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The Broad Street Pump

Translating pathogen genomics into improved public health outcomes: Prospective evaluation of the effectiveness of genome-sequencing guided investigation of outbreaks

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Salmonellosis, listeriosis and tuberculosis are high-burden and life-threatening diseases worldwide and in Australia, but have significant potential for prevention. Currently in NSW, investigations into potential sources of outbreaks or person-to-person transmission of these diseases are guided by using MLVA or MIRU bacterial typing to identify clusters of cases caused by closely-related isolates.

Recent retrospective studies of whole genome sequencing (WGS) using isolates from known outbreaks have demonstrated the superior resolution power of WGS compared to other typing methods currently in use. However, critical questions about routine implementation of this technology into disease control systems remain unanswered. In 2016, CIDM-PH, NSW Health Pathology and Health Protection NSW embarked on a two year project to address these questions by integrating WGS-based characterisation into surveillance of salmonellosis, listeriosis and tuberculosis in NSW, and prospectively evaluate the contribution it makes to outbreak detection and investigation.

Inside this issue

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Translating pathogen genomics into improved public health outcomes: Prospective evaluation of the effectiveness of genome-sequencing guided investigation of outbreaks...*continued from page 1*

Methods

All isolates of *Salmonella* Typhimurium (STm), *Listeria monocytogenes,* and *Mycobacterium tuberculosis* submitted to the NSW Enteric Reference Laboratory and NSW Mycobacterial Reference laboratory undergo whole genome sequencing and genomic analysis in parallel with current typing methods. All specimens collected between October 2016 to March 2018 will be sequenced.

Single nucleotide polymorphism (SNP) differences are used to define clusters. For *Salmonella*, a cluster is any two or more isolates with ten or fewer SNPs different, and for *M.tuberculosis* the cutoff is five SNPs. Due to the high number of cases, only selected clusters of STm are investigated. Clusters are prioritised based on the number of cases, the temporal and geographic distribution of the cases, and any unusual features such as a high proportion of cases being small children. All clusters of TB are investigated to identify transmission events, and contacts who may have been exposed.

Data management and data sharing

Compared to traditional typing methods which allocate each bacterial isolate a type 'name' that may only be a few characters long, whole genome sequencing produces enormous amounts of data. This project is acting as a pilot to determine how much data storage would be required if WGS were to be integrated into surveillance permanently. There are also questions around ownership of data, access requirements, and data compatibility with existing laboratory and public health databases that need to be resolved. The analytic methods and cluster nomenclature used so far in the project are not comparable with other jurisdictions, so trials of other analytic approaches are planned.

Results

Since October, over 1,700 bacterial isolates have been sequenced and analysed (Table 1).

