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The Broad Street Pump

A Centre for Infectious Diseases and Microbiology - Public Health (CIDM-PH) and Marie Bashir Institute for Infectious Diseases & Biosecurity (MBI) publication

The genomic landscape of *Legionella pneumophila*

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The recent Sydney outbreak

In 2016 Sydney was the epicentre of several outbreaks of legionellosis that spanned three geographical regions, the CBD, Kogarah and Burwood [1]. The CBD was affected twice, once during March 2016 (named the CBD March period) and then from 28th of April to the 11th of May 2016 (named the CBD May period). For the CBD March period, five towers were found to be *Legionella pneumophila* serogroup 1 (Lp1) positive and despite the cleaning of these towers, legionellosis returned in late April (CBD May period). The aplication of whole genome sequencing (WGS) on Lp1 isolates obtained in the CBD March period revealed that several patients were infected with the same strain of Lp1. Further, the *L. pneumophila* sequence based typing scheme (SBT) labelled the dominant Lp1 strain as ST211 [2]. When cases of Legionellosis were detected in the CBD May period that were also ST211, WGS was used on isolates from newly sampled towers and the environmental source of the Lp1 was found.

However, between March and April, legionellosis also occurred in Kogarah and Burwood, suburbs 18kms and 16.5 km away from the CBD, respectively. The Lp1 strain found in Kogarah was also ST211 and was isolated from patients and water cooling towers. Without the use of WGS, this connection would not have been identified however the reasons for this connection remained unclear. Were the water cooling towers in the CBD and Kogarah contaminated from a common source or is ST211 ubiquitous in Sydney?

Inside this issue

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What we know of ST211

ST211 was thought to exclusively reside in the colder climate of Canada where it was first identified in 1989 [3] and it was initially thought to be contributing to sporadic cases. On further investigation, it was found to be responsible for 12.5% of isolates. ST211 has since become one of the most persistent and predominant STs in Ontario and along with other STs, has almost completely replaced the previously dominant Lp1 ST1. Yet despite its dominance as a disease causing clone, this ST has never been reported outside Canada.

The *Legionella* genus

Since the first description of Legionnaires disease in 1976 when a large outbreak of pneumonia occurred at an American Legion conference in Philadelphia [4], *L. pneumophila* has been implicated in sporadic cases and community outbreaks worldwide. The infection commonly affects males over 50 years of age, people with co-morbidities and smokers [5]. However the sporadic nature of legionellosis outbreaks reflects the environmental distribution of these pathogens. The genus *Legionella* contains over 60 species and is ubiquitous in the environment with a natural habitat of water, amoebae and biofilms. All species are found in the environment and about half of these can cause a life-threatening illness in humans. *L. pneumophila* is responsible for the majority of disease and is further subdivided into 16 serogroups with the majority (80-84%) of legionellosis cases caused by Lp1. However, this serogroup 1 dominance is not reflected in the environmental makeup of *L. pneumophila* [6][7][8].

Genomics of *L. pneumophila*

L. pneumophila are highly recombinant and readily share and exchange DNA between strains. The high recombination rate provides the species with a suite of mobile genetic elements, including plasmids and pathogenicity islands [9]–[12]. These mobile genetic elements encode virulence and fitness factors necessary for intracellular replication and survival in both environment and host and even help in the resistance to detergents [13][14]. This high level of recombination contributes to the difficulty in typing *L. pneumophila*.

While 7-allelle gene sequencing based typing (SBT) methods have been used for outbreak investigation in the past [15], the limited resolution of SBT has led to a wider application of WGS. WGS is universally acknowledged to be a powerful tool that can provide high resolution information on strain type, transmission pathways, outbreak origin and antibiotic resistance for a growing number of disease outbreaks both locally [2] and internationally [16][17]. For example, the application of WGS in the USA as an outbreak surveillance tool for severe foodborne outbreaks caused by *Listeria monocytogenes* saw the number of solved outbreaks increase from two, prior to WGS, to nine after WGS was implemented [18].

What can WGS show us?

