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Meningococcal carriage among Georgia and Maryland high school students

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Abstract

Background—Meningococcal disease incidence in the U.S. is at an all-time low. In a previous study of Georgia high school students, meningococcal carriage prevalence was 7%. The purpose of this study was to measure the impact of a meningococcal conjugate vaccine on serogroup Y meningococcal carriage and to define the dynamics of carriage in high school students.

Methods—This was a prospective cohort study at 8 high schools, 4 each in Maryland and Georgia during a school year. In each state, 2 high schools were randomized for participating students to receive MCV4-DT at the beginning of the study and 2 at the end. Oropharyngeal swab cultures for meningococcal carriage were performed three times during the school year.

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Disclosures

Dr. Harrison previously received research support from Sanofi Pasteur and lecture fees from Sanofi Pasteur and Novartis Vaccines. He previously served on scientific advisory boards for GlaxoSmithKline, Merck, Novartis Vaccines, Pfizer, and Sanofi Pasteur. All relationships with industry were terminated before Dr. Harrison became a member of the Advisory Committee on Immunization Practices on July 1, 2012.

Previous presentations

This study was presented in part at the 16th International Pathogenic Neisseria Conference, Rotterdam, The Netherlands, September 2008.

Results—Among 3,311 students, prevalence of meningococcal carriage was 3.21%–4.01%. Phenotypically non-groupable strains accounted for 88% of carriage isolates. There were only 5 observed acquisitions of serogroup Y strains during the study; therefore, the impact of MCV4-DT on meningococcal carriage could not be determined.

Conclusions—Meningococcal carriage rates in U.S. high school students were lower than expected and the vast majority of strains did not express capsule. These findings may help explain the historically low incidence of meningococcal disease in the U.S.

Background

Adolescents are at increased risk for infection with *Neisseria meningitidis*, an important cause of serious invasive disease including meningitis and septicemia in the U.S. (1). Since 2005, the Advisory Committee on Immunization Practices has recommended quadrivalent meningococcal conjugate vaccine that covers serogroups A, C, W, and Y (MCV4) for all adolescents (2,3).

Nasopharyngeal carriage of *N. meningitidis*, a prerequisite for the development of invasive meningococcal disease, is in most cases asymptomatic and results in strain-specific immunity (4). Meningococcal carriage rates are variable by age, with adolescents and young adults having the highest prevalence (5–14). Because of high rates of carriage and evidence for transmission within families, adolescents are considered to be a primary reservoir for transmission to other groups including young children and infants (15).

Polysaccharide-protein conjugate vaccines against *Haemophilus influenzae* type b and *Streptococcus pneumoniae* prevent acquisition of carriage, which interrupts transmission and leads to protection of unvaccinated persons (16,17). With the implementation of a national serogroup C vaccination program in the U.K., substantial reductions in serogroup C meningococcal carriage and disease were observed in vaccinated persons, with similar declines in both carriage and disease occurring in the unvaccinated (5,18–20). Similarly, early results from the African meningitis belt suggest that the recently-introduced serogroup A conjugate vaccine has led to interruption of transmission of serogroup A meningococcal carriage (21). Such herd protection can dramatically enhance the public health benefits of conjugate vaccination programs. As a result, the ability of conjugate vaccines to prevent meningococcal carriage has become an important post-licensure evaluation question.

The main purpose of this study was to assess the impact of MCV4 on serogroup Y meningococcal carriage among Georgia and Maryland high school students. Serogroup Y was chosen because it caused approximately one-third of meningococcal disease reported in the U.S. from 1996–2007, and was the most frequently isolated serogroup in a previous carriage study among Georgia high school students (22). We also sought to study the dynamics of carriage in this population. At the time of the study, the incidence of invasive meningococcal disease in the U.S. was falling to historically low levels (1). During 2006–2007, MCV4 coverage rates among children 13–17 years old were estimated to be 11%–32% (23,24).

Methods

Study design

This study was approved by the institutional review boards of the CDC, Maryland Department of Health and Mental Hygiene, Johns Hopkins Bloomberg School of Public Health, and Georgia Department of Human Resources. Written informed consent was obtained for all participating students. For students <18 years old, consent was also obtained from the parent or guardian as well as student assent. Students were paid \$10 for full participation at each survey. Full participation at each survey included a pharyngeal swab and completion of a study questionnaire. MCV4 was offered to consenting non-vaccinated study participants either at the initial survey or at the final survey depending on the school's randomization.

