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Case Report

Resurgence of *Neisseria meningitidis* serogroup W ST-11 (cc11) in Madagascar, 2015–2016

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SUMMARY

The resurgence of invasive meningococcal disease caused by *Neisseria meningitidis* serogroup W with sequence type ST-11 (cc11) was observed in Madagascar in 2015–2016. Three cases were investigated in this study. Molecular characterization of the strains suggests the local transmission of a single genotype that may have been circulating for years.

1. Introduction

Meningococcal disease due to *Neisseria meningitidis* serogroup W isolates has been reported in Africa since 1982.[1] In 2000, there was a Hajj-associated meningococcal outbreak in Saudi Arabia, caused by isolates belonging to the hypervirulent *N. meningitidis* serogroup W sequence clonal complex ST-11/ET-37 (cc11). These cc11 isolates were present in Africa prior to 2000, and the Hajj-linked isolates correspond to clonal expansion within these cc11 isolates. After declining in Africa since 2004, serogroup W/cc11 then re-emerged in 2010.[1] Thanks to the use of whole genome sequencing (WGS), greater resolution has been achieved and has shown that W/cc isolates worldwide correspond to several lineages – a feature that is in favour of the multifocal emergence of these isolates.[2] In Madagascar, W/cc11 isolates were last reported in 2002.[3] From 2003 to 2011, no data were available until the implementation of hospital-based sentinel surveillance for paediatric bacterial meningitis. During the period 2012 to 2015, no *N. meningitidis* W was identified in 1354 cerebrospinal fluid (CSF) specimens (Robinson A, personal communication). Three cases of *N. meningitidis* infection caused by cc11 serogroup W, very likely occurring as a result of local transmission in Antananarivo, the capital city of Madagascar, are described herein.

2. Case report

In February 2015 and February 2016, two adults and one infant were admitted to the Infectious Diseases and Paediatric wards in Antananarivo for invasive meningococcal disease. The first case reported on February 8, 2015, was a 23-year-old male farmer presenting with febrile acute meningitis and purpura of the lower limbs. One year later, on February 8, 2016, a very similar case

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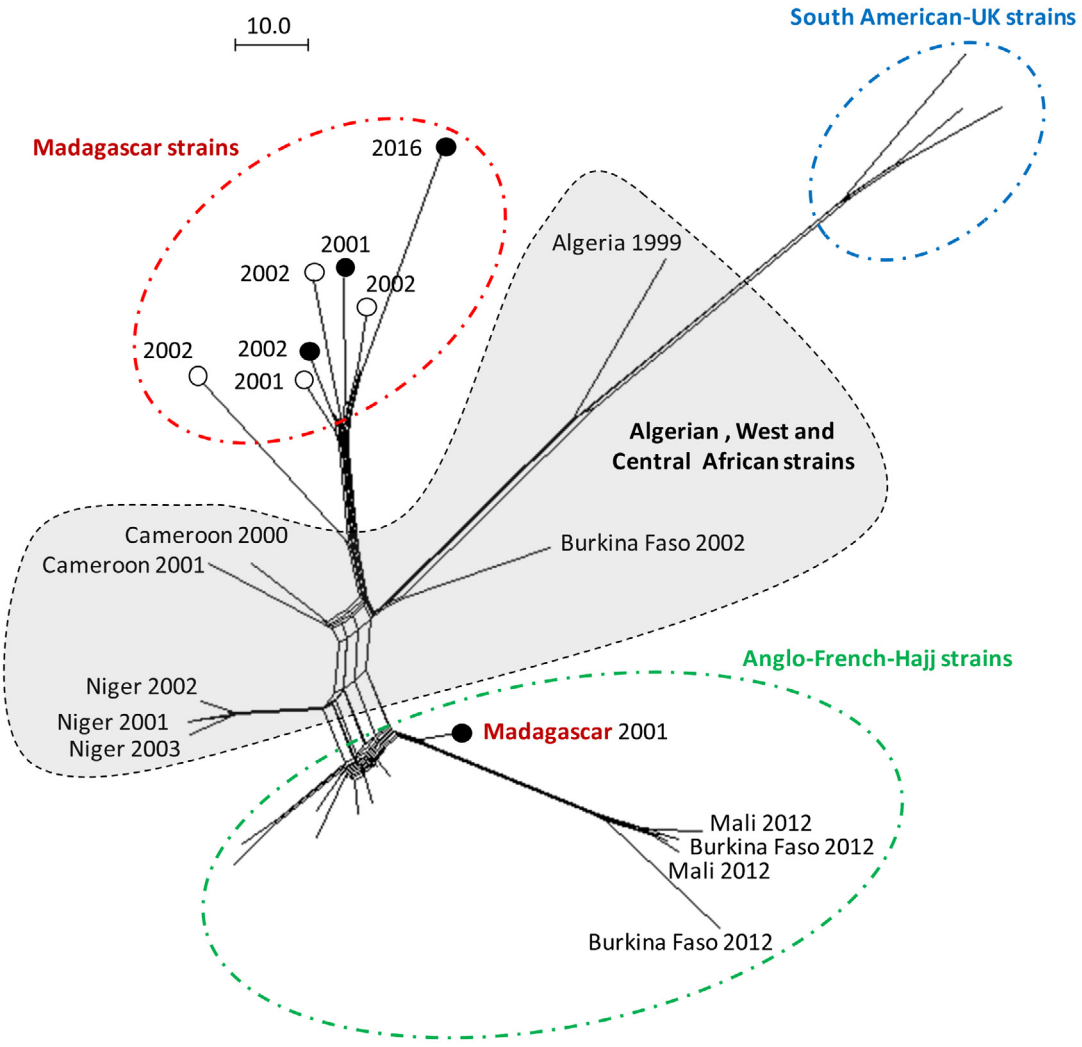