Table 1. Isolates sequenced and clusters detected

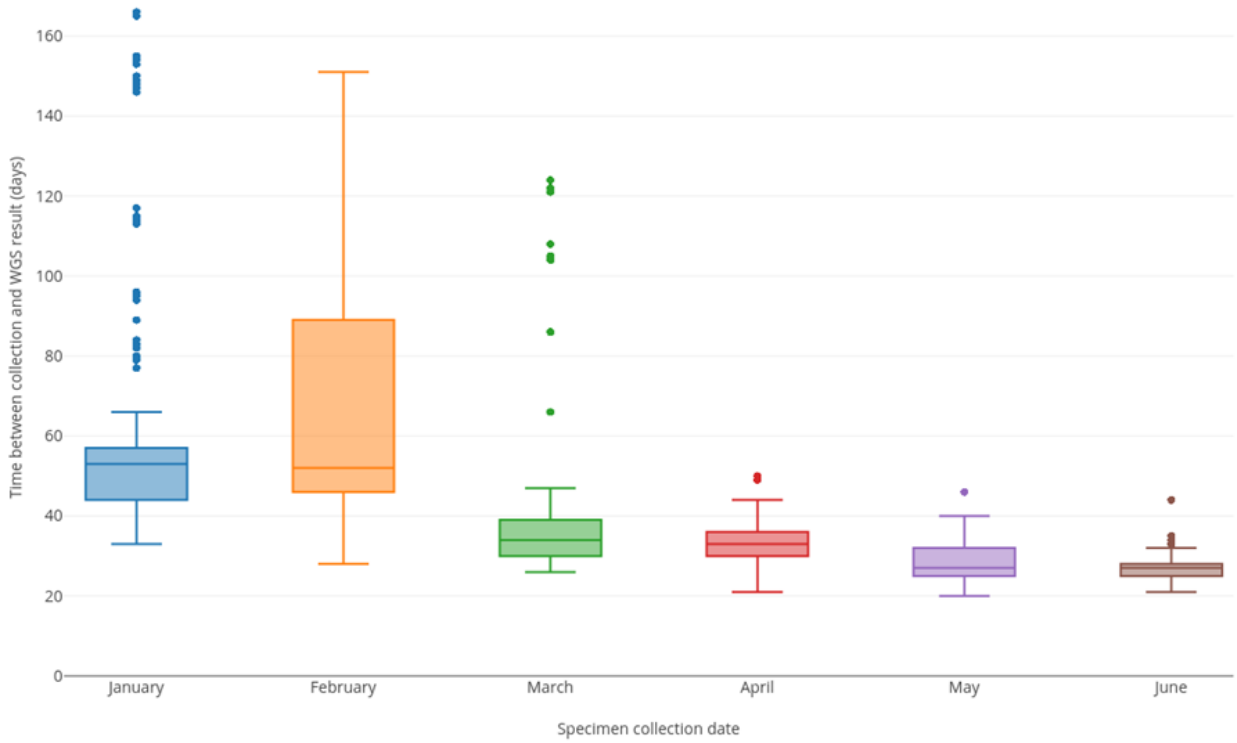
Organism	Number sequenced	Clusters detected	Median number of cases per cluster
Salmonella Typhimurium	1369	137	3
Listeria monocytogenes	7 human / 23 environmental	0	0
Mycobacterium tuberculosis	321	21	2

Salmonella Typhimurium (STm)

One of the main challenges of this project is scaling up sequencing and analysis procedures to handle high case numbers, while still providing robust results to Health Protection NSW in a timely manner to inform public health action. Turnaround times are monitored, and adjustments made to laboratory protocols to improve efficiency. Between January and June 2017, the median turnaround time between specimen collection date and distribution of a cluster report decreased from 53 to 27 days (Figure 1). The longer turnaround time in February was caused by the delay in sequencing due to a global issue of the reagent quality from the manufacture (Figure 1).

Translating pathogen genomics into improved public health outcomes: Prospective evaluation of the effectiveness of genome-sequencing guided investigation of outbreaks...*continued from page 2*

Figure 1. Time between specimen collection and whole genome sequencing based cluster report, *Salmonella* Typhimurium



Genomic analysis to identify clusters is performed weekly, and a report is submitted to Health Protection NSW. Any new clusters, clusters that have increased in size since the last report, or clusters that contain isolates submitted by the NSW Food Authority are reviewed. Demographic and exposure information about cases is then compiled into a combined genomic and epidemiological summary, which is discussed in a weekly teleconference between Health Protection NSW, Enteric Reference Laboratory, CIDM-PH and the NSW Food Authority.

To date, eight WGS clusters of *Salmonella* Typhimurium have met the criteria for further investigation. WGS provides additional information above that provided by traditional typing (MLVA). Some examples:

- In a large outbreak associated with eggs, WGS was retrospectively used to confirm that isolates obtained during inspection of an egg farm and a restaurant serving eggs from that farm were indistinguishable from isolates from people who ate egg dishes at the restaurant prior to becoming ill.
- A small cluster of people who lived in the same part of Sydney and had STm of the same MLVA type was identified through routine typing. WGS was used to link an additional case who lived on the other side of Sydney to the cluster. Although the source of the outbreak could not be determined, this genomic link was supported by epidemiologic evidence as the additional case worked in the area where the initial cases lived.
- An apparent cluster of cases with the same MLVA type was identified. In these investigations, no epidemiological links were identified between the cases. WGS analysis subsequently demonstrated that the isolates were not closely related enough to suggest a single shared source.

