AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Comparison of Phenotypic and Genotypic Approaches to Capsule Typing of *Neisseria meningitidis* by Use of Invasive and Carriage Isolate Collections

C. Hal Jones,[a] Naglaa Mohamed,[a] Eduardo Rojas,[a] Lubomira Andrew,[a] Johanna Hoyos,[a] Julio C. Hawkins,[a] Lisa K. McNeil,[a] Qin Jiang,[a] Leonard W. Mayer,[b] Xin Wang,[b] Rodica Gilca,[c,d,e] Philippe De Wals,[c,d,e] Louise Pedneault,[a] Joseph Eiden,[a] Kathrin U. Jansen,[a] Annaliesa S. Anderson[a]

Pfizer Vaccine Research and Development, Pearl River, New York, USA[a]; Meningitis and Vaccine Preventable Diseases Branch, Division of Bacterial Diseases, National Center for Immunizations and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA[b]; Institut National de Santé Publique du Québec, Quebec City, Canada[c]; Laval University, Quebec City, Canada[d]; CHU de Québec, Quebec City, Canada[e]

***Neisseria meningitidis* serogroup B (MnB) is a leading cause of bacterial meningitis; however, MnB is most commonly associated with asymptomatic carriage in the nasopharyngeal cavity, as opposed to the disease state. Two vaccines are now licensed for the prevention of MnB disease; a possible additional benefit of these vaccines could be to protect against disease indirectly by disrupting nasopharyngeal carriage (e.g., herd protection). To investigate this possibility, accurate diagnostic approaches to characterize MnB carriage isolates are required. In contrast to invasive meningococcal disease (IMD) isolates, which can be readily serogrouped, carriage isolates often lack capsule expression, making standard phenotypic assays unsuitable for strain characterization. Several antibody-based methods were evaluated for their abilities to serogroup isolates and were compared with two genotyping methods (real-time PCR [rt-PCR] and whole-genome sequencing [WGS]) to identify which approach would most accurately ascertain the polysaccharide groups associated with carriage isolates. WGS and rt-PCR were in agreement for 99% of IMD isolates, including those with coding sequences for MnB, MnC, MnW, and MnY, and the phenotypic methods correctly identified serogroups for 69 to 98% of IMD isolates. In contrast, only 47% of carriage isolates were groupable by genotypic methods, due to mutations within the capsule operon; of the isolates identified by genotypic methods, ≤43% were serogroupable with any of the phenotypic methods tested. These observations highlight the difficulties in the serogrouping and capsular genogrouping of meningococcal carriage isolates. Based on our findings, WGS is the most suitable approach for the characterization of meningococcal carriage isolates.**

Neisseria meningitidis, a Gram-negative bacterium that causes both epidemic and endemic life-threatening disease, is also an obligate human commensal organism that colonizes the nasopharyngeal mucosa with no or minimal harm to the host, a phenomenon known as carriage (1). Asymptomatic pharyngeal colonization with *N. meningitidis* in young adults is relatively common, and these asymptomatic carriers represent a potential reservoir for the transmission of pathogenic isolates in the community (2, 3). The rates of asymptomatic carriage in the United States and Europe are highest among adolescents and young adults, peaking at the age of 19 years, and are estimated to be 10% to 35% (4–6). Rates of transmission and carriage are higher in closed and semiclosed populations, such as university students living in dormitories and military recruits housed in barracks (7). Higher rates of carriage are also found among people in close contact with patients with active meningococcal infections (8). For the majority of people, carriage is an immunizing process that results in systemic, serogroup-specific, protective antibody responses (9, 10). Invasive meningococcal disease (IMD) usually occurs shortly after the onset of colonization of a susceptible host, when the bacteria penetrate the mucosal membranes, overcome host defenses, and invade the bloodstream or meninges, leading to septicemia or meningitis, respectively (11).

IMD is cyclical and varies by age group, being more common among children, especially those <5 years of age. A second peak in disease occurs in adolescence, with rates declining during early adulthood and subsequently decreasing for older age groups (12, 13). Asymptomatic carriers represent a potential source of virulent isolates that cause disease; however, the factors that lead to a change in the carriage state of the organism, resulting in invasive disease, are still poorly understood. Approximately 450 cases of meningococcal disease occurred in the Unites States in 2012, yielding a rate of 0.15 cases/100,000 population (14). The overall incidence rates in Canada (2011 data) and Europe (2012 data) were 0.55 cases/100,000 population and 0.68 cases/100,000 population, respectively (15, 16). In outbreak and epidemic settings, the case rates are considerably higher, i.e., 100 cases/100,000 population to 1,000 cases/100,000 population (17, 18).

The polysaccharide capsule is an important meningococcal

Received 29 May 2015 **Returned for modification** 11 July 2015
Accepted 20 August 2015

Accepted manuscript posted online 26 August 2015

Citation Jones CH, Mohamed N, Rojas E, Andrew L, Hoyos J, Hawkins JC, McNeil LK, Jiang Q, Mayer LW, Wang X, Gilca R, De Wals P, Pedneault L, Eiden J, Jansen KU, Anderson AS. 2016. Comparison of phenotypic and genotypic approaches to capsule typing of *Neisseria meningitidis* by use of invasive and carriage isolate collections. J Clin Microbiol 54:25–34. doi:10.1128/JCM.01447-15.

Editor: D. J. Diekema

Address correspondence to C. Hal Jones, hal.jones@pfizer.com.

Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.01447-15.

virulence factor that protects the bacterium from complement-mediated bactericidal activity of human serum, as well as opsonophagocytic killing in the bacteremic stages of meningococcal disease (19–21). *N. meningitidis* strains are classified into serogroups based on structural differences in their capsular polysaccharides (7, 22, 23). Twelve serogroups have been described, with six (i.e., *N. meningitidis* serogroup A [MnA], MnB, MnC, MnW, MnX, and MnY) being implicated in the majority of IMD (24–27).

Capsule antigens (polysaccharide and polysaccharide conjugate) are potent and efficacious in preventing IMD when formulated as vaccines (28, 29). However, the common sialic acid-containing epitope of the MnB capsule is also present on human neural tissue, which may explain the poor immunogenicity of the MnB capsule; therefore, out of an abundance of caution, the MnB polysaccharide has not been considered a viable vaccine candidate (30). As a result, protein antigens have been employed in the development of MnB vaccines (31). Two vaccines have recently been licensed for the prevention of MnB disease, namely, Trumenba (bivalent rLP2086) (32) and Bexsero (4CMenB) (33).

In areas in which MnC conjugate vaccines were introduced and had widespread implementation, including receipt of the vaccines by very large proportions of adolescents, disease incidence in nonvaccinated populations was dramatically affected due to indirect prevention of disease through reductions in carriage rates, e.g., herd protection/immunity (34–37). This effect was not observed with the use of nonconjugated polysaccharide vaccines. It will be important to study whether MnB vaccines have this additional benefit (38). A recent study demonstrated similar levels of herd protection for protein-based (4CMenB) and non-protein-based (MenACWY-CRM) meningococcal vaccines (39). Meningococcal carriage studies can be challenging, due to a combination of reduced capsule expression by carriage strains and a lack of standardized approaches to evaluate carriage isolates in order to identify their associated capsule polysaccharide groups.

Invasive disease isolates are traditionally serogrouped by slide agglutination serogrouping (SASG) assays (23, 40), which use commercial polyclonal antibody reagents raised against prototypical *N. meningitidis* isolates representing the important disease-associated serogroups. The reagents and laboratory methods used for SASG assays are not standardized across laboratories, especially because the endpoint of the assay is read subjectively with the unaided eye (23, 40). Other serogrouping approaches have employed capsule-specific monoclonal antibodies (MAbs) applied to different platforms, such as dot blotting (41), whole-cell enzyme-linked immunosorbent assays (ELISAs) (42), and flow cytometry (43). Large proportions (70 to 80%) of invasive isolates can be successfully serogrouped using expression-based phenotypic assays (40). In contrast, high percentages of isolates recovered from healthy carriers are reported as nonserogroupable in phenotypic assays; the general explanation for this finding has been the low levels or complete absence of expression of the capsule genes during carriage (44–47), which can be explained by several mechanisms, including phase variation of capsule expression genes (44), insertion of genetic mobile elements (48), or a complete absence of the genes required for capsule production, leading to a capsule-null (*cnl*) phenotype (49, 50). The *cnl* mutation was reported for ~16% of nasopharyngeal meningococci collected from healthy children and young adults in two independent carriage studies (45, 50). A recent report by Loh et al. (51) described a complex regulatory mechanism linking growth temperature to capsule gene expression. Molecular mechanisms underlying capsule biosynthesis have been elucidated; polysaccharide synthesis and translocation genes are clustered at a single chromosomal locus called *cps*. The *cps* locus consists of six different regions, i.e., A to D, D′, and E (52). Of particular interest for the present study are genes in region A, which encode the enzymes responsible for polysaccharide synthesis (53, 54), and genes in region C, which encode proteins that facilitate polysaccharide transport across bacterial membranes (55, 56).

Capsular genogrouping of carriage isolates can be achieved using rapid sensitive DNA-based methods (40). A number of PCR approaches have been developed to detect targets within the *ctrA* (capsule transport) gene (52, 57), as well as genes required for serogroup-specific capsule biosynthesis (8, 40, 58–61). For serogroup prediction (capsular genogroup assignment), PCR using primers designed for serogroup-specific genes can be applied (62–66). Fluorescence-based real-time PCR (rt-PCR) assays were recently developed to confirm the capsule typing of meningococcal isolates (59, 67, 68). Whole-genome sequencing (WGS) data also are being used increasingly for epidemiological typing of bacterial pathogens (69–71). In this study, we compared different capsular serogrouping methodologies for the assignment of invasive and carriage isolates to capsular polysaccharide serogroups, in order to determine a methodology for the characterization of carriage isolates that is broadly applicable for different laboratory settings and has the capacity to be standardized.

