Re-emergence and laboratory diagnosis of Pertussis

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Pertussis (whooping cough) cases have experienced a recent resurgence despite high vaccine coverage against the main etiologic agent, *Bordetella pertussis*. In the pre-vaccination era, pertussis was the main cause of infant death due to infectious disease in the first year of life. Recent reports of an increase in pertussis cases especially among adults and adolescents is therefore of concern, given this group can serve as a reservoir and source of infection for infants who have not completed their vaccination course. Table 1 shows that the number of cases per age group has altered since the pre-vaccinated and whole cell vaccine era compared to recent epidemics, with the ≥15 age group now reporting a higher proportion of pertussis cases. In addition, asymptomatic infections among household contacts of notified cases can be common (up to 46%).
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Table 1: The proportion (%) of pertussis cases in each age group pre-vaccination (1933-1939), during whole cell vaccination (1978-1981) and the start of acellular vaccination (1997-2000) in the U.S.

<table>
<thead>
<tr>
<th>Years</th>
<th>&lt;1</th>
<th>1-4</th>
<th>5-9</th>
<th>10-14</th>
<th>≥15</th>
</tr>
</thead>
<tbody>
<tr>
<td>1933-1939</td>
<td>7.5%</td>
<td>41.1%</td>
<td>46%</td>
<td>4.1%</td>
<td>0.9%</td>
</tr>
<tr>
<td>1978-1981</td>
<td>53.5%</td>
<td>26.5%</td>
<td>8.2%</td>
<td>5.4%</td>
<td>6.5%</td>
</tr>
<tr>
<td>1997-2000</td>
<td>29.4%</td>
<td>11.1%</td>
<td>9.8%</td>
<td>29.4%*</td>
<td>20.4%#</td>
</tr>
</tbody>
</table>

*B. pertussis* has a suite of mechanisms to evade and suppress the immune response and cause disease, some of which are used in the five component acellular vaccine. These include, pertussis toxin (PT), pertactin (Prn), filamentous haemagglutinin (FHA) and fimbriae (Fim2 and Fim3). Changes in the genotype of circulating strains may contribute to the resurgence of pertussis in countries with high acellular vaccine coverage, although the mechanisms are not yet clear. In a recent outbreak, changes in the PT promoter region were initially thought to be the main driver, however it was the replacement of the resident *fim3-1* allele with the new *fim3-2* allele that correlated most strongly to the resurgence of pertussis in this outbreak. In addition, strains have emerged that do not express one or more components of the acellular vaccine, such as Prn. In a study on the global population structure of *B. pertussis* that included strains from Australia, vaccination was suggested to be a major force in driving changes in *B. pertussis* populations.

In Australia, epidemics of pertussis occurred in 2009 and 2012 while an upward trend is also occurring this year with 2866 notifications up to May compared to a total of 3131 for last year (http://www0.health.nsw.gov.au/data/diseases/pertussis.asp). While *B. pertussis* continues to be the main cause of whooping cough, other pathogenic Bordetella, namely, *B. parapertussis* and *B. holmesii* and more rarely *B. bronchiseptica*, have been reported to cause significant outbreaks of pertussis, albeit in a milder form. *B. parapertussis* is responsible for a milder form of pertussis but there is very little evidence of it causing significant disease in Australia. One study found *B. parapertussis* by PCR in 25 out of 14135 nasopharyngeal swabs collected from patients in Western Australia and the Northern Territory.

*B. holmesii* is usually an infection in immunocompromised patients, however several recent outbreaks have reported up to 11.5%-29% of cases initially diagnosed as pertussis are actually *B. holmesii* infections. In contrast, some countries have not found *B. holmesii* infection despite active surveillance. *B. holmesii* is found predominantly in the 10-19 year old age group and usually causes less severe disease, therefore most cases of *B. holmesii* infection may go unnoticed. Like *B. pertussis*, *B. holmesii* epidemics appear to cycle every 3-5 years, yet the cycles may not coincide with *B. pertussis* and in the large Australian *B. pertussis* epidemic of 2011, *B. holmesii* numbers had dropped after an initial peak in 2010. It is unknown if infection by *B. holmesii* contributes to cases of vaccine failure, although the estimated vaccine effectiveness is thought to be lower for *B. holmesii* infection compared to *B. pertussis* infection. Most PCR tests designed to detect *B. pertussis* will also amplify *B. holmesii* DNA with the latter distinguishable only if a second PCR is performed.