Translating pathogen genomics into improved public health outcomes: Prospective evaluation of the effectiveness of genome-sequencing guided investigation of outbreaks...*continued from page 3*

Listeria monocytogenes

Genomic detection of *Listeria* clusters in Australia is performed at the Microbiological Diagnostic Unit (MDU), Melbourne. Currently all isolates are forwarded to MDU for sequencing and analysis. This project will involve validating sequencing performed at ICPMR against sequencing performed at MDU. Once validated, NSW will be able to send sequences to MDU for inclusion in the national surveillance program instead of cultures, which will reduce the time and costs involved.

Tuberculosis

An understanding of local patterns of transmission is essential for tuberculosis control in NSW. The typing method currently used, MIRU-24, is not discriminatory enough to determine whether two related cases likely represent person-to-person transmission or infection with related strains from different sources. As well as prospective sequencing of all new isolates, older isolates that match to new isolates by MIRU are undergoing whole genome sequencing to resolve the degree of relatedness. Whole genome sequencing has not identified any additional clusters that were not identified by MIRU typing or case follow-up.

In addition to cluster detection, WGS is being conducted in *M. tuberculosis* investigations to identify mutations conferring antibiotic resistance. To date, genomic results are completely concordant with phenotypic antimicrobial resistance testing, but results are usually available sooner.

Conclusion

In the first nine months of the project, great progress has been made in integration of whole genome sequencing into surveillance of salmonellosis, listeriosis and tuberculosis in NSW. While many of the practical issues around data management and reporting are yet to be resolved, the use of WGS in cluster investigations to date demonstrate value of the additional information that genomic analysis provides when compared to traditional typing methods.

*Pictured left to right: Director of CIDM-PH Westmead **A/Prof Vitali Sintchenko,** former NSW Minister for Medical Research **Pru Goward**, Director CRE in Critical Infectious Diseases **Prof Jon Iredell**, former NSW Health Minister **Jillian Skinner** MP and **Geoff Lee** MP, Seat of Parramatta.*

Upcoming events...

27 September 2017:

A nested environmental approach to typhoid epidemiology in Central Division, Fiji

CIDM-PH & MBI Seminar: Dr Aaron Jenkins

Westmead Institute for Medical Research

https://aaronjenkinsseminar.eventbrite.com.au

28-29 September 2017:

Short Course in Critical Infection

Westmead Education & Conference Centre

http://sydney.edu.au/medicine/criticalinfection/events

20 October 2017 :

Advances in Microbial Genomics

Westmead Education & Conference Centre

https://symposiummicrobialgenomics.eventbrite.com.au

17 November 2017:

MBI Colloquium

New Law Lecture, University of Sydney

register online

24 November 2017:

CIDM-PH Colloquium

Westmead Education & Conference Centre

https://2017cidmphcolloquium.eventbrite.com.au

Differentiation between enteroinvasive *Escherichia coli* (EIEC) and *Shigella* using multiplex PCR

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Shigella is one of the main pathogens responsible for bacterial dysentery or non-bloody diarrhoea (1) and all species of *Shigella* affecting humans (i.e. *Shigella sonnei*, *S. flexneri*, *S. boydii* and *S. dysenteriae*) can cause shigellosis (2). Isolation or detection of *Shigella* species has been recognised as the definitive laboratory evidence, however, their molecular detection remains a challenge due to their similarities with evolutionary relatives (3). One of such relatives is enteroinvasive *Escherichia coli* or EIEC. They share many similarities such as invasive pathogenic mechanisms, ability to cause dysentery, and similar biochemical properties (2, 4, 5). Importantly, culture-independent diagnostic tests (CIDT) used to screen for *Shigella* in stool samples rely on *ipaH* gene target which is also present in EIEC (6, 7).

Recent increases in the number of positive CIDT PCR assays which are confirmed by *Shigella* culture have raised concerns about the specificity of shigellosis CIDT and the need for the development of additional molecular markers differentiating *Shigella* from EIEC. One of the examples of this is the development of the test method using β-glucuronidase (*uidA*) and lactose permease (*lacY*) gene (8). However, according to one study, this duplex PCR may not be effective in differentiation of these pathogens (2) clearly indicating need of development of more effective assay. Our team at the CIDM-Public Health aimed at developing a PCR assay that can rapidly distinguish *Shigella* and EIECs in cultures from CIDT positive samples. We developed an assay based on two multiplex PCR reactions targeting a unique combination of genetic markers in EIEC and *Shigella* allowing their differentiation.

