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Genetic Characterization of a *Neisseria meningitidis* cluster in Queensland, Australia

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Conflicts of interest: none

Abstract

Neisseria meningitidis serogroups B and C have been responsible for the majority of invasive meningococcal disease in Australia, with serogroup B strains causing an increasing proportion of cases in recent years. Serogroup Y has typically caused sporadic disease in Australia. In 2002, a cluster of four cases was reported from a rural region in Queensland. Three of these cases were serogroup C, with one case diagnosed by molecular detection only, and the fourth case was identified as a serogroup Y infection. Genomic analysis, including antigen finetyping, MLST and cgMLST demonstrated that the serogroup Y case, though spatially and temporally linked to a serogroup C disease cluster, was not the product of a capsule switch, and that one of the serogroup C isolates had a deletion of the entire *porA* sequence.

Key Words: *N. meningitidis*, outbreak, whole genome sequencing, *porA* mutant

Neisseria meningitidis, a Gram negative commensal bacterium, causes invasive meningococcal disease (IMD) in humans. Strains of *N. meningitidis* undergo genetic recombination, through mechanisms such as recombination of flanking repeat regions or IS1301 disruption of capsule biosynthesis genes, generating changes to the capsular serogroup, referred to as capsule switching (Hilse et al. 1996; Mustapha et al. 2016; van der Ende et al. 1999) . As the polysaccharide capsule is an important target of the host serogroup-specific immune response and a component of most *N. meningitidis* vaccines, capsule switching assists in immune evasion (Rishishwar et al. 2012). Strains may also lose expression of outer membrane proteins such as PorA as an alternate mechanism for immune evasion (Harrison et al. 2006; van der Ende et al. 1999).

In 2002, four cases of IMD from a rural central Queensland region occurred over a 41 day period, which met the national guideline criteria of a disease cluster; a community vaccination program for the defined risk age group of 18-40 years old was subsequently implemented (Pugh et al. 2003). Of the four cases, three serogroup C and the other serogroup Y, one of the serogroup C cases was diagnosed by molecular detection therefore no isolate was available for further characterization. At the time of the disease cluster, serosubtyping and serotyping determined the two serogroup C isolates were C:2a:P1.5 and C:2a:NST respectively, while the serogroup Y isolate was Y:NT:P1.5 (Programme 2003; Pugh et al. 2003). This information in combination with epidemiological linkages led to speculation that the serogroup Y isolate had arisen via a capsule switch from the serogroup C strain responsible for the other cases (Pugh et al. 2003). This study has revisited this disease cluster, using whole genome sequencing to characterize the genetic lineages and to establish if capsule switching had occurred.

DNA was prepared by the Ion Plus Fragment library kit and sequenced on an Ion Torrent PGM using the Ion PGM IC 200 Kit, Ion Chef and 316v2 chips (Life Technologies) according to the manufacturer's instructions. FASTQ sequences were *de novo* assembled using the built-in assembler in Geneious R7. Assemblies ranged from 122 to 361 contigs and 103-150 average depth. Sequences were deposited in ENA under study PREJEB13900, and isolate records in the PubMLST isolate database (Table 1).

In silico molecular antigenic profiling and MLST analysis performed at pubMLST (<http://pubmlst.org/neisseria/>) (accessed 18/08/2016) revealed that the two serogroup C isolates shared MLST and antigen profiles, except that *porA* was absent in M35809. M35044 was typed as Y:P1.5-1,10-4:F4-1:ST-23, sharing only the same PorA type as M35007 which was a C:P1.5-1,10-4:F3-6:ST-11 type. The C:P1.5-1,10-4:F3-6:ST-11 genotype has not been reported for Australian strains in the time that national genotyping data has been made available (2010-2013) nor in Queensland post-2008 (2004-2015 unpublished data) (Lahra and Enriquez 2012, 2013, 2014; Programme 2011). Due to the absence of molecular antigenic profiling surveillance in 2002, it is unclear whether this particular genotype was prevalent in Australia at the time of this disease cluster. The serogroup Y isolate from the 2002 cluster was determined to be Y:P1.5-1,10-4:F4-1:ST-23. This genotype has been previously reported circulating in the UK in a 2007-2009 study and also has been noted in low levels in Australia (1/10 serogroup Y cases in 2012 and 3/14 cases in 2013) (Lahra and Enriquez 2013, 2014).

High resolution gene typing was performed in RidomSeqSphere+ (Ridom GmbH, Germany) using the *N. meningitidis* cgMLST v1.0 scheme (Bratcher et al. 2014) (Table 1). Figure 1 shows a neighbor-joining tree with the three isolates and the closest related strains to each respectively available on pubMLST. Isolates were compared using the PhyloTree tool on pubMLST and

iTOL visualization (Letunic and Bork 2016). As expected from the MLST and antigen profiling, the serogroup Y isolate did not share significant core genome similarity to the serogroup C isolates (Figure 1). The serogroup C isolates differed at 13/1605 core genes and fit with sublineage 11.2 (Lucidarme et al. 2015). At the time of writing, there are no other isolates on the pubMLST database with 25 or fewer cgMLST loci differences to either of these two isolates.