MATERIALS AND METHODS

Meningococcal isolates. Invasive isolates ($n = 97$) representing serogroups MnA, MnB, MnC, MnW, and MnY were selected from the Pfizer Invasive Disease collection, which contains isolates from Europe (Czech Republic, France, Germany, Netherlands, and United Kingdom) and the United States (64% of isolates were from the United States) that were collected between 2000 and 2008 (Table 1) (72). Although these isolates had been previously characterized by SASG assays for serogroup determination, they were subjected to blinded operator evaluations in this study. Meningococcal carriage isolates ($n = 93$) were obtained from pharyngeal swabs from healthy ninth grade students and college/university students living in dormitories and participating in a 1-year-long epidemiological study in Quebec City (in 2010 to 2012) (Table 2) (73).

Isolate collection and preparation. Carriage isolates were cultured on modified Thayer-Martin plates (Remel/Thermo Fisher Scientific, Lenexa, KS) for up to 48 h at 37°C in 5% CO_2. Suspect colonies were subcultured on trypticase soy agar (TSA) II blood agar (Remel) overnight at 37°C in a 5% CO_2 incubator. All isolates were identified as *N. meningitidis* by Gram staining (BD BBL, Sparks, MD) and oxidase testing (DrySlide; BD BBL, Cockeysville, MD) and were identified to the species level with API NH tests (bioMérieux, Hazelwood, MO). IMD isolates were selected from the Pfizer global IMD collection and cultured on TSA II blood agar as described above.

Genotypic methods. (i) Whole-genome sequencing analysis. Bacterial isolates were grown overnight on chocolate agar. A 10-μl bacterial loop was used to harvest the growth from one-eighth of a plate into 100 μl of GCK medium (GC medium base [Difco] with 0.042% $NaHCO_3$ and Kellogg's supplements) (74), and the mixture was incubated at 95°C for 5 min to prepare cell suspensions. Genomic DNA (gDNA) was extracted from cell suspensions using magnetic bead technology, as described (Agencourt Genfind V2 system; Beckman Coulter, Brea, CA). The final elution of gDNA was in 100 μl of 10 mM Tris-1 mM EDTA buffer (pH 8). DNA concentrations were measured using the Qubit double-stranded DNA (dsDNA) high-sensitivity assay kit (Life Technologies, Carlsbad, CA) and adjusted to a final value of 0.2 ng/μl with distilled water. The NexteraXT DNA sample preparation protocol (Illumina, San Diego, CA)

TABLE 1 Invasive disease isolates

Isolate	Capsular genogroup by WGS	Sequence type[a]	Clonal complex[b]
PFE-I-0001	MnW	174	ST174 complex
PFE-I-0008	MnW	1158	ST22 complex
PFE-I-0009	MnY	11	ST11 complex/ET-37 complex
PFE-I-0011	MnB	136	ST41/44 complex/lineage 3
PFE-I-0012	MnB	1260	ST103 complex
PFE-I-0002	MnB	4701	ST32 complex/ET-5 complex
PFE-I-0003	MnB	162	ST162 complex
PFE-I-0004	MnB	NST	NST
PFE-I-0005	MnB	162	ST162 complex
PFE-I-0097	MnW	NST	NST
PFE-I-0098	MnY	11	ST11 complex/ET-37 complex
PFE-I-0006	MnW	11	ST11 complex/ET-37 complex
PFE-I-0013	MnA	1	ST1 complex/subgroup I/II
PFE-I-0014	MnC	345	NST
PFE-I-0015	MnY	23	ST23 complex/cluster A3
PFE-I-0016	MnY	23	ST23 complex/cluster A3
PFE-I-0017	MnY	23	ST23 complex/cluster A3
PFE-I-0018	MnY	23	ST23 complex/cluster A3
PFE-I-0019	MnA	5	ST5 complex/subgroup III
PFE-I-0020	MnC	11	ST11 complex/ET-37 complex
PFE-I-0021	MnC	1011	ST231 complex
PFE-I-0022	MnC	NST	NST
PFE-I-0023	MnW	185	ST174 complex
PFE-I-0024	MnB	269	ST269 complex
PFE-I-0025	MnB	40	ST41/44 complex/lineage 3
PFE-I-0026	MnB	1162	NST
PFE-I-0027	MnB	213	ST213 complex
PFE-I-0028	MnB	3010	NST
PFE-I-0029	MnB	3279	ST213 complex
PFE-I-0030	MnB	33	ST32 complex/ET-5 complex
PFE-I-0031	MnB	457	ST35 complex
PFE-I-0032	MnB	213	ST213 complex
PFE-I-0033	MnB	457	ST35 complex
PFE-I-0034	MnB	41	ST41/44 complex/lineage 3
PFE-I-0010	MnB	213	ST213 complex
PFE-I-0035	MnB	136	ST41/44 complex/lineage 3
PFE-I-0036	MnB	5100	NST
PFE-I-0037	MnB	803	ST32 complex/ET-5 complex
PFE-I-0038	MnB	11	ST11 complex/ET-37 complex
PFE-I-0039	MnB	162	ST162 complex
PFE-I-0040	MnB	32	ST32 complex/ET-5 complex
PFE-I-0041	MnB	2976	ST269 complex
PFE-I-0042	MnB	136	ST41/44 complex/lineage 3
PFE-I-0043	MnB	2048	NST
PFE-I-0044	MnC	11	ST11 complex/ET-37 complex
PFE-I-0045	MnC	11	ST11 complex/ET-37 complex
PFE-I-0046	MnC	11	ST11 complex/ET-37 complex
PFE-I-0047	MnC	11	ST11 complex/ET-37 complex
PFE-I-0048	MnC	11	ST11 complex/ET-37 complex
PFE-I-0049	MnC	NST	NST
PFE-I-0050	MnC	11	ST11 complex/ET-37 complex
PFE-I-0051	MnC	2048	NST
PFE-I-0052	MnC	11	ST11 complex/ET-37 complex
PFE-I-0053	MnC	11	ST11 complex/ET-37 complex
PFE-I-0054	MnC	11	ST11 complex/ET-37 complex
PFE-I-0055	MnC	11	ST11 complex/ET-37 complex
PFE-I-0056	MnC	11	ST11 complex/ET-37 complex
PFE-I-0057	MnC	11	ST11 complex/ET-37 complex
PFE-I-0058	MnC	11	ST11 complex/ET-37 complex
PFE-I-0059	MnC	11	ST11 complex/ET-37 complex
PFE-I-0060	MnC	11	ST11 complex/ET-37 complex
PFE-I-0061	MnC	2006	ST103 complex
PFE-I-0062	MnC	11	ST11 complex/ET-37 complex
PFE-I-0063	MnC	11	ST11 complex/ET-37 complex
PFE-I-0064	MnY	23	ST23 complex/cluster A3
PFE-I-0065	MnY	23	ST23 complex/cluster A3
PFE-I-0066	MnY	23	ST23 complex/cluster A3
PFE-I-0067	MnY	23	ST23 complex/cluster A3
PFE-I-0068	MnY	23	ST23 complex/cluster A3
PFE-I-0069	MnY	23	ST23 complex/cluster A3
PFE-I-0070	MnY	23	ST23 complex/cluster A3
PFE-I-0071	MnY	23	ST23 complex/cluster A3
PFE-I-0072	MnY	23	ST23 complex/cluster A3
PFE-I-0073	MnY	23	ST23 complex/cluster A3
PFE-I-0074	MnY	1625	ST23 complex/cluster A3
PFE-I-0075	MnY	1624	ST167 complex
PFE-I-0076	MnB	32	ST32 complex/ET-5 complex
PFE-I-0077	MnB	32	ST32 complex/ET-5 complex
PFE-I-0078	MnB	32	ST32 complex/ET-5 complex
PFE-I-0079	MnB	162	ST162 complex
PFE-I-0080	MnB	162	ST162 complex
PFE-I-0081	MnB	162	ST162 complex
PFE-I-0082	MnA	1	ST1 complex/subgroup I/II
PFE-I-0083	MnA	4	ST4 complex/subgroup IV
PFE-I-0084	MnC	NST	NST
PFE-I-0085	MnC	917	ST37 complex
PFE-I-0086	MnC	11	ST11 complex/ET-37 complex
PFE-I-0087	MnC	11	ST11 complex/ET-37 complex
PFE-I-0088	MnC	11	ST11 complex/ET-37 complex
PFE-I-0089	MnW	11	ST11 complex/ET-37 complex
PFE-I-0090	MnY	NST	NST
PFE-I-0091	MnY	11	ST11 complex/ET-37 complex
PFE-I-0092	MnY	23	ST23 complex/cluster A3
PFE-I-0093	MnY	23	ST23 complex/cluster A3
PFE-I-0094	MnY	103	ST103 complex
PFE-I-0095	MnY	11	ST11 complex/ET-37 complex
PFE-I-0096	MnW	1262	ST174 complex

[a] NST, no sequence type, i.e., sequence type not assigned to gene alleles determined by multilocus sequence typing.
[b] ET, electrophoretic type.

was followed to prepare DNA libraries for WGS. Library quality was evaluated using the Agilent high-sensitivity DNA kit (product no. G2938-90321; Agilent Technologies, Santa Clara, CA). The libraries were then normalized according to the manufacturer's instructions (Illumina), and the normalized products were evaluated using the Power SYBR Green quantitative PCR assay (Life Technologies, Norwalk, CT). The material was then loaded into the MiSeq instrument (Illumina) for WGS analysis, and genomic data were obtained using the Illumina sequencing platform followed by *de novo* assembly (CLC Bio, Aarhus, Denmark) to generate contiguous sequences (contigs). Assembled contigs were deposited in the Bacterial Isolate Genome Sequencing Database (BIGSdb) (pubmlst.org/software/database/bigsdb) with the isolate sources and names and other demographic information, after which contigs were scanned for genes of interest and alleles and capsule groups were assigned (75). WGS data were inspected for the presence of all *cps* genes for polysaccharide synthesis and transport for a given genogroup, in order to define the genogroup; the absence of a complete coding sequence for any required gene resulted in a nongroupable (NG) designation.