The Bordetella can be difficult to detect, particularly as the disease progresses. There are many diagnostic tests available with PCR being the most widely used. Serological diagnosis however, can be useful with increasing duration of disease, as not only is the sensitivity of the PCR assay dependent on sample type (nasopharyngeal swab or aspirate is preferred to the throat swab), sample collection and bacterial load, but also on the duration of symptoms. The optimum time to take a sample for PCR detection is within the first 3-4 weeks of cough. Despite these obstacles, PCR, particularly RT-PCR, is the most sensitive and specific test in which to detect pathogenic Bordetella. Most assays target insertion sequences (IS) for which multiple copies exist thereby increasing the sensitivity of the test. Table 2 outlines the common IS for each pathogenic Bordetella species and the number of copies that are usually present.
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Table 2: The insertion sequences of pathogenic Bordetella that are used for diagnosis.

<table>
<thead>
<tr>
<th>Marker</th>
<th>B. pertussis</th>
<th>B. parapertussis</th>
<th>B. holmesii</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS481*</td>
<td>Present (50-238 copies)</td>
<td>Not present</td>
<td>Present (8-10 copies)</td>
</tr>
<tr>
<td>hIS1001</td>
<td>Not present</td>
<td>Not present</td>
<td>Present (3-5 copies)</td>
</tr>
<tr>
<td>pIS1001</td>
<td>Not present</td>
<td>Present (20 copies)</td>
<td>Not present</td>
</tr>
</tbody>
</table>

*Some (1-5%) B. bronchiseptica contain this IS.

As PCR detection is the most relied upon, there are less bacterial isolates available for the monitoring of epidemics and strain typing, hence, CIDM-Public Health is currently developing new methods to detect and type pathogenic Bordetella direct from clinical samples. We encourage our partners to exclude B. holmesii infections when investigating recent outbreaks of pertussis, especially in hospital settings, and would be happy to accept requests for B. holmesii testing. We have sequencing facilities to perform whole genome sequencing in suspected outbreaks in order to gain information on currently circulating strains and therefore monitor how effective the current vaccine components are likely to be.

References


**OBJECTIVES:** Detection of pyrazinamide (PZA) resistance in *Mycobacterium tuberculosis* isolates presents significant challenges in settings with no dominant clonal lineages, such as Australia. We assessed the utility of whole-genome sequencing (WGS) versus standard PCR amplification assays for the characterization of PZA resistance in multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB) isolates identified in New South Wales, Australia, over an eight-year period.

**METHODS:** PCR-amplicon sequencing was used to identify molecular markers associated with antibiotic resistance in pyrazinamide-resistant MDR-TB isolates recovered by the New South Wales (NSW) Mycobacterium Reference Laboratory between 2007 and 2014. WGS was subsequently performed on two isolates for which *pncA* amplification failed.

**RESULTS:** WGS identified two novel genomic deletions associated with *in vitro* resistance to pyrazinamide in MDR-TB. One isolate also carried a second deletion involving the genes *dfrA* and *thyA* associated with resistance to para-aminosalicylic acid.


