

Toward a Global Genomic Epidemiology of Meningococcal Disease

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Whole-genome sequencing (WGS) is invaluable for studying the epidemiology of meningococcal disease. Here we provide a perspective on the use of WGS for meningococcal molecular surveillance and outbreak investigation, where it helps to characterize pathogens, predict pathogen traits, identify emerging pathogens, and investigate pathogen transmission during outbreaks. Standardization of WGS workflows has facilitated their implementation by clinical and public health laboratories (PHLs), but further development is required for metagenomic shotgun sequencing and targeted sequencing to be widely available for culture-free characterization of bacterial meningitis pathogens. Internet-accessible servers are being established to support bioinformatics analysis, data management, and data sharing among PHLs. However, establishing WGS capacity requires investments in laboratory infrastructure and technical knowledge, which is particularly challenging in resource-limited regions, including the African meningitis belt. Strategic WGS implementation is necessary to monitor the molecular epidemiology of meningococcal disease in these regions and construct a global view of meningococcal disease epidemiology.

Keywords. Meningitis belt; *Neisseria meningitidis*; next generation sequencing; whole genome sequencing; genomics; metagenomics; surveillance; outbreaks; epidemics; molecular epidemiology.

Rapid and effective public health responses to bacterial meningitis require ongoing and timely detection and characterization of the causative pathogens. While real-time polymerase chain reaction (PCR) analysis and microbiological methods can quickly and efficiently identify bacterial meningitis pathogens and guide epidemic response, whole-genome sequencing (WGS) efficiently provides more-extensive characterization of bacterial isolates. By replacing multiple molecular typing methods and, in some instances, substituting for phenotypic testing methods [1], WGS permits laboratories to retire multiple pathogen-specific methods that each require their own reagents, equipment, and training. Furthermore, as sequencing technologies advance and become less expensive, WGS has the potential to be a leapfrog technology for lower-income countries that do not currently have the public health laboratory infrastructure for these methods. In many cases, a single sequencing protocol can be applied across a wide range of bacterial species, simplifying laboratory processes and creating flexibility

for response to surges in disease caused by different pathogens. Finally, metagenomic sequencing technology, which is not limited to pathogen-specific tests, can be used in studies to detect pathogens in clinical specimens that would otherwise go undetected, including previously undiscovered pathogens [2, 3].

Here, we provide a perspective on how these developments are transforming the capabilities of public health laboratories (PHLs) to perform surveillance and investigate outbreaks, and we consider the infrastructure and technology development required to expand WGS capabilities at PHLs, including specific challenges for countries in the meningitis belt. The cost of WGS implementation for bacterial meningitis pathogens can be reduced by optimizing WGS workflows and by developing computational systems for data integration, analysis, visualization, and sharing. Combined with investments in laboratory infrastructure and the increasing accessibility of sequencing technologies, these developments will create an opportunity to perform global genomic surveillance for bacterial meningitis pathogens.

GENOMIC SURVEILLANCE

Genomic surveillance allows public health scientists to track the spread of strains within their countries and rapidly assess the traits of novel strains. While conventional molecular typing methods rely on sequencing a few individual genes (eg, multilocus sequence typing [MLST] and fine typing [4]), WGS of bacterial isolates provides draft genome assemblies with full-length, high-confidence sequences for most genes

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[5, 6], including genes that affect important phenotypes, such as serogroup, antimicrobial susceptibility, and vaccine antigen expression.

WGS effectively identifies relationships among bacteria, first at the species level [7] and then at higher resolution with analyses based on ribosomal MLST, core genome MLST, whole-genome MLST [8], or single-nucleotide polymorphisms [9]. These methods reliably assess the genetic diversity of strains and track their spread, whereas conventional molecular surveillance methods such as MLST often cannot distinguish among strains collected in different countries over several years. For example, WGS-based methods have revealed the origin, evolution, and spread of pandemic *Neisseria meningitidis* serogroup A strains in the 20th century [10] and serogroup W strains after the 2000 Hajj-related outbreak [11–13], clarifying when new strains were introduced to the countries in the meningitis belt and also identifying genetic changes that may have contributed to the epidemic potential of these strains. WGS has also been used to trace the evolution of pathogenicity within lineages of *Neisseria meningitidis*. For example, the recently emerged serogroup C strains in the meningitis belt were shown to have acquired virulence genes as they evolved from a less virulent strain already present in the meningitis belt [14, 15]. Likewise, a urethritis-causing clade of *N. meningitidis* in the United States [16] was shown to be distinct from previously described *N. meningitidis* urethritis isolates in Europe [17] and to have acquired *Neisseria gonorrhoeae* gene variants that likely allow it to establish urethral infections.

