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Volume 38 Number 3 September 2017

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Contents

Cover image: Salmonella *Typhimurium (red) replicating within infected RAW264.7 mouse macrophages (yellow/blue). Image acquired at 18 hours post infection using a Zeiss LSM 700 laser scanning confocal microscope. Photo credit: Joshua Newson.*

Roy Robins-Browne
President of ASM

I recently returned from Hobart, where I attended another hugely successful Annual Scientific Meeting of our Society. The conference attracted many outstanding international and Australian participants, who spoke on a wide range of topics. The overall theme of 'Planetary Health' worked well with a number of speakers alluding to this vitally important issue in their presentations.

General microbiology meetings of this type appear to be falling out of favour, partly because specialist conferences are luring potential attendees away. I believe, however, that general meetings, in particular smaller ones with around 500 participants, still have much to offer. Where else could you hear leading authorities speak on the latest developments in active learning, the pathogenesis of listeriosis, the microbiology of coral reefs, the biophysical properties of biofilms, novel diagnostic method in clinical micro, and strategies to develop more effective meningococcal vaccines, within a few hours of each other? I also felt that the new format of combining three 20-minute symposium talks with three 10-minute proffered papers into a single 90-minute session worked well. This is likely to be become the standard format for many symposium sessions in future.

The social program was another a big hit, highlighted by a 'Tastes of Tasmania' Welcome Function and a Rubbo Celebration, with a DJ and photo booth.

Meetings of this type are the result of hard work by many people. Although it may be unfair to single out individuals, I must give special mention to Anthony Barker, the LOC Chair; John Bowman and Tom Ross, the Scientific Program Coordinators, and Dena Lyras, Chair of our National Scientific Advisory Committee (NSAC) for making this meeting so successful.

I also want to thank those members who attended the Annual General Meeting and voted unanimously for changes to our Constitution. The key changes are intended to make our Society more inclusive by smoothing the pathway to MASM, and by allowing Associate Members to vote at General Meetings. Other changes will allow more flexibility in setting the program for the Scientific Meeting and in the determining the membership of NSAC.

Next year's Annual Scientific Meeting in Brisbane promises to be another memorable one. Do yourself a favour and mark the dates 1–4 July 2018 in your diary now. I look forward to seeing you there.

Issues of *Microbiology Australia* usually have a specific microbiology theme; however, this issue is different. It resulted from a call from the Editorial Board to Early Career Researchers of *The Australian Society for Microbiology* for expressions of interest to contribute to an issue showcasing upcoming leading Australian research, over a year ago. After submission, the Editorial Board then invited full submissions from Early Career Researchers who had 'breaking' research to present. These went through the normal process of peer review and revision before acceptance.

The completed articles proudly showcase the achievements of our younger ASM members, including a number who are still students, illustrating the diversity of studies that our students are involved in.

Martina Jelocnik with Adam Polkinghorne present a study of *Chlamydia pecorum*, which is affecting one of Australia's iconic animals – the koala. They discuss the occurrence of the pathogen in Australia's livestock. Research on a necrotic disease of bivalve larvae, affecting our economically prized Australian seafood industry, is presented by Tzu Kwan.

The remaining articles focus on pathogens affecting us, and our loved ones, including viral, bacterial and fungal pathogens. Sushama Telwatte reports on her work in California with Steven Yukl exploring HIV latency using transcription profiling. Lucy Furfaro with Barbara Chang and Matthew Payne report on serotype prevalence and distribution of maternal Group B *Streptococcus*, highlighting differences in Perth versus the rest of Australia. Caroline Firacative, with colleagues Luciana Trilles and Wieland Meyer describe research leading to a better understanding of cryptococcosis and its aetiological agent, *Cryptococcus* spp. Joshua Newson discusses the understanding and controlling of *Salmonella* infections in Australia.

Outbreaks of infections are always a concern. Claire Gorrie discusses the hidden reservoirs of hospital-associated infections, specifically of *Klebsiella pneumoniae*. Felise Adams reports on a key regulatory mechanism of antimicrobial resistance in pathogenic *Acinetobacter baumannii.* Leah Roberts shows how whole genome sequencing is helping to rapidly characterise nosocomial bacterial outbreaks, and Shakeel Mowlaboccus reveals new insights on *Neisseria meningitidis* that are emerging from whole genome sequencing.

Finally, Erin Shanahan, along with colleagues Gerald Holtmann and Mark Morrison report on life in the small intestine, which can be regarded as the forgotten microbiome.

In the ASM Affairs section Anthony Baker recaps on the recent ASM National Conference in Hobart, and gives details of the latest awards and accolades for ASM members. Congratulations to all the ASM award recipients.

Microbiology Australia continues to hold a valued position as a source of communication and a source of peer-reviewed information. The journal is listed by Thompson Reuter's Emerging Sciences Citation Index (ESCI) since 2015 and applications are underway for the journal to be listed by Scopus and Web of Science.

Microbiology Australia's open access policy ensures wide readership and access to articles. The ASM Executive and Editorial Board are very encouraged by the widespread readership and we can see the effect of ASM ambassadors as they promote the journal. Turkey, Brazil, Iran and Russia have appeared in the top 10 lists of countries accessing *Microbiology Australia*, and this appears to be attributable to outreach by our Editorial Board members and University of Tasmania to these countries.

Finally, the Editorial Board would like to take this opportunity to congratulate the early career researchers and their mentors for their excellent contribution to this issue. We also reiterate Professor Roy Robins-Browne's thanks in the last Vertical Transmission for the long service given by Professor Peter Coloe and Chris Burke to *Microbiology Australia*. We also welcome the new Editorial Board members, Ross Barnard, Sam Manna, Chris Owens and Erin Shanahan.

We strongly encourage suggestions for content and feedback from all of our ASM members and readers of *Microbiology Australia*. The Editorial Board is keen to have similar themed issues, and welcomes your submissions and ideas.

From the Editorial Board of *Microbiology Australia*:

Ipek Kurtböke (Chair), Ian Macreadie (Editor-in-Chief), Ross Barnard, Mary Barton, Linda Blackall, Chris Burke, Narelle Fegan, Gary Lum, Sam Manna, John Merlino, Wieland Meyer, Chris Owens, William Rawlinson, Roy Robins-Browne (ASM President), Paul Selleck, Erin Shanahan, David Smith, Helen Smith and Jack Wang.

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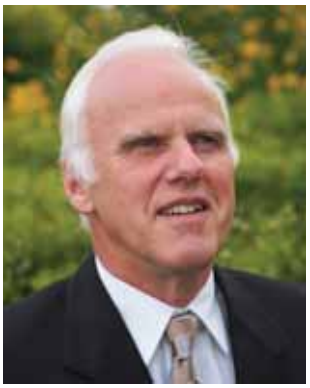

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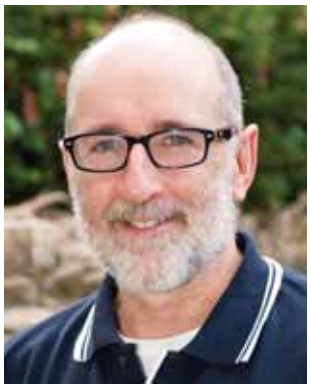

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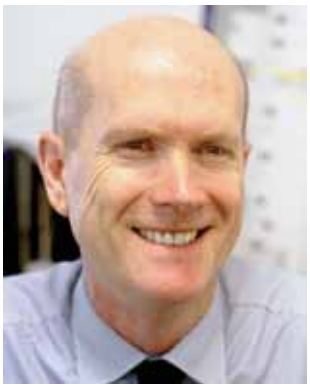

William Rawlinson

Roy Robins-Browne (ASM President)

Paul Selleck

Erin Shanahan

David Smith

Helen Smith

Jack Wang

Chlamydia pecorum: successful pathogen of koalas or Australian livestock?

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In Australia, the obligate intracellular bacterium *Chlamydia pecorum* is best known as the notorious koala pathogen that causes debilitating ocular and urogenital tract disease. While globally published data suggests that this species is essentially ubiquitous in livestock, little is known about the epidemiology of livestock *C. pecorum* infections here in Australia. My research is focused on investigating the genetic diversity and transmission patterns of *C. pecorum*, and why it causes disease. Using our newly developed *C. pecorum*-specific molecular epidemiology typing scheme we provided the first epidemiological data on infections in sheep and cattle in Australia, identifying strains associated with a range of diseases in livestock, and uncovering an unexpected level of diversity for this pathogen. Most importantly, we observed that the same strain can infect koala and sheep, indicating on ongoing cross-host transmission and 'spill-over' risks to wildlife. Further, by dissecting koala, sheep, cattle and pig *C. pecorum* strains genomes, we have also identified novel virulence-associated factors that could be explored as vaccine candidates for both livestock and koala infections.

Chlamydiae, known for their obligatory parasitic lifestyle and distinct biphasic life-cycle[1], are diverse and enigmatic bacteria that infect an astonishing range of hosts[2]. This phylum includes *Chlamydia pecorum*, a species that constantly remains in the spotlight due to its role as a major threat to the survival of the iconic koalas. In koalas, highly prevalent *C. pecorum* infections cause ocular disease that can eventually lead to blindness, and urogenital disease that can lead to infertility[3,4]. The pathogenic potential of *C. pecorum* in economically significant livestock species (cattle, sheep, goats and pigs) has been well documented in Europe and USA, and to a lesser degree elsewhere including Australia. The spectrum of *C. pecorum* infections in livestock hosts ranges from subclinical to acute disease manifestations such as polyarthritis, sporadic bovine encephalomyelitis, pneumonia and conjunctivitis, with (asymptomatic) gastrointestinal shedding as the common feature[5]. *C. pecorum* infections in free-range ruminants such as Alpine ibex and chamois from Europe have also been reported[6,7].

The curious case of *Chlamydia pecorum* infections in Australia

While the koala chlamydial infections are easily the most intensively studied of any wildlife species, almost nothing is known about the prevalence and epidemiology of *C. pecorum* infections in Australian livestock. Veterinarians from agriculturally productive areas throughout Australia regularly report cases of chlamydiosis in sheep and cattle[8,9], but the information about the genetic diversity of strains infecting livestock is lacking. A range of previous molecular studies suggested that *C. pecorum* strains infecting koala are genetically diverse[10,11]; however, none of the studies investigated how and whether these strains are related to livestock strains. It has been hypothesised that the origin of koala *C. pecorum* infections is associated with the import of 'presumably *C. pecorum* infected' livestock in the late 1780s[3]. The encroachment of koala habitats by sheep and cattle farming along the east coast of Australia is common and raises serious questions over the relationships and potential role that cross-host transmission may have in the epidemiology and origin of chlamydial disease in koalas[3]. Despite the ongoing field reports, the epidemiology,

transmission, and reservoirs of *C. pecorum* in Australian sheep and cattle remain poorly understood[5,12].

C. pecorum molecular epidemiology

Multi-locus sequence typing (MLST) is a widely used epidemiological tool for strain identification and estimation of intra-species relationships for many pathogens. Even today, with whole genome sequencing readily available, MLST is still often used as the first point to barcode a strain[13]. In an effort to evaluate the overall genetic diversity and relationships between Australian koala, sheep and cattle *C. pecorum* strains, we developed and applied a *C. pecorum*-specific MLST to a range of isolates and *C. pecorum*-positive clinical samples from koalas, Australian sheep and cattle[14–16].

The newly developed MLST proved to be an effective fine-detailed molecular epidemiology tool in characterising novel *C. pecorum* genotypes infecting livestock and koalas, highlighting an unexpected level of diversity for this pathogen. The genetic diversity of *C. pecorum* extends from the individual to the population level with repeated molecular evidence that: a) a single host can harbour two distinct *C. pecorum* strains at different anatomical sites; b) discrete populations can be infected with a mix of genetically diverse strains and c) the same strain can infect two different hosts (e.g. koala and sheep; sheep and cattle)[14] (Figure 1*a*). This observation was also repeated in the recent MLST of *C. pecorum* infecting koalas from French Island in Victoria. In this study, it was also observed that the certain koala strains were more similar to livestock strains than they were to other koala strains[17].

C. pecorum-specific MLST and Bayesian phylogenetic analyses of strains infecting Australian sheep with conjunctivitis, polyarthritis, or with no clinical signs of disease and cattle with sporadic encephalomyelitis revealed important observations of the genetic identity and relationships between *C. pecorum* strains found at different anatomical sites and the range of associated diseases[15,16]. We observed two distinct *C. pecorum* genotypes associated with disease, one denoted sequence type (ST) 23 found in association to sheep with polyarthritis and conjunctivitis and cattle with encephalomyelitis, and the other denoted ST 69 found in association with ovine conjunctivitis as well as previously detected in koala urogenital tract and ocular infections (Figure 1*a*). The majority of *C. pecorum* strains detected at vaginal and rectal sites of clinically healthy as well as diseased animals clustered together in a distinct and separate clade, away from strains associated with disease. *C. pecorum* ST 23 strains are also readily found in association with disease in European and USA livestock hosts, indicating a potential global spread of such clonal 'pathogenic' *C. pecorum*[14,18].

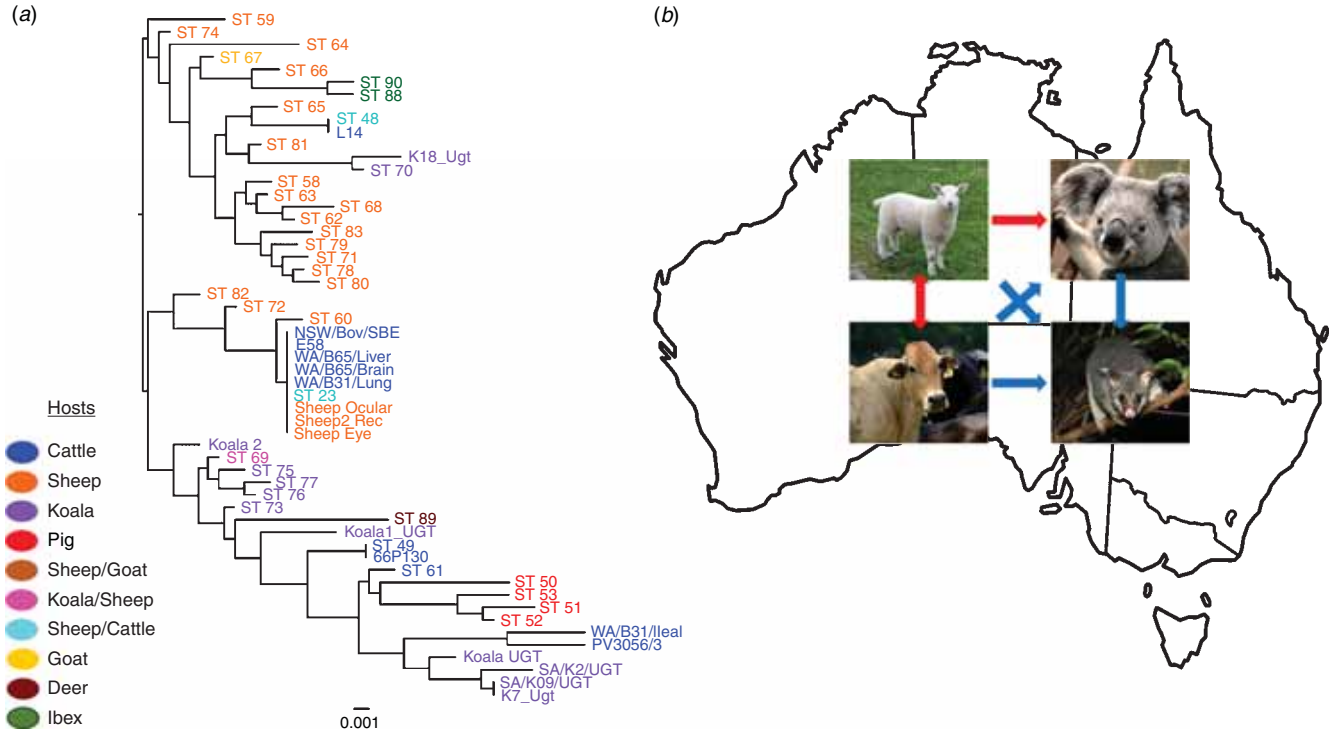

Figure 1. Genetic relationships of *C. pecorum* strains found in various hosts. (*a*) Phylogenetic analyses of the concatenated seven MLST gene fragments sequences from the previously described *C. pecorum* STs available from *Chlamydiales* PubMLST (http://pubmlst.org/chlamydiales/) and newly generated *C. pecorum* STs from our database. The mid-point rooted phylogenetic tree was generated using FastTree, as implemented in Geneious 9.1 software. Hosts are denoted by different colours in the legend. (*b*) Schematic representation of possible and present cross-host transmission of *C. pecorum* infections in livestock, koalas and other wildlife hosts (depicted by a possum). Red arrows indicate the most likely (molecularly evidenced) routes of cross-host transmission (e.g. sheep and cattle, and sheep and koala), while blue arrows indicate likely (hypothesised) routes of cross-host transmission (e.g. cattle and koalas, cattle or sheep and other native wildlife, and between wildlife animals).

Similarly, genetically identical the 'non-pathogenic' vagino-rectal strains characterised in our studies were also detected in the faeces of clinically healthy sheep from Europe (Figure 1*a*).

Chlamydial infections can spread rapidly in a flock/herd, potentially developing into diseases such as polyarthritis, conjunctivitis and encephalomyelitis, leading to increased morbidity and mortality on farms[19]. Detection of the genetically different *C. pecorum* strains at various anatomical sites in a single host and a plausible association of certain genotypes with disease/virulence in our and other studies[18,20] raises questions over the existence of 'pathogenic and non-pathogenic' *C. pecorum* and their role in pathogenesis of disease and impact on an animal's health[12].

The role of cross-host transmission in the epidemiology and origin of *C. pecorum* infections

The repeated evidence that identical or closely related *C. pecorum* strains infect koalas and livestock raises serious questions over the role of contemporary and historic spill over of this pathogen from domesticated animals to koalas[3,21] (Figure 1*b*). Ongoing work in our laboratory also provided evidence of *C. pecorum* infections in other Australian native marsupials *(unpublished observations)*, raising the possibility of cross-host transmission to the native wildlife as well (Figure 1*b*). Although ecologically distinct, livestock and wildlife host species could have an indirect contact allowing an opportunity for infection spill-over, most likely via the faecal-oral route[12,22]. For livestock *C. pecorum* infections, sheep and cattle are strong reservoir candidates as evidenced by ubiquitous faecal shedding of presumably 'non-pathogenic' strains[16,22] (Figure 1*b*). Evidence for cross-host transmission was also observed in our typing study of *C. pecorum* strains detected in European free-range ruminants[7] where, in limited examples, we observed a phylogenetic relatedness of the Alpine ibex *C. pecorum* with sheep faecal strains.

The lessons from ongoing *C. pecorum* genomics

While the MLST provided us with preliminary characterisation of *C. pecorum* strains from various hosts, comparative genomics provides valuable insights into the lifestyle, within-host adaptation, virulence factors and population structure[12,23]. The ongoing

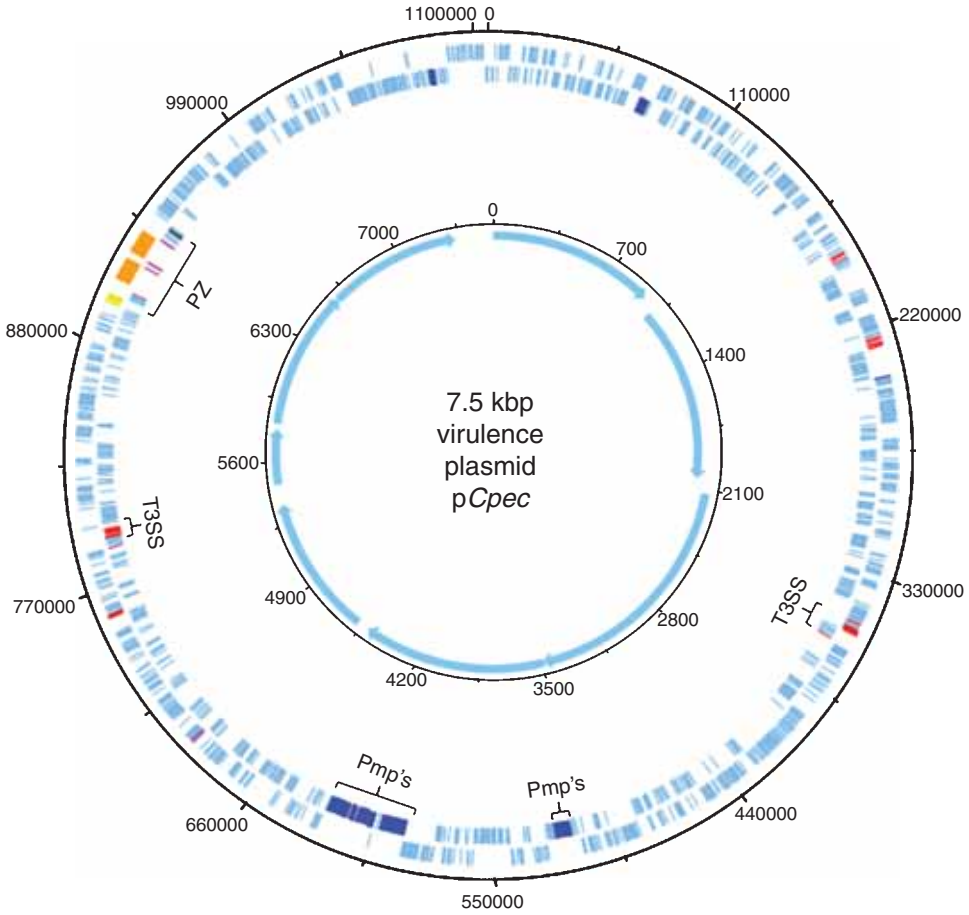

Figure 2. *C. pecorum* genome and plasmid composition. Circular representation of the *C. pecorum* chromosome: First ring denotes CDSs (in light blue) in forward direction, while the second ring denotes CDSs in reverse direction. Genomic location of important genomic elements such as Type III secretion system (T3SS) in red, polymorphic membrane proteins (*pmp*'s) in blue as well as the PZ (with cytotoxin genes in orange, and Phospholipase D genes in pink) are also outlined on the *C. pecorum* genome plot. Inset: Circular representation of the chlamydial plasmid (pCpec), where present in the genome. Image was generated with the DNAplotter.

