Transmission Dynamics and Microevolution of *Neisseria meningitidis* During Carriage and Invasive Disease in High School Students in Georgia and Maryland, 2006–2007

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Background. The mechanisms by which *Neisseria meningitidis* cause persistent human carriage and transition from carriage to invasive disease have not been fully elucidated.

Methods. Georgia and Maryland high school students were sampled for pharyngeal carriage of *N. meningitidis* during the 2006–2007 school year. A total of 321 isolates from 188 carriers and all 67 invasive disease isolates collected during the same time and from the same geographic region underwent whole-genome sequencing. Core-genome multilocus sequence typing was used to compare allelic profiles, and direct read mapping was used to study strain evolution.

Results. Among 188 *N. meningitidis* culture-positive students, 98 (52.1%) were *N. meningitidis* culture positive at 2 or 3 samplings. Most students who were positive at >1 sampling (98%) had persistence of a single strain. More than a third of students carried isolates that were highly genetically related to isolates from other students in the same school, and occasional transmission within the same county was also evident. The major pilin subunit gene, *pilE*, was the most variable gene, and no carrier had identical *pilE* sequences at different time points.

Conclusion. We found strong evidence of local meningococcal transmission at both the school and county levels. Allelic variation within genes encoding bacterial surface structures, particularly *pilE*, was common.

Keywords. *Neisseria meningitidis*; meningococcal disease; carriage; transmission; *pilE*; type 4 pili.

Neisseria meningitidis are gram-negative, obligate human commensal bacteria carried asymptomatically, with adolescents and young adults having the highest rates of carriage [1]. Meningococci can cross the protective barriers of the pharynx to cause invasive meningococcal disease [2].

Meningococcal transmission occurs via contact with secretions or large respiratory droplets in persons in close contact with a pharyngeal carrier [3]. Transmission is more intense in crowded settings, such as households, schools, and dormitories. Other risk factors for meningococcal transmission and disease are cigarette smoking, intimate kissing, and frequenting social gatherings [4]. Meningococcal carriage isolates are genetically highly diverse, but invasive disease isolates comprise a small

number of hypervirulent lineages. The genetic basis for heightened virulence among these hyperinvasive lineages remains a subject of intense study [5].

Meningococcal genomes are highly dynamic. Variation at some loci are likely adaptive responses to host immunity and have been observed in both carriage and invasive disease isolates [6]. Homologous recombination is associated with emergence and persistence of epidemic strains through changes in capsule group [7] and outer membrane proteins (OMPs) [8]. Similarly, the major type IV pilin subunit gene *pilE* undergoes rapid allelic variation through recombination with nearby *pilS* cassettes [9]. Phase variation—the rapid switching on and off of genes through slipped-strand mispairing—is also associated with rapid changes in gene expression of various meningococcal surface proteins [10–12].

In a previous study of students at 8 high schools in Georgia and Maryland during the 2006–2007 school year, Harrison et al [13] found a meningococcal carriage prevalence of 3.2%–4.0%. In the current study, we applied whole-genome sequencing to investigate the evolution and transmission of meningococci among students who were positive for carriage at multiple sampling time points

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throughout the school year, and we compared the meningococcal carriage and invasive disease isolates collected through population-based surveillance from the same period and geographic locations.

METHODS

Study Setting and Sources of Meningococcal Isolates

Meningococcal carriage isolates were obtained from a previous study of students from 8 high schools before meningococcal conjugate vaccine introduction in Baltimore County, Maryland, and Douglas County, Georgia [13]. Three meningococcal carriage surveys involving 3311 students were conducted in 2006–2007. A total of 321 carriage isolates from 188 students were included in the present study (Table 1 and Supplementary Table 1). One isolate was collected per carrier per study round; no carrier developed invasive disease during the carriage study period [13]. Invasive meningococcal isolates comprised all available isolates collected through the Maryland and Georgia Active Bacterial Core surveillance from case patients with meningococcal disease in the state of Maryland (n = 45) and 20 counties that comprise the Atlanta metro area in the state of Georgia (n = 22) for the period of 2006–2008 (Table 1 and Supplementary Table 1).