By examining the presence of and variation in capsule biosynthesis genes, WGS data can accurately predict serogroup in *N. meningitidis* [18] and serotype in both *Haemophilus influenzae* (C. Potts et al, unpublished data) and *Streptococcus pneumoniae* [19]. Similarly, antimicrobial susceptibility in several species can be predicted from genomic data [20–22]. While *N. meningitidis* isolates in the meningitis belt and globally are typically susceptible to the antimicrobial drugs used for treatment and chemoprophylaxis [23, 24], strains have been identified with reduced susceptibility to penicillins, cephalosporins, and quinolones [25, 26]. In addition, many *N. gonorrhoeae* strains have been identified with reduced antimicrobial susceptibility [22], raising concern that resistance could be transferred to closely related species such as the recombinogenic *N. meningitidis* [16]. Genomic surveillance provides a cost-effective means of detecting known antimicrobial resistance variants across many loci in the *N. meningitidis* population, thereby providing an early warning for the emergence of antimicrobial resistance.

Furthermore, WGS data have been used to predict the potential strain coverage by a vaccine. For example, WGS provides the sequences of genes encoding vaccine antigens, such as the serogroup B meningococcal vaccine antigens factor H binding protein (FHbp), *Neisseria* adhesin A (NadA), and *Neisseria* heparin binding antigen (NhbA) [27]. While the protein-based

serogroup B meningococcal vaccines were designed with the control of serogroup B disease in mind, they may provide additional protection against other serogroups, including serogroup X [28], which causes disease in the meningitis belt and for which a polysaccharide-protein conjugate vaccine is in development [29]. The sequences of these genes and their promoter regions have been used to predict vaccine protection against a given strain and overall strain coverage [30–32]. Coverage predictions should improve as understanding of the genetic bases for expression of the targeted proteins increases. Currently, expression of vaccine antigens is used to predict serum bactericidal antibody values [33], which in turn correlate with vaccine protection. As additional proteins are explored as candidate vaccine targets, WGS data from public health surveillance programs provide a critical resource for estimating the frequency and diversity of the candidate proteins among disease-causing strains in each country [34].

OUTBREAK AND EPIDEMIC INVESTIGATIONS

The detailed information provided by WGS is proving invaluable for investigating meningococcal outbreaks. Genomic comparisons enable tracing the spread of distinct meningococcal strains among geographic regions and establishing whether a single strain is responsible for multiple cases among members of a community and their close contacts [9, 35]. Additionally, confirmation that a hyperinvasive lineage is spreading in a community can guide the decision to initiate a mass-vaccination campaign to prevent additional cases of disease [36, 37] and inform countries' outbreak-preparedness plans.

Countries of the African meningitis belt experience seasonal epidemics, which prompt reactive vaccination campaigns when the number of suspected meningitis cases reaches the alert threshold, suggesting that disease risk in a community has increased [38, 39]. While real-time PCR analysis is best suited for confirming bacterial meningitis cases during an epidemic, genomic surveillance can identify and track the spread of hyperinvasive *N. meningitidis* lineages, potentially helping to identify communities with an elevated risk for epidemics, based on surveillance data from the previous year. Multiyear studies of *N. meningitidis* genomic diversity within countries are beginning to reveal the dynamics of strain replacement within communities (eg, serogroup A strains in northern Ghana [40]) and spread within countries (eg, serogroup X strains in Burkina Faso [41]). Understanding the genomic differences associated with strain replacement provides a foundation for determining how genetic variation among *N. meningitidis* strains, such as the evolution of noncapsular antigens [40, 42], influences disease risk when combined with human and environmental factors.

WGS is applicable even when bacterial culture is not available, which may be needed at times to detect and characterize outbreaks [2, 3]. For example, metagenomic shotgun sequencing of clinical specimens demonstrated that a single

lineage of *N. meningitidis* was responsible for an initially unrecognized meningococcal disease outbreak in Liberia [43]. The *N. meningitidis* DNA sequences identified in specimens from 6 cases were compared to a diverse *N. meningitidis* genome library, demonstrating that these individuals were infected with the same lineage of *N. meningitidis* that caused large serogroup C outbreaks and epidemics in Nigeria and Niger during the same period [14, 42, 43]. Further development of culture-independent WGS methods is showing that high-quality genome data can be produced from cerebrospinal fluid specimens by enriching *N. meningitidis* DNA sequences, which is described in detail below.