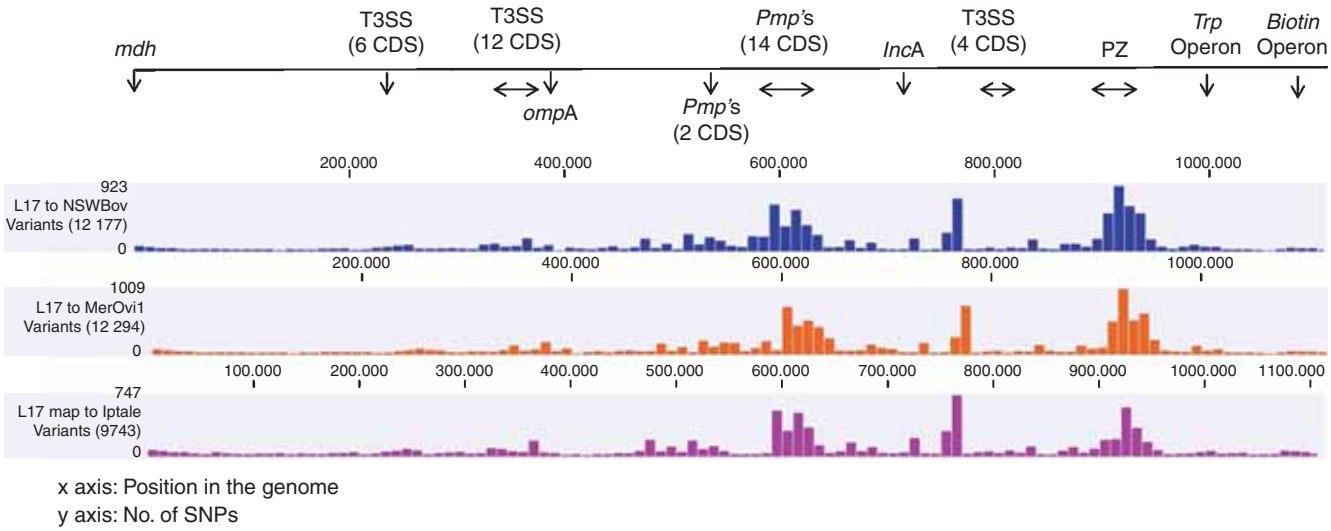

Figure 3. SNPs distribution in *C. pecorum* genomes. Genomic position of T3SS, *pmp*'s, inclusion proteins (*Inc*), major outer membrane protein (*omp*A), PZ, tryptophan (*Trp*) and biotin (*bio*) operons are indicated on a line above genomes, while the number and distribution of SNPs across the pig *C. pecorum* genome in comparisons to cattle (in blue), sheep (in orange) and koala (in pink) genomes are depicted as histograms. SNPs, represented as histograms of a 10 kb size, were determined in CLC Genomics (CLCBio, Qiagen) platform. All genomes start from malate dehydrogenase (*mdh*) gene.

comparative genomics of koala, sheep, cattle and pig *C. pecorum* genomes are building the global population framework of this enigmatic species[20,23–25]. Highly conserved and syntenic, the main genetic differences between *C. pecorum* strains are limited to single nucleotide polymorphisms (SNPs), mainly accumulating in the highly polymorphic membrane protein (*pmp*'s) genes and the plasticity zone that harbours virulence factors, such as cytotoxin, phospholipase D and Mac-Perforin genes[25] (Figures 2 and 3). The unexpected genomic finding in our studies was the identification of a chlamydial virulence plasmid (p*Cpec*) (Figure 2). p*Cpec* seems to be highly distributed in strains, however plasmidless strains are also common, raising questions over its function in disease pathogenesis[26]. Culture-independent genome sequencing of sheep, cattle and koala *C. pecorum* positive clinical swabs demonstrated that not only could multiple strains infect a single host but that a single anatomical site can harbour multiple *C. pecorum* strains[27], somewhat confirming observations from molecular epidemiology but also reiterating complex epidemiology of these infections. *C. pecorum* genome-derived phylogenies highlighted the polyphyletic history of the pathogen itself, and provided important clues about the evolutionary origins of koala strains, giving more confidence to our hypothesis that the origin of *C. pecorum* infections in Australia is associated with importation of domesticated animals into Australia with European colonisation[3,25,27].

Future directions

Drawing on the knowledge acquired in our studies, we have the opportunity to minimise cross-host transmission and improve on the diagnosis and management of these infections. We have recently developed a prototype anti-*C. pecorum* sheep[28] and improved koala vaccine formulation[29] using novel antigens identified in our genomic studies that could be used as a valuable tools to manage and control *C. pecorum* infections in the future. Improvements to diagnostic testing are now possible and laboratory and Point-of-Care assays for use in the lab and field are now also in development.

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Biographies

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Associate Professor Adam Polkinghorne is a molecular microbiologist and the Director of the University of the Sunshine Coast's Centre for Animal Health Innovation. His work is primarily focussed on understanding the diagnosis, epidemiology and control of chlamydial infections in a variety of wild and domesticated animal species.

Advances in the understanding of the *Cryptococcus neoformans* and *C. gattii* species complexes and cryptococcosis



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The rising incidence of cryptococcosis, a potentially fatal fungal infection affecting both immunocompromised and immunocompetent humans and animals, and the emergence of disease outbreaks, has increased the need for more in-depth studies and constant vigilance of its two etiological agents, the cosmopolitan and well known *Cryptococcus neoformans* and its sibling species *C. gattii*. As a result, a global scientific network has established formal links between institutions to gain better insights into *Cryptococcus* and cryptococcosis, enabling collaborations amongst researchers with different backgrounds, perspectives and skills. Interdisciplinary projects include: (1) the study of the ecology and geographical distribution of the agents of cryptococcosis; (2) the application of new alternative methodologies for the rapid and accurate identification of the two sibling species and major molecular types/possible cryptic species (VNI-VNIV and VGI-VGIV); (3) the use of different animal models of infection to assess cryptococcal pathogenesis and virulence factors; and (4) population genetics studies directed towards the discovery of virulence/tissue tropism associated genetic signatures. These studies enrich the knowledge and understanding of the epidemiology of this mycosis and help to better comprehend fungal virulence, genetics, pathogenesis, antifungal susceptibility, as well as investigating the regional and global spread, to improve treatment options of the disease caused by these important emerging pathogenic yeasts.

The encapsulated basidomycetes yeasts *C. neoformans* and *C. gattii* are the causative agents of cryptococcosis worldwide, causing pneumonia and meningoencephalitis in both, immunocompetent and immunocompromised hosts, resulting in a high morbidity and mortality. With almost 250 000 people affected with cryptococcal meningitis per year, this fungal infection is still responsible for more than 180 000 deaths anually[1]. By combining data from different studies carried out in numerous laboratories from around the world, it has been possible to define the geographical distribution of human clinical, animal and environmental cryptococcal isolates and to characterise different aspects of their biology.

Taxonomy, epidemiology and ecology

Within the currently accepted two species, four serotypes, distinguished by the capsular polysaccharide, and eight major molecular types, which are possible cryptic species, have been recognised. These major molecular types have been identified by different genotyping methodologies including PCR fingerprinting[2], amplified fragment length polymorphisms (AFLP)[3], restriction fragment

length polymorphisms (RFLP)[4] and multilocus sequence typing (MLST)[5]. In *C. neoformans* the serotype A, comprises the molecular types VNI (VNB) and VNII, the serotype D, molecular type VNIV, and the AD hybrid between serotypes A and D, molecular type VNIII. In *C. gattii* the serotypes B and C comprise both the molecular types VGI, VGII, VGIII and VGIV. From these, seven molecular types have recently been proposed to be raised to species level[6], although the issue of species definition is still controversial amongst the cryptococcal research community, resulting in the recognition of *C. neoformans* and *C. gattii* as species complexes[7].

Epidemiological studies have shown that *C. neoformans* molecular type VNI causes the majority of the cases of cryptococcosis and that this species is also most commonly isolated from the environment worldwide, except in Australia and Papua New Guinea, where VGI is the most common type[8–10]. VNII is an uncommon molecular type, which is reported from all the continents but in very low percentages[9,11]. VNIII AD hybrids are also recognised but their frequency seems to be strictly related to the presence of VNIV molecular type. In Europe and in the USA, where the frequency of isolation of VNIV strains is higher than in other geographical areas, a similar percentage of VNIII isolates has been observed, suggesting that in these regions hybridisation between the haploid VNI and VNIV populations is occurring[9,11]. Although *C. gattii* is less frequently recovered and it was considered to be geographically restricted to tropical and sub-tropical regions of the world, new studies have reported its extent to temperate regions and additional ecological reservoirs[12–16]. Besides the molecular type VGII isolates reported extensively from the Vancouver Island outbreak and its expansion to the Pacific Northwest of the USA[16,17], this molecular type has also been reported from Brazil, Colombia and Australia, with some cases in Europe[10–12,15]. The molecular type VGIII has mostly been recovered from Mexico, Colombia and the USA[11,13,18], while it is very rare in other continents[11]. Apart from Oceania, VGI is found in Asia, North, Central and South America, and Europe, where it counts for less than 10% of the isolates and is rare in Africa[9]. The molecular type VGIV has been reported from Southern Africa, India, Colombia and Mexico[9,11] (Figure 1).

Unlike *C. neoformans*, which predominantly affects immunocompromised hosts, *C. gattii* most often affects patients with no apparent risk factors[1,18–21]. In addition, in both species, the same genotypes have been found to be recovered from patients and from the patients' environment[19,22], which supports the theory of the acquisition of the infection from environmental sources and reaffirms the saprophytic and arboreal sources of these yeasts. Both species have also been isolated frequently from air, and swabs from trees, from decaying wood in hollows of many tree species, either individually or together[14,23]. Regarding the epidemiology of clinical cases of cryptococcosis, male patients are more commonly reported, as it is thought that male immune response may be less efficient in controlling cryptococcal infection[24], whilst cases in children and older patients are still rare in most countries[18,19]. In domestic animals from North America, *C. neoformans* molecular type VNI has been found to affect mostly dogs whereas *C. gattii*

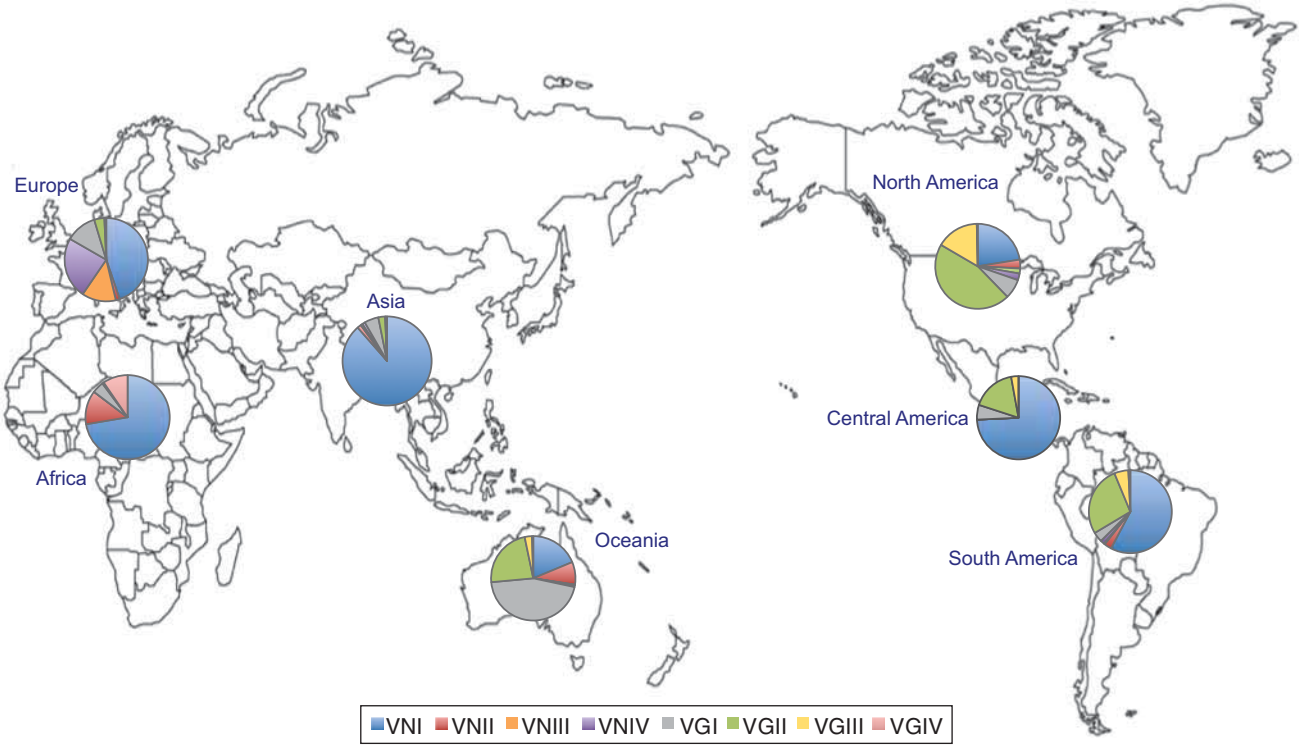

Figure 1. Geographical distribution of the major molecular types within the *Cryptococcus neoformans* and *C. gattii* species complexes.

molecular type VGIII predominantly infects cats. Genotypic analysis of such veterinary cases found as well that they are closely related to human and environmental cases and that VGII isolates presented higher minimum inhibitory concentrations (MICs) for most antifungal drugs than *C. neoformans* and other molecular types in *C. gattii*[25].

Molecular type and species identification

Between and within the major molecular types/species, there are differences in the ecology, epidemiology, clinical manifestations and antifungal drugs susceptibility that emphasise the need for rapid and accurate differentiation of *Cryptococcus* spp. Two methodologies have been assessed and adopted as a possible alternative for a distinction of the species and major molecular types of *C. neoformans* and *C. gattii*, compared with the currently used conventional techniques, such as PCR fingerprinting, AFLP and RFLP. Matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF MS) has been shown to be a suitable tool for inter- and intra-specific differentiation of human, animal and environmental strains of both cryptococcal species and also allowed for the detection of hybrid strains (Figure 2)[26]. As a result of this study, an in-house spectral library was constructed with the obtained spectra made available for use in a clinical diagnostic laboratory setting. Given the high costs of the initial setup of MALDI-TOF MS, a hyper-branched rolling circle PCR (HRCA) assay was developed as a substitute, a fast and specific method applicable in resource restricted countries. This method, which is based on the detection of specific single nucleotide polymorphisms in the phospholipase B gene among all major molecular types, proved to be fast, specific and highly sensitive and reproducible, for the differentiation of the molecular types and identification of hybrids within *C. neoformans* and *C. gattii* from human clinical samples (Figure 2)[27].

Animal models for the study of cryptococcosis

High (VGIIa and VGIIc) and low (VGIIb) virulent subtypes within *C. gattii* molecular type VGII are associated with outbreaks of infection in British Columbia, Canada and the Pacific Northwest of the USA[16,17] but less is known about the virulence of the major molecular types of *C. gattii*. A number of animal models, including a mouse and two invertebrate models of infection, have been used to determine whether or not there is a relationship between the molecular type and the virulence of the strains. The mouse model is a very well established system to study virulence and uses inoculation via the respiratory route. However, *Drosophila melanogaster* and larvae of the wax moth *Galleria mellonella* are gaining prominence as alternative models to study pathogenesis, given that insects can be used and maintained in large quantities with low costs, are easy to handle and their utilisation does not require ethical permission. In addition, fungal virulence factors involved in mammalian pathogenesis, such as capsule enlargement and melanin production, are also important during infection and death of insects (Figure 3*a*, *b*). In a *D. melanogaster* model, VGIII was found to be the most virulent molecular type when grown at 30°C, while VGII had the higher MICs against fluconazole and VNI grew most rapidly at 37°C, synthesised more melanin at 30°C and was more resistant to H_2O_2. This model showed, however, that temperature played a significant role in the flies' survival[28]. In a *G. mellonella* model, performed at 37°C, no differences amongst the major molecular types were observed in larvae survival and the expression of virulence factors, including capsule production, melanin synthesis and growth rate at 37°C. However, isolates with enhanced virulence were recognised among VGI, VGIII and VGIV, which importantly suggests that virulence might be associated with specific attributes of the strains that need further characterisation[29], and which may not be associated with the temperature at which the experiments are carried out in the different model systems. After intranasal infection of Balb/C mice with the highly virulent strains identified in the insect models, comparable results were found and, in addition, formation of multiple granulomas in the lungs was observed, which supported pulmonary cryptococcosis as the main outcome of infection (Figure 3*c*, *d*).

Whole genome sequencing-based population genetics to discover genetic differences relevant to pathogenicity and tissue tropism

In order to detect speciation and genetic differences within the distinct emergent populations of *C. gattii*, whole genome sequencing (WGS) has been performed on 134 *C. gattii* VGII isolates from five continents, including the subtypes VGIIa, VGIIb and VGIIc[16,17], which were previously studied by MLST analysis. Although the VGII subtypes were found to be completely clonal, the overall VGII population showed a high genetic diversity. In addition, several mutations, deletions, transpositions and recombination events have been identified within VGII strains, and this pointed to a South American origin[30]. Using WGS to compare subtypes, various genetic differences have been recognised, which are potentially related to habitat adaptation, virulence, and pathology[30]. For example, a set of ~50 genes has been identified being either present or absent in lung or brain infecting strains[30], offering the possibility to identify biomarkers, which should be able to guide clinical treatment to reduce mortality in patients based on the early detection of strains with a specific tissue tropism.

Similarly, to gain insights in the emerging *C. gattii* molecular type VGIII, more than a hundred isolates from endemic areas and sporadic cases have been characterised. The VGIII population was

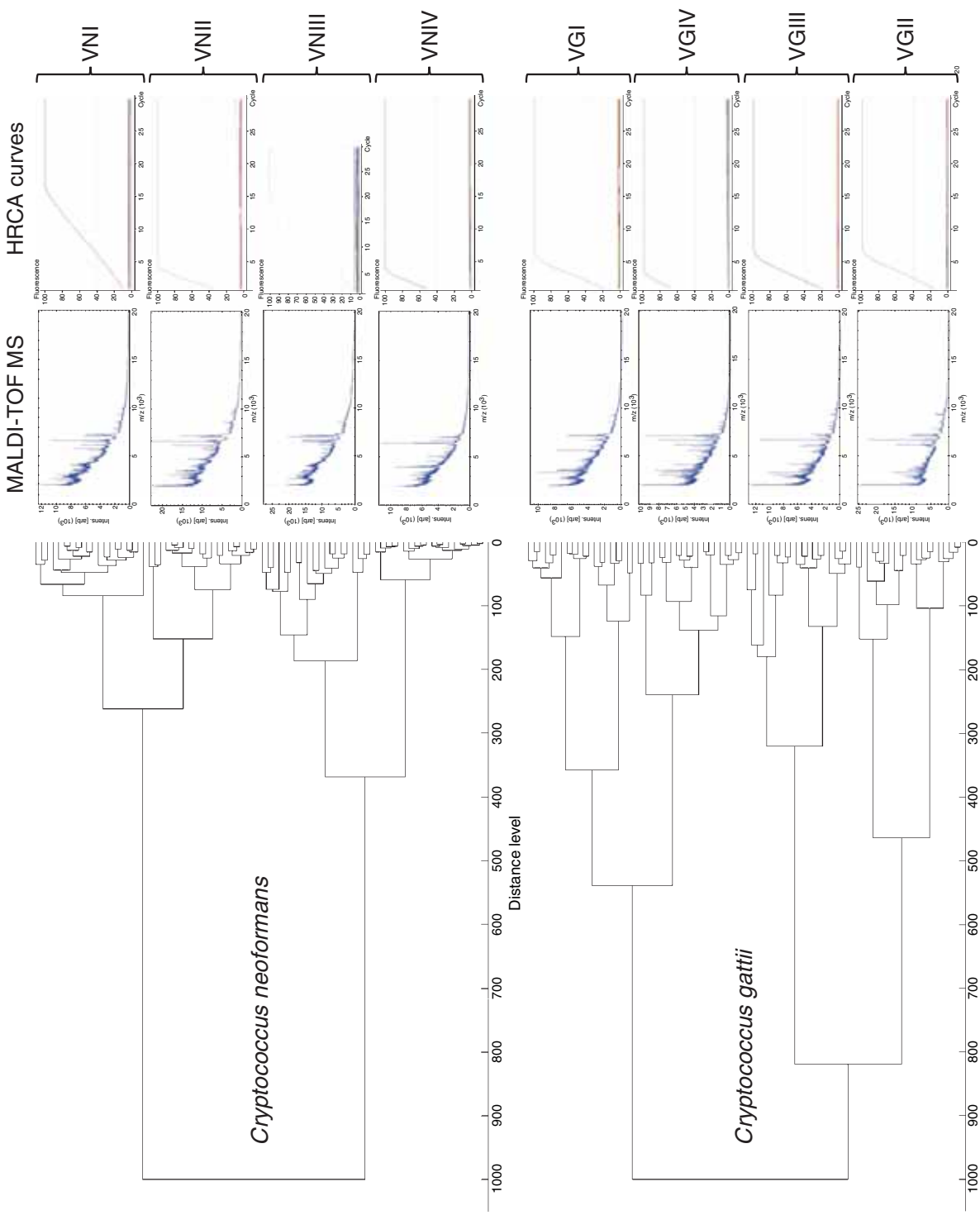

Figure 2. Differentiation of the major molecular types of *Cryptococcus neoformans* and *C. gattii* species complexes by MALDI-TOF MS and hyperbranched rolling circle PCR (HRCA). Mass spectra of total proteins generated by Bruker MALDI Biotyper and HRCA amplification curves of representative strains of each major molecular type are shown after the dendrogram, respectively.