M35809 sequences reads were mapped to the reference strain FAM18 (AM421808) using CLC Genomic Workbench 8, with no reads aligning to the *porA* gene region of FAM18. Alignment of the *de novo* assembly to the FAM18 genome, using Geneious 7 with the Mauve 2.3.1 plugin, demonstrated a 1013 bp deletion in the *porA* region of M35809, corresponding to the region between nucleotides 1 355 993 and 1 358 164 of FAM18. This deletion is flanked by repeat regions and encompasses the entire *porA* gene as well as sequences upstream and downstream.

Antigenic profiling revealed that the serogroup Y isolate belonged to a different genetic lineage than the two serogroup C isolates and was not the result of a capsule switch during the outbreak as originally speculated (Pugh et al. 2003). Furthermore, although the serogroup C isolates, M35007 and M35809 were similar by antigenic profiling, analysis revealed that these isolates not only differed in the *porA* locus but also at 13 other core genes. It is likely that genetic variability in 14 different core genes represents sufficient genetic distance that these two isolates are not indicative of close transmission, although inter-host variability cannot be ruled out as only single colonies were stored for each case. Comparatively, 6 isolates that differed by 6 or less cgMLST v1.0 alleles, were considered to belong to the same Canadian MSM outbreak of sublineage 11.2, while a French MSM outbreak strain LNP27256 (pubMLST ID 26733) was 36 alleles different from a fellow 'outbreak' isolate (pubMLST ID 26821), leading the authors to suggest that the level of genetic variation did not constitute an outbreak, but rather a close

transmission network (Lucidarme et al. 2015; Tsang et al. 2003; Veyrier et al. 2013). We speculate that while the two serogroup C cases were considered sufficiently spatially and temporally linked to meet the criteria of a disease cluster, they are likely the result of a strain circulating in a geographical area rather than a directly linked transmission event.

The non serosubtypeable phenotype (C:2a:NST) of M35809 was due to the deletion of the *porA* region. Loss of *porA* has been reported in a low number of incidences and is thought to be either a host immune response evasion mechanism, or the result of multiple subcultures of the isolate *in vitro* (van der Ende et al. 1999, 2000). The M35809 isolate was isolated 41 days after that for the M35007 specimen and stored at -80°C within a few days. Repeat elements, present throughout the *N. meningitidis* chromosome, have been demonstrated to play a role in deletion of genes, including *porA*, through recombination (Schoen et al. 2008; van der Ende et al. 1999). In M35809, repeat sequences were identified upstream and downstream of *porA*, and it is likely that the deletion of *porA* occurred via recombination between these repeat regions. This isolate is one of only three *porA* negative isolates detected in Queensland IMD cases in the last fifteen years and the only belonging to serogroup C.

This 2002 cluster of four IMD cases resulted in a significant public health response, including a targeted vaccination program utilising a polysaccharide (ACWY) vaccine. Epidemiological information and phenotypic typing data available at the time lead to speculation that the serogroup Y case was caused by a capsule switch from a serogroup C strain. This study has utilised whole genome sequencing to demonstrate that the serogroup Y isolate was not derived by a capsule switch but most likely a sporadic case of IMD that occurred spatially and temporally related to a cluster of serogroup C disease. Furthermore, genetic analysis revealed that the two serogroup C cases were caused by isolates of the same lineage, with high genetic

similarity but with variation in a small number of genes, including the loss of the entire *porA* gene in one isolate.

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Figure 1: Neighbor-joining tree based on 1605 cgMLST alleles for isolates from this study and closest neighbours in PubMLST database.

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Table 1: Isolates sequenced in this study.

Isolate	PubMLST ID	Number of cgMLST loci used in analysis	Phenotype	Genotype	ENA Sample number
M35007	41845	(1583/1605)	C:2a:P1.5	C:P1.5-1,10-4:F3-6:ST-11 (cc11)	ERS1146380
M35809	41847	(1584/1605)	C:2a:NST	C:P1.Δ:F3-6:ST-11 (cc11)	ERS1146381
M35044	41846	(1573/1605)	Y:NT:P1.5	Y:P1.5-1,10-4:F4-1:ST-23 (cc23)	ERS1146382

Note. NST = Not subtypeable, NT = Not serotypable, Δ= gene determined to be absent

Figure 1: Neighbor-joining tree based on 1605 cgMLST alleles for isolates from this study and closest neighbours in PubMLST database.

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