We analysed 6,432 genomes of *Shigella,* 13,147 genomes of non-invasive *E. coli*, and 88 genomes of EIECs stored in the NCBI GenBank. We have included 12 and 48 clinical isolates of EIEC and *Shigella*, respectively, for test evaluation. We have employed 21 *Shigella* and 4 EIEC genomes of various diversity in terms of serotypes/sequence types. 21 *Shigella* and 4 EIEC genomes were sequenced in-house using Illumina NextSeq500 platform. The fastq files containing raw sequences of these bacteria were assembled *de novo* into draft contigs using SPAdes Genome Assembler and annotated with RAST. The genes were compared using gene IDs in RAST and the unique gene IDs from EIEC absent from *Shigella* sp. exported to SQL server where they were joined to their sequences. The fasta files containing these EIEC specific sequences were used to filter duplicates and to confirm the absence/presence of genetic loci using BLAST. We have identified the set of unique genes which can differentiate various *Shigella* species from EIEC.

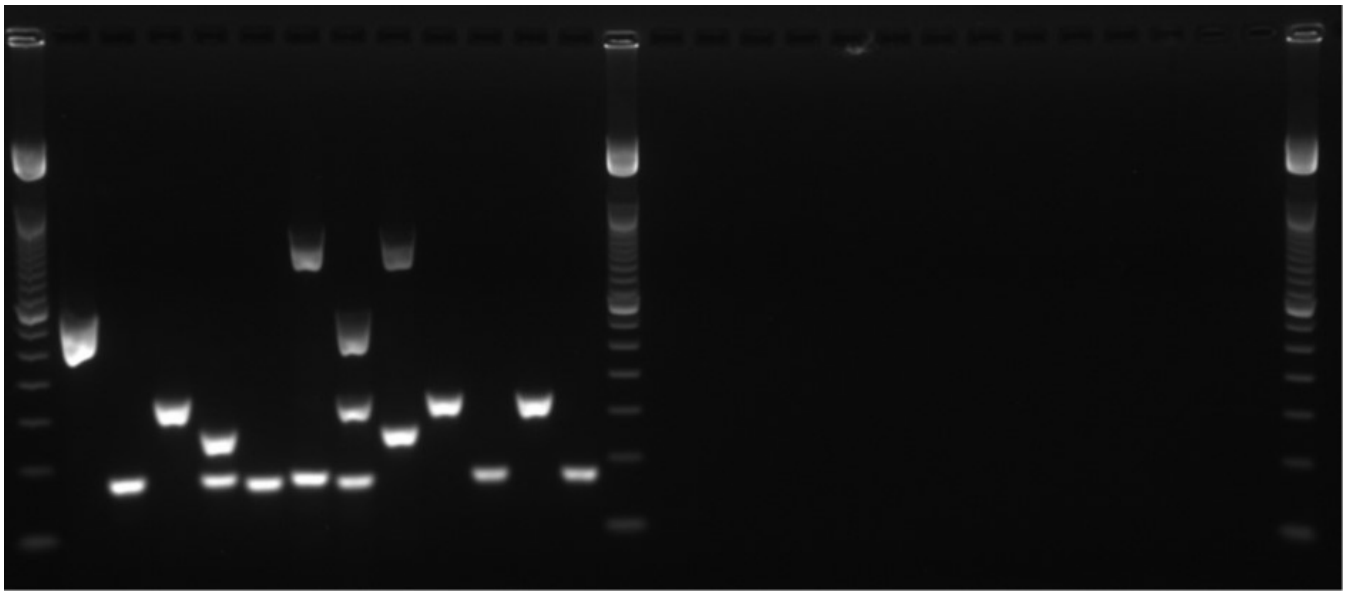
The multiplex-PCR assay was developed after testing these markers *in vitro* and *in silico*. The *in silico* test was done using 6,432 *Shigella* and 13,147 EIEC genomes. Two multiplex PCR systems A and B were developed with each system including 3 primer sets. PCR products were visualised by 2% agarose gel electrophoresis. The interpretation of result was based on the presence or absence of amplicons. For *ipaH* positive isolates, EIEC showed the presence of two or more bands in agarose gel after PCR and *Shigella* showed only one or no band. Analytical sensitivity of the assay was also determined by performing PCR on the serial dilution of cell suspension from 10^9 to 10^0 cells/mL. Furthermore, the combination of loci present in the EIEC isolates was compared with the phylogeny of previous work by Lan et al. 2004, Pettengill et al. 2015 and Hazen et al. 2016 where the same genomes had been used for studying phylogeny.