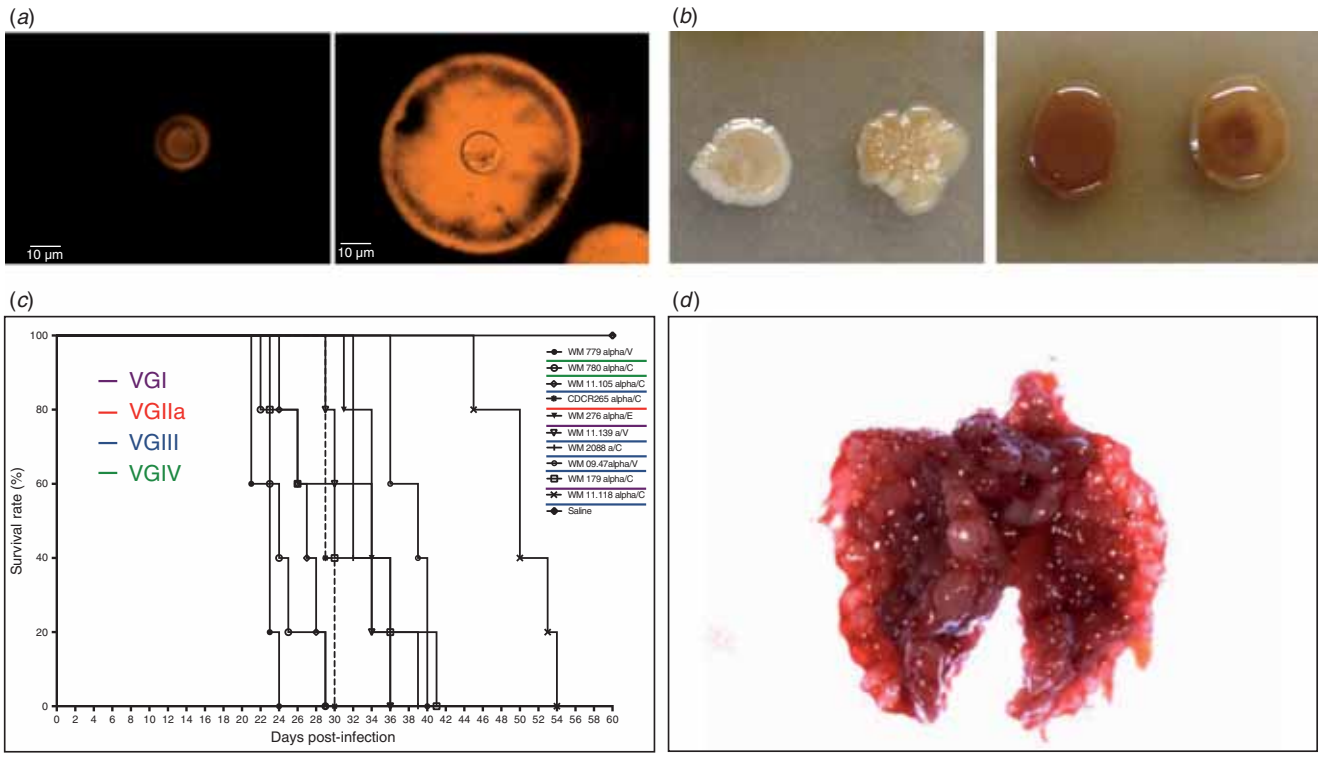

Figure 3. In a *Galleria mellonella* model of cryptococcosis, capsule enlargement (*a*) and melanin production (*b*) of the strains are observed. Highly virulent strains of *Cryptococcus gattii* are recognised after intranasal inoculation of BALB/C mice (*c*) resulting in granuloma formation in the lungs (*d*). Strains are organised according to the number of days required to cause the death of all mice after infection. C: clinical, E: environmental, V: veterinary strains.

found to have a high genetic diversity but clustered into two main groups, which correspond with either serotype B or C. WGS revealed Mexico and the USA as a likely origin of the serotype B VGIII population, and Colombia as a possible origin of the serotype C VGIII population. Importantly, serotype B isolates were found to be more virulent than serotype C isolates in a mouse model of infection, while serotype C showed to be less susceptible to fluconazole and azoles than serotype B isolates. These results emphasise the importance of the molecular characterisation of the isolates causing cryptococcosis in order to choose an appropriate and timely treatment for cryptococcal infection[22].

In conclusion, the rising occurrence of cryptococcosis in humans and animals, the emergence of outbreaks and the decreased susceptibility to the commonly used antifungal drugs, and the expansion of the ecological niche of cryptococcal species highlight the importance of a constant interaction between researchers with different backgrounds, perspectives and skills, enabling increased vigilance of these pathogenic yeasts. Differences regarding the epidemiology, virulence, and antifungal susceptibility between and within species are relevant to disease outcome and fungal therapy. Thus, the correct and fast recognition of the major molecular types within *C. neoformans* and *C. gattii* and their phenotypic and genotypic characterisation is essential, as it will most certainly decrease the time from diagnosis to treatment, to lead to a more effective treatment and reduced mortality rates. Continuous collaborative studies on the ecology, genetics and pathogenesis of these medically important yeasts are necessary to contribute, integrate and enrich our knowledge of this important emerging mycosis.

Acknowledgements

This work results as part of the continuous efforts and collaboration among multiple institutions and research groups from around the world, working together and devoted to study *Cryptococcus* and cryptococcosis. It was supported by grants from the National Health and Medical Research Council (NHMRC) of Australia (APP1031943), The National Institute of Health (NIH) USA (R21AI098059), and the program science without borders (CAPES) Brazil (098/2012) to WM and by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior' (CAPES) of Brazil (098/2012) to LT.

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Biographies

Dr Carolina Firacative is a biologist, with a PhD in Medicine from The University of Sydney, Australia. Her research focuses on different aspects of medical mycology, including fungal virulence and pathogenicity, the detection of nosocomial outbreaks and the identification of human and animal pathogenic fungi. She recently finished a post-doctoral fellowship from the Alexander von Humboldt Foundation, at the Institute of Immunology, University of Leipzig, Germany, where she was working on the identification of cryptococcal allergens that may contribute to immunodiagnostic and immunotherapies in fungal infection. Currently, she is the recipient of a University of Sydney Fellowship for postdoctoral researchers to work at the MBI, Sydney Medical School, on functional genomics of *Cryptococcus gattii* to elucidate new niche adaptations and the emergence of novel clinical phenotypes.

Dr Luciana Trilles is a biologist, with a PhD in infectious diseases at the National Institute of Infectious Diseases Evandro Chagas (INI), Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil. She is currently Curator of the Pathogenic Fungi Culture Collection (CFP), Professor in the PG Program in Clinical Research on Infectious Diseases and coordinates the environmental investigations of systemic mycosis outbreaks at the National Reference Laboratory on Medical Mycology at INI.

Professor Wieland Meyer is a leading molecular medical mycologist, with a PhD in fungal genetics from the Humboldt University of Berlin, Germany, a postdoctoral experience in yeast phylogeny and population genetics at Duke University Medical Center, Durham North Carolina, USA. He is a professor at Sydney Medical School, The University of Sydney, and a guest professor at Fundação Oswaldo Cruz (FIOCRUZ) Rio de Janeiro, Brazil, heading the MMRL within CIDM, Westmead Institute for Medical Research. His research focuses on phylogeny, molecular identification, population genetics, molecular epidemiology and virulence mechanisms of human and animal pathogenic fungi. He is the Convener of the Mycology Interest Group of ASM, the General Secretary of the International Society of Human and Animal Mycology (ISHAM) and a member of the Executive Committee of the International Mycological Association (IMA).

Salmonella in Australia: understanding and controlling infection

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The bacterium *Salmonella* causes a spectrum of foodborne diseases ranging from acute gastroenteritis to systemic infections, and represents a significant burden of disease globally. In Australia, *Salmonella* is frequently associated with outbreaks and is a leading cause of foodborne illness, which results in a significant medical and economic burden. *Salmonella* infection involves colonisation of the small intestine, where the bacteria invades host cells and establishes an intracellular infection. To survive within host cells, *Salmonella* employs type-three secretion systems to deliver bacterial effector proteins into the cytoplasm of host cells. These bacterial effectors seek out and modify specific host proteins, disrupting host processes such as cell signalling, intracellular trafficking, and programmed cell death. This strategy of impairing host cells allows *Salmonella* to establish a replicative niche within the cell, where they can replicate to high numbers before escaping to infect neighbouring cells, or be transmitted to new hosts. While the importance of effector protein translocation to infection is well established, our understanding of many effector proteins remains incomplete. Many *Salmonella* effectors have unknown function and unknown roles during infection. A greater understanding of how *Salmonella* manipulates host cells during infection will lead to improved strategies to prevent, control, and eliminate disease. Further, studying effector proteins can be a useful means for exploring host cell biology and elucidating the details of host cell signalling.

Salmonella infections in Australia

Australia experiences an unusually high number of reported *Salmonella* infections, comprising a significant baseline of individually acquired infections, combined with a string of well-publicised foodborne outbreaks[1]. Typically, outbreaks are associated with bacterial contamination of livestock and animal products in farms and factories, which are then carried on through to consumers. Several high-profile outbreaks have been linked to contamination of animal products: the outbreak at the Langham hotel in Melbourne in 2015 was linked to raw egg mayonnaise[2], while chicken and pork products were linked to an outbreak at a Sydney bakery in 2016[3]. Interestingly, several recent outbreaks have been linked to contaminated fruits and vegetables: *Salmonella* Anatum in bagged salads[4], *Salmonella* Saintpaul on beansprouts[5], and *Salmonella* Hvittingfoss on rockmelons[6]. These outbreaks translate to a significant economic impact to the affected industries, in addition to the medical burden caused to the affected individuals[7,8].

Australia enjoys a high standard of public health surveillance, and *Salmonella* infections are notifiable and publicly reported through the National Notifiable Diseases Surveillance System. These reports indicate more than 17 000 cases of *Salmonella* infection were reported in 2016[9], and already more than 10 000 reported cases in 2017 at the time of writing[10]. These numbers are derived from a symptomatic person presenting to a healthcare professional, and so the true number of infections is likely to be significantly higher[11]. *Salmonella* therefore represents a significant public health concern.

Salmonella causes a range of disease states

At first glance, the phylogeny of *Salmonella* appears relatively simple, with the genus comprising only two species. *S. enterica* is the species most frequently associated with disease states in humans and other animals, while *S. bongori* lacks several important virulence factors and consequently rarely causes disease in humans[12]. Within *S. enterica* however lies an astonishing variety of serologically distinct serovars, some of which have undergone selective pressures resulting in host adaptation and acquisition of additional virulence factors. Serovars within *S. enterica* can cause drastically different disease states: *S. enterica* serovars Typhimurium and Enteritidis are frequently the causative agents of gastroenteritis outbreaks, while *S. enterica* serovar Typhi causes typhoid fever, a systemic infection with broadly different clinical outcomes[13]. Even within serovars, variation and selection can result

in strikingly different strains. For example, the globally disseminated *S. enterica* Typhimurium strain ST19 causes self-limiting gastroenteritis, while the genetically similar *S. enterica* Typhimurium strain ST313 is causative of bloodstream infections resulting in significant mortality rates in Sub-Saharan Africa[14].

In Australia, the burden of disease arises from a variety of *Salmonella* serovars. Individually acquired infections and outbreak-associated infections are most typically caused by serovar *S.* Typhimurium[15], which is a generalist strain capable of infecting different animals. Some serovars are specialised to infect certain livestock animals: *S.* Gallinarum and *S.* Pullorum are adapted to chickens, while *S.* Choleraesuis is associated with pigs. Occasionally, outbreaks are associated with rarer serovars, such as *S.* Anatum and *S.* Hvittingfoss. Nearly all cases of *S.* Typhi infection in Australia occur in travellers that have contracted the bacteria while overseas[1].

Ultimately, the diversity of *Salmonella* serovars and spectrum of diseases that it causes demonstrate a bacterium that is adept at persisting through the food supply chain, from contamination of farms and factories to restaurants and household kitchens, despite stringent food safety standards and public health surveillance.

Salmonella subverts host cells to achieve infection

Infection by *Salmonella spp.* follows ingestion of live bacteria, which survive passage through the stomach and attach to the gastrointestinal epithelium. The infection strategy of *Salmonella* involves the invasion of host cells (either by localised disruption of the membrane of non-phagocytic epithelial cells, or by endocytic uptake into professional phagocytes) followed by the establishment of an intracellular replicative niche[16–18] (Figure 1). These activities are heavily mediated by the use of type-three secretion systems (T3SS), which function as molecular syringes to penetrate host cells and deliver bacterial effector proteins into the host cytoplasm (Figure 2). These effector proteins typically recognise specific host proteins, and disrupt the normal function of these proteins often by catalysing post-translational modifications[19,20].

Salmonella employs two distinct type-three secretion systems at different stages of infection. These secretion systems translocate distinct cohorts of effector proteins into the host cell to achieve different goals for the bacterium. At early stages of infection, *Salmonella* uses the SPI-1 secretion system (encoded on the genomic region termed *Salmonella* pathogenicity island 1) to achieve uptake into non-phagocytic epithelial cells. Effector proteins translocated by this secretion system manipulate the host

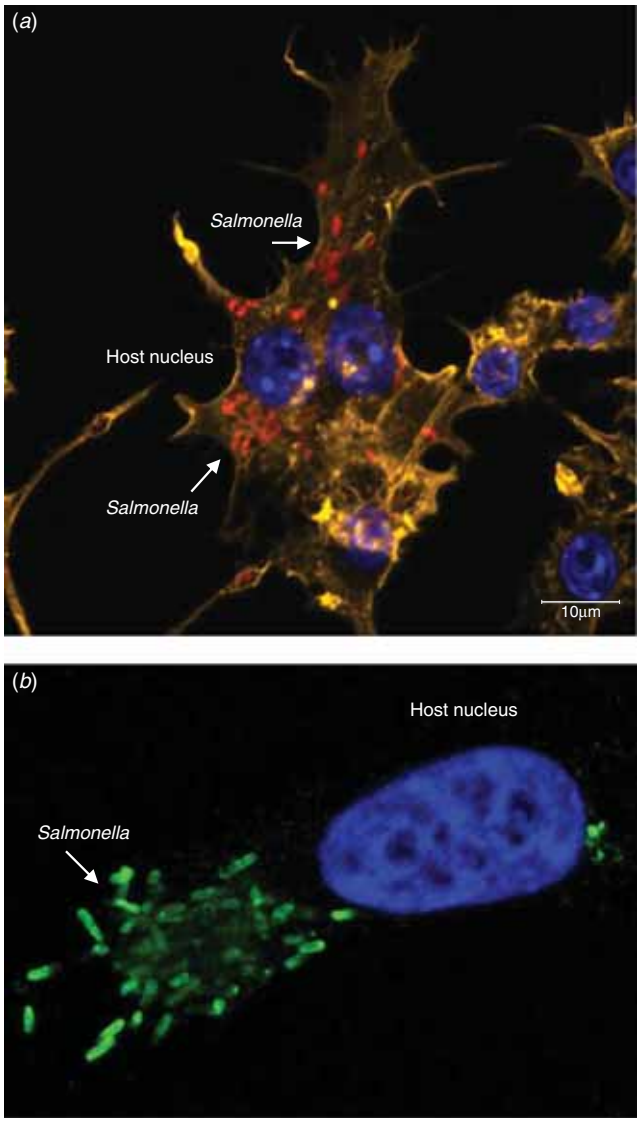

Figure 1. Immunofluorescence micrographs of Salmonella replicating within infected host cells. (*a*) RAW264.7 murine macrophages infected with *Salmonella* Typhimurium for 18 hours. (*b*) HeLa cell infected with *Salmonella* Typhimurium for 16 hours. Arrows indicate *Salmonella* (red in panel (*a*), green in panel (*b*)), as visualised with a polyclonal antibody to the *Salmonella* common structural antigens. Host cell nuclei visualised with Hoescht stain (blue). Host cells visualised in (*a*) with Phalloidin–Tetramethylrhodamine B isothiocyanate (yellow).

actin cytoskeleton to induce membrane ruffling, which enables bacterial uptake[21]. Shortly after invasion, the SPI-1 secretion system is downregulated and the bacterium is taken up into a phagocytic vacuole. Sensing the acidification of the vacuole, *Salmonella* then deploys the SPI-2 secretion system, which delivers another set of effectors into the host cytoplasm. Broadly, these effectors are responsible for remodelling the vacuole into a replication-permissive space (Figure 3), translocating and extending the vacuole along host microtubules, and interfering with the secretion of inflammatory cytokines[22,23]. Together, these activities enable *Salmonella* to survive within both epithelia and phagocytic cells, to replicate to high numbers, and to disseminate to adjacent cells and on to new hosts.

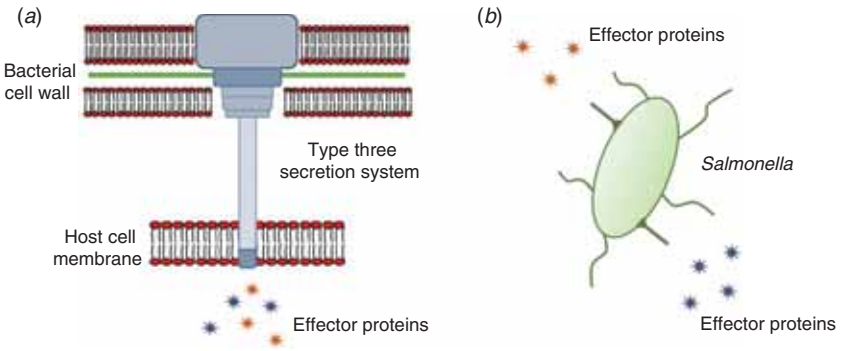

Figure 2. Schematic representation of the type three secretion system. (*a*) Representative structure of the type three secretion system, comprised of a basal body embedded in the bacterial cell wall, and a needle and tip complex which penetrates the host cell membrane. The complex forms a conduit from bacterial to host cytoplasm, allowing translocation of effector proteins. (*b*) Cartoon representation of *Salmonella* demonstrating translocation of distinct cohorts of effector proteins.

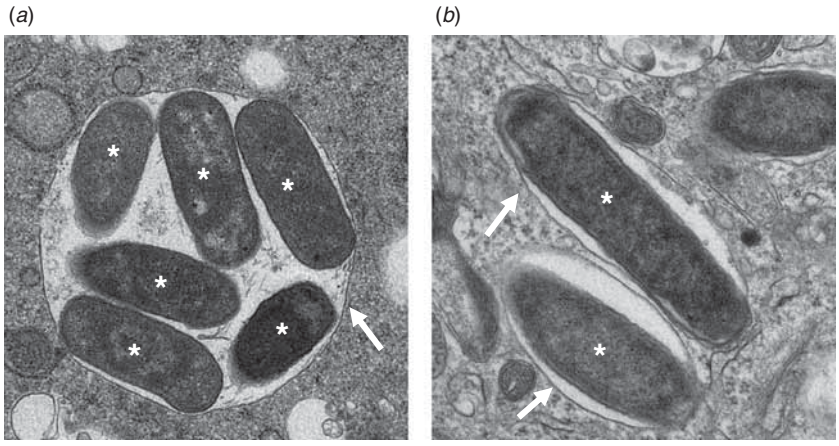

Figure 3. Electron micrograph of vacuole-bound *Salmonella* surviving within infected epithelial cells. Arrows denote the perimeter of the *Salmonella*-containing vacuole. Asterisks denote vacuolar *Salmonella*, residing in either (*a*) communal or (*b*) individual vacuoles. Images acquired by Vicki Bennett-Wood.

Discovering the function of *Salmonella* effector proteins

It is well established that *Salmonella* relies on effector protein translocation to achieve infection – mutant strains lacking type-three secretion systems do not invade epithelia effectively, and are deficient in intracellular persistence[24]. However, the contribution of individual effector proteins is less well defined. Some effector proteins are relatively well described, others are poorly understood, and many have no known function and no known role during infection. Our research aims to characterise the poorly understood effector proteins of the SPI-2 secretion system.

Classical methods for identifying interactions between a given protein and its potential binding partner rely on the strength of the interaction between the two. Approaches such as yeast two-hybrid screening and immunoprecipitation have found success in identifying the host substrates of many *Salmonella* effector proteins. However, these approaches are unsuitable if the binding affinity between two proteins is weak or transient. For instance, effector proteins that are predicted to be enzymes are not likely to have strong or stable binding affinity for their host substrates.

Our research focuses on the enzymatic activity of a family of *Salmonella* effector proteins. These effectors are known substrates of the SPI-2 secretion system, and are predicted to be glycosyltransferases. Therefore, we have screened the proteome of infected host cells to detect changes in glycosylation activity. This approach combines custom immunoprecipitation techniques with highly sensitive mass spectrometry to detect glycosylated peptides during infection. Our approaches are not limited by the binding affinity of an effector for its substrate, and also allow for interrogating the activity of effector proteins without overexpression or ectopic expression. Preliminary results suggest *Salmonella* engages in a blockade of apoptotic cell death signalling, a finding that expands our understanding of how bacteria survive within host cells.

Directions and applications

Antibiotic treatment is generally ineffective at reducing the impact of *Salmonella* infection, and vaccines that are effective and economically viable are still in development[25]. The successful control and prevention of *Salmonella* infection requires a more robust understanding of the interaction between the pathogen and the

host cell. Given that type-three secretion mutants are significantly attenuated during infection, it stands to reason that interfering with effector translocation represents a viable opportunity for developing novel anti-infective therapeutics. As we move towards a more complete understanding of the *Salmonella*–host interaction, it is likely that new opportunities will arise to antagonise the intracellular lifestyle of the bacteria. Ultimately, effective control and prevention of *Salmonella* infection will require a combination of human and animal vaccination, public health surveillance and food safety compliance, and effective therapeutic options.

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Biography

Joshua Newson is a PhD student at the Doherty Institute, The University of Melbourne. His research involves identifying mechanisms by which *Salmonella* survive within infected immune cells, and elucidating the function of translocated effector proteins.

Correction:
The banner on page 62 of the last print issue (Volume 38, Issue 2) should read *Advertisement*. *Microbiology Australia* welcomes reviews of microbiology texts from ASM members.

Life in the small intestine: the forgotten microbiome?



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The gastrointestinal (GI) microbiota is now widely accepted to be an important modulator of our health and well-being. The microbes colonising the GI tract aid in promoting gut and immune homeostasis, while alterations in the composition and/or density of these microbes, often referred to as dysbiosis, have been implicated in many intestinal and extra-intestinal disorders. As a result, the GI microbiota is of increasing interest as a therapeutic target. This is particularly the case in the context of GI disorders linked to chronic inflammation of the mucosa. In this article, we focus on the small intestinal microbiota, which in many senses can be considered the 'forgotten' gut microbiome.

Much of the current knowledge of the microbiota is from studies of faecal samples, which are relatively easy to access, and contain a high microbial biomass that is amenable to widely used culture-independent approaches. However, the faecal microbiota may be subject to physiological and environmental factors such as intestinal transit time and dietary variation. In contrast, those microbes that colonise the GI mucosa may be less subject to short term environmental variations, and have greater capacity to affect mucosal barrier functions and host immune responses. Assessment of the mucosa-associated microbiota (MAM) has therefore received increasing attention, and these sub-communities in the large bowel show a defined biogeography in healthy subjects[1] as well as variations associated with particular disease states[2].

Conversely, there has been relatively scant attention paid to the small intestine, particularly the proximal regions (duodenum and jejunum). This is despite the fact that the small intestine represents the greatest surface area of the bowel, estimated to be ten times greater than that of the large intestine[3]. The small intestine is also an important site for digestion, nutrient absorption and immunological functions. Clinically, up to one-third of the population experience some form of upper GI digestive disease or disorder. Yet we understand very little about the structure-function relationships inherent to the proximal GI microbiota, constraining our diagnosis and treatment of these conditions, many of which are associated with chronic inflammation. If we are to fully appreciate the role and potential clinical management of the gut microbiota, we need to better understand the host-microbe interactions of the small intestine.

