulation. Therefore, many isolates are misidentified as non-sporulating strains of *Microsporum canis*. Confirmed identification of these isolates can be made with DNA sequencing of the internal transcribed spacer (ITS) region.

The increased emergence of *Microsporum audouinii* in Australia highlights the importance of detection and treatment in order to prevent possible future outbreaks.

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The National Centre for Antimicrobial Stewardship – A One Health Approach

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The National Centre for Antimicrobial Stewardship (NCAS) was established in 2015 as a Centre for Research Excellence and is based at the Peter Doherty Institute for Infection and Immunity in Melbourne.

NCAS comprises brings together many of the essential groups in the scientific and medical community that have an interest in antimicrobial stewardship and antimicrobial resistance. It takes a 'One Health' approach to antimicrobial use and interventions occur across the five streams of acute care, aged care, general practice, companion animals and food producing animals. The aims of NCAS are to understand current prescribing behaviour, build workforce capacity and to develop, implement and evaluate practical strategies to improve the way that antimicrobials are used within Australia.

This presentation will describe some key programs and researching findings across the human and animal streams.