Six loci were selected after bioinformatics analyses on whole genome sequences and *in silico* PCR testing. These six genetic loci appeared highly discriminatory. At least two out of six target loci were found in 34 out of 35 (97.2%) EIEC strains used for assay design. Only 6 out of 6,432 (0.09%) *Shigella* and 2 out of 13,147 *E. coli* showed at least 2 loci while none of target loci were present in the majority of *Shigella* and *E. coli*. All clinical isolates of EIEC available to us showed at least 2 loci while none of 48 clinical isolates of *Shigella* showed any loci. Various patterns demonstrated by EIEC isolates in agarose gel are shown in Figure 1. The limit of detection for all bands representing loci in agarose gel was 10^5 bacterial cells/mL.

Differentiation between enteroinvasive *Esherichia coli* (EIEC) and *Shigella* using multiplex PCR

continued from page 5

Figure 1. Differentiation of EIEC and *Shigella*. Three lanes (leftmost, middle and rightmost) of 50-bp DNA ladder are shown. The lanes between the leftmost and middle lane belong to EIEC isolates and those between the middle and rightmost lane belong to *Shigella*. Starting from the second lane, each two lanes belong to one isolate. For each isolate, the first lane belongs to multiplex PCR system A and the second lane belongs to multiplex PCR system B. EIEC isolates can be genotyped based on the type assigned based on the combination of loci present.



The genomes of EIEC and *Shigella* used in this study are diverse in origin, sequence types and serotypes. This method of differentiation of EIEC and *Shigella* is cost effective and can complement serotyping of *Shigella* when EIEC is suspected. If EIEC is positive, the serotyping of *Shigella* may not be required. We compared the combination of loci present in the EIEC isolates with the phylogeny of previous three studies where the same genomes had been employed and found good correlation with our multiplex PCR results. Our evaluation is continuing and we will be grateful for referrals of *ipaH* positive clinical isolates of *Escherichia coli* for further identification and characterisation.

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Welcome to Dr Aaron Jenkins

Name: Dr Aaron Jenkins

Position: Research Fellow, Sydney School of Public Health and MBI, University of Sydney and Edith Cowan University's Centre for Ecosystem Management

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In April, MBI researcher Aaron Jenkins started a joint appointment with Sydney School of Public Health and Edith Cowan University's Centre for Ecosystem Management as the first Research Fellow in Planetary Health for the region. His role is designed to strengthen the breadth and depth of research on ecological determinants of health within these institutions, MBI and the region. The emerging field of Planetary Health is transforming the field of public health to consider the broader natural ecosystem and recognize ecosystem transformation effects on emerging threats, not only to human health and wellbeing, but also to the "sustainability of our civilization" and to the "natural and human-made systems that support" humanity.

Aaron assessing reef health in Fiji

Aaron is well placed to undertake this challenge, with 20 years' experience throughout the Asia-Pacific region in crosscutting development themes including integrated conservation and development, wetland management for health, nutrition and climate change mitigation, WASH and waterborne disease management, intersection of climate change, natural disaster, land and water management on health and sustainable fisheries.

He is currently assisting the Sydney Research Excellence Initiative (SREI) on Climate Change and Health to investigate the role of extreme storm (i.e. tidal and non-tidal flooding) and heat events on vector abundance and vector-borne disease risk using established surveillance programs in NSW coastal and remote interior sites. He is also involved in ongoing research in Fiji, integrating data from a set of studies linking environmental, microbiological and physicochemical findings to patterns of typhoid disease at catchment, residential and household scales. He is also involved in integrated drinking water quality and health surveillance for emergency recovery and response in Fiji, helping to design integrated surveillance methodologies with UNICEF, WHO and Fiji National agencies to use in prioritizing recovery and response post natural disaster. He is also currently guest lecturing for the University of Sydney Sustainability Program.