(ii) TaqMan rt-PCR. All *N. meningitidis* isolates were tested using an rt-PCR assay targeting the *porA* (encoding the class 1 or PorA protein) and

TABLE 2 Carriage isolates

Isolate	Capsular genogroup by WGS	Sequence type[a]	Clonal complex[b]
PFE-C-0001	NG-*ctr*Aneg	53	ST53 complex
PFE-C-0002	NG-*ctr*Apos	269	ST269 complex
PFE-C-0003	NG-*ctr*Aneg	53	ST53 complex
PFE-C-0004	NG-*ctr*Aneg	198	ST198 complex
PFE-C-0005	MnW	184	ST22 complex
PFE-C-0006	NG-*ctr*Aneg	823	ST198 complex
PFE-C-0007	MnB	NST	NST
PFE-C-0008	MnW	184	ST22 complex
PFE-C-0009	NG-*ctr*Aneg	53	ST53 complex
PFE-C-0010	NG-*ctr*Aneg	43	ST41/44 complex/lineage 3
PFE-C-0011	MnY	23	ST23 complex/cluster A3
PFE-C-0012	NG-*ctr*Aneg	53	ST53 complex
PFE-C-0013	NG-*ctr*Aneg	53	ST53 complex
PFE-C-0014	NG-*ctr*Aneg	823	ST198 complex
PFE-C-0015	NG-*ctr*Aneg	43	ST41/44 complex/lineage 3
PFE-C-0016	MnB	NST	NST
PFE-C-0017	MnY	23	ST23 complex/cluster A3
PFE-C-0018	MnBpartial	269	ST269 complex
PFE-C-0019	MnY	23	ST23 complex/cluster A3
PFE-C-0020	MnY	23	ST23 complex/cluster A3
PFE-C-0021	NG-*ctr*Aneg	53	ST53 complex
PFE-C-0022	MnY	23	ST23 complex/cluster A3
PFE-C-0023	NG-*ctr*Aneg	53	ST53 complex
PFE-C-0024	NG-*ctr*Aneg	823	ST198 complex
PFE-C-0025	MnBpartial	6473	ST41/44 complex/lineage 3
PFE-C-0026	NG-*ctr*Aneg	1136	ST1136 complex
PFE-C-0027	MnC	944	ST41/44 complex/lineage 3
PFE-C-0028	NG-*ctr*Aneg	43	ST41/44 complex/lineage 3
PFE-C-0029	NG-*ctr*Aneg	198	ST198 complex
PFE-C-0030	NG-*ctr*Aneg	53	ST53 complex
PFE-C-0031	NG-*ctr*Aneg	53	ST53 complex
PFE-C-0032	MnY	23	ST23 complex/cluster A3
PFE-C-0033	MnY	23	ST23 complex/cluster A3
PFE-C-0034	MnB	6473	ST41/44 complex/lineage 3
PFE-C-0035	MnB	43	ST41/44 complex/lineage 3
PFE-C-0036	MnC	944	ST41/44 complex/lineage 3
PFE-C-0037	NG-*ctr*Aneg	1117	ST1117 complex
PFE-C-0038	NG-*ctr*Apos	NST	NST
PFE-C-0039	MnY	23	ST23 complex/cluster A3
PFE-C-0040	NG-*ctr*Aneg	823	ST198 complex
PFE-C-0041	MnY	23	ST23 complex/cluster A3
PFE-C-0042	MnW	184	ST22 complex
PFE-C-0043	MnB	2726	ST32 complex/ET-5 complex
PFE-C-0044	MnB	2726	ST32 complex/ET-5 complex
PFE-C-0045	NG-*ctr*Apos	23	ST23 complex/cluster A3
PFE-C-0046	NG-*ctr*Aneg	1136	ST1136 complex
PFE-C-0047	MnY	23	ST23 complex/cluster A3
PFE-C-0048	MnB	269	ST269 complex
PFE-C-0049	MnY	23	ST23 complex/cluster A3
PFE-C-0050	NG-*ctr*Aneg	823	ST198 complex
PFE-C-0051	NG-*ctr*Aneg	1117	ST1117 complex
PFE-C-0052	MnB	6473	ST41/44 complex/lineage 3
PFE-C-0053	NG-*ctr*Aneg	823	ST198 complex
PFE-C-0054	NG-*ctr*Aneg	198	ST198 complex
PFE-C-0055	NG-*ctr*Aneg	53	ST53 complex
PFE-C-0056	NG-*ctr*Apos	269	ST269 complex
PFE-C-0057	MnB	2726	ST32 complex/ET-5 complex
PFE-C-0058	NG-*ctr*Aneg	53	ST53 complex
PFE-C-0059	NG-*ctr*Aneg	198	ST198 complex
PFE-C-0060	MnB	2726	ST32 complex/ET-5 complex
PFE-C-0061	MnY	23	ST23 complex/cluster A3
PFE-C-0062	NG-*ctr*Aneg	198	ST198 complex
PFE-C-0063	NG-*ctr*Aneg	1117	ST1117 complex
PFE-C-0064	MnW	5389	ST22 complex
PFE-C-0065	MnW	5389	ST22 complex
PFE-C-0066	MnB	6473	ST41/44 complex/lineage 3
PFE-C-0067	MnY	23	ST23 complex/cluster A3
PFE-C-0068	NG-*ctr*Aneg	53	ST53 complex
PFE-C-0069	MnY	23	ST23 complex/cluster A3
PFE-C-0070	MnB	43	ST41/44 complex/lineage 3
PFE-C-0071	MnW	184	ST22 complex
PFE-C-0072	NG-*ctr*Aneg	823	ST198 complex
PFE-C-0073	MnW	184	ST22 complex
PFE-C-0074	MnB	2726	ST32 complex/ET-5 complex
PFE-C-0075	NG-*ctr*Aneg	53	ST53 complex
PFE-C-0076	NG-*ctr*Aneg	823	ST198 complex
PFE-C-0077	NG-*ctr*Aneg	823	ST198 complex
PFE-C-0078	MnB	NST	NST
PFE-C-0079	MnB	6473	ST41/44 complex/lineage 3
PFE-C-0080	MnC	944	ST41/44 complex/lineage 3
PFE-C-0081	MnW	184	ST22 complex
PFE-C-0082	MnY	23	ST23 complex/cluster A3
PFE-C-0083	MnB	6473	ST41/44 complex/lineage 3
PFE-C-0084	NG-*ctr*Apos	NST	NST
PFE-C-0085	MnY	NST	NST
PFE-C-0086	MnY	23	ST23 complex/cluster A3
PFE-C-0087	MnW	184	ST22 complex
PFE-C-0088	MnC	278	ST35 complex
PFE-C-0089	MnY	23	ST23 complex/cluster A3
PFE-C-0090	NG-*ctr*Aneg	1117	ST1117 complex
PFE-C-0091	NG-*ctr*Aneg	823	ST198 complex
PFE-C-0092	NG-*ctr*Aneg	823	ST198 complex
PFE-C-0093	NG-*ctr*Aneg	1136	ST1136 complex

[a] NST, no sequence type, i.e., sequence type not assigned to gene alleles determined by multilocus sequence typing.
[b] ET, electrophoretic type.

*ctr*A genes (76, 77). All isolates (regardless of *porA* and *ctrA* results) were evaluated further in rt-PCR assays specific for the eight capsule serogroups, as described by Rojas et al. (68). Isolates that were *ctrA* negative or did not give positive results in any of the serogroup-specific assays were characterized as nongroupable (NG).

Phenotypic methods. (i) Slide agglutination serogrouping. *N. meningitidis* isolates were serogrouped by SASG assays using commercial antisera (Difco, Sparks, MD), as described previously (40). For the carriage isolates, SASG1 and SASG2 assays were performed at two different laboratories, using independently supplied commercial reagents. In the case of the IMD isolates, SASG1 and SASG2 assays were performed in the same laboratory with two different lots of commercial reagents. Briefly, for each isolate, a few colonies were picked from an overnight culture plate to make a suspension in 0.1 to 0.2 ml of 1× phosphate-buffered saline (PBS). On a sectioned glass microscope slide, 100 μl of bacterial suspension was mixed with 20 to 100 μl of test serum; the slide was rocked gently for 1 min. The slide was observed for evidence of clumping, indicating a positive agglutination reaction. A saline control was used to detect nonspecific autoagglutination.

(ii) Dot blotting. Dot blot assays were conducted as described previously (78). Briefly, one loopful of each overnight culture (at 37°C in 5% CO_2) was harvested from chocolate agar (Remel), transferred into 200 μl saline, and incubated at 56°C for 30 min. Two-microliter cell suspensions were dotted on nitrocellulose membranes (0.45-μm pore size; Bio-Rad

Laboratories, Hercules, CA). After 30 min of drying at room temperature, membranes were blocked with 1% bovine serum albumin (BSA) in 1× PBS. MAbs specific for MnA, MnB, MnC, MnW, and MnY were added directly into the blocking buffer, at various dilutions of 1:200 to 1:1,000. Blots were incubated for 1 h at room temperature with gentle rocking. Membranes were washed separately four times with 20 mM Tris (pH 7.5)-180 mM NaCl plus 0.05% Tween 20 (vol/vol) and then were incubated for 1 h with goat anti-mouse IgG conjugated to alkaline phosphatase (1:1,000 dilution; Sigma-Aldrich, St. Louis, MO). Each membrane included type isolate controls. Monoclonal antibody reagents for serogroups MnA, MnC, MnW, and MnY were described previously (78), and anti-MnB was provided by the National Institute of Biological Standards and Control (NIBSC) (South Mimms, United Kingdom).

(iii) Flow cytometry. Serogrouping by fluorescence-activated cell sorting (FACS) analysis was conducted as described previously (43). The MAbs utilized were as described for the dot blot assay.