*Salmonella enterica* serovar Typhimurium is the most common *Salmonella* serovar causing food borne infections in Australia and many other countries. Twenty one *S. Typhimurium* strains from *Salmonella* reference collection A (SARA) were analyzed using Illumina high-throughput genome sequencing. SNPs in 21 SARA strains range from 46 SNPs to 11,916 SNPs with an average of 1,577 SNPs per strain. Together with 47 selected from publicly available *S. Typhimurium* genomes, the *S. Typhimurium* core genes (STCG) was determined. The STCG consists of 3,846 genes, which is much larger than the set of 2,882 *Salmonella* core genes (SCG) found previously. The STCG together with 1,576 core intergenic regions (IGRs) was defined as the *S. Typhimurium* core genome. Using 93 *S. Typhimurium* genomes from 13 epidemiologically confirmed community outbreaks, we demonstrated that typing based on *S. Typhimurium* core genome (STCG+ core IGRs) provides superior resolution and higher discriminatory power than that based on SCG for outbreak investigation and molecular epidemiology of *S. Typhimurium*. Both STCG and STCG+ core IGRs typing achieved 100% separation of all outbreaks in comparison to SCG typing which failed to separate isolates from two outbreaks from background isolates. Defining the *S. Typhimurium* core genome allows standardization of genes/regions to be used for high resolution epidemiological typing of *S. Typhimurium* for genomic surveillance.


**OBJECTIVES:** Phenotypic drug susceptibility testing (DST) for *Mycobacterium tuberculosis* takes several weeks to complete and second-line DST is often poorly reproducible, potentially leading to compromised clinical decisions. Following a fatal case of XDR TB, we investigated the potential benefit of using whole-genome sequencing to generate an in silico drug susceptibility profile.

**METHODS:** The clinical course of the patient was reviewed, assessing the times at which phenotypic DST data became available and changes made to the therapeutic regimen. Whole-genome sequencing was performed on the earliest available isolate and variants associated with drug resistance were identified.

**RESULTS:** The final DST report, including second-line drugs, was issued 10 weeks after patient presentation and 8 weeks after initial growth of *M. tuberculosis*. In the interim, the patient may have received a compromised regimen that had the potential to select for further drug resistance. The in silico susceptibility profile, extrapolated from evolving evidence in the literature, provided comparable or superior data to the DST results for second-line drugs and could be generated in a much shorter timeframe.

**CONCLUSIONS:** We propose routine whole-genome sequencing of all MDR *M. tuberculosis* isolates in adequately resourced settings. This will improve individual patient care, monitor for transmission events and advance our understanding of resistance-associated mutations.
Abstracts of selected recent publications by CIDM-Public Health investigators


BACKGROUND: Shigellosis is an acute, severe bacterial colitis that, in high-income countries, is typically associated with travel to high-risk regions (Africa, Asia, and Latin America). Since the 1970s, shigellosis has also been reported as a sexually transmitted infection in men who have sex with men (MSM), in whom transmission is an important component of shigellosis epidemiology in high-income nations. We aimed to use sophisticated subtyping and international sampling to determine factors driving shigellosis emergence in MSM linked to an outbreak in the UK.

METHODS: We did a large-scale, cross-sectional genomic epidemiological study of shigellosis cases collected from 29 countries between December, 1995, and June 8, 2014. Focusing on an ongoing epidemic in the UK, we collected and whole-genome sequenced clinical isolates of Shigella flexneri serotype 3a from high-risk and low-risk regions, including cases associated with travel and sex between men. We examined relationships between geographical, demographic, and clinical patient data with the isolate antimicrobial susceptibility, genetic data, and inferred evolutionary relationships.

FINDINGS: We obtained 331 clinical isolates of S flexneri serotype 3a, including 275 from low-risk regions (44 from individuals who travelled to high-risk regions), 52 from high-risk regions, and four outgroup samples (ie, closely related, but genetically distinct isolates used to determine the root of the phylogenetic tree). We identified a recently emerged lineage of S flexneri 3a that has spread intercontinentally in less than 20 years throughout regions traditionally at low risk for shigellosis via sexual transmission in MSM. The lineage had acquired multiple antimicrobial resistance determinants, and prevailing sublineages were strongly associated with resistance to the macrolide azithromycin. Eight (4%) of 206 isolates from the MSM-associated lineage were obtained from patients who had previously provided an isolate; these serial isolations indicated atypical infection patterns (eg, reinfection).

INTERPRETATION: We identified transmission-facilitating behaviours and atypical course(s) of infection as precipitating factors in shigellosis-affected MSM. The intercontinental spread of antimicrobial-resistant shigella through established transmission routes emphasises the need for new approaches to tackle the public health challenge of sexually transmitted infections in MSM.