The study design was a field trial comprising three sequential, cross-sectional pharyngeal carriage surveys spanning the 2006–2007 school year (clinical trials registration NCT00119080). Eight public high schools were included: four in Baltimore County, Maryland and four in Douglas County, Georgia. Two schools at each site were randomized for their participating students to be offered free immunization at the time of initial carriage survey toward the beginning of the school year (vaccination schools), while participants at the other two schools at each site were offered free immunization at the third survey toward the end of the school year (control schools). The baseline carriage survey and school-based vaccination clinic for vaccination schools, were completed at the same visit within 1 to 2 months of the start of the school year, with subsequent surveys completed at approximately 3 and 6 to 7 months later.

A questionnaire was also completed at the time of each survey to identify potential risk factors for meningococcal carriage including demographic and household variables, recent illness, smoking and antibiotic use.

In a previous meningococcal carriage study among Georgia high school students, the prevalence of serogroup Y carriage was 2.5%–3.5% (22). Therefore, for the present study, we estimated a baseline prevalence of serogroup Y carriage of 2%. To detect a 50% reduction in serogroup Y carriage, we estimated that approximately 2,000 study subjects in each group would be required from vaccination and control schools at each round.

Data and specimen collection

Oropharyngeal swab specimens were collected from participating students by trained study workers who swabbed both tonsillar pillars and the oropharynx. Swabs were inoculated and streaked for incubation by trained laboratory personnel directly onto culture plates containing Thayer Martin Improved medium (R01886; Remel, Inc., Lenexa, KS) at each high school and placed in Mitsubishi boxes to generate a CO₂-enriched atmosphere during holding and transport. During and at the end of each survey session, culture plates were transported to participating laboratories for incubation and primary identification. To ensure that *N. meningitidis* was not lost in transport, a control plate was inoculated each day with *N. meningitidis* and transported with the study plates before incubation; good growth was noted on all control plates.

MCV4 immunization

Students in vaccination schools received a single intramuscular dose of A, C, W, Y meningococcal conjugate vaccine that uses diphtheria toxoid as the protein carrier (MCV4-DT) (Menactra, Sanofi Pasteur), the only licensed MCV4 at the time, during the first survey. Students in control schools were offered vaccination at the third survey.

Laboratory methods

Species and phenotypic serogroup identification for serogroups A, B, C, E, W, X, Y, Z (8 of 12 meningococcal serogroups) were performed using standard biochemical tests and slide agglutination, respectively. To determine the genotypic capsular group, serogroup-specific (SGS)-PCR was performed using an assay that determines the genetic capsular type for the six serogroups that cause invasive meningococcal disease: A, B, C, W, X, and Y (25). An isolate was defined as nongroupable for the phenotypic or genotypic assays when it did not react in either the phenotypic or SGS-PCR assays, respectively. Genotypic capsular group was determined independently of phenotypic serogroup. Further isolate characterization was performed using multi-locus sequence typing (MLST), and *porA*, *porB*, and *fetA* genotyping (outer membrane protein genotyping) as previously described (26).

Data analysis

Analyses were performed using SAS 9.3 (SAS Institute, Cary, NC). The impact of vaccination on both prevalent and incident carriage of serogroup Y meningococcus was evaluated. We compared the proportion of students carrying serogroup Y in vaccination schools to the proportion carrying serogroup Y in control schools in each swab survey using Fisher's exact test. We also compared the rate of acquisition of serogroup Y carriage among students in vaccination and control schools. Rates of acquisition were measured as new carriers of serogroup Y meningococcus identified in rounds two and three per 1,000 students who had participated in more than one round. Rate ratios were calculated for serogroup Y carriage at vaccination versus control schools at the time of each survey.

For the purpose of further defining the dynamics of carriage in this population, we assessed strain evolution and carriage with different strains among students with carriage on at least two surveys. Strain evolution was defined as carriage of an isolate of the same clonal complex (CC) but a different sequence type and/or change in the outer membrane protein genotype. Strain replacement was defined as carriage of an isolate of a different CC. Genotyping was repeated on isolates to confirm suspected strain evolution.