(iv) Live-cell phenotypic assay. Monoclonal antibodies (MAbs) specific for MnA, MnB, MnC, MnW, and MnY capsular antigens were adsorbed batchwise onto protein G magnetic beads (Magne; Promega, Madison, WI), to a concentration of 0.16 μg IgG per μl, and were rinsed in Dulbecco's phosphate-buffered saline (DPBS) to remove unbound antibody. Beads (3 μl) were distributed in duplicate into separate wells of a low-protein-binding, white, 96-well plate (Greiner, Monroe, NC). Control wells received beads adsorbed with normal mouse IgG or no beads, to monitor specificity and nonspecific binding, respectively. *N. meningitidis* test isolates were prepared by scraping a 2-cm area from a freshly grown plate (chocolate agar; Remel) with a 10-μl loop and suspending the cells in 1 ml of GC medium base supplemented with Isovitalex (BD), in the wells of a 96-deep-well block. Two 10-fold serial dilutions of the cells were prepared in the same medium, and 100 μl of the 1:100 dilution was transferred to the reaction plate containing antibody beads. The suspension was incubated for 45 min at 37°C in 5% CO_2 with shaking (300 rpm). A Tecan HydroSpeed plate washer equipped with a magnetic base station was used to remove unbound cells (six washes). Cells remaining bound to the antibody beads were detected using the luciferase-based BacTiter-Glo lysis reagent (Promega, Madison, WI), which measures ATP released from live bacteria via its associated luminescence. After incubation with the reagent at 37°C for 10 min at room temperature, the plate was sealed with transparent tape and results were read on a luminometer (Spectramax M5e; Molecular Devices, Sunnyvale, CA). Results were corrected for nonspecific binding by dividing the average of replicate reads observed with antigen-specific antibodies by the average of reads obtained for mouse IgG controls. Values ≥2-fold greater than the mouse IgG baseline value were reported as positive results.

Statistical analysis. Concordance between methods was determined using a concordance rate for matching assay results for each isolate; the denominator was the total number of samples tested for the paired methods. The 95% confidence intervals (CIs) for the concordance rates were calculated using the Clopper-Pearson exact method (79).

RESULTS

Genotypic characterization by WGS. Two isolate collections (invasive, $n = 97$; carriage, $n = 93$) were subjected to whole-genome sequencing (WGS) to determine whether the isolates had complete *cps* pathways, to serve as positive controls for the capsular genogrouping and serogrouping assays under evaluation (Tables 1 and 2). Using WGS, the isolates were then placed into one of several categories, i.e., (i) complete *cps* operon, (ii) incomplete *cps* operon missing essential synthesis or transport genes, with or without an insertion sequence (IS) element, (iii) incomplete *cps* operon missing one or more of the accessory genes (e.g., *O*-acetyltransferase), with or without an IS element, or (iv) *cps* operon deletion (capsule null [*cnl*]).

The invasive isolates were initially scanned for the presence of *porA*, *ctrA*, and *sodC* as *N. meningitidis* species-specific markers; two invasive isolates had *por*A gene deletions, and one isolate had a partial *porA* deletion. All 97 invasive isolates were positive for both *ctrA* and *sodC* and were assigned to capsular polysaccharide groups by WGS based on the *cps* locus. Nine isolates had an incomplete *cps* locus with genetic lesions (insertions/deletions) in accessory genes. Four MnW isolates had truncated *cssF* (formerly *oatWY*) genes, two of which had insertions within the *cssF* gene, identified as IS*1016* and IS*1301* (one each) (80–82). One MnY isolate had a truncation in *cssF* with an IS*1016* insertion. Three MnC isolates had IS*1301* insertions in the *cssE* gene (formerly *oatC*), with an additional MnC isolate having a truncation of the *cssE* gene (see Table S1 in the supplemental material). These results indicated that all 97 invasive isolates should be groupable by the rt-PCR assay and serogroupable by the phenotypic assays, because the accessory genes (*cssE* and *cssF*) are not essential for capsule expression (81).

Similarly, WGS was conducted on the carriage isolates ($n = 93$), and isolates were screened for the presence of the *porA*, *ctrA*, and *sodC* genes; one isolate had a partial *porA* deletion, and three isolates were negative for *sodC*. In contrast to the invasive isolates, only 35 carriage isolates (38%) had a complete *cps* locus by WGS (capsular serogroup MnB [18%], MnC [4%], or MnY [15%]). The remainder of the isolates ($n = 58$) had an incomplete *cps* locus, with 40% of the isolates missing *ctrA*. Thirty-nine (67%) of the 58 isolates were *ctrA* negative; of those, 32 isolates lacked capsule biosynthesis and capsule transport genes and thus were classified as capsule operon-null (*cnl*) isolates (83, 84). Fifteen isolates had insertions bearing coding sequences from IS*1301* (80–82), including nine in *cssF* (MnW) and three each in *cssA* (MnY) and *csb* (MnB). An additional isolate had an IS*30*-family insertion sequence associated with truncation of the *cssA* gene (MnY) (85) (see Table S1 in the supplemental material). These findings suggest that 44 carriage isolates should be groupable by the rt-PCR assay and serogroupable by the phenotypic assays. With this baseline genotypic information established, the accuracy of five diagnostic assays was assessed to determine which technique (genotypic or phenotypic) was best able to determine the capsular group (genogroup or serogroup).

Diagnostic methodologies. Figure 1 shows the distributions of the various serogroups and capsular genogroups in the invasive and carriage isolate collections, as determined by each of the methodologies. Tables 3 and 4 show the agreement between the five methods for the invasive and carriage isolate collections, respectively, using WGS as the standard method. Table 5 shows the agreement between the methods for the isolates in the two isolate sets that harbored a capsule operon sufficient to express capsule.

Characterization of invasive disease isolates. All 97 invasive isolates were positive for *porA* and *ctrA* by the TaqMan rt-PCR assay. Capsule group assignments by rt-PCR were in agreement with WGS results for 96 of the isolates (Table 3). One isolate (PFE-I-0010) was nongroupable by rt-PCR, as it gave positive results with both the MnA and MnB primer sets. The WGS data showed that a complete MnB locus, including the capsule biosynthesis genes *cssA-cssC and csb*, was present in this isolate. WGS analysis did not identify any sequences for the MnA operon, indicating a false-positive result by rt-PCR for this isolate. In addition, two isolates that lacked the *porA* gene by WGS gave false-positive results by rt-PCR.

Using the phenotyping approaches, we were able to serogroup

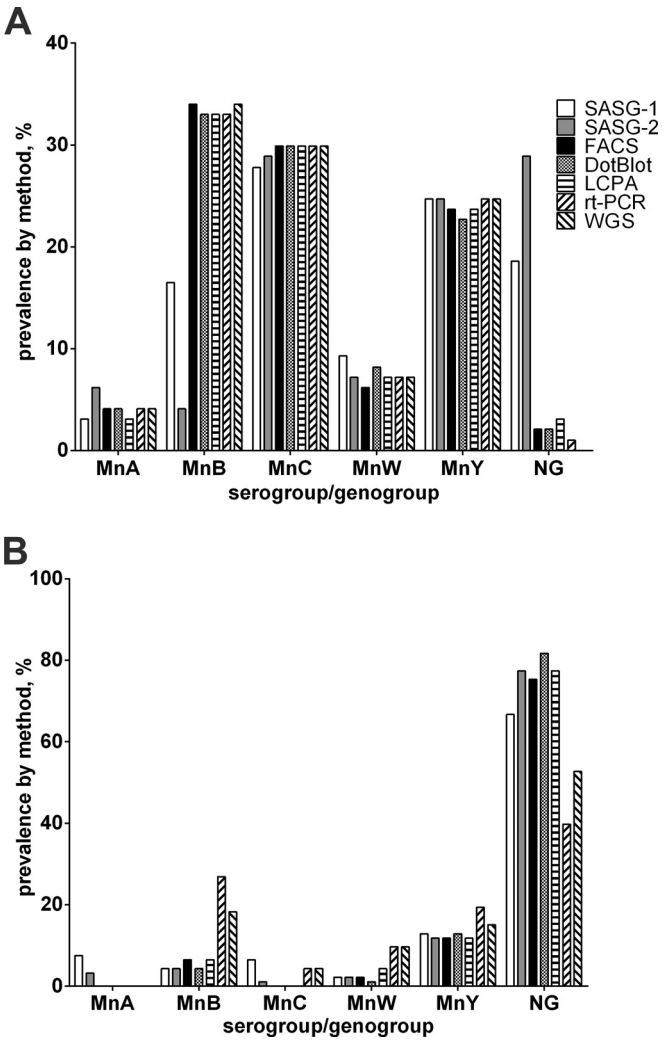

FIG 1 Serogroup distributions among invasive isolates ($n = 97$) (A) and carriage isolates ($n = 93$) (B) determined by different diagnostic methods. Isolates underwent serogrouping by SASG1 and SASG2 assays, LCPA, dot blotting, and FACS and capsular genogrouping by rt-PCR and WGS. Proportions of isolates in each serogroup, as identified by the indicated assays, are presented.

between 69% and 97% of invasive isolates, with the methods using monoclonal antibody reagents (FACS, dot blotting, and live-cell phenotypic assay [LCPA]) being the most sensitive and specific (Tables 3 and 5). As expected, all nine isolates with an incomplete

TABLE 3 Agreement between genotypic and phenotypic methods used for grouping invasive isolates ($n = 97$)

WGS capsular genogroup assignment	rt-PCR capsular genogroup agreement (%)	Phenotypic method agreement (%)[a]				
		SASG1	SASG2	Dot blot	FACS	LCPA
MnA ($n = 4$)	100	75	100	100	100	75
MnB ($n = 33$)	97	48	12	94	97	94
MnC ($n = 29$)	100	93	97	100	100	100
MnW ($n = 7$)	100	100	100	100	86	100
MnY ($n = 24$)	100	100	100	96	96	96

[a] Pairwise concordance rates (Clopper-Pearson exact method) for WGS, rt-PCR, FACS, LCPA, and dot blotting were ≥96.9% (95% CI, 91.2 to 100%).

TABLE 4 Agreement between genotypic and phenotypic methods used for grouping carriage isolates ($n = 93$)

WGS capsular genogroup assignment	rt-PCR capsular genogroup agreement (%)	Phenotypic method agreement (%)[a]				
		SASG1	SASG2	Dot blot	FACS[b]	LCPA
MnB ($n = 17$)	100	24	24	24	35	35
MnC ($n = 4$)	100	0	0	0	0	0
MnW ($n = 9$)	100	0	0	11	22	22
MnY ($n = 14$)	100	79	71	86	79	79
NG, *ctrA* negative ($n = 39$)	72	72	79	100	100	92
NG, *ctrA* positive ($n = 10$)	10	60	100	100	100	100

[a] Pairwise concordance rates (Clopper-Pearson exact method) for serogroup assignment by FACS, dot blotting, or LCPA versus WGS were ≤20.4% (95% CI, 11.0 to 30.1%) and that for rt-PCR versus WGS was 47.3% (95% CI, 36.9 to 57.9%).
[b] Four samples were not tested by FACS.

cps locus (four MnW, four MnC, and one MnY) were found to express the capsule by the various serogrouping approaches, as the loss of the functional *O*-acetyltransferase genes did not affect capsule expression or detection in the serogrouping assays (81).