BUILDING WGS INTO PUBLIC HEALTH MICROBIOLOGY

Establishing WGS capacity for surveillance and outbreak investigation in a laboratory involves multiple steps, starting with specimen collection, DNA extraction and sequencing, and sequence data analysis and reporting (Table 1). An effective specimen referral network is needed for providing high-quality specimens to the sequencing laboratory. The quantity and type of specimens to be sequenced has a major influence on the overall cost of WGS. Sequencing DNA from bacterial isolates rather than clinical specimens decreases the cost of sequencing by providing abundant, pure, and undamaged pathogen DNA, which produces reliable sequence data using high-throughput sample preparation and sequencing methods. In contrast, sequencing DNA directly from clinical specimens typically produces less complete and reliable results, even when laboratories dedicate additional effort to sample preparation and sequencing.

The quantity and type of specimens also determine the most efficient sequencing platform needed by the laboratory. Many sequencing technologies are provided on a

range of sequencing platforms; higher-throughput platforms (eg, the Illumina HiSeq platform) provide a lower cost per sample in exchange for higher fixed costs, relative to lower-throughput platforms (eg, the Illumina MiSeq platform). For consideration, the widely used MiSeq platform can readily sequence the genomes of 50 *N. meningitidis* isolates per week, possibly enabling 1 reference laboratory to produce WGS data for representative surveillance isolates provided from several locations across the meningitis belt. This same equipment could be used to sequence isolates and clinical specimens from outbreaks. Depending on the resource availability and quantity of sequencing performed annually, laboratories can choose to build sequencing capacity at different levels. Resource-limited laboratories will likely rely on the sequencing capacity in regional or global reference laboratories in the near future. For laboratories that do not have bioinformatics capacity, they may establish the ability to generate sequence data, while collaborating with reference laboratories on data analysis.

Comparison of WGS data globally requires standardized sequencing methods, analysis tools, and quality-control practices. Many laboratory methods needed to perform WGS on bacterial cultures are already well established and standardized, which facilitates training of laboratory scientists and standardization across different laboratories. Training is further simplified when a workflow can be applied to multiple pathogens. While bacterial growth conditions and DNA extraction methods can vary by pathogen, basic WGS methods such as library preparation and sequencer operation typically apply to a wide range of organisms. This allows laboratorians to be trained in a single method even if they work with multiple organisms (eg, as with PulseNet, for enteric bacterial pathogens [44]) and specialize in WGS methods including library preparation and sequencer operation.

Table 1. Considerations When Establishing Whole-Genome Sequencing (WGS) Capabilities and Challenges Faced in Low-Resource Settings

Capability	Sequencing Considerations	Challenges
Specimen collection	Culture-based sequencing is less expensive and more reliable than culture-free sequencing	Preserving specimens during transport, establishing culture capacity near the point of care, compiling clinical and epidemiological data
Sample preparation	Multistep preparation of sequencing libraries requires equipment, reagents, and training DNA enrichment is required for culture-free sequencing of pathogens	Importing specialized reagents that require cold supply chain Recruiting microbiologists with WGS experience, maintaining specialized reagents and expertise for DNA enrichment
Sequencing platform	Technologies vary in accuracy and completeness Sequencing platforms vary in throughput, fixed costs, and cost per sample	Building laboratory space with climate control and steady electricity Enlisting technicians to maintain and repair sequencing instruments
Data management and analysis	Online genomic analysis platforms can provide standardized, easy-to-interpret results and facilitate data sharing Local genomic analysis requires high-performance computers, information technology, and bioinformatics expertise	Establishing affordable high-speed Internet service in laboratories Enlisting information technology support.