Risk factors for carriage were evaluated by comparing questionnaire answers from students who were carriers and those who were non-carriers. Risk factors were first assessed using stratified univariate logistic regression using school as the stratification variable to adjust for clustering within schools on demographic, household and symptom variables. Factors with a p-value less than 0.2 in the univariate analysis were eligible for entry into a stratified multivariable stepwise logistic regression model. Factors that remained significant in the multivariable model were checked for interactions.

Results

In total, 3,311 students were enrolled. In the first survey round, 1,731 students were included from the four vaccination schools and 1,543 from the control schools. In Georgia, there were 13 students at control schools and 24 at vaccination schools who did not participate in the first survey but did participate in the second. From the first to the third survey, loss to follow-up was 12.7%, with 11.7% loss in vaccination schools and 13.8% in control schools. In Georgia, study participation by school among eligible students ranged from 23%–31% and in Maryland participation was 20%–27%.

The median age of participants was 16 years, with comparable representation achieved among the four school grades. Of all participants, 45.2% were male, 61.6% were white, and 7.2% were Hispanic. Demographic characteristics were generally comparable between students in vaccination and control schools, although students in control schools were more likely to be white and smokers and to have less paternal education (Table 1).

Prevalence of carriage and serogroup distribution

In the first survey, the prevalence of meningococcal carriage was 3.21%, with 3.52% in vaccination schools and 2.85% in control schools ($p=0.32$) (Table 2). For that survey, carriage prevalence in Maryland was 3.91% compared to 2.68% in Georgia ($p=0.06$). In Maryland and overall, carriage prevalence trended upward in both vaccination and control schools over the course of the school year. This was largely driven by phenotypic non-groupable meningococcal carriage. A similar trend was not observed in Georgia. By the final survey, overall carriage prevalence in Maryland was 5.91% vs. 2.63% in Georgia ($p < 0.0001$).

In total, 325 meningococcal isolates were identified during all 3 survey rounds: 138 from Georgia and 187 from Maryland students. Of these, 285 (88%) were non-serogroupable, 26 (8%) were serogroup Y, and 14 (4%) were serogroup B. No serogroup C isolates were identified. Serogroup Y was more common in Maryland than in Georgia, while serogroup B was not encountered in Maryland (Table 2). Among the 285 non-serogroupable isolates, 60 (21%), 5 (2%), and 39 (14%) were genotypic capsular groups B, C, and Y, respectively. Based on the combination of both phenotypic and genotypic typing of the 325 isolates, 20% were group Y, 23% were group B and 1.5% were group C.

Impact of MCV4-DT on prevalent and incident serogroup Y carriage

The proportions of students with carriage of serogroup Y strains at baseline in the vaccination and control schools were 0.35% and 0.19%, respectively ($p=0.51$) (Table 2). Over the course of the three survey rounds, the prevalence of serogroup Y carriage did not change substantially in either group. Only 5 acquisitions of serogroup Y carriage were observed, 3 (1.79 per 1000 students) in the vaccination schools and 2 (1.34 per 1000 students) in the control schools (rate ratio 1.34, 95% confidence interval 0.18 to 11.39). In an analysis of genotypic group Y carriage, 10 students (5.97 per 1000 students) in the vaccinated schools and 10 students (6.69 per 1000 students) in the control schools acquired

genotypic group Y carriage over the course of the three survey rounds (rate ratio 0.89, 95% confidence interval 0.34 to 2.31).

Risk factors for meningococcal carriage

On univariate analysis adjusted for clustering by school, risk factors for carriage were white race, being in grades 11 or 12, older age, being a current smoker, and living in a household with other smokers (Table 3); gender, Hispanic ethnicity, housing type; parental education, symptoms in the past two weeks, antibiotic use in the past 30 days, and household crowding were not associated with carriage (data not shown). Meningococcal carriage was observed during at least one survey round in 8.0% of white students versus vs. 2.1% of non-white students ($p<0.0001$), 8.0% of students in grades 11 or 12 versus 3.4% in grades 9 or 10 ($p<0.0001$), 12.1% for current smokers versus 5.0% for non-smokers ($p<0.0001$), and 7.4% for students who lived in a household with other smokers versus 4.6% of those who did not ($p=0.012$).