Therefore we sought to contextualise the 2016 Lp1 outbreak in Sydney as several questions remained unanswered. First, was ST211 an emergent clone or had it always existed in Sydney? Which STs of *L. pneumophila* were responsible for past outbreaks? Is there any information in the genomes of outbreak strains that we can use to identify future outbreaks? To answer these questions, Lp1 isolates from 1992 to the present were subjected to WGS to determine the landscape of Lp1 in Sydney, establish if it had changed over time and interrogate the Lp1 genomes for any genomic structures that have been present in past outbreaks.

Surprisingly, the ST211 strain has been a resident of Sydney since at least 1994 (Figure). According to our information, it has been responsible for other outbreaks in the greater Sydney area. The idea that one clone is dominant in most of the outbreaks in a city has also been raised in Melbourne where the strain responsible for the largest Legionella outbreak in Australia was also involved in other outbreaks in that city [19]. In future work we will determine more accurate cluster assignment without the reliance on the 7-allelle SBT scheme given its limited resolution and utility. Genome wide association studies can also be performed to uncover any genomic elements that are present in outbreak versus non outbreak strains.

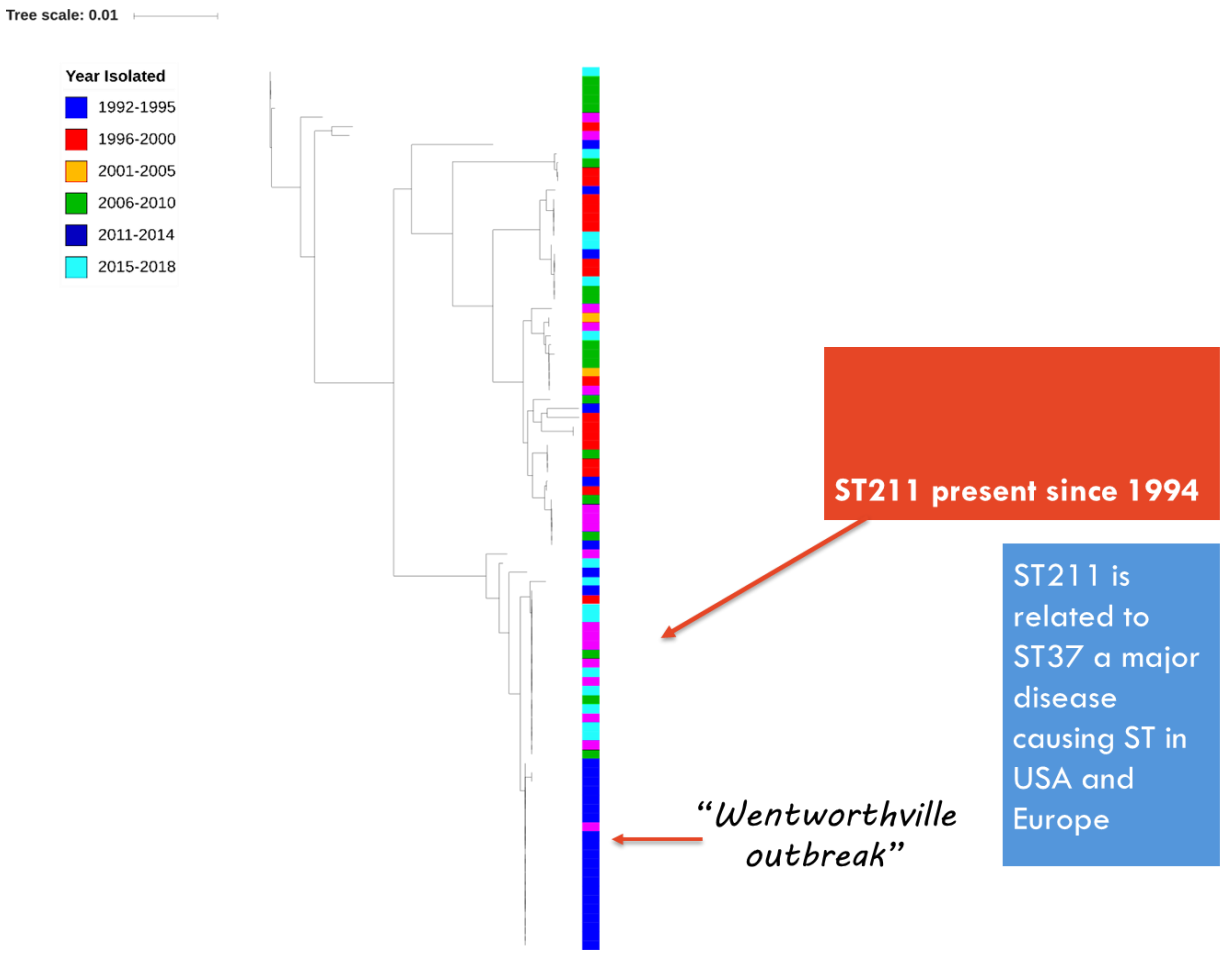

Figure: Phylogenetic tree showing comparison of Lp1 strains from 1992-2016.
Coloured bar corresponds to the year the Lp1 strain was isolated according to the key.

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Visit to Harvard University Boston

Dr Nathan Bachmann

Last October, Nathan spent 4 weeks as a visiting research fellow to the Harvard University in Boston. He was working with Dr Ashlee Earl's research team. Ashlee is the senior leader for the Bacterial Genomics Group at the Broad Institute of MIT and Harvard. The purpose of the visit was to analyse the large collection of genomic data for Mycobacterial species that has been collected since the advent of next-generation whole genome sequencing.

The goal of the project was to investigate the evolution of mycobacterium species, in particular the emergence of slow growing pathogenic Mycobacteria (such as *M. tuberculosis*) from environmental rapid growing species. During his visit at the Broad Institute, Nathan performed analysis on the genome sequences of 155 Mycobacterial species of different growth rates. The Pathogen Genomics team at the Broad Institute provided expertise and custom software to assist with the analysis. Figure 1 shows the phylogenetic relationship of the 155 species, which indicated that ancestral species were rapid growers with subsequent distinct genetic separation between rapid and slow grower phenotypes. The tree also distinguishes between the 5 recently classified subgenera of Mycobacteria with the Mycobacteroides, including *M. abscessus*, identified as an outlier group that separated prior to the evolutionary split between ancestral rapid growing species and slow growers. Apart from the Mycobacteroides, all other rapid growing species are located in the Mycolicibacterium genus. While the majority of slow growing species are members of the other three genera Mycobacteria, Mycolicibacillus and Mycolicibacter, located on the other arm of the major phenotypic split.