Table 5 shows the percent agreement in serogroup assignment in comparisons of the five phenotypic assays versus WGS. Dot blotting, LCPA, and FACS demonstrated ≥97% agreement with WGS and also demonstrated that the one isolate (PFE-I-0010) that was called NG (positive for both MnA and MnB) by rt-PCR expressed a MnB capsule. The SASG2 and SASG1 assays showed 69% and 79% overall agreement, respectively, with WGS data. The SASG1 and SASG2 assays had 100% agreement with WGS in assigning serogroups W and Y (Table 3); however, the rates of agreement for serogroup B determination were much lower (48% and 12%, respectively) and differed greatly between the two SASG panels. The SASG1 and SASG2 assays resulted in NG results for ~20% and ~30% of the isolates tested, respectively. For the SASG1 panel, 89% of the NG assignments were determined by the other phenotypic assays to be MnB, in agreement with the WGS results. For the SASG2 panel, 100% of the NG assignments were determined to be MnB by the other phenotypic methods and WGS. The incorrect identifications by the phenotypic methods are presented in Table S2 in the supplemental material.

Characterization of *N. meningitidis* carriage isolates. All 93 carriage isolates were *porA* positive, with 65 (70%) of the isolates testing positive for *ctrA* by rt-PCR. A capsule genogroup was as-

TABLE 5 Agreement in serogroup assignments for *cps*-positive isolates

Type of classification and assay	Agreement for groupable isolates[a] (%)	
	Invasive isolates ($n = 97$)	Carriage isolates ($n = 44$)
Serogroup		
SASG1	79	32
SASG2	69	29
FACS	98	43
Dot blot	97	39
LCPA	97	39
Capsular genogroup		
rt-PCR	99	100

[a] Isolates determined by WGS to have complete capsule operons.

signed to 56 (60%) of the *ctrA*-positive isolates by rt-PCR. Of the capsular serogroupable isolates identified by WGS ($n = 44$), dot blotting, LCPA, and FACS correctly grouped 17 isolates (39%), 18 isolates (41%), and 19 isolates (43%), respectively (Table 5). Specifically for MnB isolates, these methods correctly identified 4 (24%), 6 (35%), and 5 (29%), respectively, of the 17 MnB isolates genogrouped by WGS. Conversely, rt-PCR incorrectly identified 12 isolates (8 MnB and 4 MnY); the *cps* locus carried by these isolates was incomplete, with partial or interrupted *csb* and *synA* genes, respectively. Dot blotting, LCPA, FACS, SASG2 assays, and the genotypic methods were in agreement on NG assignments for all 10 nongroupable *ctrA*-positive isolates. Similarly, dot blotting, FACS, and the genotypic methods were in agreement on NG assignments for all 39 nongroupable *ctrA*-negative isolates. SASG1 and SASG2 assays correctly assigned serogroups to 14 (32%) and 13 (29%) of the 44 serogroupable isolates, respectively, as determined by rt-PCR and confirmed by WGS. The incorrect identifications by the phenotypic methods are presented in Table S3 in the supplemental material.

DISCUSSION

The meningococcal capsule is a virulence factor, as exemplified by the low frequency of unencapsulated *N. meningitidis* isolates causing invasive disease (86, 87). The capsule protects the bacterium from bactericidal activity during invasion and dissemination (51, 88–90). In this study, we demonstrated that ≥97% of invasive disease isolates expressed capsule *in vitro* and could be readily characterized by both genotypic and phenotypic methodologies. Serogrouping of carriage isolates phenotypically, as well as by rt-PCR, was challenging; this was particularly true for MnB isolates. This is due to reduced or no capsule expression in the carriage state, which has been reported to be the result of phase variation and/or genetic deletions or insertions in the *cps* locus (44–47). A subset of nongroupable isolates has been described in the literature, with deletion of the complete *cps* locus yielding capsule-null (*cnl*) mutants (45, 50, 84). WGS confirmed the loss of the *ctr* (capsule transport) operon and capsule biosynthesis genes in 32 (34%) of the carriage isolates tested. In agreement with published work, the capsule-null isolates in our collection were restricted to the following clonal complexes: sequence type 53 (ST53) (38%), ST198 (41%), ST1117 (12%), and ST1136 (9%) (91). Additionally, 10% of the carriage isolates were negative by rt-PCR for serogroup-specific genes, although they did carry the *ctrA* gene. Although carriage isolates may be missing the capsule locus or harbor loci with insertions or deletions, this does not necessarily predispose such isolates to be nonvirulent, as *N. meningitidis* is naturally genetically competent and has the capacity to obtain the *cps* pathway genes through horizontal genetic exchange (92–94). In the carriage state in the human nasopharynx, cocolonization with other pathogenic and nonpathogenic *N. meningitidis* strains has the potential to lead to genetic exchange and capsule switching (95, 96). Furthermore, although IMD caused by *cnl* meningococci is rare, there have been reports of *cnl* isolates causing disease, mainly in immunocompromised patients (86, 87).

WGS identified 44 carriage isolates (47%) that contained a complete *cps* operon; of those isolates, less than one-half were phenotypically positive by the various phenotypic diagnostics tests (Table 5). Phenotypic assays were more effective in serogrouping invasive isolates than carriage isolates; ≥95% of invasive isolates were successfully grouped by FACS, dot blotting, and LCPA (Table 5). Although the capsule is an important virulence factor, *cps* expression is regulated at different stages of growth and infection (47). The recent study by Loh et al. (51) demonstrated that environmental factors (e.g., temperature) play roles in the expression of capsule and surface antigens through a thermosensor. Those authors identified an 8-bp repeat motif whose deletion resulted in dysregulation of the *cssA* promoter. In the present study, alignment of the intergenic region containing the *ctrA* and *cssA* promoters from carriage isolates that failed to express the capsule and capsule-positive control isolates did not reveal any clues, at the sequence level, to explain the lack of capsule expression by the carriage isolates (data not shown). Isolates in our collection were shown to have as many as three of the 8-bp repeat motifs described by Loh et al. (51); additional studies will be required to determine the role of these sequences in capsule expression *in vivo*.

WGS is rapidly becoming the gold standard for bacterial epidemiological studies (69–71). In this study, we have highlighted its potential usefulness for the characterization of meningococcal carriage isolates. Although the rt-PCR assay appears to be highly predictive of capsule genogrouping, especially for invasive *N. meningitidis* isolates (99%), this approach relies on the detection of a small fragment (<120 bp) of one gene in the *cps* operon for each capsular genogroup, and assay sensitivity can be affected by the high degree of genetic plasticity, which we confirmed especially when evaluating carriage isolates. Selection of the target sequences is critical and may be different in different geographical locations.

Several targets (*porA*, *ctrA*, and *sodC*) have been employed as *N. meningitidis*-specific diagnostic targets for rt-PCR assays (97, 98). In this study, we looked at all three targets by WGS in both of our isolate collections. As prior studies have found (50, 84), *ctrA* is absent in a large percentage of carriage isolates; in our collection, only 54/93 carriage isolates (58%) were positive for *ctrA*, whereas 100% of invasive isolates were positive for this gene. Both *porA* (99% and 97%, respectively) and *sodC* (97% and 100%, respectively) were well represented in the carriage and invasive isolate collections. Our data confirm that either of these target genes could be a reliable *N. meningitidis*-specific marker for an rt-PCR diagnostic assay.

The carriage and IMD isolate collections were not contemporary or from the same geographical locations. However, data collected over the past 5 years from diverse geographical locations, which showed that the levels of capsule expression in carriage isolates were the same (data not shown), suggested that geographical locations may not affect our final conclusions. Additionally, the IMD isolates were previously characterized by SASG assays; therefore, the isolates tested were not from an unbiased set, although all assays were conducted in a blinded fashion. As reported previously, IMD isolates are efficiently serogrouped by SASG assays; therefore, this was not thought to affect the overall conclusions of the study (40).

With the introduction of vaccines targeting MnB, carriage studies are essential for monitoring the ability of the new vaccines to prevent carriage acquisition and/or to shorten the duration of carriage episodes and for assessing the development of herd protection (12). Identification of the appropriate diagnostic tools to conduct surveillance following vaccination will be critical. The present study demonstrates the utility of using WGS to accurately type bacterial isolates recovered during clinical studies. Based on

our field study, rt-PCR diagnostic assays seems unlikely to provide the reliability necessary for carriage surveillance, due to a large number of misclassified isolates with a mutated or deleted *cps* locus. Our findings suggest that the use of WGS to type carriage isolates accurately, in conjunction with the use of FACS to determine the capsule phenotype, may offer a superior algorithm to characterize carriage isolates for most purposes, compared to single methods. Remaining challenges for the development of WGS as an interpretive tool for disease surveillance, confirmatory diagnosis, and case management include the development of an appropriate infrastructure for data generation, analysis, and storage. We conclude that a combination of genotypic and phenotypic methods will improve *N. meningitidis* serogroup determination and the understanding of *N. meningitidis* carriage and epidemiology.

ACKNOWLEDGMENTS

We acknowledge Alita Miller and Robert Donald for helpful conversations and Li Hao for assistance with bioinformatics analyses.

This study was sponsored by Pfizer Vaccine Research.

C.H.J., N.M., E.R., L.A., J.H., J.C.H., L.K.M., Q.J., L.P., J.E., K.U.J., and A.S.A. are current or former employees of Pfizer and may own stock in the company. R.G. and P.D.W. have received research grants and/or reimbursement of travel expenses from Pfizer. L.W.M. and X.W. have no conflicts of interest and do not receive financial support or have other interests in commercial companies.