Figure 1. Core genome MLST Neighbor-Net phylogenetic network for the temporal distribution of W/cc11 isolates from Madagascar (n = 8) and other countries (n = 27). The tree was built with Neighbor-Net SplitsTree graphs generated by SplitsTree4 (version 4.13.1). The network shows four different lineages. The major Madagascar lineage includes invasive (filled circles) and carriage (open circles) strains isolated in 2001–2002 and 2016. The Anglo-French–Hajj lineage includes strains relating to the Hajj outbreak of 2000 onwards (unlabelled nodes), West African strains from 2012, and one Madagascar invasive strain isolated in 2001. The South America–UK lineage includes strains relating to expanding endemic W/cc11 disease in South America and the UK (unlabelled nodes). The last lineage, highlighted in grey (filled area), includes 15 W/cc11 isolates from Algeria and West and Central Africa (IDs 27087, 30074, 30076, 30081, 30083, 30087, 30088, 30089, 30092, 30095, 47005, 47006, 47008, 47009, and 47010). The 12 W/cc11 isolates representing the Anglo-French–Hajj strains and the South America–UK strains (unlabelled nodes) described here were retrieved from Lucidarme et al.[2] (IDs 2290, 21573, 28158, 28159, 28161, 29360, 29992, 30066, 30067, 30075, 31164, 31165). The scale bar indicates the number of loci differing among the 1605 compared.

appeared in a 25-year-old male bricklayer experiencing seizures and displaying extensive purpura between the lower limbs and the trunk. Then, the third case was detected on February 25 of the same year in an 8-month-old girl with fever and acute aseptic meningitis. Lumbar puncture revealed turbid cerebrospinal fluid (CSF) in the adult cases, while the CSF remained clear in the infant. CSF samples were inoculated onto chocolate agar and showed microbial growth after 24 h for the first and third cases. The API NH system (bioMérieux, Marcy l'Etoile, France) revealed the presence of *N. meningitidis* isolates. An in-house multiplex real-time PCR targeting serogroup-specific genes of *N. meningitidis* performed on the CSF and colonies was negative for all serogroups tested, except for W (testing for A, B, C, X, Y, and W). All CSF samples, but only the paediatric isolate, were retrieved for further investigation.