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A nested environmental approach to typhoid epidemiology in Fiji

Dr Aaron Jenkins

Research Fellow, Sydney School of Public Health and Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, and Edith Cowan University's Centre for Ecosystem Management

Wednesday, 27 September 2017

12noon - 1pm

Level 2 Seminar Room,

Westmead Institute for Medical Research, Westmead

Typhoid fever is a serious disease threat in the South Pacific region, with Fiji reporting the highest annual number of cases, yet risk factors in this setting have been poorly studied. While localised behaviours have dominated perspectives on typhoid transmission, interactions between distal ecological conditions, conditions of the residential environment and localised behaviour deserve greater attention for their potential to influence transmission. This presentation demonstrates a nested approach to typhoid epidemiology utilising geospatial, case-control, microbiological, physicochemical and observational methodologies to explore how regional, river basin, residential, socio-cultural and behavioural subsystems influence the risk of typhoid transmission in Central Division, Fiji. Significant risk factors are synthesized within and across nested subsystems and several intervention scenarios are explored using a Bayesian Network approach. This study demonstrates how a nested environmental approach to studying and interrupting waterborne disease transmission extends the testing of causal assumptions beyond the domestic domain, enhances traditional case-control approaches and provides evidence for multi-scale interventions on drivers of disease and environmental degradation.

About Dr Aaron Jenkins

Dr Aaron Jenkins is the inaugural Research Fellow in Planetary Health research with the Sydney School of Public Health, Marie Bashir Institute for Infectious Diseases and Biosecurity, and Edith Cowan University's Centre for Ecosystem Management. He has highly regarded expertise in crosscutting development themes including integrated conservation and development, wetland management for health, nutrition and climate change mitigation and waterborne disease management. This expertise stems from 20 years of professional experience in international development working with international, regional agencies, governments, NGOs, donors, universities and communities across Asia-Pacific. He has high-level engagement in international and regional affairs, and is an elected board member of the International Association of Ecology & Health. His current research focus explores the socio-ecological determinants of waterborne diseases.

Event Details

This is a free event co-hosted by Centre for Infectious Diseases & Microbiology- Public Health and the Marie Bashir Institute .

Registration

To register https://aaronjenkinsseminar.eventbrite.com.au

Congratulations…..

The **Centre for Infectious Diseases and Microbiology - Public Health** was successful in their application for Round 5 of the ***NSW Health Prevention Research Support Program (PRSP) 2017 –2021***. PRSP provides funding to NSW research organisations conducting prevention and early intervention research that aligns with NSW Health priorities. PRSP funding supports research infrastructure and strategies to build research capability and translate evidence from research into policy and practice.

A/Prof Vitali Sintchenko and CIDM-PH team at Westmead

The Minister for Health and the Minister for Medical Research, Hon. Brad Hazzard announced the successful recipients of round two of the ***Translational Research Grants Scheme*** on 5 June 2017. The projects were funded based on their innovation and potential to translate quickly into treatments that could benefit the lives of NSW patients.

Dr Matthew O'Sullivan , a senior investigator of CIDM-PH and Staff Specialist for NSW Health Pathology, was one of the fifteen recipients to be awarded funding for his project "A state-wide typing network for rapid detection of outbreaks of healthcare associated infection".

Professor David Lewis, a senior investigator of CIDM-PH and Director of Western Sydney Sexual Health Centre, was recently awarded the ***Gold Medal of the International Union against Sexually Transmitted Infections***, at the World STI & HIV Congress in Rio de Janeiro, for promoting international co-operation in the fight against STIs.

This prestigious medal is only the sixth to have been awarded, and is the first from the Southern Hemisphere.

Congratulations David!

CONTACT US

For more information on any articles or CIDM-PH & MBI events, or to join the e-lists and receive regular updates, please contact us at:

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