REFERENCES

1. **Caugant DA, Maiden MCJ.** 2009. Meningococcal carriage and disease: population biology and evolution. Vaccine **27**(Suppl 2):B64–B70. http://dx.doi.org/10.1016/j.vaccine.2009.04.061.
2. **MacLennan J, Kafatos G, Neal K, Andrews N, Cameron JC, Roberts R, Evans MR, Cann K, Baxter DN, Maiden MC, Stuart JM.** 2006. Social behavior and meningococcal carriage in British teenagers. Emerg Infect Dis **12:**950–957. http://dx.doi.org/10.3201/eid1206.051297.
3. **Yazdankhah SP, Caugant DA.** 2004. *Neisseria meningitidis*: an overview of the carriage state. J Med Microbiol **53:**821–832. http://dx.doi.org/10.1099/jmm.0.45529-0.
4. **Caugant DA, Hoiby EA, Magnus P, Scheel O, Hoel T, Bjune G, Wedege E, Eng J, Froholm LO.** 1994. Asymptomatic carriage of *Neisseria meningitidis* in a randomly sampled population. J Clin Microbiol **32:**323–330.
5. **Claus H, Maiden MC, Wilson DJ, McCarthy ND, Jolley KA, Urwin R, Hessler F, Frosch M, Vogel U.** 2005. Genetic analysis of meningococci carried by children and young adults. J Infect Dis **191:**1263–1271. http://dx.doi.org/10.1086/428590.
6. **Stephens DS.** 1999. Uncloaking the meningococcus: dynamics of carriage and disease. Lancet **353:**941–942. http://dx.doi.org/10.1016/S0140-6736(98)00279-7.
7. **Cartwright K.** 1995. The clinical spectrum of meningococcal disease, p 115–146. *In* Cartwright K (ed), Meningococcal disease. John Wiley & Sons, Chichester, United Kingdom.
8. **Olcen P, Kjellander J, Danielsson D, Lindquist BL.** 1981. Epidemiology of *Neisseria meningitidis*: prevalence and symptoms from the upper respiratory tract in family members to patients with meningococcal disease. Scand J Infect Dis **13:**105–109. http://dx.doi.org/10.3109/inf.1981.13.issue-2.05.
9. **Kremastinou J, Tzanakaki G, Levidiotou S, Markou F, Themeli E, Voyiatzi A, Psoma E, Theodoridou M, Blackwell CC.** 2003. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in northern Greece. FEMS Immunol Med Microbiol **39:**23–29. http://dx.doi.org/10.1016/S0928-8244(03)00174-3.
10. **Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM.** 2001. Meningococcal disease. N Engl J Med **344:**1378–1388. http://dx.doi.org/10.1056/NEJM200105033441807.
11. **Edwards EA, Devine LF, Sengbusch GH, Ward HW.** 1977. Immunological investigations of meningococcal disease. III. Brevity of group C acquisition prior to disease occurrence. Scand J Infect Dis **9:**105–110.
12. **Christensen H, Trotter CL, Hickman M, Edmunds WJ.** 2014. Re-evaluating cost effectiveness of universal meningitis vaccination (Bexsero) in England: modelling study. BMJ **349:**g5725. http://dx.doi.org/10.1136/bmj.g5725.
13. **Anderson AS, Hao L, Jiang Q, Harris SL, Jones TR, Perez JL, York L, Eiden J, Jansen KU.** 2013. Potential impact of the bivalent rLP2806 vaccine on *Neisseria meningitidis* carriage and invasive serogroup B disease. Hum Vaccin Immunother **9:**471–479.
14. **Centers for Disease Control and Prevention.** 2013. Active Bacterial Core Surveillance report, Emerging Infections Program Network, *Neisseria meningitidis*, 2012. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/abcs/reports-findings/survreports/mening12.pdf.
15. **Li Y, Tsang R, Desai S, Deehan H.** 2014. Enhanced surveillance of invasive meningococcal disease in Canada, 2006–2011. Can Commun Dis Rep **40:**160.
16. **European Centre for Disease Prevention and Control.** 2015. Annual epidemiological report 2014: vaccine-preventable diseases: invasive bacterial diseases. European Centre for Disease Prevention and Control, Stockholm, Sweden. http://ecdc.europa.eu/en/publications/Publications/AER-VPD-IBD-2014.pdf.
17. **Dwilow R, Fanella S.** 2015. Invasive meningococcal disease in the 21st century: an update for the clinician. Curr Neurol Neurosci Rep **15:**2. http://dx.doi.org/10.1007/s11910-015-0524-6.
18. **McNamara LA, Shumate AM, Johnsen P, MacNeil JR, Patel M, Bhavsar T, Cohn AC, Dinitz-Sklar J, Duffy J, Finnie J, Garon D, Hary R, Hu F, Kamiya H, Kim HJ, Kolligian J, Jr, Neglia J, Oakley J, Wagner J, Wagner K, Wang X, Yu Y, Montana B, Tan C, Izzo R, Clark TA.** 2015. First use of a serogroup B meningococcal vaccine in the US in response to a university outbreak. Pediatrics **135:**798–804. http://dx.doi.org/10.1542/peds.2014-4015.
19. **Tzeng YL, Stephens DS.** 2000. Epidemiology and pathogenesis of *Neisseria meningitidis*. Microbes Infect **2:**687–700. http://dx.doi.org/10.1016/S1286-4579(00)00356-7.
20. **Kahler CM, Martin LE, Shih GC, Rahman MM, Carlson RW, Stephens DS.** 1998. The (α→8)-linked polysialic acid capsule and lipooligosaccharide structure both contribute to the ability of serogroup B *Neisseria meningitidis* to resist the bactericidal activity of normal human serum. Infect Immun **66:**5939–5947.
21. **Vogel U, Frosch M.** 1999. Mechanisms of neisserial serum resistance. Mol Microbiol **32:**1133–1139. http://dx.doi.org/10.1046/j.1365-2958.1999.01469.x.
22. **Branham SE.** 1953. Serological relationships among meningococci. Bacteriol Rev **17:**175–188.
23. **Craven DE, Frasch CE, Robbins JB, Feldman HA.** 1978. Serogroup identification of *Neisseria meningitidis*: comparison of an antiserum agar method with bacterial slide agglutination. J Clin Microbiol **7:**410–414.
24. **Khatami A, Pollard AJ.** 2010. The epidemiology of meningococcal disease and the impact of vaccines. Expert Rev Vaccines **9:**285–298. http://dx.doi.org/10.1586/erv.10.3.
25. **Harrison LH.** 2006. Prospects for vaccine prevention of meningococcal infection. Clin Microbiol Rev **19:**142–164. http://dx.doi.org/10.1128/CMR.19.1.142-164.2006.
26. **Maiden MC, Begg NT.** 2001. Overview: epidemiology, surveillance, and population biology, p 121–130. *In* Pollard AJ, Maiden MCJ (ed), Methods in molecular medicine, vol 67: Meningococcal disease methods and protocols. Humana Press, Totowa, NJ.
27. **World Health Organization.** 2011. Laboratory manual for the diagnosis of meningitis caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*, 2nd ed. World Health Organization, Geneva, Switzerland.
28. **Borrow R, Abad R, Trotter C, van der Klis FR, Vazquez JA.** 2013. Effectiveness of meningococcal serogroup C vaccine programmes. Vaccine **31:**4477–4486. http://dx.doi.org/10.1016/j.vaccine.2013.07.083.
29. **Trotter CL, Ramsay ME.** 2007. Vaccination against meningococcal disease in Europe: review and recommendations for the use of conjugate vaccines. FEMS Microbiol Rev **31:**101–107. http://dx.doi.org/10.1111/j.1574-6976.2006.00053.x.
30. **Finne J, Leinonen M, Makela PH.** 1983. Antigenic similarities between brain components and bacteria causing meningitis: implications for vaccine development and pathogenesis. Lancet **ii:**355–357.
31. **Zlotnick GW, Jones TR, Liberator P, Hao L, Harris S, McNeil LK, Zhu D, Perez J, Eiden J, Jansen KU, Anderson AS.** 2015. The discovery and development of a novel vaccine to protect against *Neisseria meningitidis* serogroup B disease. Hum Vaccin Immunother **11:**5–13. http://dx.doi.org/10.4161/hv.34293.