Harvard University Boston

Dr Nathan Bachmann

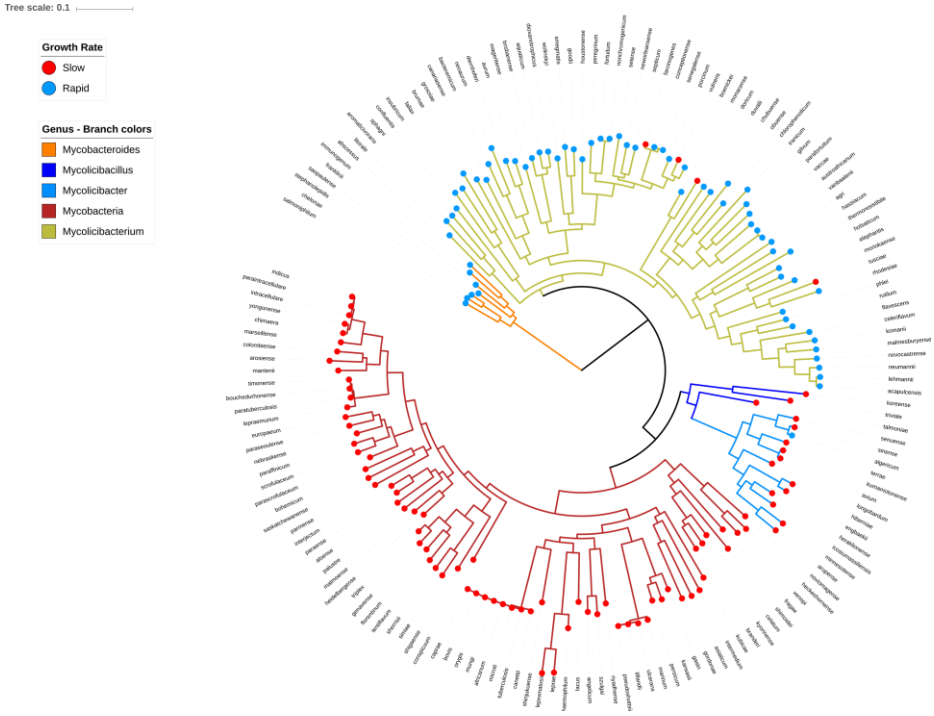


Figure 1: Phylogenetic tree of Mycobacteria species showing the distribution of slow growing and rapid growing species.

Pangenome comparisons were performed to identify genes that are unique to each of the five genera, which may be potential genetic features that are linked to growth rate. One such feature is the five-gene operon that encodes for an amino acid transporter. Homology matches, as shown in Figure 2, reveal that these genes are found exclusively in the ancestral rapid growing species of the Mycobacteroides and Mycolicibacterium genera. The ability to uptake various metabolites are likely to be important for the rapid growth rate of *Mycobacterium* species, hence the presence of this transport system that is absent in slow growers.