One method that is still being standardized is culture-free sequencing, which is particularly important for countries that are at high risk for bacterial diseases but have a low bacterial culture rate. Culture-free sequencing is challenging because the data obtained for pathogen DNA can be limited by wasteful sequencing of human DNA in clinical specimens. The efficiency of culture-free sequencing can be further improved by enriching pathogen DNA in the clinical specimen, by either removing human DNA or enriching targeted pathogen DNA. The feasibility of this method for *N. meningitidis* has recently been demonstrated with 2 different methods: capture of *N. meningitidis* fragments from a sequencing library using 120mer, biotinylated RNA bait [45] and selective whole-genome amplification (SWGA) of *N. meningitidis* genomic DNA by using heptamer DNA primers (M. Itsko, unpublished data). Both methods produced standard molecular typing results from several cerebrospinal fluid specimens, while SWGA was also demonstrated to work on urine specimens. Further development of meningococcal DNA-enrichment methods will likely improve reliability and affordability, thereby providing a method that can be implemented by bacterial meningitis reference laboratories.

Standardization of user-friendly bioinformatics pipelines is critical to allow laboratory scientists to quickly obtain results from WGS and metagenomic data without the need for bioinformatics specialists. An efficient way to deploy and update standardized bioinformatics analysis tools is through an Internet-accessible genomic analysis platform. Several organizations provide websites that facilitate WGS data analysis, reducing the need for on-site computers and bioinformatics expertise in all laboratories, and include PubMLST [46], EDGE

[47], and PATRIC [48]. While these websites provide flexible tools for sequence analysis, currently none is configured with full analytic pipelines for bacterial meningitis pathogens that start from raw sequence reads generated by a sequencer and ultimately produce results that are relevant to public health microbiologists and epidemiologists. To fill this gap, the Bacterial Meningitis Genomic Analysis Platform (BMGAP) is being developed by the Centers for Disease Control and Prevention to provide public health laboratories with access to multiple species-specific genomic analysis pipelines that are configured for standardized WGS workflows and supported by a secure data repository to facilitate integration of WGS laboratory and epidemiological data (Figure 1).

BMGAP allows laboratory scientists to obtain results from WGS data by producing a quality-controlled genome assembly for each isolate and identifying the species [7], which automatically triggers species-specific analysis tools. For *N. meningitidis*, the pipeline uses *Neisseria* gene nomenclature provided by PubMLST.org to report MLST results; FetA, PorA, and PorB types; peptide sequence identifiers for the vaccine antigens FHbp, NadA, and NhbA; and serogroup prediction based on capsule locus genes [17]. Ongoing development will allow BMGAP to report the presence of antimicrobial resistance determinants and also provide an analytic pipeline customized for culture-free WGS (Itsko, unpublished data). In addition to describing the genomes of individual isolates, BMGAP incorporates tools to compare the genome of pathogens from multiple cases of disease, which is important for detecting outbreaks and tracking the geographic spread of strains. Users can search the BMGAP database to identify genomes that are similar to any given genome and then perform a detailed phylogenetic analysis of these genomes.

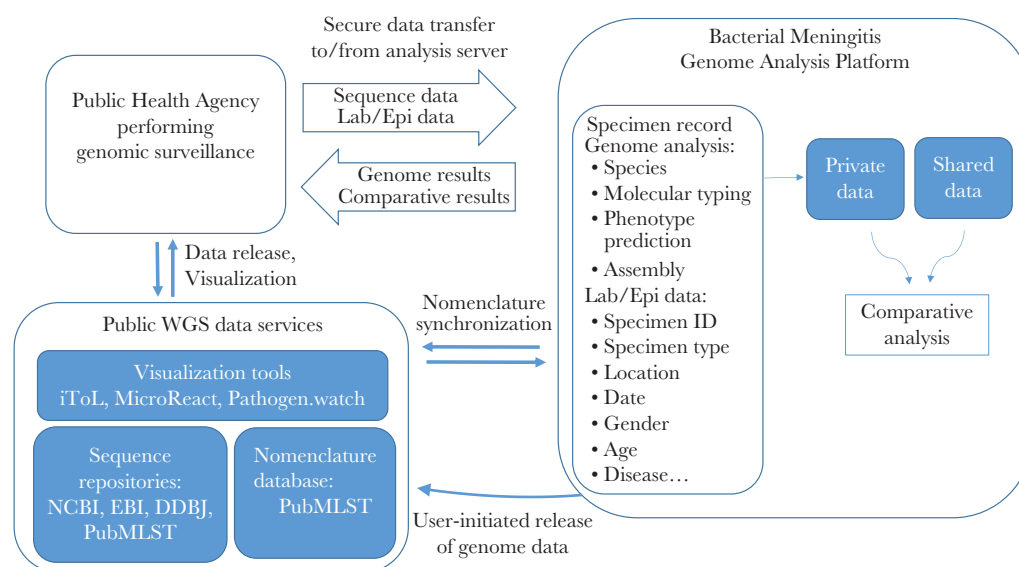


Figure 1. Process for data analysis and sharing using the Bacterial Meningitis Genomic Analysis Platform. DDBJ, DNA Data Bank of Japan; EBI, European Bioinformatics Institute; ID, identifier; iTOL, Interactive Tree of Life; NCBI, National Center for Biotechnology Information; WGS, whole-genome sequencing.