The small intestinal niche and its 'forgotten microbiota'

The small intestine represents a unique series of niches within the digestive tract (Figure 1). An important difference relative to the large intestine is the existence of an oxygen gradient, with the

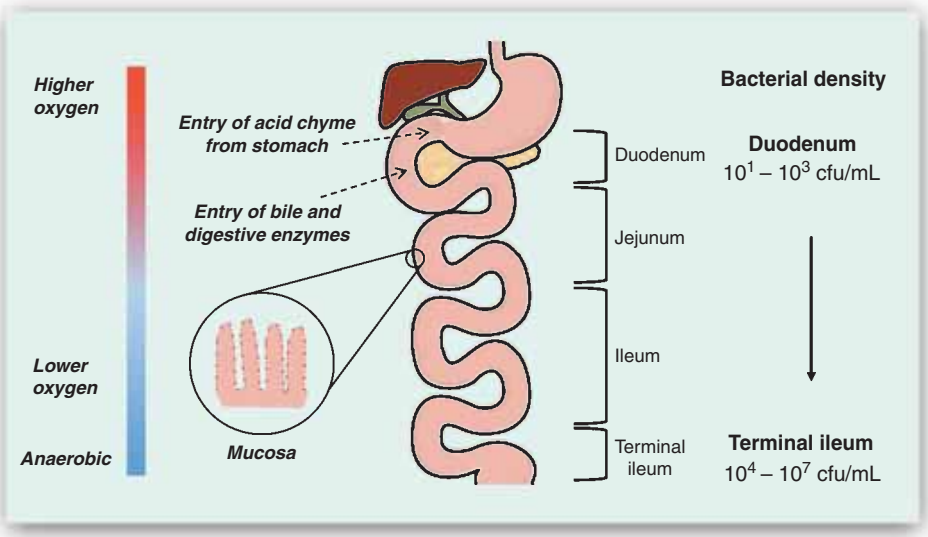

Figure 1. The small intestine. A gradient in oxygen levels and bacterial load exists along the length of the tract. The proximal small intestine (duodenum and jejunum) are particularly influenced by the entry of acid chyme, as well as bile and digestive enzymes. The small intestinal mucosa is lined with villi, which project into the lumen and create a vastly increased surface area.

proximal regions more oxygenated, and only the terminal portion being anaerobic, sharing greater similarity with the large bowel. It is a relatively harsh environment characterised by influxes of acidic chyme from the stomach, and the presence of bile and digestive enzymes. The effect of gut motility on digesta residence time is also a strong selective pressure on the microbiota. Thus microbial colonisation of the small intestine, and the proximal regions in particular, is a challenge in comparison to the more hospitable environments of the lower gut. These factors, along with the sporadic presence of host ingested nutrients, mean mucosal colonisation is of particular importance for small intestinal microbes.

For these reasons, the proximal small intestine, was long considered sterile. Early culture-based studies found that in most cases, very limited numbers of bacteria could be isolated[4], and were often speciated as either oral or colonic/faecal bacteria[5–7]. The general consensus was that bacteria were not resident but rather contamination from other sites, such as the oral cavity, and were of little relevance. These assumptions were also driven, in no small part, by the fact that the proximal small bowel is difficult to reach and therefore sample. It is only possible to sample this site during medical procedures, primarily endoscopy. Standard endoscopic procedures are only able to reach the duodenum and collection of tissues during these procedures is also inherently susceptible to contamination, via exposure to the oral cavity and lumen. Thus it can be difficult to distinguish between the MAM and non-resident organisms picked up during sampling[8].

However, as the *Helicobacter pylori* story so aptly demonstrates, the mucosa of such harsh regions of the GI tract can be stably colonised by niche adapted organisms, and these previously overlooked microbes can have important implications for health, with the role of *H. pylori* in ulcers and gastric cancer clearly established. Interestingly, emerging evidence also suggests the importance of other microbes, resident in the gastric mucosa, along with *H. pylori*, in the progression to gastric cancer[9]. Thus it is clear that the mucosal microbiota of the upper GI tract cannot be assumed to be flow through or contamination.

A developing picture of the small intestinal microbiota

The development of new molecular, sequencing and computational technologies has driven the renewed interest in the complex communities of microbes associating with the human body. In particular, these technologies are facilitating analysis of previously overlooked body sites. Thus there are emerging data describing the microbiota present in the small intestinal mucosa, in both an inflamed and non-inflamed gut. These studies have revealed the proximal bacterial community is principally comprised of Firmicutes, Bacteroidetes and Proteobacteria affiliated taxa, dominated by the genera *Streptococcus, Prevotella, Granulicatella, Veillonella, Neisseria, Haemophilus, Gemella* and *Rothia*[10–17]. From these studies there appear to be overlaps with the oral microbial community, at broader taxonomic levels[18], as well as some similarity to the microbiota of the large bowel, although few studies have directly compared the two regions in the same patients[13]. While the proximal small bowel lacks many of the highly abundant large bowel groups including *Faecalibacterium*, *Ruminococcus*, *Bacteroides* and *Clostridium*, there are some overlaps, notably *Prevotella*. However, whether the *Prevotella* spp. observed at the

two sites are the same, or represent unique/niche-adapted strains, has not been characterised.

In the context of disease, a primary focus for the small bowel has been coeliac disease. When comparing patients to non-coeliac controls, lower microbial diversity and reductions in the relative abundance of *Streptococcus* and *Prevotella* have been reported, along with higher levels of Proteobacteria[10,12,14], although not all studies have identified such differences[11,16]. In irritable bowel syndrome, a single study has compared the jejunal mucosal microbiome, which is similar in composition to that of the duodenum, in patients and healthy volunteers, however no substantive differences in microbial profiles were observed[19].

In other regions of the GI tract, increasing interest and attention is being directed towards the further components of the microbiota: fungi, archaea, viruses, bacteriophage and protozoa[20]. However, in the small intestine, our understanding of these microbes as interacting members of the mucosal microbiota is very limited. In the context of the proximal small bowel, we are aware of only one study to date that has profiled the non-bacterial mucosal community. This study focused on the mycobiome (the fungal microbiota), and identified two predominant phyla, Ascomycota and Basidiomycota, with the most represented classes being *Tremellomycetes*, *Saccharomycetes*, *Basidiomycetes*, *Agaricomycetes* and *Dothideomycetes*[21]. Given the recent identification of small intestinal fungal overgrowth, associated with otherwise unexplained gastrointestinal symptoms[22], further efforts to characterise the mycobiome, as well as other microbiota components, in the proximal gut mucosa are warranted.

Ongoing challenges

Such profiling studies are not without their challenges, and this is particularly the case when working with human tissue samples, of limited size, that have relatively low microbial loads and high levels of human DNA. Typically, these tissues are subject to profiling studies that involve amplification of a marker gene, such as the bacterial 16S rRNA gene, next-generation sequencing, and bioinformatics procedures to align sequence reads to a database and assign taxonomy. However, bias may be introduced through sample collection methods, primer choices and bioinformatics methodologies[23,24], taxonomic assignment beyond genus level can be problematic, and care must be taken to avoid amplification of spurious products, such as those present in common laboratory reagents[25] (Table 1).

Metagenomics, in which total DNA is sequenced, rather than an amplicon, can overcome some of these issues. The risk of amplification bias is greatly reduced, and obtaining partial, to near complete, microbial genomes can enable more specific taxonomic assignments, such as to species level; as well as facilitate assessment of the functional capacity of the microbiota[24]. However, in low microbial load mucosal samples, performing metagenomics is technically challenging[26] and it can be difficult to obtain sufficient microbial biomass for accurate profiles.

The limited number of studies investigating the proximal small intestinal MAM are constrained by a number of other factors, including relatively small numbers of participants. Factors such as diet and medication use have also not yet been considered in detail in these studies. In particular, the widespread use of proton pump inhibitors (PPIs), their impact on gastric acid secretion, and recent observations that the use of PPIs results in changes to the stool microbiota, namely increases in the presence of oral-like organisms[27,28]; indicates these are likely to also impact the proximal small intestinal microbiota. A further complication when investigating the healthy microbiota in these sites is the unlikelihood of a symptom-free, healthy individual undergoing a medical procedure such as an endoscopy. Understanding the relative contributions of the microbiota in the various niches of the GI tract, to various disease states, is also an important consideration, which has not been well addressed. Given the burden of GI disorders in which the small intestine is implicated, more comprehensive studies are required to address these issues.

The 'Brisbane approach' to the small intestinal microbiota

We are interested in the mucosal microbiota in the proximal small bowel in the context of functional gastrointestinal disorders, specifically functional dyspepsia (FD) and irritable bowel syndrome. Patients with these disorders experience often severe GI symptoms such as meal related pain, fullness, indigestion, bloating and altered bowel habits. These symptoms are associated with mild chronic inflammatory responses but the drivers of inflammation are unknown[29,30]. The microbiota is suggested to play a key role. However, to understand the potential of host-microbe interactions in driving these symptoms, the constraints limiting the prior studies of the proximal small intestinal MAM must be overcome.

We have recently described an approach in which a sheathed biopsy forceps, designed to remain free of luminal and oral material during sampling of the mucosa, was utilised during endoscopy procedures to sample the small intestinal mucosa[31]. This type of technique is not routinely utilised when sampling in the GI tract, and has facilitated a targeted assessment of the duodenal MAM using molecular and culture-based approaches (Figure 2). Through these

Table 1. Challenges associated with microbial profiling studies based on amplicon sequencing. Working with human tissues, with low microbial load, presents additional challenges compared to high biomass samples such as stool.

	General considerations	Additional considerations for low microbial load tissue samples
Sampling	– Consistency in sample collection and storage prior to processing – Collection of sufficient number of samples to accurately profile tissue – Inclusion of sufficient study participants to overcome inter-individual variations	– Collection method that specifically samples intended microbiota target
Nucleic acid extraction	– Cell lysis methods that can effectively lyse all members of the microbial community – Avoid introduction of batch effect (e.g. avoid processing all control samples in a single batch)	– Strategies to avoid contamination of low microbial load samples from the laboratory environment – Methods to enrich for microbial DNA
Amplification and Sequencing	– Choice of primer set targeting region of interest (e.g. 16S rRNA gene variable region) – Amplification bias	– Primer set that avoids amplification of human DNA – Include multiple negative controls to account for potential amplification of low level nucleic acids in laboratory reagents
Bioinformatics	– Pre-processing to retain only informative, high quality sequence read data – Methods for alignment of sequences and database used for taxonomic assignment – Accuracy of taxonomic assignment from short sequence reads – Assessment of required sequencing depth	– Removal of non-target reads
Statistical analyses	– Accounting for differences in sequencing depth (number of sequence reads per sample) – Methods for data normalisation – Applying statistical methods to sparse (many zeros) and relational (relative abundance) data – Integrating microbial profiles with clinical data and patient characteristics	

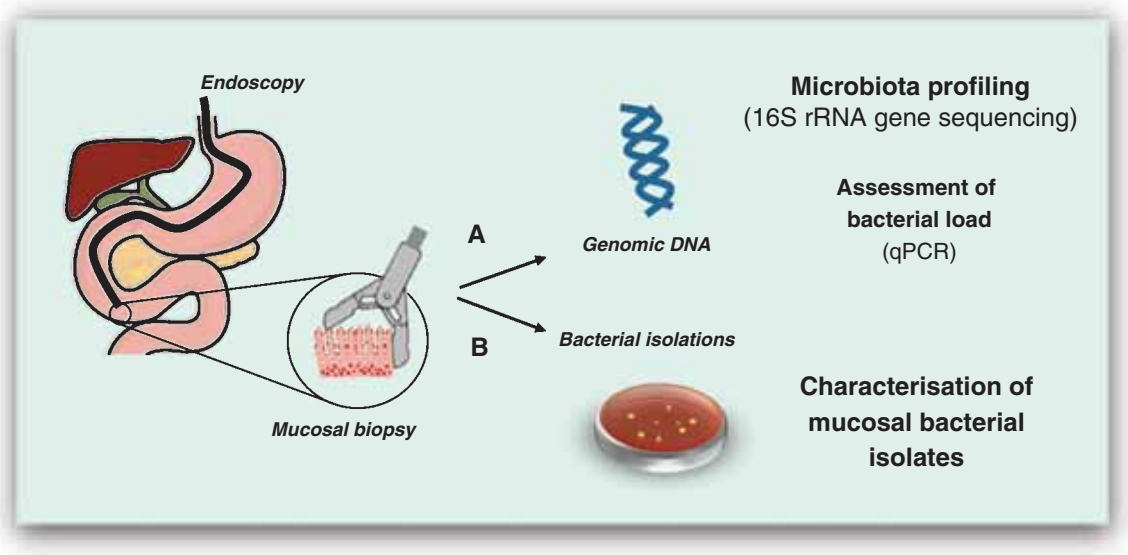

Figure 2. Analysis of duodenal mucosal biopsies. The mucosa of the small intestine is sampled using a biopsy forcep during a patient's endoscopy procedure. The mucosal biopsies are processed via two methods. (A) For nucleic acid based analyses, the biopsy is stored frozen until use. Genomic DNA is extracted utilising optimised methods and utilised for Illumina 16S rRNA gene amplicon sequencing, and quantitative PCR analysis for approximation of bacterial load. (B) For culture based analyses, the biopsy is transferred, within the biopsy forceps, immediately to an anaerobic chamber, homogenised and stored frozen in anaerobic glycerol. A variety of rich culture media are utilised, as well as aerobic and anaerobic incubation conditions, to isolate bacteria directly from the biopsy tissue.

techniques, we have identified correlations between bacterial load and the specific symptoms experienced by FD patients[32]. Specifically, the density of mucosal bacteria in the duodenum was positively correlated with meal-related symptoms and negatively correlated with patient quality of life scores. Indeed, bacterial load appears to be a stronger driver of FD symptoms than any specific alteration in the taxonomic profile of the duodenal microbiota. These findings are supported by the recent publication of a placebo-controlled trial in which treatment with the poorly absorbed antibiotic rifaximin was found to reduce symptoms in FD patients[33].

While profiling studies are useful in regards to identifying the overall structure of a microbial community, the data is limited in its ability to provide functional or mechanistic insights, or indeed reflect the presence of live, niche adapted organisms. To that end, we are currently developing a collection of contamination-free tissue for microbe isolation and culture. Using this approach, new duodenal isolates of *Streptococcus, Veillonella, Fusobacterium, Actinomyces* and *Neisseria* spp. have been obtained[34]. We are now sequencing the genomes of these isolates, and testing their pro-inflammatory activity, with a view to further defining taxonomic and functional specificities, not unlike the approaches used to date to identify colonic bacteria considered relevant to the progress of inflammatory bowel diseases[35].

Conclusion

In summary, the small intestine represents an important component of our GI tract, and yet we know relatively little regarding the microbial communities inhabiting this region. Developments in molecular microbiology have opened a window on the organisms residing in the mucosa; however, further research is required, particularly in regards to understanding the microbiota as a whole, encompassing bacteria, along with fungi, archaea, protozoa and viruses. Innovations in sampling techniques, and further well-designed studies targeting the MAM of the proximal small intestine, will allow us to better understand the niche-adapted microbes present in this region, and the host-microbe interactions that are likely to be important in disease processes. This provides potential for improved diagnoses and more targeted interventions that will enable treatment of GI disorders, rather than simply management of ongoing patient symptoms.

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A key regulatory mechanism of antimicrobial resistance in pathogenic *Acinetobacter baumannii*

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***Acinetobacter baumannii* is a Gram-negative bacterial pathogen that has become a pressing global health issue in recent decades. Although virulence factors for this pathogen have been identified, details of how they are regulated are largely unknown. One widely employed regulatory mechanism that bacteria, such as *A. baumannii*, have adopted is through two component signal transduction systems (TCS). TCS consist of two proteins; a histidine kinase and response regulator. The histidine kinase allows the bacterium to sense alterations in the extracellular milieu, transmitting the information to the response regulator which prompts the cell to modify gene expression levels accordingly. Bacteria can encode multiple TCS, where each system can mediate specific responses to particular conditions or stressors. Identifying those conditions in which these TCS are expressed, and the genes they regulate known as their 'regulon', is vital for understanding how *A. baumannii* survives and persists within the hospital environment or the human host during infection. As we enter the post-antibiotic era, knowledge of TCS could prove to be invaluable, as they offer an alternative target for the treatment of multidrug resistant bacterial infections.**

Acinetobacter baumannii is a Gram-negative opportunistic 'superbug' that causes a diverse range of nosocomial infections, primarily in patients whom are immunocompromised, such as those within intensive care units[1]. The *Acinetobacter* species is one of the leading causes of bacterial pneumonia within the hospital environment[2,3] and have been responsible for numerous outbreaks of nosocomial infections, worldwide[4]. Within Australia, *A. baumannii* isolates are not only confined to hospitals but are also seen within community-based settings, primarily in tropical regions of Northern Australia[5,6]. These community-acquired infections have been shown to be predominant in individuals whom have underlying medical conditions such as diabetes mellitus or other risk factors including excessive alcohol consumption[6].

High levels of innate and acquired multidrug resistance mechanisms represent a leading factor in the pathogenic success of this organism. Intrinsic resistance mechanisms include low outer membrane permeability, production of chromosomally-encoded antibiotic inactivating enzymes and efflux pump systems[7,8]. Acquired mechanisms contributing to resistance include the uptake of foreign resistance determinants via horizontal gene transfer[9,10] and over-expression of resistance genes through introduction of insertional elements or mutations particularly within their regulators[11,12]. This ability of the bacterium to acquire and modulate expression of an array of antimicrobial resistance determinants provides a strong ecological advantage to survive the selective pressures typically found within the hospital setting, resulting in the growing emergence of multidrug resistant *A. baumannii* lineages[1].

Infections caused by multidrug resistant *A. baumannii* isolates, particularly those resistant to carbapenems, have been linked to increased morbidity and mortality, as well as a significant rise in hospital associated costs[13]. Recently, the World Health Organization recognised the impending threat of carbapenem resistant *A. baumannii* isolates, listing them as one of the top three critical priorities for research and development towards new therapeutic treatments for antibiotic-resistant bacterial species (excluding Mycobacteria)[14]. Despite the risk this bacterium poses to susceptible individuals and our healthcare facilities, virulence traits including antimicrobial resistance, are not completely understood. Furthermore, the regulatory mechanisms that modulate these characteristics are even more ill-defined.

One simple yet highly sophisticated mechanism that bacteria utilise to effectively regulate the expression of virulence factors employs TCS[15,16]. TCS consist of two proteins; a histidine kinase which

senses variations in the extracellular milieu and a response regulator which alters gene expression upon activation by its cognate histidine kinase[17] (Figure 1). TCS not only modulate virulence-associated mechanisms but also fundamental biological processes such as pathways involved in metabolism[18,19] and osmoregulation[20]. The proportion of genes coding for TCS within a bacterial genome is thought to be dependent on a range of factors, including genome size and the diversity of different environments the bacterium may encounter[21]. Generally, histidine kinase and response regulator genes are co-transcribed in an operon, but they can also exist in the genome separated from their cognate partner, and are defined as orphans[22]. In *A. baumannii*, genomic analyses have identified 12 response regulator genes in an avirulent isolate that increases to 16 to 19 in pathogenic isolates[23]. Of these, only a handful have been experimentally examined[24–29].

An aspect of my research has focused on the AdeRS TCS which was originally identified as a regulator of the AdeABC three component efflux system in many *A. baumannii* clinical isolates (Figure 2*a*). When overexpressed, AdeABC exports a broad range of structurally-unrelated antimicrobials including antibiotics, biocides and dyes[25,30–34]. Importantly, within this group of substrates are compounds from the carbapenem class of antibiotics as well as tigecycline, one of the last lines of defence against *A. baumannii*[35]. Analysis of many multidrug resistant *A. baumannii* isolates has shown a high incidence of mutations or the presence of insertional elements in the AdeRS regulatory system, deeming this TCS to be a significant contributor to the observed multidrug resistance phenotype[11,36,37]. At a genetic level, the *adeRS* genes lie adjacent to *adeABC*, but are divergently transcribed (Figure 2*b*). The AdeR response regulator protein binds to a 10 base-pair direct repeat DNA sequence and modulates *adeABC* expression[38]; however, the external signal(s) that interact with the AdeS histidine kinase remain unknown. Aside from changes in antimicrobial resistance, deletion of *adeRS* seen in some clinical isolates, has identified significant alterations in persistence strategies, such as

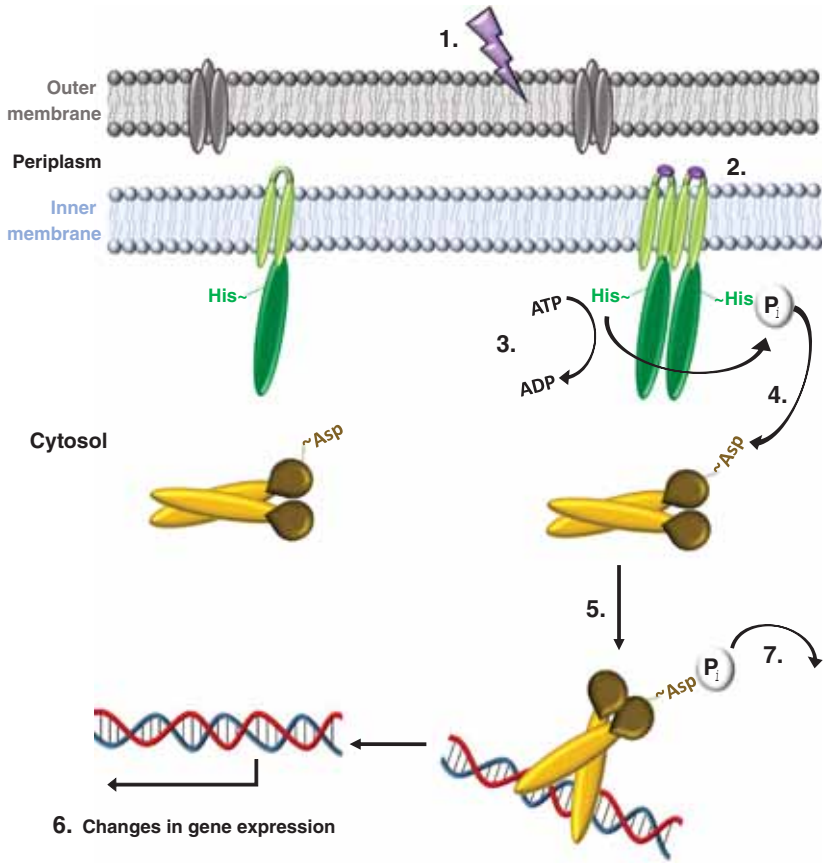

Figure 1. Schematic representation of a typical two component signal transduction system cascade in Gram-negative bacteria. Two component signal transductions systems consist of a histidine kinase (HK) and response regulator (RR) protein (green and yellow, respectively). HK proteins are generally localised in the inner membrane and possess a variable N-terminal sensing domain and a highly conserved C-terminal kinase region (light and dark green, respectively). In contrast, cytosolic RR proteins contain a highly conserved N-terminal domain and a variable C-terminal output domain (brown and yellow, respectively). The HK detects the presence of an external stimulus (1). Binding of the stimulating agent induces a conformational change in the HK (2) resulting in trans-autophosphorylation between HK homodimers whereby one monomer catalyses phosphorylation using ATP of the conserved histidine (His) residue in the second monomer (3). This phosphate (P_i) is subsequently transferred to the highly conserved aspartate (Asp) residue on the RR protein (4). Phosphorylation of the RR induces conformational changes that alters its DNA binding properties (5) modulating target gene expression (6). Resetting the system to pre-stimulus state is attained by de-phosphorylation of the RR (7) through phosphatase activity of the HK or by other phosphatase enzymes.