Infant botulism is a potentially life-threatening paralytic disease that can be associated with high levels of morbidity if not rapidly diagnosed and treated. Disease results from the production of botulinum neurotoxin (BoNT) by Clostridium botulinum, following the ingestion of spores which can germinate and grow in the immature intestine. Four infants were treated for infant botulism in New South Wales, Australia, between June 2011 and September 2013. This was an unusually high incidence rate in this setting. All cases were culture positive and clustered the four NSW isolates together. However, the extra resolution provided by whole-genome SNP comparisons showed that all four isolates were genomically distinct as they were separated from each other by >7000 SNPs. WGS analyses indicated that the four infant botulism cases had all acquired different isolates of C. botulinum that were unlikely to have originated from a common environmental source. Isolates did however cluster together, when compared with international isolates, suggesting that C. botulinum from environmental reservoirs throughout NSW have descended from a common ancestor. Toxigenic properties have been governed by the acquisition of mobile genetic elements. The presence of identical BoNT/A2 neurotoxin clusters in three of the isolates suggested that it had been horizontally acquired by each of the isolates from a common reservoir.
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In April 2013, a Public Health Unit in Sydney was notified of three patients in different tertiary hospitals testing positive for listeriosis within eight days of each other. A public health investigation concluded that it was most likely that the three patients acquired their infections from eating a hospital dessert supplied by a specific company. Near real-time molecular typing techniques and rapid review of hospital menus using an electronic menu database were used together to quickly identify a source of infection and implement control measures, which is likely to have successfully prevented further cases. Listeriosis can be a serious illness in vulnerable hospital populations. Hospital-acquired listeriosis can occur; hospital food safety plans therefore need to ensure the risk of listeriosis to patients is minimised.

Staff Profile

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Verlaine studied Microbiology at the University of NSW before working overseas as a clinical scientist for the Public Health Laboratory Service (UK), Leeds General Infirmary (UK) and Statens Serum Institute (Denmark) researching the gastric bacterium Helicobacter pylori. She returned to the University of NSW for her PhD in Medical Microbiology on pathogenic Mycobacterial sp. Here she developed an interest in fastidious bacteria and the interplay between humans and the pathogens that have challenged us for centuries.

Her current project on the re-emergence of pathogenic Bordetella sp. extends this interest and she hopes to gain more insight into this group using whole genome sequencing techniques. In particular, Verlaine is interested in how this pathogen is evading current vaccination strategies and how other Bordetella sp. are contributing to pertussis outbreaks.

CONTACT US

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The 2015 Ross River virus outbreak: A reminder of local mosquito-borne disease threats

Cameron E Webb
Department of Medical Entomology, CIDMLS, Pathology West – ICPMR Westmead, New South Wales, Marie Bashir Institute for Infectious Diseases and Microbiology, University of Sydney, New South Wales

There has been much interest in the future public health risks associated with mosquitoes in Australia. Concern has mostly focused on the threats posed by the potential introduction of exotic mosquitoes and their pathogens and the enhancement of those threats that may accompany climate change. However, an outbreak of mosquito-borne Ross River virus along the east coast of Australia has refocused the attention of local health authorities on potential changes to local health risks.

The increasing activity of mosquito-borne dengue and chikungunya viruses across SE Asia and the Pacific has increased awareness of the threats these pathogens may pose to Australia. While only one mosquito species, *Aedes aegypti*, able to transmit these viruses currently exists on mainland Australia, the discovery of a second potential nuisance-biting pest and vector, *Aedes albopictus*, in the Torres Strait has put authorities on alert. Unlike *Aedes aegypti* that is currently restricted in its distribution to north Queensland, modelling has suggested that if *Aedes albopictus* is introduced into mainland Australia, it could become established in our southern metropolitan centres and represent a potential health threat.3,4,5