32. **The Medical Letter.** 2015. Trumenba: a serogroup B meningococcal vaccine. Med Lett Drugs Ther **57:**5–6.
33. **Food and Drug Administration.** 2015. FDA approves a second vaccine to prevent serogroup B meningococcal disease. Food and Drug Administration, Washington, DC. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm431370.htm.
34. **McIntyre PB, O'Brien KL, Greenwood B, van de Beek D.** 2012. Effect of vaccines on bacterial meningitis worldwide. Lancet **380:**1703–1711. http://dx.doi.org/10.1016/S0140-6736(12)61187-8.
35. **Trotter CL, Maiden MC.** 2009. Meningococcal vaccines and herd immunity: lessons learned from serogroup C conjugate vaccination programs. Expert Rev Vaccines **8:**851–861. http://dx.doi.org/10.1586/erv.09.48.
36. **Bijlsma MW, Brouwer MC, Spanjaard L, van de Beek D, van der Ende A.** 2014. A decade of herd protection after introduction of meningococcal serogroup C conjugate vaccination. Clin Infect Dis **59:**1216–1221. http://dx.doi.org/10.1093/cid/ciu601.
37. **Sadarangani M, Scheifele DW, Halperin SA, Vaudry W, Le Saux N, Tsang R, Bettinger JA.** 2014. The impact of the meningococcal serogroup C conjugate vaccine in Canada between 2002 and 2012. Clin Infect Dis **59:**1208–1215. http://dx.doi.org/10.1093/cid/ciu597.
38. **Roderick M, Finn A.** 2014. Advances towards the prevention of meningococcal B disease: a multidimensional story. J Infect **68**(Suppl 1)**:**S76–S82. http://dx.doi.org/10.1016/j.jinf.2013.09.026.
39. **Read RC, Baxter D, Chadwick DR, Faust SN, Finn A, Gordon SB, Heath PT, Lewis DJ, Pollard AJ, Turner DP, Bazaz R, Ganguli A, Havelock T, Neal KR, Okike IO, Morales-Aza B, Patel K, Snape MD, Williams J, Gilchrist S, Gray SJ, Maiden MC, Toneatto D, Wang H, McCarthy M, Dull PM, Borrow R.** 2014. Effect of a quadrivalent meningococcal ACWY glycoconjugate or a serogroup B meningococcal vaccine on meningococcal carriage: an observer-blind, phase 3 randomised clinical trial. Lancet **384:**2123–2131. http://dx.doi.org/10.1016/S0140-6736(14)60842-4.
40. **Mothershed EA, Sacchi CT, Whitney AM, Barnett GA, Ajello GW, Schmink S, Mayer LW, Phelan M, Taylor TH, Jr, Bernhardt SA, Rosenstein NE, Popovic T.** 2004. Use of real-time PCR to resolve slide agglutination discrepancies in serogroup identification of *Neisseria meningitidis*. J Clin Microbiol **42:**320–328. http://dx.doi.org/10.1128/JCM.42.1.320-328.2004.
41. **Zollinger WD, Moran EE, Connelly H, Mandrell RE, Brandt B.** 1984. Monoclonal antibodies to serotype 2 and serotype 15 outer membrane proteins of *Neisseria meningitidis* and their use in serotyping. Infect Immun **46:**260–266.
42. **Abdillahi H, Poolman JT.** 1988. Typing of group-B *Neisseria meningitidis* with monoclonal antibodies in the whole-cell ELISA. J Med Microbiol **26:**177–180.
43. **McNeil LK, Murphy E, Zhao XJ, Guttmann S, Harris SL, Scott AA, Tan C, Mack M, DaSilva I, Alexander K, Mason K, Jiang HQ, Zhu D, Mininni TL, Zlotnick GW, Hoiseth SK, Jones TR, Pride MW, Jansen KU, Anderson AS.** 2009. Detection of LP2086 on the cell surface of *Neisseria meningitidis* and its accessibility in the presence of serogroup B capsular polysaccharide. Vaccine **27:**3417–3421. http://dx.doi.org/10.1016/j.vaccine.2009.01.075.
44. **Hammerschmidt S, Muller A, Sillmann H, Muhlenhoff M, Borrow R, Fox A, van Putten J, Zollinger WD, Gerardy-Schahn R, Frosch M.** 1996. Capsule phase variation in *Neisseria meningitidis* serogroup B by slipped-strand mispairing in the polysialyltransferase gene (*siaD*): correlation with bacterial invasion and the outbreak of meningococcal disease. Mol Microbiol **20:**1211–1220. http://dx.doi.org/10.1111/j.1365-2958.1996.tb02641.x.
45. **Sadler F, Fox A, Neal K, Dawson M, Cartwright K, Borrow R.** 2003. Genetic analysis of capsular status of meningococcal carrier isolates. Epidemiol Infect **130:**59–70. http://dx.doi.org/10.1017/S0950268802007987.
46. **Tzeng YL, Swartley JS, Miller YK, Nisbet RE, Liu LJ, Ahn JH, Stephens DS.** 2001. Transcriptional regulation of divergent capsule biosynthesis and transport operon promoters in serogroup B *Neisseria meningitidis*. Infect Immun **69:**2502–2511. http://dx.doi.org/10.1128/IAI.69.4.2502-2511.2001.
47. **Von Loewenich FD, Wintermeyer E, Dumig M, Frosch M.** 2001. Analysis of transcriptional control mechanisms of capsule expression in *Neisseria meningitidis*. Int J Med Microbiol **291:**361–369. http://dx.doi.org/10.1078/1438-4221-00142.
48. **Hammerschmidt S, Hilse R, van Putten JP, Gerardy-Schahn R, Unkmeir A, Frosch M.** 1996. Modulation of cell surface sialic acid expression in *Neisseria meningitidis* via a transposable genetic element. EMBO J **15:**192–198.
49. **Hoang LM, Thomas E, Tyler S, Pollard AJ, Stephens G, Gustafson L, McNabb A, Pocock I, Tsang R, Tan R.** 2005. Rapid and fatal meningococcal disease due to a strain of *Neisseria meningitidis* containing the capsule null locus. Clin Infect Dis **40:**e38–e42. http://dx.doi.org/10.1086/427875.
50. **Claus H, Maiden MC, Maag R, Frosch M, Vogel U.** 2002. Many carried meningococci lack the genes required for capsule synthesis and transport. Microbiology **148:**1813–1819. http://dx.doi.org/10.1099/00221287-148-6-1813.
51. **Loh E, Kugelberg E, Tracy A, Zhang Q, Gollan B, Ewles H, Chalmers R, Pelicic V, Tang CM.** 2013. Temperature triggers immune evasion by *Neisseria meningitidis*. Nature **502:**237–240. http://dx.doi.org/10.1038/nature12616.
52. **Frosch M, Weisgerber C, Meyer TF.** 1989. Molecular characterization and expression in *Escherichia coli* of the gene complex encoding the polysaccharide capsule of *Neisseria meningitidis* group B. Proc Natl Acad Sci U S A **86:**1669–1673. http://dx.doi.org/10.1073/pnas.86.5.1669.
53. **Swartley JS, Liu LJ, Miller YK, Martin LE, Edupuganti S, Stephens DS.** 1998. Characterization of the gene cassette required for biosynthesis of the (α1→6)-linked *N*-acetyl-D-mannosamine-1-phosphate capsule of serogroup A *Neisseria meningitidis*. J Bacteriol **180:**1533–1539.
54. **Edwards U, Muller A, Hammerschmidt S, Gerardy-Schahn R, Frosch M.** 1994. Molecular analysis of the biosynthesis pathway of the α-2,8 polysialic acid capsule by *Neisseria meningitidis* serogroup B. Mol Microbiol **14:**141–149. http://dx.doi.org/10.1111/j.1365-2958.1994.tb01274.x.
55. **Frosch M, Edwards U, Bousset K, Krausse B, Weisgerber C.** 1991. Evidence for a common molecular origin of the capsule gene loci in Gram-negative bacteria expressing group II capsular polysaccharides. Mol Microbiol **5:**1251–1263. http://dx.doi.org/10.1111/j.1365-2958.1991.tb01899.x.
56. **Frosch M, Muller D, Bousset K, Muller A.** 1992. Conserved outer membrane protein of *Neisseria meningitidis* involved in capsule expression. Infect Immun **60:**798–803.
57. **Ala'Aldeen DA, Neal KR, Ait-Tahar K, Nguyen-Van-Tam JS, English A, Falla TJ, Hawkey PM, Slack RC.** 2000. Dynamics of meningococcal long-term carriage among university students and their implications for mass vaccination. J Clin Microbiol **38:**2311–2316.
58. **Borrow R, Claus H, Chaudhry U, Guiver M, Kaczmarski EB, Frosch M, Fox AJ.** 1998. *siaD* PCR ELISA for confirmation and identification of serogroup Y and W135 meningococcal infections. FEMS Microbiol Lett **159:**209–214. http://dx.doi.org/10.1111/j.1574-6968.1998.tb12862.x.
59. **Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarski EB.** 2001. Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. J Clin Microbiol **39:**1553–1558. http://dx.doi.org/10.1128/JCM.39.4.1553-1558.2001.
60. **Lewis C, Clarke SC.** 2003. Identification of *Neisseria meningitidis* serogroups Y and W135 by *siaD* nucleotide sequence analysis. J Clin Microbiol **41:**2697–2699. http://dx.doi.org/10.1128/JCM.41.6.2697-2699.2003.
61. **Taha MK, Alonso JM, Cafferkey M, Caugant DA, Clarke SC, Diggle MA, Fox A, Frosch M, Gray SJ, Guiver M, Heuberger S, Kalmusova J, Kesanopoulos K, Klem AM, Kriz P, Marsh J, Molling P, Murphy K, Olcen P, Sanou O, Tzanakaki G, Vogel U.** 2005. Interlaboratory comparison of PCR-based identification and genogrouping of *Neisseria meningitidis*. J Clin Microbiol **43:**144–149. http://dx.doi.org/10.1128/JCM.43.1.144-149.2005.
62. **Probert WS, Bystrom SL, Khashe S, Schrader KN, Wong JD.** 2002. 5′ Exonuclease assay for detection of serogroup Y *Neisseria meningitidis*. J Clin Microbiol **40:**4325–4328. http://dx.doi.org/10.1128/JCM.40.11.4325-4328.2002.
63. **Drakopoulou Z, Kesanopoulos K, Sioumala M, Tambaki A, Kremastinou J, Tzanakaki G.** 2008. Simultaneous single-tube PCR-based assay for the direct identification of the five most common meningococcal serogroups from clinical samples. FEMS Immunol Med Microbiol **53:**178–182. http://dx.doi.org/10.1111/j.1574-695X.2008.00406.x.
64. **Borrow R, Claus H, Guiver M, Smart L, Jones DM, Kaczmarski EB, Frosch M, Fox AJ.** 1997. Non-culture diagnosis and serogroup determination of meningococcal B and C infection by a sialyltransferase (*siaD*) PCR ELISA. Epidemiol Infect **118:**111–117. http://dx.doi.org/10.1017/S0950268896007261.
65. **Taha MK.** 2000. Simultaneous approach for nonculture PCR-based identification and serogroup prediction of *Neisseria meningitidis*. J Clin Microbiol **38:**855–857.
66. **Pollard AJ, Probe G, Trombley C, Castell A, Whitehead S, Bigham JM, Champagne S, Isaac-Renton J, Tan R, Guiver M, Borrow R, Speert DP,**

