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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: ≤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: ≥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
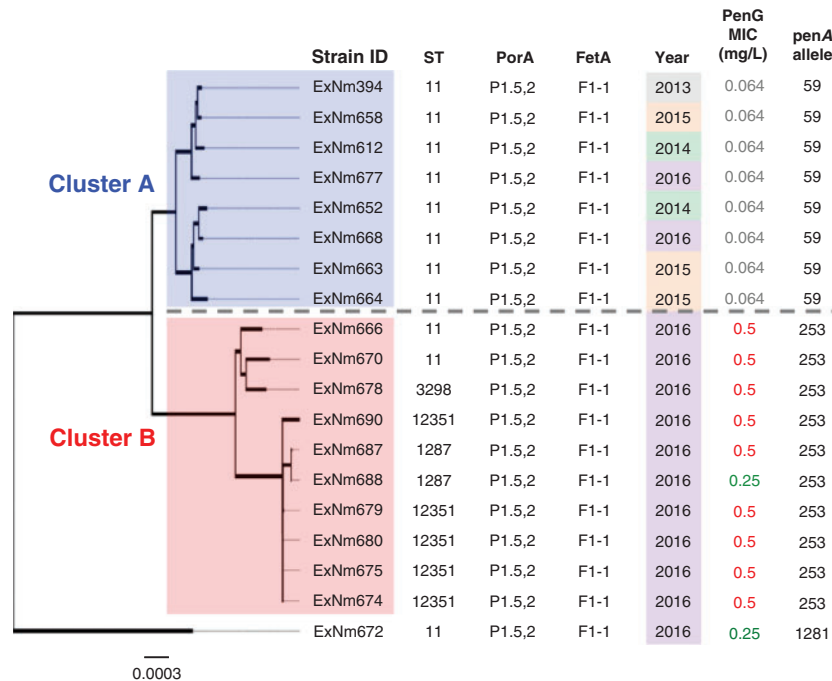
Figure 1. Neighbour-joining tree using the core genome of invasive MenW:cc11 strains isolated in Western Australia during 2013–2016. The dendrogram represents a neighbour-joining tree (500 bootstraps) generated using the core genome sequences of the meningococci. All isolates possessed the same PorA:FetA 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.

Acknowledgements

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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.

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