While these “exotic” mosquito and pathogen risks have attracted much attention, a number of years of below average activity of endemic mosquito-borne disease in coastal Australia has resulted in some complacency regarding the mosquito-borne Ross River virus. Ross River virus infects around 5,000 people per year across Australia resulting in flu-like symptoms typically including fever, rash, arthritic joint pain and fatigue. The severity of these symptoms varies and may persist from a few days to several months. While not fatal, the disease has the potential to be severely debilitating.6,7

In the first six month of 2015, there has been a significant increase in the number of human notifications of Ross River virus infection. The national average has already been exceeded with a total of 7,706 cases reported to 7 July 2015.8 The majority of those cases have been in QLD and NSW with a total of 6,466 cases reported over that period.9 There is now a strong indication that this will be one of the largest outbreaks of Ross River virus across the two states in close to twenty years.

Understanding the environmental and entomological factors that drive outbreaks of Ross River virus is difficult. Unlike dengue viruses, where only one mosquito species is involved in transmission, there are over 40 different mosquito species implicated in the transmission of Ross River virus.7 These mosquitoes include species found in estuarine and freshwater habitats as well as backyard water-holding containers. In addition, as native macropods, such as kangaroos and wallabies,10 are considered the most important reservoir hosts of the virus, understanding their role in outbreaks is required. There is currently a paucity of information available on the importance of local wildlife along the east coast.

While a changing climate is often suspected to drive southern movement of “tropical” mosquitoes and pathogens,11 perhaps health authorities should work to understanding the relationships between increased temperatures, changing rainfall patterns, sea level rise and increasing frequency and severity of extreme weather events on wetlands, wildlife and mosquitoes with a view to developing improved surveillance and strategic responses to increased public health risks.

The changing face of vector-borne disease will be a focus of a free forthcoming symposium hosted by CIDM-PH and MBI on 11 September 2015 at Westmead Hospital. The symposium will cover emerging issues in medical entomology, from mosquitoes to ticks and from fleas to bed bugs. How can we better understand, and hopefully manage, the potential public health risks posed by both local and introduced arthropods.

For more information on the symposium, please email: WSLHD-CIDM-PH@health.nsw.gov.au

References

Upcoming Events....

Beating the bite of emerging pest and vector-borne disease threats in Australia

Date: Friday, 11th September 2015
Time: 8.30am - 4.00pm
Location: Lecture Theatre 3, Westmead Hospital, Sydney
Program: Coming soon
Enquiries: WSLHD-CIDM-PH@health.nsw.gov.au

Emerging public health threats can take many forms. As urbanisation and a changing climate influences the local environmental, the pest and public health risks associated with mosquitoes, ticks, fleas and bed bugs will rise. Australia has recently experienced one of its worst outbreaks of mosquito-borne Ross River virus disease; the threat of exotic mosquito-borne pathogens such as dengue and chikungunya viruses continue; ticks remain a major health concern at the fringes of our metropolitan regions; and the parasites of our pets may represent an emerging risk in our homes. The news isn’t all bad as researchers are now finding ways that arthropods may provide some positive outcomes for human health through their use in medicine. This full day symposium, jointed presented by CIDM-PH and MBI, will explore the emerging and re-emerging vector-borne disease risks in Australia and discuss the development of surveillance programs and strategic responses to infectious disease threats in light of the latest research in medical entomology.

MBI Colloquium
Date: Friday, 6th November 2015
Time: 8.30am - 4.30pm
Location: New Law Lecture Theatre 101, Main Campus, University of Sydney
Program: Coming soon
Enquiries: mbi@sydney.edu.au

CIDM-PH Colloquium
Date: Friday, 27th November 2015
Time: 8.30pm - 4.00pm
Location: Lecture Theatre 2, Westmead Hospital, Sydney
Program: Coming soon
Enquiries: WSLHD-CIDM-PH@health.nsw.gov.au

International Conference on the Politics and Ethics of Infection, PEI 2015
Date: 10th - 12th December 2015
Location: University of Sydney

Reducing the burden of TB and lung disease by increasing and expanding regional partnerships
Date: 31st August - 2nd September 2015
Location: Sydney
Enquiries: www.aprunion2015.com