Whole-Genome Sequencing, Assembly and Phylogenetic Analyses

Genomic DNA was extracted from pure overnight cultures of single bacterial colonies using a Qiagen DNAeasy Blood and Tissue Kit (Qiagen). Library construction and sequencing were conducted using the Illumina Nextera DNA Sample Prep Kit with 150–base pair paired-end reads, and libraries were sequenced on the MiSeq platform (Illumina). A total of 29 isolates from 19 carriers and 10 disease case patients were resequenced using long-read DNA sequencing technology (Oxford Nanopore Technology) [14].

Table 1. Students With Invasive Meningococcal Disease Case and Carriers by State, 2006–2007

Disease or Carriage Status ^a	Students, No.		
	Georgia	Maryland	Total
Invasive disease (n = 67)	22	45	67
Carriers			
Positive in 1 survey round (n = 90)			
Round 1	20	11	31
Round 2	7	9	16
Round 3	12	31	43
Positive in 2 survey rounds (n = 63)			
Rounds 1 and 2	12	14	26
Rounds 1 and 3	6	7	13
Rounds 2 and 3	13	11	24
Positive in all 3 survey rounds (n = 35)	12	23	35

^aCarriage round 1 was September–November 2006; round 2, November 2006 to February 2007; and round 3, April–May 2007.

Genome analyses were performed using a number of publicly available software. Raw sequencing reads were trimmed using trim_galore software v0.4 and were assembled using SPAdes software v3.13 [15] for Illumina data and Unicycler v0.4 [16] for hybrid assembly of Illumina and Oxford Nanopore data. Genomes were annotated using prokka software v1.14 [17]. Core-genome phylogenetic trees were constructed using shared single-nucleotide polymorphisms relative to the capsular group B reference genome MC58 using snippy software v4.4 (github.com/tseemann/snippy). Recombinant single-nucleotide polymorphisms were removed using ClonalFrameML software v1.11 [18], and maximum likelihood phylogenetic trees were constructed using RAxML software v8.0 [19] under the general time-reversible model with a categorical model of rate heterogeneity.

Defining Core-Genome Multilocus Sequence Type, OMP Allelic Profiles and Capsule Genotypes

Assembled genomes were submitted to the *Neisseria* PubMLST database (pubmlst.org/neisseria/), where alleles were assigned to each genome [20]. Allele designations for conventional multilocus sequence type (MLST), major OMP antigen genes (*porA* [VR1, VR2], *porB*, *fetA* VR, and FHbp peptide), and core-genome MLSTs (cgMLSTs) were downloaded from PubMLST. The cgMLST loci comprised 1605 single-copy core genes curated by the PubMLST database [21]. Capsule genotypes were determined based on the presence and organization of capsule biosynthesis genes [22, 23]. Capsule genotypes (genogroups) were compared with capsular group (serogroup), obtained using latex agglutination test.

PilE Sequence Analysis

To determine the presence and sequence diversity of the PilE protein, we used tblastn/blastp software v2.9 to search each annotated genome assembly for the signature conserved peptide sequences associated with class 1 (MNTLQKG/EYYLN) and class 2 (MKAIQKG/EYYSD/EYYSN) PilE proteins [9]. We also considered additional evidence of gene synteny, whereby the class I *pilE* gene is located upstream of the *lpxC* gene, while the class II *pilE* is situated between *katA* and *prlC* genes [9, 24, 25]. To assess the accuracy of *pilE* gene detection by automated searches of genome assemblies, we conducted additional analyses by mapping and visual inspection of Illumina reads to the closest hybrid assembled reference genome using breseq v0.33 [26], MEGA v7 [27], and CLC Genomics Workbench v11.0 (Qiagen) software.

Defining Transmission and Transition to Invasive Disease

To measure evolutionary distances within our genome collection, we calculated cgMLST distances for each genome to all other genomes in the same sequence type (ST) in our study. The cgMLST distance measured dissimilarity between a given pair of genomes by counting the number of shared genes that had ≥1 nucleotide change between the 2 genomes [21].

We then compared cgMLST distances for genome pairs binned into 4 epidemiologic categories. The *same-person* group represented genome pairs from the same student collected at different time points; the *same-school* group, genome pairs from 2 students attending the same school; the *same-county* group, pairs of genomes collected from different students attending different schools in the same county; and the *different county* group, genome pairs from students in different states. Finally, we used the distribution of cgMLST distances among persistent carriers to define a cgMLST cutoff for determining transmission. We defined (indirect) transmission as any instance in which 2 genomes from different individuals had cgMLST distances <20, within the 95th percentile of all distances observed among persistent carriage isolates.