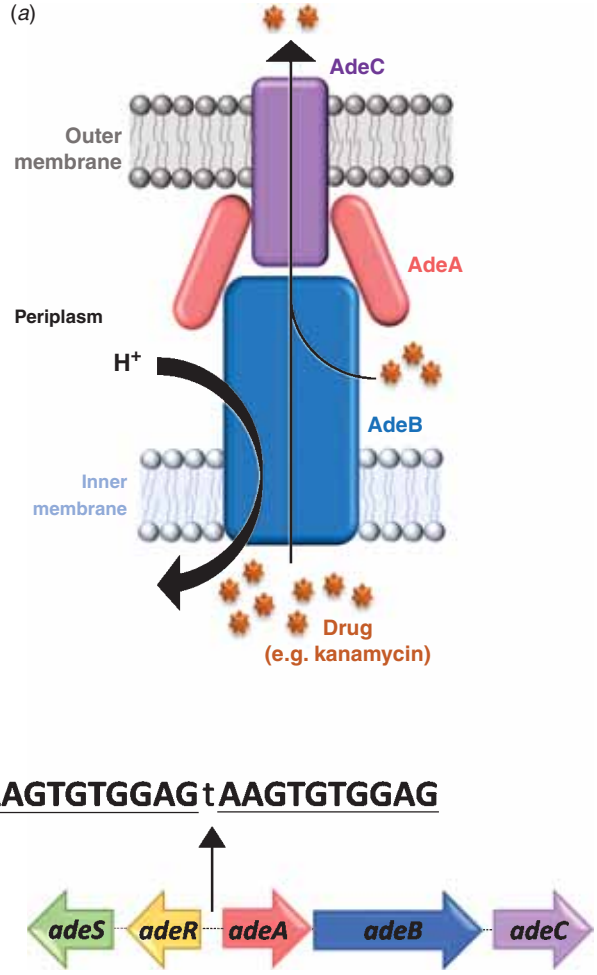

Figure 2. Composition of the AdeABC tripartite pump and genetic organisation of the *adeRS* and *adeABC* operons typically found in the *Acinetobacter baumannii* membrane and chromosome, respectively. (*a*) The pump is constituted of three proteins; AdeB the cytoplasmic membrane transporter (blue), AdeA the membrane fusion protein (pink) and AdeC the outer membrane protein (purple), that come together to form a functional complex. Activity of the AdeABC pump is coupled to the proton gradient (H^+), where substrates of the pump, e.g. kanamycin (orange stars), can be directly expelled into the external environment. (*b*) The *adeRS* genes lie adjacent to the *adeABC* genes and are divergently transcribed (arrows indicate the coding sequence and the direction of transcription). Located within the intergenic region between *adeR* and *adeA* is a 10 base-pair direct repeat, separated by a thymine nucleotide. This repeat is predicted to be where the AdeR response regulator binds to modulate *adeABC* gene expression.

the production of an extracellular protective matrix known as a biofilm[32,33].

Many clinical *A. baumannii* isolates harbour different genetic arrangements of the *adeRS* and *adeABC* operons[39]. Examination into one *A. baumannii* clinical isolate identified that insertional-inactivation of the outer membrane component of the pump (AdeC) did not affect resistance towards two previously identified AdeABC substrates[25]. It was suggested that in the absence of AdeC, AdeAB can utilise an alternative outer membrane protein to form a functional tripartite complex[25]. Given that AdeC may not be essential to confer antimicrobial resistance, and the diverse genetic arrangements of these operons across clinical *A. baumannii* isolates, a key aim of my studies was to (1) ascertain whether clinical isolates which naturally lack *adeC* also confer antimicrobial resistance and to (2) determine if the previously observed regulatory properties governed by AdeRS are also maintained. The well characterised *A. baumannii* 'type' strain ATCC 17978[40] isolated from an infant with fatal meningitis, which does not carry *adeC*, was chosen for genetic manipulation. Through double homologous recombination techniques, mutant derivatives targeting either *adeRS* or *adeAB* genes were generated and compared to the ATCC 17978 parent. Antibiogram analysis of the *adeAB* mutant identified changes in resistance to a subset of structurally related antimicrobials, including a commonly utilised clinical disinfectant. The role of the AdeRS TCS in modulating expression of *adeABC* is of current debate. As deletion of *adeRS* exhibited similar resistance levels to the *adeAB* deletion strain, my research supports the hypothesis that the AdeRS TCS activates expression of the *adeAB*(*C*) operon[25]. These research findings shed new light on the resistance capabilities of the AdeABC pump, questioning the views that AdeABC does not contribute towards the intrinsic resistance of *A. baumannii* and that antibacterial efflux can only occur when AdeABC is constitutively over-expressed[32].

To identify the effects of the deletion of *adeRS* on the global transcriptional landscape, RNA-sequencing methodologies were employed. Numerous changes in gene transcription levels were identified including expression of *adeAB*. Additionally, other genes known to be important for virulence, such as iron sequestering and pilus assembly operons were differentially expressed. AdeR has previously been found to bind to a 10 base-pair direct repeat only found within the intercistronic region between the *adeRS* and *adeABC* operons[38]. Genomic analyses within ATCC 17978 also support this finding. Therefore, in the *adeRS* deletion strain, aside from alterations in *adeAB* expression, the transcriptional changes in the aforementioned virulence associated genes are likely to be indirect. Interestingly, these direct/indirect transcriptional changes differed from an *adeRS* deletion mutant constructed in a different *A. baumannii* clinical isolate[33], emphasising that changes in the global transcriptional landscape are dependent on the isolate under investigation.

With a lack of currently effective antimicrobial treatments and a less than promising pipeline for the generation of new antibiotics, research into novel antimicrobial treatments is of significant interest. Histidine kinases of TCS are seen as attractive targets, primarily due to their presence in many pathogenic bacterial species[16] and the absence of homologues in higher eukaryotes, including humans[41]. A number of novel inhibitors towards some TCS regulatory cascades present across a number of clinically relevant

pathogenic bacterial species have been identified[42]; however, no inhibitors have been recognised for TCS found within *A. baumannii*. Results from this research area have made promising leads but progress is slow and many challenges still remain[42].

In recent decades, *A. baumannii* has fast become an extremely problematic hospital-acquired pathogen, propelled by its ability to flourish within hostile clinical environments and accrue resistance to the current armamentarium of therapeutic treatments. The AdeABC efflux system is a known contributor to the multidrug resistance phenotype displayed by this organism. My research into this system has identified that AdeAB in ATCC 17978 is functional despite the absence of AdeC and can provide intrinsic antimicrobial resistance, albeit to a limited substrate range. Antibacterial drug research efforts over recent decades have highlighted the eligibility of targeting TCS regulatory cascades for the development as an alternate therapy to treat bacterial infections. In light of this research, the AdeRS system holds particular interest due to its direct role in regulating a key aspect of multidrug resistance in many clinical *A. baumannii* isolates. Further examination into the AdeRS TCS is required, particularly identifying the activating stimuli of this system. This knowledge may be instrumental in the identification of novel inhibitors, which could aid in the future treatment of infections caused by this formidable pathogen.

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Biography

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From isolate to answer: how whole genome sequencing is helping us rapidly characterise nosocomial bacterial outbreaks

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The occurrence of highly resistant bacterial pathogens has risen in recent years, causing immense strain on the health-care industry. Hospital-acquired infections are arguably of most concern, as bacterial outbreaks in clinical settings provide an ideal environment for proliferation among vulnerable populations. Understanding these outbreaks beyond what can be determined with traditional clinical diagnostics and implementing these new techniques routinely in the hospital environment has now become a major focus. This brief review will discuss the three main whole genome sequence techniques available today, and how they are being used to further discriminate bacterial outbreaks in nosocomial settings.

In recent decades, society has witnessed a rapid and alarming increase in highly resistant pathogens causing human disease, to the extent that the World Health Organization (WHO) has labelled antimicrobial resistance (AMR) as, 'a serious threat to global public health'[1]. The challenge of AMR is arguably greatest in health-care facilities, which present a unique environment for pathogens to proliferate and infect those most vulnerable. Once within the hospital, these pathogens can be readily spread due to the close proximity of patients and the mass of shared vectors in the environment (including bathrooms, wards, healthcare workers, trolleys, etc.). With limited treatment options, patient outcomes during these nosocomial outbreaks decline, leading to increased mortality and a substantial economic burden[2–4]. Traditional techniques employed in routine hospital diagnostics focus mainly on phenotypic and gene-centric analyses, which are useful for inexpensive and rapid surveillance in the preliminary stages of an outbreak. However, these techniques are unable to render fine-scale analyses capable of detecting transmission pathways and otherwise unexpected epidemiological connections. As such, new high-resolution technologies, such as whole genome sequencing (WGS), are now being used in response to outbreak investigations. This article will discuss the three main WGS technologies available today and briefly review their existing and future impact in clinical settings.

Short-read sequencing in nosocomial settings

Many hospitals worldwide already appreciate the power of WGS analysis, having integrated it into several published investigations[5–7]. The advantages of WGS are most accessible through the use of short-read sequencing as exemplified by the Illumina platform. In addition to being both high-throughput and cost-effective, analysis tools specific for short-read sequencing are well established, making it a useful and reliable research tool. One of the main advantages of WGS is the ability to detect single nucleotide variants (SNV). Detection of SNV can allow prediction of phenotypic changes, such as enhanced resistant to antibiotics (for example, via the loss of a functional outer membrane porin cause by indels or point mutations[8–10]). When coupled with metadata, SNV data can also be used to predict transmission pathways by inferring transmission directionality via the accumulation of SNV over time. Short-read data are also routinely used to determine presence or absence of genes using read mapping or short-read assembly techniques.

Carbapenem-resistant Enterobacteriaceae (CRE) are among the most prevalent clinically relevant organisms, designated as an urgent threat by the Centers for Disease Control and Prevention[11]. Snitkin *et al.*[12] were amongst the first to apply WGS to identify transmission pathways in a CRE outbreak that could not be resolved using traditional epidemiological investigations alone. Recently, we have used WGS to investigate a suspected CRE outbreak in a Brisbane hospital[13]. Using Illumina short-read sequencing, we were able to determine transmission of a carbapenemase-producing *Enterobacter cloacae* sequence type (ST) 90 strain within the intensive care unit (ICU) between three patients over a three-month period. Of the 10 isolates analysed, we detected only

4 SNV overall, indicative of direct transmission. Patients 1 and 3 were found to have identical isolates at the core genome level, despite not being in the ICU at the same time. This suggested a probable environmental source of the infection, rather than direct patient-to-patient transmission. This was supported by comparison of the isolates to publicly available genomes, which identified a near-identical *E. cloacae* from 2013 (isolated in the same ward and hospital). This *E. cloacae* differed by only one SNV from the 2015 isolates, suggesting a reservoir in the hospital environment since at least 2013. Despite efforts to identify the environmental source in the hospital through extensive screening of all possible environmental vectors (excluding healthcare workers), a source was not found. Overall, WGS was able to accurately determine the relationship of *E. cloacae* over the course of the outbreak, providing unambiguous evidence of environmental cross-transmission over at least two years, prompting further surveillance and re-imposing infection control standards.

Beyond clonal transmission: tracking mobile genetic elements with long-read sequencing

The most significant drawback of using short-read sequencing is the inability to accurately characterise mobile genetic elements (MGE) such as insertion sequences (IS), genomic islands and plasmids. MGE are often associated with important elements such as virulence factors and antibiotic resistance genes[14–16], but are also known to comprise numerous repetitive regions, the main culprit being IS[17]. These repetitive regions are unable to be traversed by short-read sequencing, causing 'collapsed repeats' in final assemblies that ultimately impedes the contextualisation of important genomic regions[18–20]. While tools exist to patch together these assemblies, such as Bandage[21], increasing the sequence read length to span these repetitive regions is the only unambiguous solution for resolving MGEs[22].

Currently, the most established long-read sequencing technology is Pacific Biosciences (PacBio) Single Molecule Real-Time (SMRT) sequencing. This technology can now routinely provide complete bacterial chromosomes and plasmids[23], allowing contextualisation of important genomic regions, tracking of plasmids and completion of high-quality reference genomes[24]. One of the most progressive examples of PacBio integration into clinical settings comes from Mount Sinai, New York, where Sullivan *et al.*[25] successively sequenced 137 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates using PacBio to allow high-precision surveillance and outbreak control. In our investigation of the 2015 carbapenemase-producing *E. cloacae*, we used PacBio SMRT sequencing to completely characterise a large ~330 kb IncHI2 plasmid carrying a complex ~55 kb multidrug resistant (MDR) region that contained the carbapenemase gene bla_{IMP4}. Once characterised, we were able to undertake broader epidemiological surveillance for this plasmid, resulting in its identification in *E. cloacae* patient isolates (and an *Escherichia coli*) from other South-East Queensland hospitals. Comparison of our plasmid to publicly available data also found a remarkably similar plasmid carried by a *Salmonella enterica* isolate from a cat[26]. Similarly, Sheppard *et al.*[27] were able to use a combination of long- and short-read sequencing to track bla_{KPC} positive Enterobacteriaceae in a single hospital over 5 years, ultimately showing the promiscuity of bla_{KPC} within several different hosts and in several different plasmids. These studies highlight the importance of tracking not only outbreaks focused on clonal transmission, but also MGE transmission between bacterial strains or species in hospital settings (Figure 1).

While this technology is effective at producing complete genomes, it does come at a price. A single bacterial genome with PacBio SMRT sequencing can cost 20 times that of an Illumina sequence[28]. Additionally, the amount of DNA required, the time needed for the library preparation (Figure 2), as well as the relatively low throughput system, makes routine sequencing with PacBio less attractive than short-read sequencing due to the time required per sample. Ultimately, to implement long-read sequencing into the clinical setting, the turn-around time from sample isolation to analysis results needs to be within a workable timeframe.

Nanopore: a new and emerging technology for real-time analysis

Oxford Nanopore MinION sequencing is a relatively new sequencing platform designed to couple long-read sequencing with a rapid turn-around time. Its portability and capacity to perform real-time analysis during sequencing has made it an attractive companion for outbreak investigations in remote regions, as was the case with the ZIBRA project[29]. Clinically, MinION sequencing has been used to retrospectively analyse *S. enterica* isolates in relation to a European-wide outbreak[30], which showed that the outbreak strain could be identified from less than 2 hours of sequencing, despite not having the resolution to elucidate transmission pathways. Viral pathogens have also been identified from clinical metagenomic samples, with a projected turn-around time of less than 6 hours[31]. While this new technology is promising, some hurdles remain before MinION sequencing can be used routinely. These include the lower base level accuracy, as well as the current difficulty in sequencing more than 12 isolates in parallel (as compared to Illumina)[28,32].

Overall, WGS technology has advanced considerably in the last decade, with an equivalently rapid decline in price[33–35]. While still

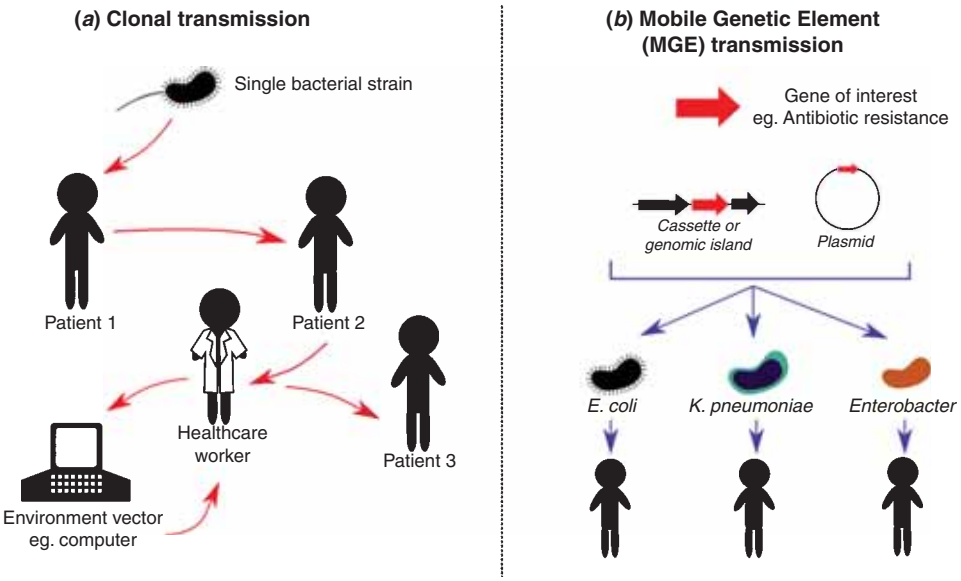

Figure 1. Comparison between clonal transmission (*a*) and transmission of mobile genetic elements between different bacterial species (*b*).

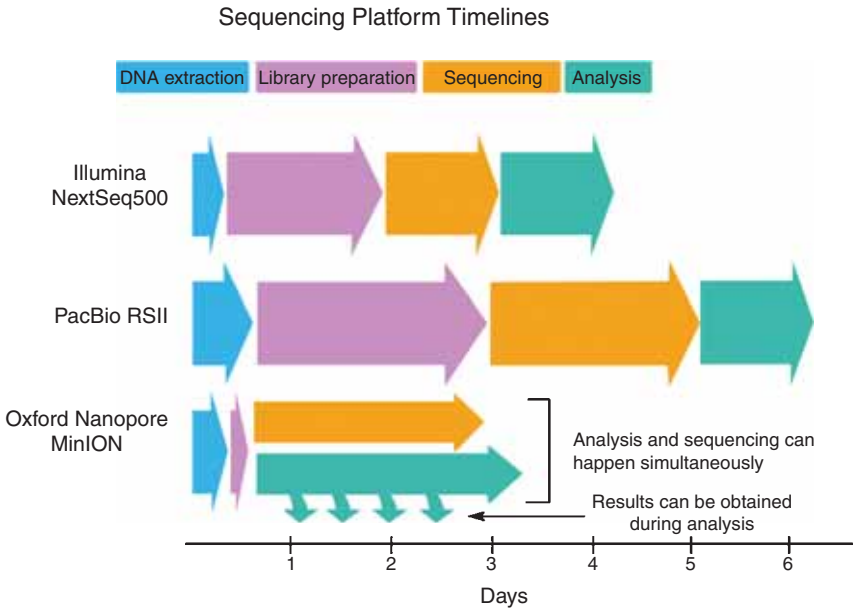

Figure 2. Approximate timelines from DNA extraction to analysis for three major sequencing platforms.

being more expensive than traditional diagnostics, if successful in preventing further transmission during an outbreak this cost becomes negligible compared to the cost of continued patient treatment and repeated infection control. WGS also retrospectively provides a large wealth of information, allowing the hospital to progressively catalogue past and ongoing infection occurrences, ultimately providing a highly detailed epidemiological map of pathogen movement in the hospital and from the community that can quickly be referred to in the case of new infections. Implementation of WGS in response to an outbreak guarantees an in-depth high-resolution analysis that cannot be determined using traditional phenotypic and genotypic methods alone. Currently, all three sequencing technologies presented in this article can be used complimentarily to produce a comprehensive outbreak understanding and provide ongoing genomic surveillance of the strain or element. Routine implementation of WGS in healthcare settings will undoubtedly become widespread in the near future, aiding clinicians, patients, infection control and researchers alike.

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Biography

Leah Roberts is currently a third-year PhD candidate completing her studies at the University of Queensland, Brisbane, Australia. Under the supervision of Associate Professor Scott A Beatson and Professor Mark A Schembri, her research interests have mainly focused on the use of whole genome sequencing and its application in clinical settings. Using a range of sequencing technologies such as Illumina, PacBio and Nanopore, Leah has analysed a variety of bacterial outbreaks, including *Acinetobacter baumannii, Klebsiella pneumoniae* and *Enterobacter cloacae*. She has presented her work at both domestic and international conferences, and has won several awards including the 2016 ASM BD award for Queensland, as well as the 2017 Applied Bioinformatics and Public Health Microbiology (ABPHM) student poster prize.

Necrotic disease in bivalve larval cultures



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The health of marine bivalve larvae is greatly affected by bacteria in the environment particularly when reared in marine hatcheries. This is generally because high stocking densities resulting in high organic loads of both food and faeces, can support increased bacterial growth and biomass levels. Increased bacterial load can lead to larval disease referred to as bacillary necrosis (BN) leading in turn to rapid larval mortality and loss of production. Despite more than 50 years since the first detailed description of BN, we still do not fully understand its causes and mechanisms. Through the manipulation of a model larval culture of the Australian blue mussels (*Mytilus galloprovincialis*), we determined that BN is linked with rapid and systematic changes in the bacterial community.

Early investigation of larval mortality in bivalve larval cultures in the 1950s reported mortality associated with infection by gram negative bacilli that necrotised larval tissues, leading to the descriptive term bacillary necrosis (BN)[1]. The disease is capable of causing total collapse of larval cultures (larval crash) in a period of 24–48 hours and is today, the most prevalent hatchery disease worldwide, affecting more than 20 bivalve species. Whilst it is difficult to quantify the impact of BN in shellfish hatcheries, frequent recurrence can severely impact hatchery production with repercussions often felt throughout the supply chains.

The prevailing view that BN is an opportunistic disease leads to the emphasis on sound husbandry practices primarily to reduce excess build-up of organic matter. However, whether and how enriched organic conditions are linked with development of BN is unclear. Efforts to study BN have also been complicated by the unpredictable nature of the outbreaks. To address this problem, we deliberately overfed a series of identical small-scale larval cultures with microalgae to create an environment that would increase the incidence of the disease. In cultures that developed mass mortalities, automated ribosomal intergenic spaces analysis (ARISA) demonstrated that BN involves rapid and systematic changes in the bacterial community, firstly in the seawater, then rapidly proceeding to the larvae as the disease progresses and necrosis occurs (Figure 1). This study shows that, at least within the system analysed here, BN is a condition of abnormal changes in seawater-associated communities that are capable of affecting the larvae, suggestive of seawater-to-larvae infectivity. The similarity of bacterial communities in seawater and larvae at the onset of mortality suggest swamping by outgrowth of particular bacteria. Bacterial diversity examination using Illumina MiSeq sequencing of 16S rRNA amplicons showed that mortality in the model systems was linked with a bacterial community increasingly dominated by *Psychroserpens*, *Polaribacter*, *Marinomonas*, and members of the Candidatus phylum *Gracilibacteria*.