In multivariable logistic regression analysis adjusted for clustering, factors independently associated with carriage were white race (odds ratio 3.2, 95% CI 2.1–4.9), being a current smoker (OR 1.6, 1.1–2.4), and older age (OR 1.3, 1.2–1.5).

Duration of carriage

Among 2,799 students who participated in all three survey rounds, 163 (5.8%) were positive during at least one survey. Of these, 36 (22.1%) were positive for carriage at all three surveys (approximately 6 months or more of continuous carriage), 11 (6.8%) at survey 1 and 3, 13 (8.0%) at surveys 1 and 2 (2–3 months), 23 (14.1%) at surveys 2 and 3 (3–5 months), and 25 (15.3%), 11 (6.8%), 44 (27.0%) at surveys 1,2, or 3 only, respectively (total 49.1% positive at only one survey). Among those who were positive for carriage on at least one survey, there was no statistically significant difference in the frequency of positive carriage isolates by race, age, Hispanic ethnicity, high school grade, gender, whether the strain was encapsulated, or phenotypic or genotypic serogroup (data not shown).

Molecular characterization of carriage isolates

Among 325 carriage isolates from 189 students, 76 sequence types (STs) representing 18 CCs were identified (Figure 1 and Supplemental Table 1); 20 isolates could not be assigned to a CC. Thirteen STs comprised more than 5 isolates each, and represented 64% of all isolates recovered. Thirty-five STs were represented by only one isolate each. Among CCs with more than 10 isolates, the most common CCs and the serogroup that is often associated with each CC (pubmlst.org/neisseria), were ST-198, non-groupable capsule null locus (*cnl*) locus (73 isolates); ST-23 (61 isolates), serogroup Y; ST-41/44 (40 isolates), serogroup B; ST-60 (33 isolates), serogroup E; ST-1157 (21 isolates), encapsulated but non-groupable; ST-35 (19 isolates), serogroup B; and ST-53 (10 isolates), non-groupable *cnl* (Figure 1). Among 98 students who had carriage on at least two occasions, strain evolution was observed in 5 students: 3 had a change in the *porA* allele, and 1 each had a change in the *porB* or *fetA* allele (Table 4). Two students were observed to have strain replacement.

Discussion

The most remarkable finding of this study is that the prevalence of meningococcal carriage among Georgia and Maryland high school students was around 3%, which was much lower than expected based on previous studies. For example, in a study among Georgia high school students in 1998 using similar methodology, overall carriage rates were 6.1%–7.7% (22). Our observed carriage rate was also much lower than has been reported for U.K. and German high school students and U.K. university students (5,27–29).

Another striking finding is that the proportion of isolates that were phenotypically non-groupable was 88%, higher than in previous studies, in which approximately 30% of U.S. and 60% of U.K isolates from high school students were phenotypically non-groupable (5,22). Thus, the little carriage that we found was caused mostly by commensal meningococci that were not expressing capsule and therefore unlikely to cause invasive disease. In addition, of the 7 CCs represented by more than 10 carriage isolates, only CC-41/44 and CC-23 were common causes of invasive disease in the U.S. during 2000–2005 (26). A limitation of our study is that the phenotypic and SGS-PCR assays detected 8 and 6, respectively, of the 12 meningococcal serogroups and therefore some of the isolates that we classified as nongroupable may have belonged to serogroups that rarely cause invasive disease (30).

These findings may at least in part explain the incidence of invasive meningococcal disease in the U.S., which peaked in the mid-1990's due in large part to the emergence of serogroup Y and which has now declined to historically low levels (1). The low prevalence of carriage of meningococci and phenotypic encapsulated meningococci observed in this study among high school students may underlie the dramatic declines in disease incidence, probably through the mechanisms of natural immunity and reduced meningococcal transmission in the U.S. Similar to other studies in high school students, we found that smoking was a risk factor for meningococcal carriage (6,29).

Commensurate with the overall low observed carriage prevalence and the decline in disease incidence, much less serogroup Y carriage was observed than the 2.5%–3.5% that we considered in our sample size calculations. Furthermore, few instances of phenotypic serogroup Y carriage acquisition were observed. In addition, our enrollment of approximately 3,300 students was lower than anticipated. Therefore, the study did not have sufficient statistical power to assess the impact of MCV4-DT on meningococcal carriage. This experience indicates a need for pilot studies to determine the required sample size before large-scale carriage studies are undertaken. Such a pilot study would likely have demonstrated that a larger study was needed to assess the impact of MCV4-DT on carriage.