Transition pairs assess genomic changes associated with the transition from carriage to invasive disease. For each genome from an invasive disease isolate, we identified the closest matching carriage isolate of the same ST from the same state. A total of 25 invasive disease isolates for which we could identify a carriage genome within 75 cgMLST differences were included for further analysis. These carriage/invasive pairs came from different individuals living in the same state. A cutoff of 75 cgMLST distance falls just outside the range (0–74) seen among persistent carriers. A gene was classified as having a high frequency of change in one group when the frequency of nucleotide allelic variation for that gene was >20% in that group and <5% in a comparison group.

Data Analysis

Data were processed and visualized using custom scripts in R, Stata v15.1 and iTOL [28] on Windows 10 and BioLinux v8 platforms [29]. All genome sequence data and allele information are publicly available (<https://pubmlst.org/organisms/neisseria-spp>; Supplementary Table 1).

RESULTS

Population Structure

Phylogenetic analysis of the 388 study isolate genomes revealed a diverse population structure (Figure 1 and Table 2). Some clonal complexes, such as clonal complex (cc) 198, cc1157, cc60, and cc53, were dominant among carriage isolates and were hardly observed among invasive disease isolates. Others demonstrated overlap among carriage and invasive disease isolates (cc23, cc41/44, and cc35), while cc11 isolates were all from patients with invasive disease.

Carriage isolates were genetically highly diverse, belonging to 64 STs and 18 clonal complexes (Figure 1, Table 2, and Supplementary Table 1). The predominant clonal complexes among the 321 carriage isolates were cc198 (22.7%), cc23 (19.0%), cc41 (12.5%), and cc60 (10%). Eighteen isolates (5.6%) with 7 diverse STs had no assigned clonal complexes. More than one-third of carriage isolates (113 of 321 [35.2%]) had complete absence of the capsule gene cluster (capsule null locus [*cnl*]), while an additional 21% had a nongroupable capsule genotype resulting from gene deletion, insertion, or frame shifts within key capsule genes. Capsular groups B, Y, and C accounted for 22.4%, 20.3%, and 1.3% of 321 carriage isolates, respectively (Supplementary Table 1).

Figure 1. Population structure of 388 *Neisseria meningitidis* study isolates from Georgia and Maryland, 2006–2007, displayed as core-genome phylogenetic tree of 388 carriage and invasive genome sequences. Rings, from outer to innermost rings, show clonal complex (cc), capsule group, *pilE* gene presence, isolate source, and geographic location, respectively. Note that 11 clonal complexes with <8 isolates each are not labeled. Abbreviation: *cnl*, capsule null locus genotype.

Table 2. Carriage and Invasive *Neisseria meningitidis* Study Isolates by Clonal Complex, Georgia and Maryland, 2006–2007

Clonal Complex	Isolates, No.		Total
	Carriage	Invasive Disease	
23	61	18	79
198	73	0	73
41	40	6	46
60	32	1	33
32	7	17	24
35	18	5	23
1157	21	0	21
Unassigned	18	2	20
53	10	0	10
162	8	2	10
11	0	9	9
178	8	0	8
1117	7	0	7
167	5	1	6
865	4	0	4
1136	4	0	4
213	0	3	3
103	1	1	2
212	2	0	2
22	0	1	1
269	1	0	1
549	0	1	1
4821	1	0	1
Total	321	67	388

Invasive disease isolates (n = 67) predominantly belonged to 1 of 4 hypervirulent lineages, with cc23, cc32, cc11, and cc41/44 accounting for 26.8%, 23.9%, 14.1%, and 11.3% of all invasive disease isolates, respectively (Figure 1, Table 2, and Supplementary Table 1). The median age of case patients was 20 years (range, 2 months to 87 years) in Georgia and 26 years (1–83 years) in Maryland. In Georgia and Maryland, 45.5% and 31.1% of case patients, respectively, were female. Based on genome sequence analysis, the majority of invasive disease isolates (30 of 67 [44.8%]) belonged to capsular group B, while groups Y and C accounted for 28.4% and 20.9%, respectively. Three invasive disease isolates (5.1%) were either *cnl* (n = 1) or nongroupable (n = 2), and 1 isolate belonged to group W.