Comparative genomic analysis is particularly informative when visualized alongside epidemiological data, such as the location and date of specimen collection. BMGAP integrates epidemiological data with genomic data so that users can rapidly identify cases that involve the same strain, an essential insight during outbreak investigations that allows for informed public health decision making. Long-term changes in risk factors or virulence that result from pathogen evolution can be made visible by incorporating data on patient characteristics or disease outcomes into the platform, as well. The collection and compilation of clinical and epidemiological data are important factors to consider when planning for genomic surveillance.

The addition of epidemiological data to a WGS analysis platform requires heightened data security and access control. BMGAP authenticates users and only allows them to view data that have been explicitly shared with them, allowing the data provider to control access to the submitted data. BMGAP removes known human DNA sequences during genome assembly to ensure that these sequences are not exported or analyzed.

Understanding of the diversity and population structure of pathogenic meningococci is currently hampered by limited representative genomic data from around the world. Efforts to create publicly accessible meningococcal genome libraries have already been undertaken in multiple countries [49, 50], and a global meningococcal genome library is considered an important component of plans to eliminate meningitis epidemics by 2030 [51]. BMGAP aims to support the creation of a global meningococcal genome library by providing high-value genomic analysis tools directly to microbiologists and epidemiologists and facilitating submission of genome assemblies to public repositories such as PubMLST.org. Ultimately, a global meningococcal genome library depends on effective disease surveillance systems and sequencing capacity, especially in the countries of the meningitis belt, which may face different challenges than those countries where surveillance systems and WGS are already implemented.

CHALLENGES FOR COUNTRIES IN THE MENINGITIS BELT

Genomic surveillance of meningococcal disease in the meningitis belt is essential for identifying new strains as they are introduced to the region and for achieving a detailed understanding of epidemics. The challenges of conducting genomic surveillance starts with acquiring clinical specimens and isolates that represent the diversity of disease-causing strains in the meningitis belt, while also documenting relevant clinical data. Even when cerebrospinal fluid specimens have been collected, slow transport of specimens from clinic to laboratory results in reduced viability of the bacteria in the specimen and degradation of their DNA [52]. Effective genomic surveillance of meningococcal strains will depend on identifying

geographically dispersed sentinel clinics that can quickly and reliably transfer clinical specimens to laboratories for culturing and/or preservation.

Laboratories within countries in the meningitis belt may face several challenges when establishing WGS capacity for evaluating bacterial meningitis cases (Table 1), initially limiting WGS capabilities to a small number of laboratories. For instance, the absence of a large WGS user community in countries in the meningitis belt may reduce access to sequencing reagents and technical expertise, resulting in higher costs than in countries with established WGS user communities. Sequencing reagents may need to be imported and require a cold supply chain, similar to the challenges seen with procurement of real-time PCR reagents [52]. Without the ability to recruit experienced WGS users from the domestic workforce, these laboratories may depend on training from international partners to establish in-country expertise in sample preparation and sequencer operation or rely on regional and global reference laboratories for sequencing. Equipment maintenance provides another challenge; while some technical support may be available from equipment manufacturers remotely through Internet connections, on-site repairs may be delayed if technicians are not based within the user's country.

Furthermore, establishing WGS capacity may require laboratory facility modification and expansion, including the installation of benches that support the weight of sequencing instruments in a climate-controlled and low-vibration environment. Backup electrical generators can limit power fluctuations during sequencing runs, which often require more than a day. Finally, several pieces of small equipment are typically needed for preparation of the sequencing library, which also require laboratory space.

In addition, information technology to support WGS, including computer networking and data management to properly organize and transfer WGS data, are needed by laboratories looking to establish WGS capacity. High-bandwidth Internet connections allow laboratories to access analysis software and share data with collaborators. Data storage and analysis can be supported by an Internet-accessible genomic analysis platform, such as BMGAP, which greatly reduces the need for on-site investment in computers and informatics staffing.