This study has the potential to determine the genetic changes that occurred in Mycobacterium that lead to the emergence of pathogenic species such *M. tuberculosis* and M. *leprae* that have adapted to surviving in humans from environmental niches. The trip was useful in establishing collaborations with Ashlee Earl and members of her team at The Broad Institute, which we will continue to foster with new projects that will be submitted as NHMRC New Ideas grants. The University of Sydney Office of Global Engagement funded Nathan's visit to the Broad Institute via the Harvard University Mobility Scheme.

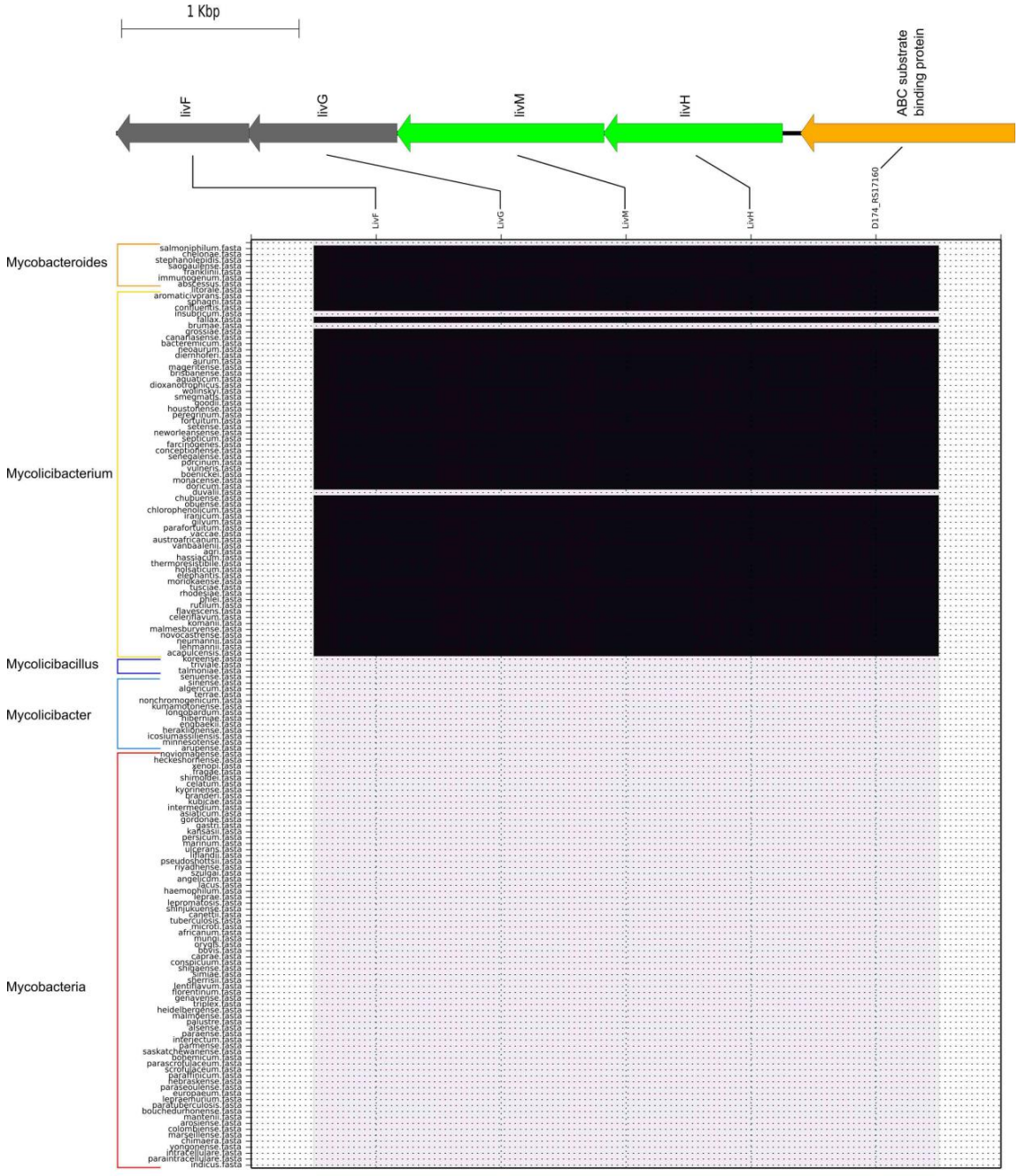
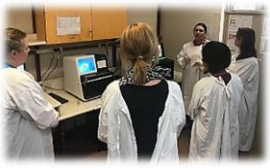