There was 100% concordance between genome-derived capsule genotype and serogroup based on latex agglutination for serogroups B, C, Y, and W (Table 3). However, the majority of phenotypically nongroupable invasive disease isolates had group B capsule genotype (7 of 10 [70%]) and more than a third of nongroupable carriage isolates (102 of 282 [36.2%]) had group B, C, or Y capsule genotypes.

Persistence of Meningococcal Carriage

A total of 188 students were culture positive for *N. meningitidis* at ≥1 survey round (Table 1) [13]. Ninety students (47.9%) had a single positive culture, whereas 98 (52.2%) were persistent

Table 3. Serogroup and Genogroup Characteristics Among Carriage and Invasive Meningococcal Isolates in Georgia and Maryland, 2006–2007

Serogroup ^a and Genogroup ^b	Isolates, No. (%)			
	Invasive		Carriage	
Both B	7	7 (100)	13	13 (100)
Both C	11	11 (100)	0	...
Both W	1	1 (100)	0	...
Both Y	16	16 (100)	26	26 (100)
NG serogroup	10		282	
Genogroup B		7 (70)		59 (21)
Genogroup C		0		4 (1)
Genogroup Y		0		39 (14)
Genogroup NG		2 (20)		67 (24)
Genogroup <i>cnl</i>		1 (10)		113 (40)
Serogroup not determined	22		0	
Genogroup B		16 (73)		0
Genogroup C		3 (14)		0
Genogroup Y		3 (14)		0

Abbreviations: *cnl*, Capsule null locus genotype; NG, nongroupable.

^aSerogroup indicates capsule phenotype as determined by latex agglutination.

^bGenogroup indicates capsule genotype as determined by whole-genome sequencing.

carriers, defined as having positive cultures at 2 (33.5%) or 3 (18.6%) of the surveys. Core-genome MLST (cgMLST), a measure of allelic differences based on sequence variation among 1605 genes found in all meningococcal genomes, provides high-resolution genotyping for detecting outbreaks and investigating strain evolution [5]. Of the 98 students who were persistent carriers, nearly all (96 of 98 [98%]) were persistent carriers of isolates of the same ST, highly similar OMP genotypes, and low allelic differences measured by cgMLST (mean [standard deviation], 10.7 [7.1] loci). The 2 remaining students had 2 same-ST isolates and 1 isolate from an unrelated clonal complex [13]. Specifically, 1 student from Maryland had ST33/cc32 isolates that differed from each another by 15 cgMLST loci in the first and second study rounds but carried a nongroupable ST823/cc198 isolate in the third study round. A second student from Georgia had ST44, cc41/44 isolate in the first round, but had ST865/cc865 isolates in the second and third rounds that differed by 7 cgMLST loci.

Transmission Dynamics

We quantified intraschool and intracounty carriage transmission by examining pairwise cgMLST distances between different epidemiologic groups. Almost all persistent carriers (93 of 98 [94.9%]) had carriage isolates that were genetically highly similar to one another (cgMLST distance <20) (Figure 2; same person). Median cgMLST among persistent carriers was 10 (range, 1–74), and the median sampling interval among persistent carriers was 99 days (49–224 days). In addition, there was no evidence of transmission between students in different states (median cgMLST differences, 152; range, 48–600) (Figure 2). In sharp contrast, more than a third of students

Figure 2. Pairwise core-genome multilocus sequence type (cgMLST) distances among meningococcal carriage isolates from Georgia and Maryland, 2006–2007. Pairwise comparisons of isolates belonging to the same sequence type are color coded by epidemiologic group. Box plots indicate median (horizontal line within box) and interquartile range (top and bottom edges of box) for each group. Dashed line denotes pairwise cgMLST distance of 20, which corresponds to 95% of all pairwise distances among pairs of isolates from persistent carriers.

(69 of 188 [36.7%]) carried meningococcal isolates that were genetically highly similar (cgMLST distance <20) to isolates from other students either in the same school or in the same county, suggesting transmission among these epidemiologic groups (Figure 2). Of the students, 17.6% (33 of 188) showed evidence of direct or indirect transmission to another student in the same school; 14.5% had evidence of indirect transmission to a student outside their school, while 13.8% carried isolates that matched isolates from students both within and outside their school. Overall, these data suggest that transmission of carriage isolates is frequent at the school level, and to a lesser degree at the county level. No comparisons of isolates sampled from students in different states fell below the 20 cgMLST cutoff, consistent with a lack of direct or indirect transmission between students in Georgia and Maryland.