The full utilization of WGS to understand the global molecular epidemiology of bacterial meningitis can be achieved through establishing a centralized database that links both clinical and sequencing data. Sequencing isolates or specimens from all regions of the world is becoming more achievable as new sequencing technologies and platforms are developed to simplify sample preparation, reduce the size and cost of sequencing equipment, and increase the robustness of sequencing reactions [3]. An easily accessible global genome library will allow public health professionals to characterize the variability of bacterial meningitis pathogens and understand

their spread during outbreaks and epidemics. The detailed information about pathogen populations provided by global genomic epidemiology will enable more-effective public health intervention and continued progress in reducing the incidence of bacterial meningitis.

Notes

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References

- Jolley KA, Maiden MC. Automated extraction of typing information for bacterial pathogens from whole genome sequence data: *Neisseria meningitidis* as an exemplar. *Euro Surveill* **2013**; 18:20379.
- Forbes JD, Knox NC, Ronholm J, Pagotto F, Reimer A. Metagenomics: the next culture-independent game changer. *Front Microbiol* **2017**; 8:1069.
- Gardy JL, Loman NJ. Towards a genomics-informed, real-time, global pathogen surveillance system. *Nat Rev Genet* **2018**; 19:9–20.
- Jolley KA, Brehony C, Maiden MC. Molecular typing of meningococci: recommendations for target choice and nomenclature. *FEMS Microbiol Rev* **2007**; 31:89–96.
- Bratcher HB, Corton C, Jolley KA, Parkhill J, Maiden MC. A gene-by-gene population genomics platform: de novo assembly, annotation and genealogical analysis of 108 representative *Neisseria meningitidis* genomes. *BMC Genomics* **2014**; 15:1138.
- Hill DM, Lucidarme J, Gray SJ, et al. Genomic epidemiology of age-associated meningococcal lineages in national surveillance: an observational cohort study. *Lancet Infect Dis* **2015**; 15:1420–8.
- Topaz N, Boxrud D, Retchless AC, et al. BMScan: using whole genome similarity to rapidly and accurately identify bacterial meningitis causing species. *BMC Infect Dis* **2018**; 18:405.
- Maiden MC, Jansen van Rensburg MJ, Bray JE, et al. MLST revisited: the gene-by-gene approach to bacterial genomics. *Nat Rev Microbiol* **2013**; 11:728–36.
- Whaley MJ, Joseph SJ, Retchless AC, et al. Whole genome sequencing for investigations of meningococcal outbreaks in the United States: a retrospective analysis. *Sci Rep* **2018**; 8:15803.
- Watkins ER, Maiden MC. Metabolic shift in the emergence of hyperinvasive pandemic meningococcal lineages. *Sci Rep* **2017**; 7:41126.
- Lucidarme J, Hill DM, Bratcher HB, et al. Genomic resolution of an aggressive, widespread, diverse and expanding meningococcal serogroup B, C and W lineage. *J Infect* **2015**; 71:544–52.
- Retchless AC, Hu F, Ouedraogo AS, et al. The Establishment and Diversification of Epidemic-Associated Serogroup W Meningococcus in the African Meningitis Belt, 1994 to 2012. *mSphere* **2016**; 1:e00201–16.
- Mustapha MM, Marsh JW, Krauland MG, et al. Genomic investigation reveals highly conserved, mosaic, recombination events associated with capsular switching among invasive *Neisseria meningitidis* serogroup W sequence type (ST)-11 strains. *Genome Biol Evol* **2016**; 8:2065–75.
- Brynildsrud OB, Eldholm V, Bohlin J, Uadiale K, Obaro S, Caugant DA. Acquisition of virulence genes by a carrier strain gave rise to the ongoing epidemics of meningococcal disease in West Africa. *Proc Natl Acad Sci U S A* **2018**; 115:5510–5.
- Sanogo YO, Guindo I, Diarra S, et al. A new sequence type of *Neisseria meningitidis* serogroup C associated with a 2016 meningitis outbreak in Mali. *J Infect Dis* **2019**; 220(Suppl 4):S190–7.
- Retchless AC, Kretz CB, Chang HY, et al. Expansion of a urethritis-associated *Neisseria meningitidis* clade in the United States with concurrent acquisition of *N. gonorrhoeae* alleles. *BMC Genomics* **2018**; 19:176.
- Taha MK, Claus H, Lappann M, et al. Evolutionary events associated with an outbreak of meningococcal disease in men who have sex with men. *PLoS One* **2016**; 11:e0154047.
- Marjuki H, Topaz N, Rodriguez-Rivera LD, et al. Whole genome sequencing to characterize capsule locus and predict serogroup of invasive meningococcal isolates. *J Clin Microbiol* **2018**; 57: e01609-18.
- Kapatai G, Sheppard CL, Al-Shahib A, et al. Whole genome sequencing of *Streptococcus pneumoniae*: development, evaluation and verification of targets for serogroup and serotype prediction using an automated pipeline. *PeerJ* **2016**; 4:e2477.