The isolate was found to be susceptible to beta-lactams (penicillin, amoxicillin, and ceftriaxone), ciprofloxacin, chloramphenicol, and rifampicin by Etest (bioMérieux, France). The patients recovered fully after treatment with a third-generation cephalosporin. They were not epidemiologically related, although living in the same area. No history of foreign travel or contact with suspected cases was reported. Prophylaxis with ciprofloxacin was administered to the patients' close contacts, relatives, and professional health workers. Therefore, no biological investigation was performed for these cases.

Molecular typing by multilocus sequence typing (MLST) using the PubMLST Neisseria database (http://pubmlst.org/neisseria) performed for the three cases revealed sequence type ST-11 (cc11) PorA (P1.5,2), and FetA (F1-1) subtypes. The cultured isolate and seven other Malagasy W/cc11 isolates (carriage n = 4 and invasive n = 3) obtained between 2001 and 2002 were subjected to WGS using Illumina NextSeq 500. The eight isolates (IDs 17191, 17192, 17193, 17194, 17195, 17196, 17197, and 42042) were uploaded to the PubMLST database and compared using core genome MLST (cgMLST) (1605 loci). Representative genomes of W/cc1 isolates (n = 27) in the PubMLST database were also included in order to identify the putative origins of the Malagasy cc11 isolates (Figure 1). One Malagasy isolate recovered from an invasive case in 2001 clustered with the Hajj outbreak. All the other isolates – including the 2016 case – were genetically linked, but remained distinct from the two major W/cc11 lineages reported previously:

the 'Anglo-French–Hajj' and 'South America–UK' clusters.[2] The Malagasy isolates also clustered independently from a lineage of W/cc11 strains that were found in Algeria and West and Central Africa in the 2000s. They shared 1355 genes among the 1505 genes of the cgMLST, while 234 genes were variable. Several genes including *galE*, *lptA*, *lgtA*, *lpxL*, and *lpxH* among these variable genes are required for the biosynthesis of meningococcal lipooligosaccharide (LOS), a group of molecules that is involved in the induction of the inflammatory and immune responses, as well as meningococcal virulence.[4]

3. Discussion

This study is the first to document the detailed biological investigation of a localized outbreak due to *N. meningitidis* W in Madagascar. Molecular characterizations and WGS of the clinical isolates found that a single strain (W:P1.5,2:F1-1:cc11) was responsible for all three cases. However, no epidemiological link between these three cases has been found. The absence of travel history to high-risk countries and contact with known susceptible or sick people, suggests that the strain has been circulating in the Antananarivo area for years. The present data are in line with this explanation, as local transmission of this strain among carriers and patients since at least 2001 was detected. This outbreak highlights the high potential for *N. meningitidis* W to spread among the population.

In this study, the two adult cases presented with meningococcemia characterized by a sudden onset of fever and purpura, whereas the paediatric case experienced non-specific signs of aseptic meningitis. These clinical findings highlight the various forms of invasive meningococcal disease and should be taken into account when performing the diagnosis.[5]

The resolution of W/cc11 isolates into 'Anglo-French–Hajj' and 'South America–UK' cluster on the basis of cgMLST analysis has been reported previously.[2] Other W/cc11 isolates found in other parts of the world – including Africa – clustered separately from the previous two lineages and were most likely related to local W/cc11 isolates. In the present study, one invasive isolate in 2001 was linked epidemiologically to the Hajj outbreak. The remaining isolates, including the 2016 case, were genetically linked, but were distinct from the two major W/cc11 lineages, thus suggesting a locally independent genotype. The present data also suggest that the LOS structure may differ between these isolates and may impact the host–pathogen interaction. Further research is needed to explore the virulence of these isolates.

In conclusion, surveillance should be enhanced in other regions of Madagascar to determine the burden of invasive meningococcal disease, both adult and paediatric cases. This could be used to guide evidence-based decision-making if meningococcal vaccines are needed in Madagascar. The lack of reported cases – especially between 2003 and 2011 – is likely the consequence of sub-optimal surveillance. This study also highlights the importance of using WGS to characterize sporadic strains and outbreak-associated strains of *N. meningitidis* W and to monitor any changes in their epidemiology.

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Conflict of interest: The authors declare that they have no conflict of interest.

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