Evolution During Carriage, Transmission, and Transition to Invasive Disease

We compared cgMLST alleles among pairs of isolates from the same person (persistent carriers), different carriers in the same transmission cluster (transmission pairs), and invasive disease isolates versus closely related carriage isolates (invasive-carriage or transition pairs; see Methods for group definitions). A strong correlation was observed between genes that had allelic changes during persistent carriage and genes that evolved during carriage transmission (Pearson correlation; $r = 0.73$) and during the transition from carriage to invasive disease ($r = 0.67$). The

carriage-invasive pairs in the transition group came from different individuals living in the same state. These data suggest that among the most prevalent clonal complexes in our collection, genes that most commonly evolved during persistent carriage also evolved during carriage transmission, as well as during the transition from carriage to invasive disease.

Genes with the highest frequencies of overall allelic change included those encoding the major type IV pilin subunit (*pilE*), opacity proteins (*opaB/opa1800*), and the restriction modification system methyltransferase (*modA12*) (Figure 3A). These genes demonstrated allelic variation in more than half of all persistent carrier, transmission, and transition pairs. Other genes encoding surface structures (*pglEAIH*, *tspA*, *cpsJ*, *nalP*), restriction modification systems (*modB*), and iron regulation enzymes (*frpC* and *hpuA*) also showed high frequencies of allelic variation (Figure 3A). A few genes had >20% frequency of allelic variation during persistence. These genes included surface proteins (*pilHTUQ*, *pglD*, *lst*, *apbC*, and *pbp2*) and several involved with iron uptake (*lbpA* and *tbpAB*), immune evasion (immunoglobulin A1 protease), and various metabolic functions (*adhA*, *clpA*, *icd*, and *pabB*). Four loci involved in essential metabolic functions (*fumC* and *adh2*) and homologous recombination (*hdsR* and *priA*) had higher rates of allelic variation during the transition from carriage to invasive disease, compared with both persistent carriers and transmission pairs.

Figure 3. Loci with the highest frequencies of allelic change among pairs of meningococcal isolates from persistent carriage, transmission, and transition from carriage to invasive disease, from Georgia and Maryland, 2006–2007. *A*, Bar graph showing frequency of allelic changes for the 20 most frequently mutated loci in our study (see Methods for group definitions); *pilE*, *opaB*, *opa1800*, and *modA12* were the most highly variable loci in the study. *B*, PilE sequence diversity by amino acid position among the 244 genomes in our study that encode PilE. PilE contains a highly conserved N-terminal region a region of intermediate diversity, and a hypervariable C-terminal region.

pilE Gene Evolution

Type IV pili play an important role in virulence by mediating the initial attachment to mucosal surfaces during carriage, and to endothelial linings of the brain during invasive disease [30]. We assessed gene presence, synteny, and sequence diversity of the type IV pilin gene *pilE*, the most variable gene in this study. The *pilES* locus is a highly variable locus that encodes a class I *pilE*, an important virulence determinant. Several *pilS* cassettes within the *pilES* locus facilitate rapid *pilE* sequence variation through homologous recombination [9]. All isolates,

even those without an intact *pilE* gene, contained some elements of the *pilES* locus. The locus was highly variable, ranging in size from 1.5 to 10 kb, and contained a median of 7 *pilS* cassettes (range, 1–11) with or without an intact *pilE* gene. We also occasionally noted an IS1106 transposase that occurred between *pilS* cassettes.

A full-length *pilE* gene was found in 242 of 388 genomes (62.3%) (Figure 1). These 242 PilE protein sequences, derived from 175 individuals, comprised 238 unique peptide sequences. Interestingly, no carrier had identical PilE peptide sequences

harbored a 252-nucleotide fragment corresponding to *pilS4* and *pilS6* (Figure 4B). These data demonstrate how genetic recombination likely led to mosaic *pilE* alleles, even among persistent carriers.