20. Metcalf BJ, Chochua S, Gertz RE, Jr., et al. Using whole genome sequencing to identify resistance determinants and predict antimicrobial resistance phenotypes for year 2015 invasive pneumococcal disease isolates recovered in the United States. *Clin Microbiol Infect* **2016**; 22:1002.e1–.e8.
21. Davis JJ, Boisvert S, Brettin T, et al. Antimicrobial resistance prediction in PATRIC and RAST. *Sci Rep* **2016**; 6:27930.
22. Demczuk W, Sidhu S, Unemo M, et al. *Neisseria gonorrhoeae* sequence typing for antimicrobial resistance, a novel antimicrobial resistance multilocus typing scheme for tracking global dissemination of *N. gonorrhoeae* Strains. *J Clin Microbiol* **2017**; 55:1454–68.
23. Harcourt BH, Anderson RD, Wu HM, et al. Population-based surveillance of *Neisseria meningitidis* antimicrobial resistance in the United States. *Open Forum Infect Dis* **2015**; 2:ofv117.
24. Hedberg ST, Fredlund H, Nicolas P, Caugant DA, Olcen P, Unemo M. Antibiotic susceptibility and characteristics of *Neisseria meningitidis* isolates from the African meningitis belt, 2000 to 2006: phenotypic and genotypic perspectives. *Antimicrob Agents Chemother* **2009**; 53:1561–6.
25. Guo Q, Mustapha MM, Chen M, et al. Evolution of sequence type 4821 clonal complex meningococcal strains in china from prequinolone to quinolone era, 1972–2013. *Emerg Infect Dis* **2018**; 24:683–90.
26. Deghmane AE, Hong E, Taha MK. Emergence of meningococci with reduced susceptibility to third-generation cephalosporins. *J Antimicrob Chemother* **2017**; 72:95–8.
27. Brehony C, Hill DM, Lucidarme J, Borrow R, Maiden MC. Meningococcal vaccine antigen diversity in global databases. *Euro Surveill* **2015**; 20:pii=30084.
28. Hong E, Giuliani MM, Deghmane AE, et al. Could the multicomponent meningococcal serogroup B vaccine (4CMenB) control *Neisseria meningitidis* capsular group X outbreaks in Africa? *Vaccine* **2013**; 31:1113–6.
29. Acevedo R, Zayas C, Norheim G, et al. Outer membrane vesicles extracted from *Neisseria meningitidis* serogroup X for prevention of meningococcal disease in Africa. *Pharmacol Res* **2017**; 121:194–201.
30. Lucidarme J, Comanducci M, Findlow J, et al. Characterization of fHbp, nhba (gna2132), nadA, porA, and sequence type in group B meningococcal case isolates collected in England and Wales during January 2008 and potential coverage of an investigational group B meningococcal vaccine. *Clin Vaccine Immunol* **2010**; 17:919–29.
31. Green LR, Lucidarme J, Dave N, et al. Phase variation of NadA in invasive *Neisseria meningitidis* isolates impacts on coverage estimates for 4C-MenB, a MenB Vaccine. *J Clin Microbiol* **2018**; 56:e00204–18.
32. Muzzi A, Brozzi A, Serino L, et al. Genetic Meningococcal Antigen Typing System (gMATS): a genotyping tool that predicts 4CMenB strain coverage worldwide. *Vaccine* **2019**; 37:991–1000.
33. Budroni S, Kleinschmidt A, Boucher P, Medini D. Pooled-sera hSBA titres predict individual seroprotection in infants and toddlers vaccinated with 4CMenB. *Vaccine* **2016**; 34:2579–84.
34. Andrae CA, Sessions RB, Virji M, Hill DJ. Bioinformatic analysis of meningococcal Msf and Opc to inform vaccine antigen design. *PLoS One* **2018**; 13:e0193940.
35. Jolley KA, Hill DM, Bratcher HB, et al. Resolution of a meningococcal disease outbreak from whole-genome sequence data with rapid Web-based analysis methods. *J Clin Microbiol* **2012**; 50:3046–53.
36. Mbaeyi SA, Blain A, Whaley MJ, Wang X, Cohn AC, MacNeil JR. Epidemiology of meningococcal disease outbreaks in the United States, 2009–2013. *Clin Infect Dis* **2018**; 68:580–585.
37. Chatt C, Gajraj R, Hawker J, et al. Four-month outbreak of invasive meningococcal disease caused by a rare serogroup B strain, identified through the use of molecular PorA subtyping, England, 2013. *Euro Surveill* **2014**; 19:pii=20949.
38. World Health Organization. Detecting meningococcal meningitis epidemics in highly-endemic African countries. *Wkly Epidemiol Rec* **2000**; 75:306–9.
39. Trotter CL, Cibrelus L, Fernandez K, Lingani C, Ronveaux O, Stuart JM. Response thresholds for epidemic meningitis in sub-Saharan Africa following the introduction of MenAfriVac®. *Vaccine* **2015**; 33:6212–7.
40. Lamelas A, Harris SR, Röltgen K, et al. Emergence of a new epidemic *Neisseria meningitidis* serogroup A Clone in the African meningitis belt: high-resolution picture of genomic changes that mediate immune evasion. *MBio* **2014**; 5:e01974–14.
41. Retchless AC, Congo-Ouédraogo M, Kambiré D, et al. Molecular characterization of invasive meningococcal isolates in Burkina Faso as the relative importance of serogroups X and W increases, 2008–2012. *BMC Infect Dis* **2018**; 18:337.
42. Kretz CB, Retchless AC, Sidikou F, et al. Whole-genome characterization of epidemic *Neisseria meningitidis* serogroup C and resurgence of serogroup W, Niger, 2015. *Emerg Infect Dis* **2016**; 22:1762–8.
43. Bozio CH, Vuong J, Dokubo EK, et al. Outbreak of *Neisseria meningitidis* serogroup C outside the meningitis belt-Liberia, 2017: an epidemiological and laboratory investigation. *Lancet Infect Dis* **2018**; 18:1360–1367.
44. Nadon C, Van Walle I, Gerner-Smidt P, et al. PulseNet international: vision for the implementation of whole genome sequencing (WGS) for global food-borne disease surveillance. *Euro Surveill* **2017**; 22:pii=30544.
45. Clark SA, Doyle R, Lucidarme J, Borrow R, Breuer J. Targeted DNA enrichment and whole genome sequencing of *Neisseria meningitidis* directly from clinical specimens. *Int J Med Microbiol* **2018**; 308:256–62.
46. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org