The observation that BN did not occur in all overfed cultures suggests variability in causation made it difficult to detect and control in small-scale larval cultures. In one instance, a replicate culture in which the initial development of BN was observed,

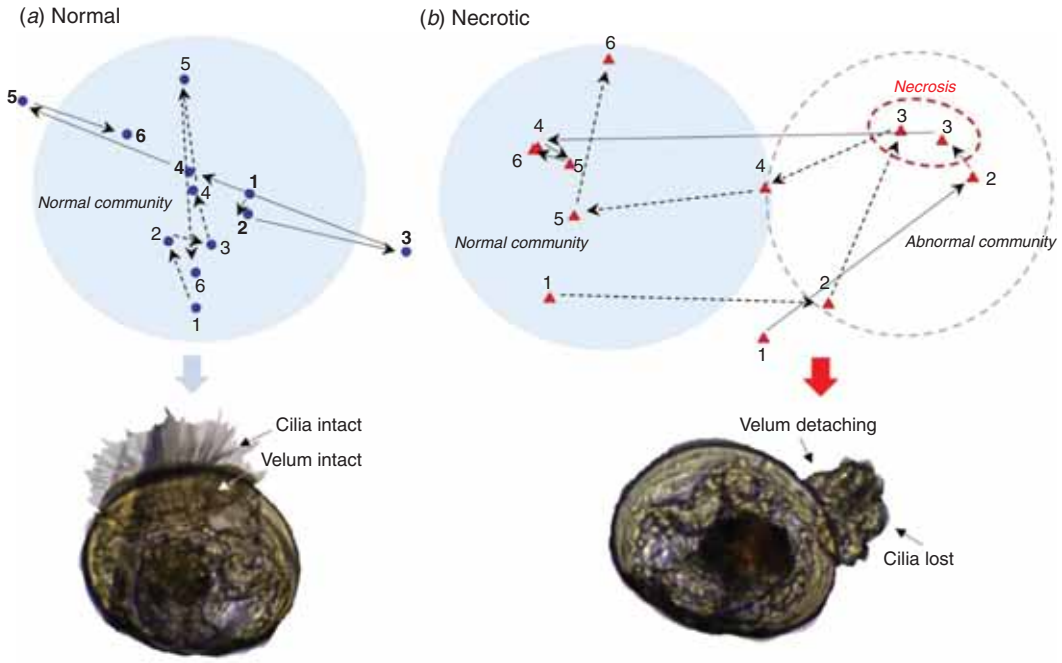

Figure 1. Comparison of bacterial communities for (*a*) normal and (*b*) necrotic conditions of larvae respectively. The solid and dotted line trajectories track shifts of bacterial community associated with seawater and larvae respectively, from day 1 to 6 of larval culture. Overfeeding can unpredictably trigger development of abnormal communities (*b*, grey dotted circle) with necrotic cultures showing communities that differ from those of normal cultures (*a*, faded blue circle). The development of necrosis (*b*) takes place first in the seawater communities where shifting occurred as early as day 1 of rearing and progressed towards the disease zone on day 2 and 3. The larval communities however, show a slower progression since at day 1 the communities were closer to the normal zone and required more than 2 days to reach the necrosis zone. Larval necrosis/mortality is characterised by the convergence of seawater and larval bacterial communities (red dotted circle) to a specific, but potentially variable disease-state community. The post mortality community was restored rapidly to the initial normal state. Necrotised larvae showed an abnormal velum that was clumped and de-ciliated and eventually detached from larvae.

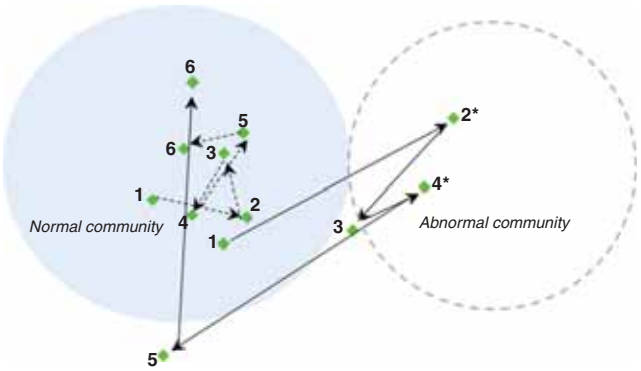

Figure 2. Bacterial communities of a larval culture that observed the initial shift of the seawater community (solid line trajectory) from normal to abnormal community zone at rearing day 1 and 2 respectively. The larval culture which was developing bacillary necrosis based on trajectories of seawater bacterial community shift was successfully mitigated after two rounds of 100% water changes at rearing day 2 and 4 (marked with asterisk; water changes were carried out after sampling for bacterial community analysis). It was observed that each of the seawater changes diverted the shift and the community progressively became closer to the normal community zone. The larval bacterial community (dotted line trajectory) remained unaffected and changed within the normal community zone.

actually had the first rearing day of normal seawater community as starting inoculum, but rapidly deviated to abnormal community in the next 24 h period (Figure 2). This suggests the seawater community characterised using ARISA fingerprinting technique may have missed the low concentration of genotypes that later emerge and drive the community to become abnormal. It appears that seawater is an important reservoir of diverse bacteria that play a critical role in the variability of BN. The link between bacterial diversity and the sporadic nature of BN is not easily established, partly due to technical limitations in resolving strain-level variation occurring in low concentration.

Currently, commercial hatchery operators still lack specific and effective means to mitigate bacillary necrosis because of the overall lack of understanding of its development process. Water changes are an important part of larval rearing activity, mainly because they help reduce organic matter build up in cultures therefore controlling the bacterial concentrations. However, there is no direct

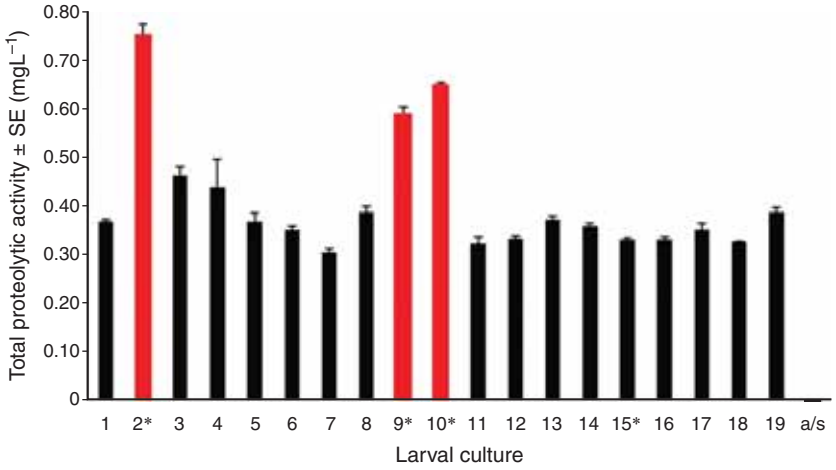

Figure 3. Protease activity (trypsin equivalent) detected in 19 replicate larval cultures. Red indicates a significant increase in protease activity (ANOVA F = 78.08, d.f. 19, $P < 0.001$) in 3 of 4 cultures suffering mortality in excess of 70% due to bacillary necrosis (marked with asterisk). Contingency analysis demonstrated significant association of total proteolytic activity and mortality (Fisher's test, $P < 0.005$). a/s denotes aged seawater. Protease activity assessed by EnzChek fluorescence protease assay kit (Thermofisher).

demonstration of how water changes can help mitigate BN. This study shows 48 h interval water changes, currently regarded as a common practice for static culture systems, can be effective if carried out before abnormal community changes are detected in the larvae (Figure 2). In cultures that suffered more rapid BN (such as Figure 1*b*), water changes did not alter the trajectory of abnormal communities.

Challenge bioassay studies demonstrate that necrotic properties of BN can be attributed to proteolytic activity of bacteria[2]. However, it is unclear how proteolytic activity is involved with BN in larval culture. A different larval culture experiment observed high microbial proteolytic activity in 3 of 4 larval cultures suffering mass mortalities (Figure 3). This suggests microbial proteolytic activity is an important disease mechanisms given that protease production in bacterial extracellular products (ECPs) is common in some seawater bacteria (such as *Polaribacter* and *Marinomonas* detected in the earlier study) and has been demonstrated to be the major virulence factors of multiple pathogenic *Vibrio* strains associated with BN[3]. However, the lack of association of proteolytic activity in one of the BN affected larval cultures (i.e. culture 15 of Figure 3) is interesting and may suggest diversity in bacterial community and in the mechanisms that lead to mortality. More work is necessary to confirm this preliminary association, particularly monitoring how seawater proteolytic activity changes with bacterial community shifts and leads/does not lead to mortality. Such work may demonstrate the potential of using protease assays as a method to assess the risk of bacillary necrosis. Once validated, protease assays could be a useful detection method to complement molecular and culturing techniques.

Based on the model larval cultures, we were able to demonstrate that BN has links with systematic bacterial community changes suggestive of a seawater-to-larvae infection. However, how this model understanding translates to commercial scale cultures requires more research. This study also described the potential of protease monitoring to aid future BN studies.

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Biographies

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Maternal Group B Streptococcus colonisation



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***Streptococcus agalactiae*, commonly known as Group B Streptococcus (GBS), is an important neonatal pathogen known to cause sepsis, meningitis and pneumonia. Australian pregnant women undergo screening during pregnancy in an effort to eradicate GBS before delivery where transmission to the neonate can occur. Preventative treatment includes intrapartum antibiotic prophylaxis and results in widespread treatment of the 10–40% of pregnant women colonised. GBS are separated into ten different capsular polysaccharide serotypes and previous studies have suggested associations between specific serotypes and disease. At present, however, minimal data exist on serotype distribution within Western Australian-pregnant women, information that may play an important role in future prophylactic treatment regimens. Our preliminary data, obtained from GBS isolated from vaginal swabs from 191 pregnant women, suggests that GBS serotype distributions in Western Australia are different to other parts of Australasia. In particular, compared to the eastern Australian states and New Zealand, in our cohort, serotype Ib prevalence was 7–17 times lower, II was 2–6 times greater and VI was 2–12 times greater. In addition, serotype IX represented 6.3% of all serotypes. Understanding which serotypes are present in our population will provide valuable data for future targeted treatment regimens such as vaccination and bacteriophage therapy.**

Group B Streptococcus during pregnancy

Neonates are among the most vulnerable forms of life, they enter this world with minimal immune defences and are faced with a vast array of opportunistic pathogens ready to colonise. One such organism is *Streptococcus agalactiae*, commonly known as Group B Streptococcus (GBS), which is responsible for morbidity and mortality in the immunocompromised, elderly and in particular, neonatal populations. GBS infection is a leading cause of sepsis and can also lead to meningitis, pneumonia, shock and even death[1,2]. It is understood that transmission of this organism can occur from a commensally colonised mother to her baby during birth, *in utero* (vertical) or alternatively through nosocomial transmission once born (horizontal)[3]. In an effort to prevent infant GBS infection, risk-based and culture-based screening of pregnant women followed by intrapartum antibiotic prophylaxis has been introduced in a number of countries globally[4]. In Australia, pregnant women are screened for presence of GBS several weeks before expected delivery to determine colonisation status. If a patient is found to carry GBS, antibiotics are administered prior to delivery in an effort to eradicate the organism before the neonate is exposed.

Serotypes

Global carriage rates among pregnant women are estimated at 10–40% which results in widespread antibiotic use in this population[4–6]. Due to contraindications of a number of drug classes during

pregnancy the antibiotics of choice include penicillin or if the woman is sensitised, cephazolin or clindamycin[4]. Penicillin resistance has rarely been described, however, clindamycin resistance is rising and has been reported recently in Australia[7]. Our current culture detection gives a presence/absence result and does not define characteristics of colonisation such as serotype. GBS are encapsulated and have a capsular polysaccharide (cps) locus that determines one of 10 serotypes (Ia, Ib, II–IX)[8–10]. Global distributions of these serotypes have shown variation in each region: for example, most countries have cps types Ia, Ib, II, III and V as the most common, although Japan has found prevalence of cps VIII, which globally is considered rare[11–13]. The capsule is considered an important virulence factor and some serotypes are associated with invasive disease more so than others[14]. For example, cps III has been observed in association with neonatal bloodstream infection, while cps V more so in cases of adult disease[15]. Understanding serotype distribution and its role in disease may improve the way we treat women during pregnancy.

GBS in Western Australia

Our research aims to determine which serotypes are prevalent amongst Western Australian pregnant women and explore alternative targeted treatment options. Our study is currently recruiting 1000 pregnant women at King Edward Memorial Hospital, Perth, Western Australia and collecting vaginal and rectal specimens at 14–22 and 34–38 weeks' gestation. The specimens are cultured and PCR tested for GBS presence and common serotypes Ia, Ib and III using our novel multiplex qPCR assay[16]. Other remaining serotypes are confirmed through methods described by Imperi *et al.*[17]. Initial retrospective studies of vaginal specimens from the UPCAN study[18] found interesting results compared to those previously reported in Australasia (Figure 1). The main differences in serotypes compared to other studies are seen for cps Ib, II, VI and IX in our WA cohort. We have identified a lower incidence of common serotype cps Ib and higher incidence of cps II, VI and IX. It must be noted, however, that a number of these previous studies had not tested for cps IX due to it only being proposed as a new cps type in 2009[10]. Comparison of cps IX to the Australia-wide study by Ko *et al.*[6] is appropriate, as testing for this new serotype was included, but no cases were detected.

Clinical impact and future directions

Monitoring of GBS strains within the pregnant community generates clinically useful information about this pathogen and can equip us for future targeted prevention and treatment strategies. For example, vaccination development targeting the capsule has now progressed with a number of candidate vaccines targeting multiple cps types such as Ia, Ib and III[24]. Knowledge of prevalent serotypes could impact our vaccination strategy as we discover differences in serotype distribution amongst different geographical populations. Another alternative targeted therapy that we are researching is bacteriophage therapy. The major principle behind this is that the specificity and lytic activity of these bacterial viruses could provide a targeted GBS treatment solution that would concurrently help to prevent emerging antibiotic resistance and microbiome dysbiosis, in addition to avoiding the unknown

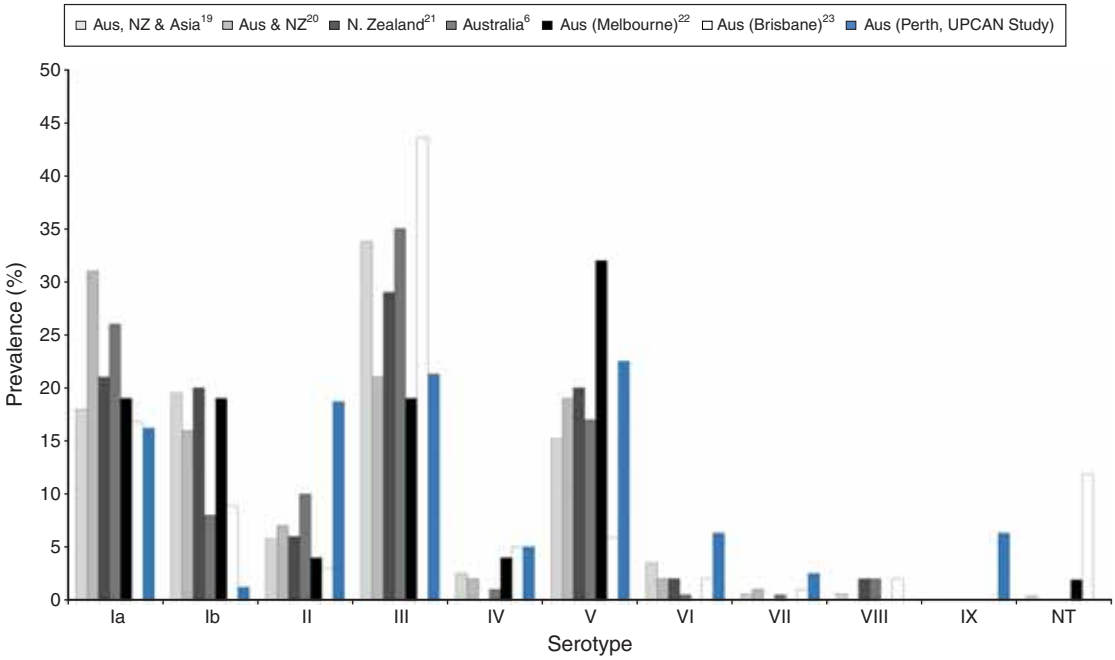

Figure 1. Prevalence of 10 *Streptococcus agalactiae* serotypes (Ia, Ib, II–IX) and non-typeable (NT) from previous studies across Australasia[6,19–23] compared to our preliminary Western Australian data.

impacts of antibiotic exposure on the newborn. We are currently isolating and testing novel bacteriophages for lytic activity against clinical GBS strains to assess future potential.

This research is all about defining our target in an effort to improve clinical detection and refining treatment strategies, to ensure we protect our vulnerable neonates.

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Biographies

Lucy Furfaro is a final year PhD candidate researching GBS dynamics in Western Australian pregnant women and a potential alternative treatment using bacteriophage therapy. She has developed a novel multiplex PCR assay to detect GBS and clinically relevant serotypes with the potential for diagnostic use.

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Exploring HIV latency using transcription profiling

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The major barrier to a cure for HIV is the existence of reservoirs consisting predominantly of latently infected CD4$^+$ T cells, which do not produce virus constitutively but can be induced to produce infectious virus on activation. HIV latency research has largely focused on peripheral blood, yet most HIV-infected cells reside in tissues, especially the gut, where differences in drug penetration, cell types, and immune responses may impact mechanisms of persistence. Exploring the differences between the gut and the blood in transcriptional blocks may reveal fundamental insights into mechanisms that contribute to HIV latency. Our novel transcriptional profiling assays enable us to determine where blocks to HIV transcription occur in various tissues and the magnitude of their contribution. These assays could also be adapted to investigate latency established by other retroviridae or even DNA viruses such as herpesviridae with a view to pinpointing mechanisms underlying latency *in vivo* and ultimately contribute to designing a cure.

Probing HIV in the gut

HIV remains a major pandemic, with more than 36 million people affected worldwide. Over 1.1 million people in the US are currently living with HIV. In Australia, increased awareness and high profile health promotion campaigns have been unsuccessful in reducing the number of new infections, which have remained steady over the last few years[1]. Despite the success of combination antiretroviral therapy (ART) in suppressing HIV-1 replication, ART is not curative and residual virus continues to cause immune activation, organ damage, and reduction in life expectancy[2,3]. HIV-1 evades ART and immune responses through latent infection of CD4$^+$ T cells[4–7]. Since these latently infected cells do not produce HIV proteins, they escape viral cytopathic effects and evade detection by the immune system. Latent HIV has been primarily found in long-lived memory CD4$^+$ T cells, which can survive for decades and expand the viral reservoir by cell proliferation[8–11]. These latently infected cells are considered to be the main barrier to HIV eradication[4] and their reactivation *in vivo* likely contributes to sustained immune activation observed during suppressive ART[12].

Although much HIV latency research utilises *in vitro* models or cells from peripheral blood, prior work has highlighted differences between the gut and blood in the phenotype of infected T and non-T cells[12,13]. Furthermore, gut and blood compartments differ in levels of T cell activation and its relationship with HIV transcription[12]. Considering that the gut harbors up to 85% of all lymphoid tissue and over 90% of all lymphocytes[14,15], it is imperative to investigate how mechanisms of HIV persistence and latency differ between gut and blood *in vivo*. To this end, we are employing a cutting-edge 'transcription profiling' approach, which features a novel panel of highly conserved, sensitive, quantitative reverse transcription droplet digital PCR (RT-ddPCR) assays. This approach quantifies the levels of HIV transcripts that suggest different mechanisms of transcriptional blockade and/or progression though various stages of HIV transcription. The levels and ratios of different HIV transcripts can be used to determine the degree to which different mechanisms contribute to reversible inhibition of HIV gene expression, and hence latency, in cells from HIV-infected individuals.

Exploiting transcriptional features of HIV

The compact genome of HIV features major coding regions, including: 1) *gag*, *pol* and *env*, common to all retroviruses, which

encode essential structural proteins (such as envelope, matrix and capsid) and critical enzymes, including protease (catalyses cleavage), reverse transcriptase (reverse transcribes RNA genome into double-stranded DNA) and integrase (mediates integration into host genome); 2) regulatory genes (*tat* and *rev)*; and 3) accessory genes (*vif*, *vpr*, *vpu* and *nef)* (Figure 1). A major putative mechanism driving HIV latency is transcriptional interference (TI), caused by ongoing transcription of host genes *in cis* that inhibit the assembly of the RNA polymerase complex on the HIV promoter region, the 5'-long terminal repeat (LTR)[16–19]. 'Read-through' transcripts (Figure 1) are suggestive of TI since they include the U3-U5 region that distinguishes them from canonical HIV transcripts. Other mechanisms that can lead to a block to HIV transcription initiation include epigenetic modification, a lack of host initiation factors[18,20], suboptimal activity of the viral transcription factor Tat[21] and integration into transcriptionally silent regions of the genome[18,22,23]. The degree of transcriptional initiation can be assessed by detection of transcripts containing the 'transactivation response' (TAR) element, which is the RNA target of Tat protein and is present in all HIV transcripts (Figure 1). Our 'TAR' assay has been specifically designed to maximise the detection of short, prematurely terminated transcripts with an efficiency equal to longer transcripts[24,25] by incorporating an additional polyadenylation step that generates an accessible priming site for reverse transcription. This strategy offers a considerable advantage over other assays, which can detect only 4% of true short transcripts and thus significantly underestimate the abundance of these transcripts[24].

Other proposed mechanisms of HIV latency include downstream blocks to elongation due to the lack of host elongation factors, the presence of inhibitory factors, nucleosome conformation and insufficient Tat activity[21,26–28]. Such mechanisms can be evaluated by targets for downstream sequences (such as 'Long LTR') that indicate elongation past the TAR loop. To assess how efficiently transcription proceeds through *pol* to the 3'end, transcripts containing *pol* and *nef* target sequence are also detected by our panel of assays. Levels of polyadenylated HIV RNA ('PolyA'), indicative of transcription completion[29], are detected using primers that span the LTR (U3) and polyA tail. Polyadenylated transcripts can act as surrogate markers for HIV protein. Similarly, multiply spliced HIV RNA ('Tat-Rev'), heralding the completion of splicing, can serve as a surrogate for productive infection[30]. The levels of each distinct transcript and the ratios between them can be used to quantify the degree to which HIV transcription is inhibited *in vivo* by TI or blocks to transcriptional initiation, elongation, completion and splicing.