DISCUSSION

In this study, high levels of meningococcal carriage transmission within high schools and, to a lesser extent, within the same county were identified through whole-genome sequencing. Crowded settings such as schools and dormitories are well-known settings for transmission of *N. meningitidis* [3, 31], so intraschool transmission was expected. On the other hand, transmission of meningococci at the county level has been less well defined. Shared isolates between students from different high schools within the same county likely reflect widespread circulation of these isolates throughout the county, rather than direct transmission events. However, the dynamics of meningococcal transmission are predominantly local, as we found no evidence of transmission between students in Georgia and Maryland [32].

We used the observed distribution of cgMLST distances among persistent carriers to define a cutoff of 20 for direct or indirect meningococcal transmission, while the median cgMLST difference among persistent carriers was 10 (range, 1–74). This number is lower than the median allelic differences of 35 (range, 11–84) observed in a study of carriers in Ethiopia sampled 2 months apart [33, 34]. These differences suggest that the rate of genomic change among persistent carriers may vary by epidemiologic setting and/or by the predominant carriage strain type. Differences in allelic changes across geography and between strain types warrant further study.

We identified a high frequency of microevolution across several genes during persistent carriage. The role of homologous recombination in the emergence and persistence of virulent meningococcal isolates is widely acknowledged [35, 36]. Genes encoding surface structures such as the type IV pilin major subunit (*pilE*), pilin glycosylation (*pglE*), and opacity proteins (*opaB/opa1800*) were among the most highly variable loci in our study, suggesting selection or evasion of immune mechanisms in the pharynx [37]. Restriction modification system genes, such as *modB* and *modA12*, also had high frequencies of allelic changes among both carriage and invasive disease isolates [36, 38]. The precise role of restriction modification systems in meningococcal virulence warrants further study.

We compared highly similar isolates found in different carriers within the same county to identify the most frequently evolved genes during transmission. Genes encoding surface proteins (*pilHTUQ*, *pglD*, *lst*, *apbC*, *pbp2*), iron uptake (*lbpA*, *tbpAB*), and immune evasion (immunoglobulin A1 protease) had higher frequencies of allelic variation during transmission. Four loci involved in essential metabolic functions (*fumC*,

adh2) and homologous recombination (*hsdR*, *priA*) had higher rates of allelic variation during the transition from carriage to invasive disease, compared with both persistent carriers and transmission pairs.

These data are in agreement with a study of persistent carriage in Ethiopia [33, 34], where a high frequency of genetic change was found among genes encoding pilin synthesis, glycosylation, restriction modification, and opacity proteins. Another study of meningococcal genome pairs isolated from the nasopharynx and bloodstream of 4 patients [39] also found allelic variation in type IV pilus biosynthesis genes (*pilE*, *pilC1*, *pglI*) and hemagglutininlike adhesion gene (*tpsA1*) associated with the transition from carriage to invasive disease. These studies demonstrate that meningococci undergo rapid genomic changes—predominantly in genes involved in type IV pilin biosynthesis, pilin modification, and homologous recombination—and that these changes may represent adaptations to enhance persistence, transmission, and transition to invasive disease.

Type IV pili are important virulence factors that are involved in attachment, aggregation and microcolony formation [30]. *PilE* encodes a surface-exposed protein that mediates attachment and interaction of meningococci with host epithelial and endothelial cells [40]. Previous studies have characterized the phase-variable nature of *pilE* and have documented rapid reshuffling facilitated by *pilS* cassettes among a limited number of reference strains [9, 24]. In this study, the use of long-read genome sequencing facilitated a detailed investigation of *pilE* diversity in a subset of isolates and revealed significant diversity of *PilE* proteins even within persistent carriers through mosaic recombination within the hypervariable region of *pilE*.

Meningococcal disease and carriage result from an active interplay of host, pathogen and environmental factors. For example, carriage isolates can quickly cause invasive disease when they encounter transmitted to a susceptible host, without necessarily any genomic changes. Additional studies will be required to delineate mechanisms by which the identified genomic changes affect bacterial processes, such as interaction with host immune defenses.

In conclusion, we found evidence of widespread circulation of *N. meningitidis* isolates at the high school and county levels. Furthermore, isolates causing persistent carriage were shown to undergo rapid microevolution within genes associated with pilus synthesis and assembly. This information will be useful in interpreting the results of outbreak investigations and can inform efforts aimed at decreasing meningococcal transmission.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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