Figure 2: Presence of the 5 gene LivFGMH operon that is found exclusively in the rapid growing Mycobacteroidies and Myclicibacterium species as shown by the black shading according to protein sequence homology.

News & Events

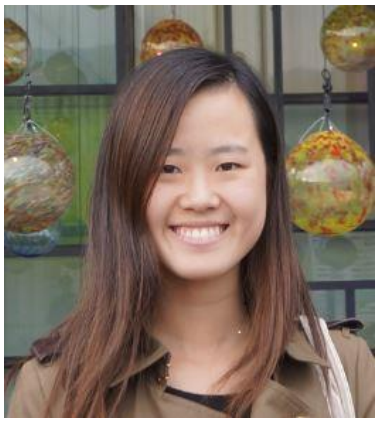
The Sydney Summer School in Pathogen Genomics and Global Health was co-hosted by the Centre for Infectious Diseases and Microbiology – Public Health (CIDM-PH) and Marie Bashir Institute (MBI). The Summer School was held at the Sydney Nanoscience Hub at Sydney University on 11th – 15th February 2019. The 5 day program included a mix of inspiring keynotes, informatics reviews, masterclasses, practical hands-on exercises and a laboratory visit to the Genome Sequencing Facility at the Centre for Infectious Diseases and Microbiology, ICPMR, Westmead.



Sydney Summer School in Pathogen Genomics & Global Health 2019

Staff Profile – Winkie Fong

Winkie Fong is a student from the University of Sydney, who did her Honours year at the Centre of Infectious Diseases and Microbiology - Public Health (CIDM-PH), where she examined the epidemiology of an emerging human pathogen, *Bordetella holmesii,* within *Bordetella pertussis* epidemics. She is currently a 3rd year PhD candidate and Research Associate at CIDM-PH, developing culture-independent and metagenomic protocols for the extraction and sequencing of *B. pertussis* from clinical specimens for diagnostic and public health purposes.



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UPCOMING EVENTS

7 June 2019:

Infection Control Symposium

Westmead Education and Conference Centre, Westmead Hospital

Registrations opening soon

2 August 2019:

Medical Entomology & Health Symposium

'Meeting the challenges of emerging mosquito-borne disease threats'

Westmead Education & Conference Centre, Westmead Hospital

Registrations opening soon

22 November 2019:

CIDM-PH Colloquium

Westmead Hospital, Sydney

Event Enquiries:

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