- website and their applications. *Wellcome Open Res* **2018**; 3:124.
47. Li PE, Lo CC, Anderson JJ, et al. Enabling the democratization of the genomics revolution with a fully integrated web-based bioinformatics platform. *Nucleic Acids Res* **2017**; 45:67–80.
 48. Wattam AR, Davis JJ, Assaf R, et al. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. *Nucleic Acids Res* **2017**; 45:D535–42.
 49. Borrow R, Alarcon P, Carlos J, et al. The Global Meningococcal Initiative: global epidemiology, the impact of vaccines on meningococcal disease and the importance of herd protection. *Expert Rev Vaccines* **2016**; 16:313–328.
 50. Meningitis Research Foundation. Using new genomic techniques to identify the causes of meningitis in UK children. <http://www.meningitis.org/research/genome>. Accessed 18 June 2019.
 51. World Health Organization. Defeating meningitis by 2030 First meeting of the Technical Taskforce, July 2018. Available at https://www.who.int/immunization/research/Defeating_meningitis_2030_TTFJuly2018_report.pdf. Accessed 24 June 2019.
 52. Feagins AR, Vuong J, Fernandez K, et al. The strengthening of laboratory systems in the meningitis belt to improve meningitis surveillance, 2008–2018: a partners’ perspective *J Infect Dis* **2019**; 220(Suppl 4):S175–81.