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Commentary

Will Genome Analysis Elucidate Evolution, Global Transmission and Virulence of *Neisseria Meningitidis* Lineages?

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Neisseria meningitidis is a frequent commensal resident of the oropharyngeal mucosa, carried by at least 10% of the human population in industrialized countries. However, *N. meningitidis* might penetrate the mucosal membranes and cause life-threatening septicemia and meningitis, commonly with a short time from onset to death. Despite appropriate treatment, the case-fatality rate for invasive meningococcal disease (IMD) remains around 10% (Goldacre et al., 2003). The bacterium uses different strategies to evade the immune system and survive in different environments, nevertheless, the only truly well-established virulence factor is the polysaccharide capsule. Thirteen serogroups differentiated based on the polysaccharide capsule have been identified, but only six of them (A, B, C, W, X and Y) account for most IMD globally. To control IMD, glycoconjugate vaccines have been developed targeting serogroups A, C, W, and Y (Cohn and Harrison, 2013). It is essential with a universal vaccine also for meningococcal serogroup B (MenB), the predominant etiology of IMD in many countries, which has not been successful due to the poor immunogenicity of the MenB capsule polysaccharide. Therefore, the development of a universal MenB vaccine has focused on conserved protein antigens (Giuliani et al., 2006) and, recent decade, reverse vaccinology (genome-based vaccine discovery) that has resulted in the 4 component meningococcus group B (4CMenB) vaccine (Bexsero; Novartis, MA, US) (O'Ryan et al., 2014). However, due to the selective pressure of 4CMenB new MenB antigenic variants may emerge and reduce the vaccine efficacy. Consequently, careful monitoring of the MenB strain population with high-resolution typing methods is crucial. The genetic diversity and population structure of *N. meningitidis* have historically been characterized by multilocus enzyme electrophoresis (MLEE) and subsequently multilocus sequence typing (MLST) based on seven slowly-evolving housekeeping genes (Maiden et al., 1998). In general, relatively few meningococcal genotypes, clonal complexes or evolutionary lineages have caused most of the IMD worldwide, and these virulent meningococcal variants diversify as they spread in human populations. As an example, since the 1970s many MenB outbreaks have been caused by strains belonging to the hyperinvasive lineage ST-32 initially described in Norway in 1969 and later described in many other countries worldwide (Caugant et al., 1987). Using whole genome sequencing (WGS), it is for the first time possible to elucidate many issues regarding pathogen outbreaks and pandemics with a high throughput, short turnaround time (using effective analysis pipeline) and ideal resolution, which might provide information even about individual transmission events.

In *E-BioMedicine*, Harrison et al. (2015) used WGS to investigate a 40-year meningococcal disease pandemic caused by the *N. meningitidis* hyperinvasive ET-5/ST-32 complex. A global collection of forty-three *N. meningitidis* isolates, including 14 different MLST STs, cultured from 1969 to 2008 was investigated, to set a baseline for the hyperinvasive ET-5/ST-32 complex. The researchers used a gene-by-gene approach and presented their effective pipeline to annotate the WGS data by combining the Bacterial Isolate Genome Sequence database (BIGSdb) (www.pubmlst.org/neisseria) and the prokaryotic annotation tool (Prokka). By comparing the WGS data with closely related reference genomes, a 'Lineage 5 pan genome' of 1940 genes and a 'Lineage 5 core genome' including 1752 genes were defined. Three distinct sub-lineages based on the 1752 core loci were also described. Interestingly, most of the European and American isolates belonged to one of two related sub-lineages and these sub-lineages had diversified before the outbreaks of ST-32 in the 1970s. However, any phylogeographical analysis might be hard to evaluate in detail because isolates from Europe and North America (USA and Canada) were representing 58% of all the investigated isolates. Accordingly, it is not clear how the investigated isolates were representative and formed a baseline for the 40 year global pandemic considering the low number of isolates (n = 43) spanning over 40 years and collected in only 20 countries. The defined pan genome included all core loci, accessory loci identified in the reference genomes, as well as loci not found in the reference genomes but found using Prokka. Interestingly, in the pan genome the researchers found a type 4 secretion system (T4SS), which has not been previously described in *N. meningitidis*, and a *Neisseria gonorrhoeae* conjugative plasmid, which most likely is consistent with horizontal genetic transfer event(s) between *N. meningitidis* and *N. gonorrhoeae*. These findings further confirm the high levels of genetic exchange between species within the *Neisseria* genus. Clearly, additional data on genomic level are crucial regarding genetic exchange between *N. meningitidis* and *N. gonorrhoeae* as well as between these two pathogenic *Neisseria* species and all commensal *Neisseria* species, especially when both the pathogens can (pharyngeal gonorrhea is relatively common in many countries) reside in the oropharynx together with commensal *Neisseria* species. Commensal *Neisseria* species have also been suggested

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to constitute reservoirs of *Neisseria* virulence alleles, and that they engage extensively in genetic exchange within the *Neisseria* genus (Marri et al., 2010). It would be most valuable to further elucidate many of these issues.

In general, WGS provides massive amount of data to analyze and interpret, and for timely translation into clinical, epidemiological, biological and scientific relevance open-access simplified pipelines for analysis will frequently be essential. Harrison et al. (2015) interpret the data using a genome-wide allelic profiling scheme with a standardized, effective, simple-to-use database. Some questions that come to mind are: Does this approach loose some of the resolution? Accordingly, would a genome-wide single nucleotide polymorphism (SNP) analysis/phylogeny, including and/or excluding recombination hot spot regions, give an increased resolution due to the coverage of the SNPs over the entire genome, relative stability over evolutionary time, ease of comparison, and inclusion of also intergenic regions (Brumfield et al., 2003; Morin et al., 2004)? Would identical sub-lineages and phylogeny be distinguished? Would the relatedness and evolutionary distances between these be similar? Might these two different approaches contradict each other, provide the same answers and/or perhaps even supplement each other?

In the future, the WGS data from hyperinvasive and additional clones in combination with transcriptomics, proteomics and appropriate genetic and phenotypic experiment have the capacity to elucidate many issues regarding evolution, virulence and general biological and transmission fitness of *N. meningitidis* lineages. Furthermore, novel targets for diagnostics, antimicrobials and vaccines will be identified and antigenic diversification of vaccine candidates over time and national and global transmission of hyperinvasive meningococcal lineages can be adequately monitored.

Conflict of Interest

The authors declare no conflicts of interest.

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