The novelty of this approach lies in the ability to simultaneously investigate multiple mechanisms of transcriptional blocks *in vivo*. Combined with RT-ddPCR, which enables absolute cDNA quantification[24], this approach provides a considerable advantage over previous work that mostly focuses on one mechanism of latency at a time and has typically utilised *in vitro* models of latency, which may not recapitulate what happens *in vivo*.[18–20,22,23,26–28]. Unlike previously employed strategies, specific blocks to transcription and the magnitude of their impact on the prevailing levels of HIV RNA can be simultaneously assessed by determining the expression of these processive transcripts. These data can then inform strategies to target latency reversal. Using matched tissues from ART-suppressed HIV-infected individuals, this transcription profiling approach is beginning to reveal differences between blood and gut in the blocks to HIV transcription, which is of particular interest due to the difficulty in accessing tissue samples and the subsequent

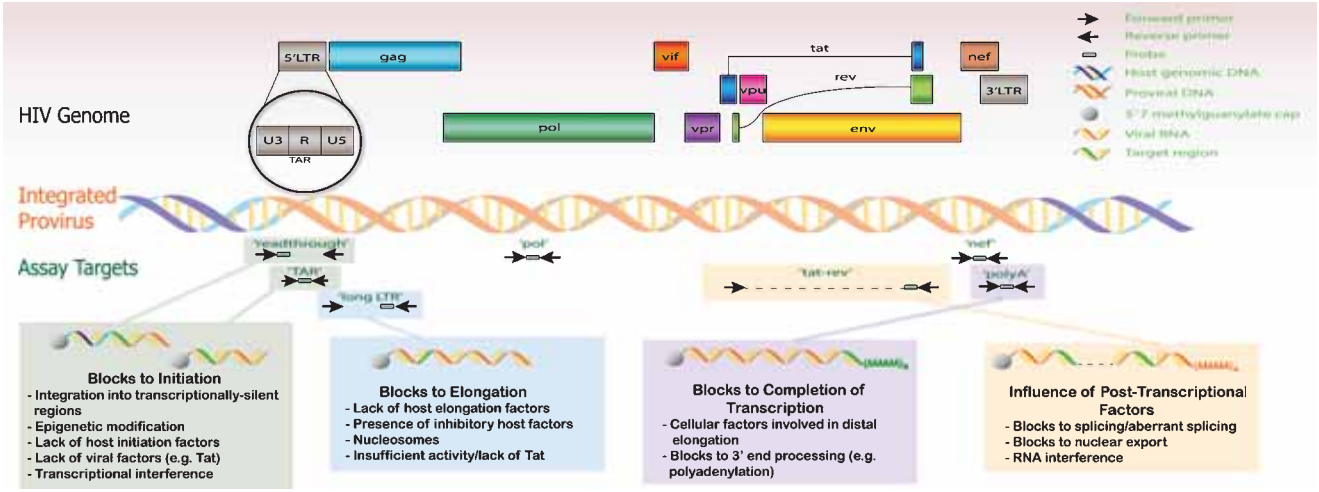

Figure 1. The HIV genome and the targets for transcription profiling assays. This schematic represents the genetic organisation of proviral HIV DNA and the HIV RNA assays that target specific sequence regions, which reveal insight into blocks to transcription. Some proposed mechanisms that underlie the blocks to transcription initiation and elongation are listed.

paucity of data examining HIV latency in the gut. This work, which contributes to elucidating the molecular mechanisms that govern HIV latency, may lead to new therapies aimed at curing HIV.

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Biographies

Sushama Telwatte is a Postdoctoral Scholar undertaking postdoctoral studies in the laboratory of Associate Professor Steven A Yukl at the Veteran Affairs Medical Center in San Francisco. Her work focuses on uncovering the mechanisms underlying HIV persistence and latency in the gut and blood *in vivo*. Sushama completed her PhD under the supervision of Associate Professor Gilda Tachedjian at the Burnet Institute/Monash University, investigating the role of synonymous mutations in HIV-1 selected during drug therapy. Sushama was awarded the ASM BD Award (Victorian Branch) and presented her PhD work at the ASM 2015 meeting.

Dr Steven Yukl is an Associate Professor of Medicine at the University of California, San Francisco (UCSF) and a staff physician at the San Francisco Veterans Affairs Medical Center. His research focuses on the mechanisms that allow HIV to persist despite immune defenses and antiretrovirals, thereby preventing HIV cure. Dr Yukl has been involved in basic, translational, and clinical research on HIV persistence since 2005, with specialisation in HIV latency, gut tissue reservoirs, cellular reservoirs, and development of ultrasensitive assays to measure HIV persistence.

Hidden reservoirs of hospital-associated infections

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***Klebsiella pneumoniae* (*Kp*) is a Gram-negative bacterium that is ubiquitous in the environment and is of increasing concern in public health. *Kp* can be carried asymptomatically as a commensal organism and can cause opportunistic infections in susceptible individuals; this is further complicated by an increasing incidence of multi-drug-resistant (MDR) strains. Given *Kp* can be carried asymptomatically, and can cause infections, it is possible that asymptomatic carriage acts as a reservoir for infection. Our recent work in Melbourne confirms this is often true. Individuals who tested positive for carriage of *Kp*, on admission to ICU, were over five times more likely to develop an infection during their hospital stay, compared to non-carriers. Whole genome sequence analyses revealed extensive diversity amongst the *Kp* infection-causing strains. These results indicate the majority of opportunistic infections are caused by patients' own microbiome strains that are already present on ICU admission. As such, screening of individuals on admission may enable clinicians to identify who is most at risk of developing infections during their hospital stay, and who is harboring drug-resistant strains that could transmit to others.**

During a one-year cohort study conducted at the Alfred hospital intensive care unit (ICU) in Melbourne, 498 patients were screened for gut carriage of *Kp* shortly following admission and were monitored for *Kp* infection[1]. The frequency of gut carriage was 10% overall, but only 6% amongst those who had no recent contact with healthcare prior to being admitted to ICU. Fifty ICU patients (1.85% of admissions) had one or more infections attributed to *Kp*, and 29 of these individuals were also screened for carriage. Bacterial isolates underwent whole genome sequencing (WGS) and comparative analyses were conducted, using strict thresholds of genomic similarity to identify potential transmission and to explore whether carriage strains matched those causing infections. Ten patients (34% of infection patients who were screened) were carrying *Kp* strains that matched their infecting strain (<0.0005% DNA sequence divergence); in six of these cases, the infection occurred at least two days after carriage was detected. Additionally, six patients developed infections with strains that were near-identical to strains previously detected in other ICU patients, consistent with intra-hospital transmission (Figure 1).

Not all infections could be attributed to prior carriage or transmission, mainly because not all ICU patients were screened for carriage (screening required informed consent from the patient or a close relative, which was not always obtainable). So for many patients it is not possible to tell whether they were carrying their infecting strain on ICU admission. However, the WGS analysis showed that these individuals were typically infected with unique strains; not closely related to strains elsewhere in the hospital. Hence it is likely that these unexplained infections were caused by the patients' own strains than new strains that they acquired in the hospital.

This study suggests that routine screening for *Kp* could identify patients at high risk of developing infections during their hospital stay. Some hospitals already have routine screening procedures of patients in place for carriage of various MDR bacteria, such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococci*, or carbapenem-resistant Enterobacteriaceae; some have reported a corresponding reduction in hospital-associated infections[2–5]. During the one-year period of this study, *Kp* infections were diagnosed by the clinical microbiology laboratory in 320 patients across Alfred Health (*unpublished observations*). Of these, 11% were caused by strains carrying extended spectrum beta-lactamase (EBSL) genes that confer resistance to third generation cephalosporins (*unpublished observations*), and a small number of these were additionally resistant to carbapenems (CP-R) (*unpublished observations*), which are typically the last line drug for treatment of Gram-negative bacterial pathogens[6]. Importantly, these genotypically ESBL and CP-R isolates displayed corresponding resistant phenotypes, when they underwent antimicrobial susceptibility profiling (*unpublished observations*). Whilst MDR infections are still in the minority, they are the most difficult to treat, having been estimated to cost billions of dollars in additional

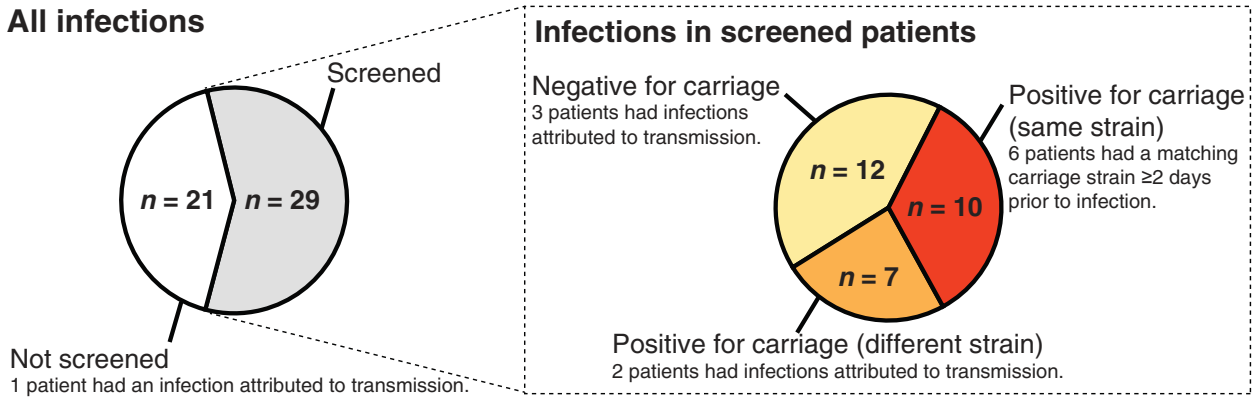

Figure 1. Infection numbers and attribution of infecting strain to carriage or transmission among individuals admitted to the ICU. Among the 50 ICU individuals with infections, six had infections that were attributed to transmission within the hospital. Ten patients had carriage and infection strains that were extremely closely related, including six patients for which the carriage strain was identified prior to infection. (Note: One infection isolate and two carriage isolates were excluded after genome sequencing and quality control.)

treatment costs and hospital stays, as well as thousands of deaths globally every year that would have been preventable had the infections not proved resistant to treatment[7–9]. Hence a screening strategy that looks for gut carriage of any MDR Gram-negative bacteria, which would detect *Kp* but also other common causes of hospital infections, could be of greatest clinical utility.

Important questions remain about how such information should be used. From an infection control standpoint, additional isolation measures could be taken to prevent transmission of MDR strains to other patients or healthcare workers. From a patient management standpoint, the drug susceptibility profile of known carriage strains could be used to guide the choice of antimicrobials used for therapy or prophylaxis[2,10]. First, this would help to avoid drugs that could select for overgrowth of resistant strains in the gut that could subsequently cause difficult-to-treat infections. Second, should an infection arise, foreknowledge of the likely strains and antimicrobial susceptibility profiles could be used to supplement empirical treatment protocols while awaiting laboratory confirmation of the susceptibility profile of the infecting organism.

The study demonstrates the value of WGS for fine-scale investigation of carriage-infection relationships, by clearly elucidating relationships between strains that cannot be distinguished by traditional laboratory typing techniques, e.g. multi-locus sequence typing or PFGE. As such, it is recommended that WGS approaches be implemented when investigating potential sources of infection, including for routine infection control as well as further research studies. Coupled with routine screening for carriage of MDR organisms, WGS could enable not just the identification of closely related strains but could also reveal the presence of mobile genes associated with antimicrobial resistance. Such genes can be readily transferred between species, hence knowledge of their presence could inform empirical treatment choices for Gram-negative infections generally and not just the isolated MDR organism. The WGS approach, with its continually developing sequencing and analyses platforms, is rapidly becoming more affordable and increasingly easy implement. Use of this technology in conjunction with, or in place of, traditional techniques could lead to much needed decreases in the numbers of hospital infections, particularly for difficult-to-treat MDR infections, leading to long term benefits both reducing the strain on the public health system and budget, but also for bettering patient outcomes.

Acknowledgements

Thank you to the team at Alfred Health who helped with various stages of this research; to the NHMRC for the funding grant that supported this work; to the team at the Wellcome Trust Sanger Institute for sequencing the genomes; and to my supervisors – Richard Strugnell, Adam Jenney, and Kathryn Holt.

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Biography

Claire Gorrie is a PhD student at The University of Melbourne. Her research combines whole genome sequencing, bacterial genomics, and epidemiology to investigate carriage-infection dynamics of *Klebsiella pneumoniae*.

Whole genome sequencing as a novel approach for characterising *Neisseria meningitidis* in Australia

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Neisseria meningitidis **(meningococcus) is the causative agent of invasive meningococcal disease that manifests as life-threatening septicaemia and/or meningitis. This review provides a brief overview of the prevention of the disease and also highlights the importance of whole genome sequencing (WGS) in detecting outbreaks of meningococci in Australia. The use of WGS in identifying the emergence of a penicillin-resistant cluster of meningococci is Western Australia is used as an example for advocating the implementation of WGS on the routine surveillance in Australia.**

Invasive meningococcal disease

Neisseria meningitidis, also known as the meningococcus (plural: meningococci), is a Gram-negative diplococcus that asymptomatically colonises the nasopharynx of approximately 10–30% of the adult population[1]. Occasionally, the bacterium crosses the epithelial layer and invades the host, causing invasive meningococcal disease (IMD) if the correct immune response is not elicited. IMD generally manifests as septicaemia and/or meningitis. Incidence of IMD follows a bimodal distribution with the first peak occurring in infants, due to lack of a mature immune system, and the second peak occurring in teenage years and early adulthood when the rate of transmission is highest due to lifestyle factors[2]. The onset of the disease is rapid and death may occur within 24 hours if the recommended antibiotics are not administered. Although IMD is rare, the mortality rate is approximately 10% and approximately 15% of survivors suffer from permanent sequelae such as limb loss and neurological disability[3,4]. Invasive strains of *N. meningitidis* express a polysaccharide capsule, which is used to classify the bacteria phenotypically into one of 12 serogroups – A, B, C, E, G, I, K, L, W, X, Y and Z[5]. IMD is predominantly caused by six serogroups (A, B, C, W, X and Y) and the distribution of these serogroups varies geographically and temporally[6,7].

Prevention of IMD

IMD can be prevented through vaccination. Although there is no vaccine available against the most recent disease-causing serogroup X, conjugate polysaccharide vaccines targeting the capsule have been used to control endemic IMD caused by serogroups A, C, W and Y for the past decades[8]. However, the majority of IMD cases in developed countries, including Australia, has been caused by meningococcal serogroup B (MenB)[9]. Vaccine development against MenB has been hampered as the capsule polysaccharide elicits autoantibodies in humans. As of now, two multi-component vaccines against serogroup B disease have been developed, which target specific sub-capsular surface antigens expressed by *N. meningitidis*. These vaccines are Bexsero® (also known as 4CMenB)[10] and Trumenba® (also known as rLP2086)[11]. Although both MenB vaccines have been licenced for use in the United States and the United Kingdom, Bexsero® is the only MenB vaccine available in Australia. Since the majority of cases (>65%) in Australia were caused by MenB post the implementation of the meningococcal C conjugate vaccine on the national immunisation programme (NIP) in 2003, inclusion of the Bexsero® vaccine on the NIP has been proposed to protect the Australian population from MenB infections. However, this focus has now shifted as there has been a switch in the predominant meningococcal serogroup

in Australia where the majority of cases are currently caused by meningococcal serogroup W (MenW)[9].

The MLST scheme

In addition to serogroup classification, meningococci can be classified genotypically into sequence types (STs) using multilocus sequence typing (MLST) of seven housekeeping genes[12]. The internal fragments of the seven genes (*abcZ*, *adk*, *aroE*, *fumC*, *gdh*, *pdhC* and *pgm*) are sequenced and an allele number is arbitrarily assigned to each different sequence for each locus. Each ST thus corresponds to a unique combination of seven integers. STs that are identical at four or more loci are grouped into the same clonal complex (cc), which is a direct measure of genetic lineage. Some clonal complexes are more commonly associated with disease than with carriage and are thus termed hypervirulent lineages. Examples of such lineages are cc1, cc5, cc11, cc32 and cc41/44[13]. In contrast to the MLST scheme, serogroup classification is not lineage specific and meningococci expressing different serogroup capsules may belong to the same cc. For instance, meningococci belonging to cc11 have been isolated as expressing either a serogroup B, serogroup C or serogroup W capsule[14].

Characterising *Neisseria meningitidis* using whole genome sequencing

In Australia, diagnostic laboratories report the serogroup of all culturable meningococci from IMD cases to the National Neisseria Network. However, MLST profiles of meningococci are not reported as this action would require the amplification of the seven housekeeping loci by polymerase chain reaction (PCR) followed by sequencing of the fragments, which is not cost-effective. Although serogroup distribution is helpful for disease surveillance, this information is inadequate in regards to monitoring outbreaks and expansion of genetically related meningococci around the country. With the advent of next generation sequencing technologies, whole genome sequencing (WGS) has become relatively inexpensive and less time-consuming compared to conventional sequencing methods. In contrast to MLST profiling, which allows for lineage identification, WGS provides a better resolution and allows strain-to-strain differentiation. Furthermore, MLST profiles can be obtained from WGS data without the need for individual PCR on the MLST loci.

Genomes of *N. meningitidis* can be analysed on the freely accessible PubMLST website (https://pubmlst.org/neisseria) using the Bacterial Isolate Genome Sequence Database (BIGSdb) platform[15]. Once the WGS data are obtained from the sequencing platform (e.g. Illumina Miseq), the nucleotide sequences need to be assembled into contigs using an assembly software (e.g. SPAdes or VELVET) before using the BIGSdb Genome Comparator tool on the website. The core genome of the meningococcus has been characterised and is defined by 1605 core loci[16]. Comparison of meningococcal core genomes has previously been used to characterise *N. meningitidis* isolates causing outbreaks and epidemics[14,17]. With the collaboration of PathWest and under the supervision of A/Prof Charlene Kahler (UWA), I was able to implement one such analysis in Australia, as described below.

WGS reveals emerging cluster of penicillin-resistant *N. meningitidis* in Australia

In our recent study[18], we used WGS as a tool to characterise the increasing number of invasive MenW isolates in Western Australia (WA). The first MenW case was recorded in 2013, which was followed by two cases in 2014 and three cases in 2015. In 2016, a significant increase in MenW incidence was detected and 13 such cases were reported then. We cultured the meningococci from all 19 cases, extracted and sequenced the genomic DNA. Analysis of the MLST loci revealed that all 19 MenW isolates belonged to the cc11 lineage although four different STs were identified. By aligning the nucleotide sequences of the core genome of each isolate, a phylogenetic tree was constructed to investigate similarities among the isolates (Figure 1). From the branching, we observed that the isolates fell into two main clusters, which we labelled as A and B. Cluster A comprised 8 isolates and Cluster B contained 10 isolates. One meningococcal strain (ExNm672), which was isolated from a traveller from Asia who had just arrived in WA, failed to cluster as its genome was significantly different from the WA isolates. Cluster A contained only ST-11 isolates whereas Cluster B contained all four STs identified in the collection. All meningococci in Cluster B were isolated in 2016. The spread of a single clone from Cluster B (ST-12351) in Kalgoorlie WA led to the one-off MenW vaccination program during Dec 2016–Apr 2017.

Interestingly, all isolates in Cluster A were sensitive to penicillin (MIC: $\leq$0.06 mg/L) whilst isolates in Cluster B showed reduced susceptibility to penicillin. The majority of isolates in Cluster B (9 out of 10) were resistant to penicillin (MIC: $\geq$0.5 mg/L). By comparing core genomes of the Cluster A isolates to the Cluster B isolates, we identified the *penA* locus that encodes a protein involved in peptidoglycan biosynthesis as the major contributor to this difference in penicillin susceptibility. Cluster A isolates harboured the *penA_59* allele whereas Cluster B isolates harboured the *penA_253* allele. These alleles differ at 101 nucleotides and the encoded peptides differ at 25 amino acid positions. Exchange of *penA_59* to *penA_253* in all Cluster A isolates resulted in reduced

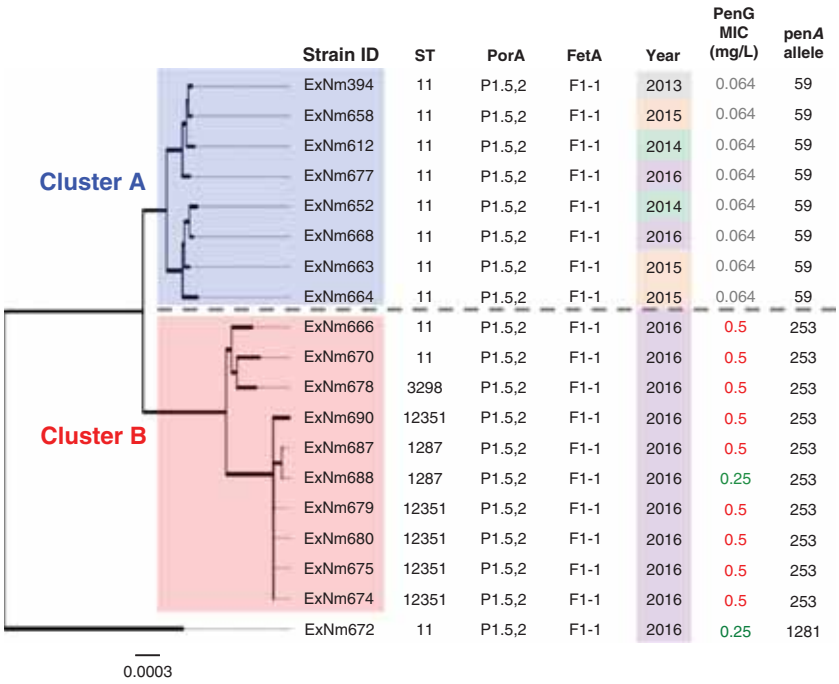

Figure 1. Neighbour-joining tree using the core genome of invasive MenW:cc11 strains isolated in Western Australia during 2013–2016. The dendrogram represents a neighbour-joining tree (500 bootstraps) generated using the core genome sequences of the meningococci. All isolates possessed the same PorA:FetA profile. The resistance phenotype to penicillin G (PenG) for each isolate is provided. The CLSI and EUCAST guidelines use the following breakpoints: sensitive (≤0.06 mg/L) shown in grey, intermediate (0.12–0.25 mg/L) shown in green and resistant (≥0.5 mg/L) shown in red. Two clusters, A and B, were observed that contain isolates that differ in penicillin resistance profile. Of the 1605 core-genome loci, a minimum of 244 loci are different between Cluster A and Cluster B. The more recent Cluster B appeared in early 2016 and contains penicillin-resistant isolates. The dendrogram is drawn to scale, with the sum of the branch lengths between two strains representing the proportion of nucleotide differences between those core genomes (~1.5 Mb) within the pairwise alignment. Source: Adapted from Mowlaboccus *et al*.[18].

susceptibility to penicillin that confirmed the role played by this allele in conferring resistance to penicillin. This finding is of global concern because the *penA_253* allele has been detected in at least five meningococcal isolates in Europe and although treatment with penicillin is still effective against penicillin-intermediate strains, low-dose treatment regimens may fail for cases involving meningococci with a penicillin MIC ≥0.5 mg/L[19].