Thomas E. 2002. Evaluation of a diagnostic polymerase chain reaction assay for *Neisseria meningitidis* in North America and field experience during an outbreak. Arch Pathol Lab Med **126:**1209–1215.
67. **Diggle MA, Smith K, Girvan EK, Clarke SC.** 2003. Evaluation of a fluorescence-based PCR method for identification of serogroup A meningococci. J Clin Microbiol **41:**1766–1768. http://dx.doi.org/10.1128/JCM.41.4.1766-1768.2003.
68. **Rojas E, Hoyos J, Oldfield NJ, Lee P, Flint M, Jones CH, Ala'Aldeen DA, Jansen KU, Anderson AS.** 2015. Optimization of molecular approaches to genogroup *Neisseria meningitidis* carriage isolates and implications for monitoring the impact of new serogroup B vaccines. PLoS One **10:** e0132140. http://dx.doi.org/10.1371/journal.pone.0132140.
69. **Jolley KA, Hill DM, Bratcher HB, Harrison OB, Feavers IM, Parkhill J, Maiden MC.** 2012. Resolution of a meningococcal disease outbreak from whole-genome sequence data with rapid Web-based analysis methods. J Clin Microbiol **50:**3046–3053. http://dx.doi.org/10.1128/JCM.01312-12.
70. **Koser CU, Holden MT, Ellington MJ, Cartwright EJ, Brown NM, Ogilvy-Stuart AL, Hsu LY, Chewapreecha C, Croucher NJ, Harris SR, Sanders M, Enright MC, Dougan G, Bentley SD, Parkhill J, Fraser LJ, Betley JR, Schulz-Trieglaff OB, Smith GP, Peacock SJ.** 2012. Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. N Engl J Med **366:**2267–2275. http://dx.doi.org/10.1056/NEJMoa1109910.
71. **Jolley KA, Maiden MCJ.** 2013. Automated extraction of typing information for bacterial pathogens from whole genome sequence data: *Neisseria meningitidis* as an exemplar. Eur Surveill **18:**pii=20379. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20379.
72. **Hoiseth SK, Murphy E, Andrew L, Vogel U, Frosch M, Hellenbrand W, Abad R, Vazquez JA, Borrow R, Findlow J, Taha MK, Deghmane AE, Caugant DA, Kriz P, Musilek M, Mayer LW, Wang X, Macneil JR, York L, Tan CY, Jansen KU, Anderson AS.** 2013. A multi-country evaluation of *Neisseria meningitidis* serogroup B factor H-binding proteins and implications for vaccine coverage in different age groups. Pediatr Infect Dis J **32:**1096–1101. http://dx.doi.org/10.1097/INF.0b013e31829aa63b.
73. **Gilca R, De Wals P, Costa JA, Nolan SM, Eiden J, Pregaldien JL, Jiang Q, Jones H, Jansen KU, Anderson AS, Pedneault L.** 2013. Longitudinal study of *Neisseria meningitidis* oropharyngeal carriage in adolescents and young adults in Québec City, Canada, abstr 10. EMGM—Eur Meningococcal Dis Soc 2013, Bad Loipersdorf, Austria, 17 to 19 September 2013.
74. **Kellogg DS, Jr, Peacock WL, Jr, Deacon WE, Brown L, Pirkle DI.** 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J Bacteriol **85:**1274–1279.
75. **Jolley KA, Maiden MC.** 2010. BIGSdb: scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics **11:**595. http://dx.doi.org/10.1186/1471-2105-11-595.
76. **Jordens JZ, Heckels JE.** 2005. A novel *porA*-based real-time PCR for detection of meningococcal carriage. J Med Microbiol **54:**463–466. http://dx.doi.org/10.1099/jmm.0.45847-0.
77. **Jordens JZ, Williams JN, Jones GR, Heckels JE.** 2002. Detection of meningococcal carriage by culture and PCR of throat swabs and mouth gargles. J Clin Microbiol **40:**75–79. http://dx.doi.org/10.1128/JCM.40.1.75-79.2002.
78. **Longworth E, Fernsten P, Mininni TL, Vogel U, Claus H, Gray S, Kaczmarski E, Borrow R.** 2002. *O*-Acetylation status of the capsular polysaccharides of serogroup Y and W135 meningococci isolated in the UK. FEMS Immunol Med Microbiol **32:**119–123. http://dx.doi.org/10.1111/j.1574-695X.2002.tb00543.x.
79. **Collett D.** 1991. Modelling binary data. Chapman and Hall, London, United Kingdom.
80. **Weber MV, Claus H, Maiden MC, Frosch M, Vogel U.** 2006. Genetic mechanisms for loss of encapsulation in polysialyltransferase-gene-positive meningococci isolated from healthy carriers. Int J Med Microbiol **296:**475–484. http://dx.doi.org/10.1016/j.ijmm.2006.05.004.
81. **Claus H, Borrow R, Achtman M, Morelli G, Kantelberg C, Longworth E, Frosch M, Vogel U.** 2004. Genetics of capsule *O*-acetylation in serogroup C, W-135 and Y meningococci. Mol Microbiol **51:**227–239.
82. **Kugelberg E, Gollan B, Farrance C, Bratcher H, Lucidarme J, Ibarz-Pavon AB, Maiden MC, Borrow R, Tang CM.** 2010. The influence of IS*1301* in the capsule biosynthesis locus on meningococcal carriage and disease. PLoS One **5:**e9413. http://dx.doi.org/10.1371/journal.pone.0009413.
83. **Schork S, Schluter A, Blom J, Schneiker-Bekel S, Puhler A, Goesmann A, Frosch M, Schoen C.** 2012. Genome sequence of a *Neisseria meningitidis* capsule null locus strain from the clonal complex of sequence type 198. J Bacteriol **194:**5144–5145. http://dx.doi.org/10.1128/JB.01099-12.
84. **Dolan-Livengood JM, Miller YK, Martin LE, Urwin R, Stephens DS.** 2003. Genetic basis for nongroupable *Neisseria meningitidis*. J Infect Dis **187:**1616–1628. http://dx.doi.org/10.1086/374740.
85. **Parkhill J, Achtman M, James KD, Bentley SD, Churcher C, Klee SR, Morelli G, Basham D, Brown D, Chillingworth T, Davies RM, Davis P, Devlin K, Feltwell T, Hamlin N, Holroyd S, Jagels K, Leather S, Moule S, Mungall K, Quail MA, Rajandream MA, Rutherford KM, Simmonds M, Skelton J, Whitehead S, Spratt BG, Barrell BG.** 2000. Complete DNA sequence of a serogroup A strain of *Neisseria meningitidis* Z2491. Nature **404:**502–506. http://dx.doi.org/10.1038/35006655.
86. **Findlow H, Vogel U, Mueller JE, Curry A, Njanpop-Lafourcade BM, Claus H, Gray SJ, Yaro S, Traore Y, Sangare L, Nicolas P, Gessner BD, Borrow R.** 2007. Three cases of invasive meningococcal disease caused by a capsule null locus strain circulating among healthy carriers in Burkina Faso. J Infect Dis **195:**1071–1077. http://dx.doi.org/10.1086/512084.
87. **Johswich KO, Zhou J, Law DK, St Michael F, McCaw SE, Jamieson FB, Cox AD, Tsang RS, Gray-Owen SD.** 2012. Invasive potential of nonencapsulated disease isolates of *Neisseria meningitidis*. Infect Immun **80:** 2346–2353. http://dx.doi.org/10.1128/IAI.00293-12.
88. **Hill DJ, Griffiths NJ, Borodina E, Virji M.** 2010. Cellular and molecular biology of *Neisseria meningitidis* colonization and invasive disease. Clin Sci **118:**547–564. http://dx.doi.org/10.1042/CS20090513.
89. **Unkmeir A, Kammerer U, Stade A, Hubner C, Haller S, Kolb-Maurer A, Frosch M, Dietrich G.** 2002. Lipooligosaccharide and polysaccharide capsule: virulence factors of *Neisseria meningitidis* that determine meningococcal interaction with human dendritic cells. Infect Immun **70:**2454–2462. http://dx.doi.org/10.1128/IAI.70.5.2454-2462.2002.
90. **Lewis LA, Ram S.** 2014. Meningococcal disease and the complement system. Virulence **5:**98–126. http://dx.doi.org/10.4161/viru.26515.
91. **Vogel U, Claus H, Frosch M.** 2004. Genetic lineages and their traits in *Neisseria meningitidis*. Int J Med Microbiol **294:**75–82. http://dx.doi.org/10.1016/j.ijmm.2004.06.027.
92. **Putonti C, Nowicki B, Shaffer M, Fofanov Y, Nowicki S.** 2013. Where does *Neisseria* acquire foreign DNA from: an examination of the source of genomic and pathogenic islands and the evolution of the *Neisseria* genus. BMC Evol Biol **13:**184. http://dx.doi.org/10.1186/1471-2148-13-184.
93. **Swartley JS, Marfin AA, Edupuganti S, Liu LJ, Cieslak P, Perkins B, Wenger JD, Stephens DS.** 1997. Capsule switching of *Neisseria meningitidis*. Proc Natl Acad Sci U S A **94:**271–276. http://dx.doi.org/10.1073/pnas.94.1.271.
94. **Kriz P, Giorgini D, Musilek M, Larribe M, Taha MK.** 1999. Microevolution through DNA exchange among strains of *Neisseria meningitidis* isolated during an outbreak in the Czech Republic. Res Microbiol **150:** 273–280. http://dx.doi.org/10.1016/S0923-2508(99)80052-7.
95. **Vogel U, Claus H, Frosch M.** 2000. Rapid serogroup switching in *Neisseria meningitidis*. N Engl J Med **342:**219–220. http://dx.doi.org/10.1056/NEJM200001203420319.
96. **Harrison LH, Shutt KA, Schmink SE, Marsh JW, Harcourt BH, Wang X, Whitney AM, Stephens DS, Cohn AA, Messonnier NE, Mayer LW.** 2010. Population structure and capsular switching of invasive *Neisseria meningitidis* isolates in the pre-meningococcal conjugate vaccine era—United States, 2000–2005. J Infect Dis **201:**1208–1224. http://dx.doi.org/10.1086/651505.
97. **Dolan Thomas J, Hatcher CP, Satterfield DA, Theodore MJ, Bach MC, Linscott KB, Zhao X, Wang X, Mair R, Schmink S, Arnold KE, Stephens DS, Harrison LH, Hollick RA, Andrade AL, Lamaro-Cardoso J, de Lemos AP, Gritzfeld J, Gordon S, Soysal A, Bakir M, Sharma D, Jain S, Satola SW, Messonnier NE, Mayer LW.** 2011. *sodC*-based real-time PCR for detection of *Neisseria meningitidis*. PLoS One **6:**e19361. http://dx.doi.org/10.1371/journal.pone.0019361.
98. **Higa FT, Fukasawa LO, Goncalves MG, Salgado MM, Lemos AP, Harrison LH, Oliveira PL, Silva CN, Sacchi CT.** 2013. Use of *sodC* versus *ctrA* for real-time polymerase chain reaction-based detection of *Neisseria meningitidis* in sterile body fluids. Mem Inst Oswaldo Cruz **108:**246–247. http://dx.doi.org/10.1590/0074-0276108022013020.