Conclusion and future direction

IMD is a debilitating disease with significant morbidity and mortality that can be controlled through vaccination. Although the meningococcal vaccine on the NIP in Australia protects only against meningococcal serogroup C, MenACWY and MenB vaccines are available on the private market. With the remarkable progress of next generation sequencing techniques, WGS has become the most cost-effective method for typing invasive meningococci for surveillance. By analysing WGS data of meningococci circulating in Western Australia, we detected the expansion of a MenW:cc11 clone in Kalgoorlie, which led to immediate vaccination of the community. Furthermore, we exploited WGS to identify the emergence of a penicillin-resistant clade of meningococci in WA that has prompted the screening of the *penA_253* allele around the world as the establishment of this allele in the meningococcal population may have an impact on treatment regimens around the world. Implementing WGS analysis as a routine surveillance for monitoring meningococci in Australia will undoubtedly allow us to detect local outbreaks instantly, which will allow urgent actions such as emergency vaccination and will also help improve intervention strategies on a global level in the event where a cluster of antimicrobial resistant meningococci is detected.

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Biography

Shakeel (Shaxx) Mowlaboccus is a doctoral candidate and a sessional lecturer at The University of Western Australia, Perth, Australia. His primary research interests include the evolution and changing epidemiology of *N. meningitidis* and investigating the mechanism of antimicrobial resistance for this pathogen. Shaxx was awarded the ASM/BD Student Travel Award to present the data illustrated in this article at the Australian Society for Microbiology Annual Scientific Meeting 2017.

Report from ASM 2017: Planetary Health

Anthony Baker

Chair of the Local Organising Committee for ASM Hobart 2017

Despite a sudden cold snap enveloping much of southern Australia during the first week of July, 408 delegates converged on the Hobart waterfront at the Hotel Grand Chancellor for the Australian Society for Microbiology's annual Scientific Meeting and Trade Exhibition. This year's gathering was themed 'Planetary Health'; a new discipline aiming to emphasise the interconnectedness of our civilisation with the natural biomes on which we depend. As microbiologists, we are acutely aware that it is often the invisible which underpin biological systems ranging from the small to a global scale.

EduCon, now in its fourth year, was held at the nearby Menzies Institute for Medical Research on the preceding Saturday. Always a great opportunity to discuss teaching practices and philosophies, it is encouraging to see EduCon growing to attract delegates from outside the field of microbiology. With a focus on emerging technology and tools in the digital era, the meeting attracted almost 20 delegates. We look forward to seeing it further develop in coming years.

Workshops running throughout Sunday were a success, with over 100 delegates in attendance. Confocal microscopy was a hit at the biofilm workshop, whilst this year saw the inclusion of a new writer's corner workshop, which showcased publication opportunities outside of the traditional scientific writing format. In addition, workshops covering virology, antimicrobials, mycobacteria and clinical serology were run throughout the day. Workshops, as always, were organised and delivered by a dedicated group of volunteers; the standard was exceptional.

The public lecture was delivered on Sunday evening by Tony Capon, Professor of Planetary Health at the University of Sydney. Tony's lecture outlined some of the enormous environmental and social challenges facing humankind. What began as a very sobering look at a potential future scenario for humankind, concluded with a message of broad optimism linked with human ingenuity and creativity to solve global problems, many of which can be tied to infectious diseases, broader public health and our other close relationships with microbes. It was pleasing to see so many members of the public in attendance at the public lecture, which speaks volumes of the community's broad interest in science.

The official meeting opened following the public lecture with the annual ASM awards ceremony. The Bazeley Oration, an ongoing award supported by the Commonwealth Serum Laboratories, this year was delivered by Professor Mariagrazia Pizza, whose discussion about reverse vaccinology was both informative and engaging.

A welcome function featuring a 'Tastes of Tasmania' showcased some of the finest boutique food suppliers in the state. Samples for tasting included Tassal's smoked salmon, Rhuby Delights chocolates, Frank's Cider, Grandvewe Cheese's sheep's milk products and preserves from the Tasmanian Gourmet Sauce Company. Combined with the poster exhibition, the social event rounded off an excellent beginning to the meeting. The following three days of microbiology featured an incredibly diverse array of meetings, functions and presentations. Of note is the Nancy Millis student and ECR breakfast and lunch (now in their fifth year). It's very pleasing to see these events being so well attended by our future scientific leaders.

The Rubbo Oration, annually supported by the Rubbo Trust, was this year delivered by Professor Pascale Cossart from the Institut Pasteur, who's decades of research in the field of intracellular pathogenesis was brilliantly and enthusiastically communicated and included a visually stunning video presentation of a quality suitable for a TV documentary. The Rubbo Celebration dinner was held immediately afterwards with mood lighting, buffet dinner and

private DJ. A digital 'photo booth' set-up outside the dinner venue complete with masks and other props had pictures of dressed up delegates streaming into the TV screens next to the dance floor. All in all, a great and fun night with many new acquaintances made. It is understood that a proportion of our delegates continued celebrations well into the night, spilling out into the streets of Hobart and on to other venues.

Delegates having fun at the Rubbo Ceremony.

All in all, the standard of scientific research and presentations at this year's meeting was truly exceptional. Symposium and proffered paper speakers delivered science of an excellent standard and showcased the diverse work that is carried out in our country. The quality and diversity of the food was outstanding and the social program was engaging and fun.

I would like to sincerely thank each of our speakers and delegates for their essential contribution to the event and the Hotel Grand Chancellor staff for their above and beyond level of service to this meeting. Furthermore, I'd like to thank our trade sponsors, whose ongoing support of the ASM is reflected in lower registration fees and an ability to bring eminent scientists from around the globe to the meeting. Many thanks go to the expert staff at ASN Events, the National Executive, Divisional Chairs, National Scientific Advisory Committee and Special Interest Groups. Finally, I'd like to personally thank my colleagues on the Local Organising Committee, for their dedication and support over the last couple of years toward making this conference happen. There have been many long discussions and late nights to pull it all together.

I look forward to catching up with you at next year's ASM meeting to be held in Queensland at the Brisbane Convention Centre from the 1st to the 4th of July. Planning is well under way and I've been assured that the weather will be a little warmer.

LOC left to right: Tom Ross, Jay Kocharunchitt, Louise Roddam, Stephen Tristram, Anthony Baker, John Bowman, Chris Burke and Belinda McEwan.

Honorary Life Membership of the Australian Society for Microbiology

Pat Blackall

University of Queensland, QLD

Cheryl Power

University of Melbourne, VIC

New Fellows of the Australian Society for Microbiology

Anna Maria Costa

Royal Children's Hospital, VIC

Dena Lyras

Monash University, VIC

ASM Distinguished Service Awards

Chris Burke

University of Tasmania, TAS

Charlene Kahler

University of Western Australia, WA

Ruth Hall

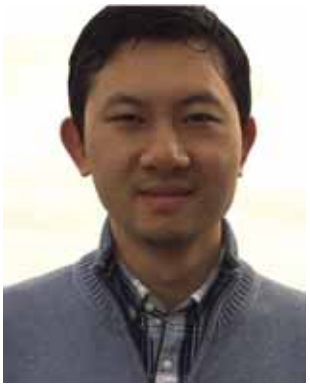
University of Sydney, NSW/NT

Clinical Travel Award: Lee-Ann Kirkham

Dr Lea-Ann Kirkham is an NHMRC Career Development Fellow at the University of Western Australia and Microbiology Lead in the Wesfarmers Centre of Vaccines and Infectious Diseases at the Telethon Kids Institute, Perth, Western Australia. Her vision is to reduce the global burden of bacterial respiratory disease from nontypeable *Haemophilus influenzae* (NTHi) and *Streptococcus pneumoniae* (pneumococcus). Using her background in bacterial pathogenesis, vaccinology and molecular diagnostics, she is developing innovative strategies to improve diagnostics and develop therapies to target these pathogens.

Her PhD research at Glasgow University led to development of a vaccine for pneumococcal pneumonia and meningitis that is currently in clinical trials. Her research into the rise in febrile convulsions after the seasonal influenza vaccine in 2010 contributed to nationwide cessation of a particular flu vaccine for all children under 5 years of age. In 2011, her team's research on identifying the predominant cause of ear infections in Australian children contributed to introduction of a new vaccine onto the National Immunisation Program. This vaccine has recently been shown to reduce ear infection rates in Indigenous Australian children.

ASM Jim Pittard Award: Si Min Man and Johanna Kenyon



Si Ming received his PhD from the University of Cambridge, United Kingdom, for his work on inflammasomes in the host defence against Salmonella infection. He obtained his postdoctoral training from St Jude Children's Research Hospital, USA, where he studied inflammasome signalling in the host response to infection and cancer. Currently, he is a Group Leader and an NHMRC RG Menzies Fellow at the Australian National University, Australia, where his laboratory focuses on innate immunity in the host defence against infectious diseases and the development of cancer and other chronic diseases.

Johanna completed her PhD in microbiology at The University of Sydney in 2012. Her PhD project, in the laboratory of Professor Peter Reeves FAA, investigating the genetics of polysaccharides produced by the bacterial pathogen, *Yersinia pseudotuberculosis*, fostered her interest in the complex genetics of bacterial surface polysaccharides. In 2012, she took up a postdoctoral position at The University of Sydney with Professor Ruth Hall OAM FAA, applying her skills in this field to a project that focused on unravelling the complexities of the genetics of surface polysaccharide production by the nosocomial pathogen, *Acinetobacter baumannii* using whole genome sequence data. Prior to this, little was known about *A. baumannii* polysaccharides, and her work established the foundations of this field. She established collaborations with specialists in Italy and Russia who were able to solve the

carbohydrate structures bringing proof to pathway predictions. In 2015, she moved to a lecturing position at Queensland University of Technology in Brisbane, and formed the Bacterial Polysaccharides Research Group. She now leads her own research team, studying the genetics, biosynthesis and structure of polysaccharides from important Gram-negative pathogens, as well as their key roles in the success of pathogens in clinical environments. She maintains collaborations with the structural collaborators and with the Hall laboratory.

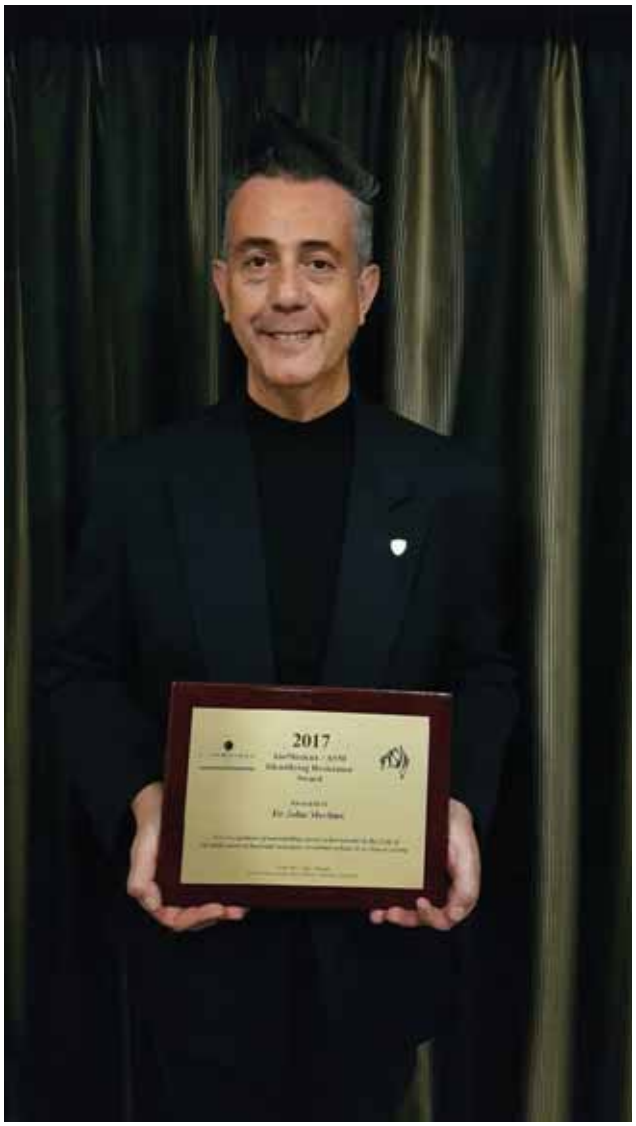
David White Excellence in Teaching Award: Karena Waller

Dr Karena Waller is a Senior Lecturer in the Department of Microbiology and Immunology at The University of Melbourne. Karena joined the Department's academic teaching staff in 2010 after 15 years in local and international malaria research. Today, Karena contributes significantly to the learning and teaching of microbiology and immunology to second and third year Bachelor of Science and Bachelor of Biomedicine students. In addition to her role as lecturer and subject coordinator, Karena is also the Academic Programs Coordinator, Majors Coordinator and the Exchange/Global Mobility Coordinator for the Department. Karena is also Chair of the Victorian Branch of the ASM and a member of the ASM NEQB Committee.

Teacher's Travel Award: Andrew Greenhill

Dr Andrew Greenhill is a Senior Lecturer in Microbiology at Federation University Australia. Andrew's academic life started as an underwhelming undergraduate student here in Hobart at the University of Tasmania. He then moved to James Cook University in Townsville for work and warm winters. At James Cook University he completed his PhD, while also gaining experience and skills in undergraduate education and post-graduate supervision. In 2008 Andrew convinced Suzie, his wife, that living in Papua New Guinea with a young family was a good idea. After four fulfilling years working at the Papua New Guinea Institute of Medical Research, Andrew and family returned to Australia to take up a role as Senior Lecturer at Monash University's Gippsland campus.

bioMérieux ASM Identifying Resistance Award: John Merlino



Dr John Merlino completed his Master of Science with Honours at Macquarie University on detection of vancomycin resistant entrococci and later completed a PhD in Medicine in the Faculty of Medicine, Department of Infectious Diseases and Immunology of the University of Sydney on detection of MRSA. He completed his FASM on antimicrobial resistance in *Staphylococcus aureus* in 2004. He lectures at the University of Sydney and is also Founding Fellow of the Faculty of Science of the Royal College of Pathologists Australasia. He received several awards on detection of antimicrobial resistance with several publications in the area. He has been the

chair and convenor of the ASM Antimicrobial Special Interest Group since 2002 – one of the largest SIG within the Australian Society for Microbiology, organising several workshops and symposia on antimicrobial resistance detection and reporting in medical laboratories.

ASM Frank Fenner Award: Hayley Newton and Catherine Satzke

Dr Hayley Newton completed her PhD at Monash University in 2007 under the supervision of Professor Liz Hartland. During her PhD, Hayley was awarded the prestigious Victoria Fellowship and AFAS-FEAST Fellowship, allowing her to extend her studies on *Legionella pneumophila* to a global network of laboratories. Subsequently, Hayley was awarded a NHMRC Training Fellowship to undertake postdoctoral research overseas. In 2009, she commenced the fellowship within the laboratory of Professor Craig Roy at Yale University, where she worked extensively to develop an understanding of the pathogenesis of the intracellular bacterial pathogen *Coxiella burnetii*. During this period, Hayley developed pioneering techniques to genetically manipulate *Coxiella burnetii* and demonstrated that the Dot/Icm type IV secretion system is an essential virulence determinant. These findings have led to a significant shift in our understanding of this mysterious intracellular pathogen.

Hayley moved to the Department of Microbiology and Immunology, University of Melbourne, at the Peter Doherty Institute in 2013. She has established a research program examining the virulence strategies of intracellular bacterial pathogens. Hayley currently holds a teaching/research academic position within the Department and is the Co-Theme leader for Host-Pathogen Interactions for the Doherty Institute.

Dr Catherine Satzke is a Senior Research Fellow at the Murdoch Childrens Research Institute and has honorary positions at The University of Melbourne, where she completed her PhD in 2007 with Professor Roy Robins-Browne. Her current research examines the impact of vaccines on pneumococcal carriage, transmission and disease, spanning both laboratory and translational science. Catherine collaborates widely with researchers from high and low-income settings, including clinicians, epidemiologists and immunologists. She leads microbiological outcomes for vaccine studies including in Fiji, Lao PDR, Mongolia and Vietnam led by Professor Kim Mulholland and A/Professor Fiona Russell within the Pneumococcal Research Group at MCRI. Other highlights include leading the development of World Health Organization guidelines for pneumococcal carriage, and establishment of global standards for pneumococcal serotyping for carriage studies with the Bill and Melinda Gates Foundation. Catherine is a NHMRC Career Development, and an inaugural veski 'inspiring women' Fellow. Her research is supported by national and international funders, the latter including the Bill and Melinda Gates Foundation, PATH, Gavi Alliance and the WHO.

ASM Lyn Gilbert Award: David Whiley

Associate Professor David Whiley is a principal research fellow at the UQ Centre for Clinical Research, The University of Queensland and research scientist at Pathology Queensland. Much of his work is aimed at enhancing the capacity of clinical laboratories to diagnose, identify and characterise pathogens, with a particular research emphasis on sexually transmitted infections and antimicrobial resistance. He has authored 146 articles and currently holds an NHMRC Career development Fellowship level 2. In recent years A/Professor Whiley has been leading the NHMRC-funded Gonorrhoea Resistance Assessment via Nucleic Acid Detection (GRAND) studies that aim to enhance gonorrhoea antimicrobial resistance surveillance and treatment strategies, particularly in remote settings.

BD ASM Student Travel Awardees

Victoria

Claire Gorrie

University of Melbourne

South Australia/Northern Territory

Matthew Macowan

University of Adelaide

New South Wales/ACT

Riti Mann

University of Technology, Sydney

Tasmania

Roger Latham

University of Tasmania

Queensland

Melinda Ashcroft

University of Queensland

Western Australia

Shakeel Mowlaboccus

University of Western Australia

BD Award Winners left to right: Roger Latham, Melinda Ashcroft, Matthew Macown, Shakeel Mowlaboccus, Riti Mann, Claire Gorrie, with Cheryl Power, Estee Madaschi (BD) and Roy Robins-Browne.

AUSTRALIAN INSTITUTE OF MEDICAL SCIENTISTS

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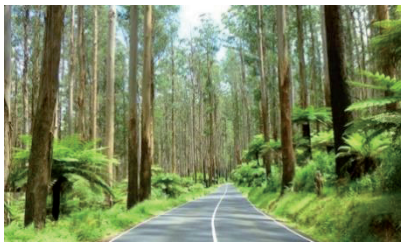
bringing Microbiologists together

The Victorian Branches of AIMS and ASM present a joint conference focusing on

Science in the Spotlight

Saturday 18th & Sunday 19th November 2017 at Vibe Hotel, Marysville VIC



What you can expect:

- A diverse and engaging scientific program focusing on current research, case studies and new technologies in the medical and microbiological sciences at a picturesque location
- Great networking opportunities
- Conference dinner opportunity on Saturday night

Keynote speaker and topic area (title TBA shortly):

- **Dr Denese Marks, Australian Red Cross Blood Service** Cryopreservation of blood products

Featured speakers and topic areas (titles TBA shortly):

- **Ms Robyn Coleman 'AIMS 2017 Travelling Orator', Sullivan Nicolaides Pathology** Coagulation topic
- **Mr David Barton, RMIT University** Presenting his PhD research on the recovery of Marysville after the 2009 Black Saturday bushfires

Confirmed speakers and topic areas (titles TBA shortly):

- **Dr Nancy Endersby-Harshman, Bio21 Institute** *Wolbachia* to reduce transmission of dengue virus
- **Ms Michelle Francis, Monash Health** Molecular microbiology topic
- **Prof Bill Heath, Doherty Institute** Breakthrough in malaria vaccine research
- **Prof Karin Jandeleit-Dahm, Baker Heart and Diabetes Institute** Diabetes and renal disease
- **Dr James Knox, Melbourne Pathology** Performance of a modified test for carbapenemase-producing Gram negative bacteria
- **Prof Johnson Mak**, **Deakin University/CSIRO** Human body's natural protein in defence against HIV
- **Dr Peter Molloy, CSIRO** Simple blood test for bowel cancer
- **Prof Greg Qiao, The University of Melbourne** Latest development in peptide-based stars as antibiotics
- **Dr Catherine Satzke, Murdoch Childrens Research Institute** Pneumococcal research topic
- **A/Prof Bayden Wood, Monash University** Resonance Raman spectroscopy for malaria diagnosis
- **Prof Matthias Ernst, Olivia Newton John Cancer Research Institute** Role of macrophages in bowel and gastric cancer
- **Ms Sridurga Mithraprabhu, Monash University/Alfred Hospital** Liquid biopsy in multiple myeloma
- **Dr Norelle Sherry, Austin Health/Melbourne Genomics Health Alliance** Genomics-based superbug tracking system

Abstract submissions: Would you like to present a 15 min talk or a poster? If so, contact Dr Ed Fox (edward.fox@csiro.au) for abstract submission instructions or go to the AIMS website.

Abstract submissions close: September 29th 2017.

CELLAVISION BD ELITechGroup ELITECH AUSTRALIA AND NEW ZEALAND Cepheid A better way.

See over page for registration details and prices.

Registration: For further details of conference registration rates / packages and to register your attendance, please go to: https://www.aims.org.au/events/event/vic-aimsasm-2017-conference
Early Bird Registration closes: Friday 29th September 2017.

Accommodation: Bookings* are to be made directly with Vibe Hotel, Marysville and can be made:

- Online: https://tinyurl.com/y86cfqry or
- Via phone: (03 5957 7700)

* When booking accommodation, please quote the Promo Code (TH9467296) to access the special conference room rates.

Registration Costs:
The FULL registration packages below include both Saturday and Sunday attendance, and the 2 course conference dinner (see below for details about the dinner).

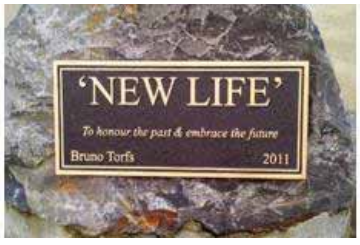
For those only attending one day of the conference, the conference dinner is an optional extra. Dinner tickets are priced at $80 per person. Extra dinner tickets may also be purchased for family members and friends to participate in this social event ($80 each).

	AIMS/ASM members:	**Non-members:**
Early Bird (before Sept 29th) FULL registration	$295.00	$355.00
FULL registration (after Sept 29th)	$345.00	$415.00
Saturday only (Dinner not included)	$155.00	$185.00
Sunday only (Dinner not included)	$155.00	$185.00
Student FULL	$245.00	$295.00
Student Saturday only (Dinner not included)	$115.00	$145.00
Student Sunday only (Dinner not included)	$115.00	$145.00

Go to the website: https://www.aims.org.au/events/event/vic-aimsasm-2017-conference to register your attendance!

Conference Dinner: A two course conference dinner with canapes and pre-dinner drinks in the Radius Bar and Grill which serves modern Australian cuisine, local wine, beer and house made bread. The restaurant has an emphasis on locally sourced produce within 100km range and the food is simple and fresh with a focus on slow cooked meats over a wood flame. This dinner is designed to bring all the delegates together to share a meal and get to know others.

Enquires: Kerryn Weekes (kerryn.weekes@monashhealth.org) or Karena Waller (klwaller@unimelb.edu.au)



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