A recent study of a quadrivalent meningococcal conjugate vaccine that uses mutant diphtheria toxin (CRM) as the conjugate protein demonstrated a 39% reduction in serogroup Y carriage 2–12 months after vaccination (31). However, given the different carrier proteins and differences in the immunogenicity of the two vaccines, those results cannot necessarily be extrapolated to MCV4-DT (32).

Among students who were carriers, approximately 22% had carriage during all three survey rounds, 22% during two consecutive rounds, 49% at one round, and 7% were intermittently positive (positive at rounds 1 and 3). In addition, 4% and 2% of students with persistent carriage had strain evolution or clonal replacement, respectively. Changes in *porA* and *fetA* alleles have been previously described (33); in our study, we also found a different *porB* allele in one student. However, it is possible that changes in strains between survey rounds were due to sampling error rather than true differences. The lack of serogroup B carriage in Maryland was an unexpected finding, particularly since 10 (29%) of 35 invasive isolates from Maryland during 2006 and 2007 were caused by serogroup B strains (data not shown).

In summary, we found a low prevalence of meningococcal carriage among high school students in Georgia and Maryland in 2006–2007 and that a high proportion of carried strains were unencapsulated. This study underscores the utility of carriage studies for better understanding the epidemiology of invasive meningococcal disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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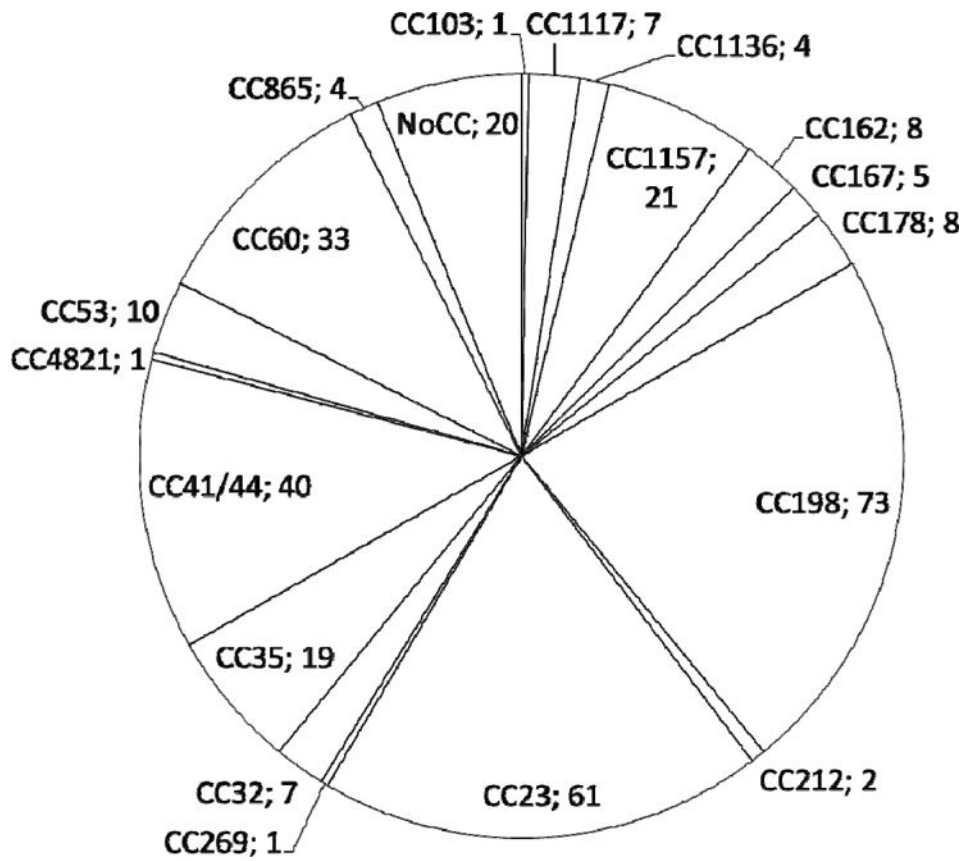


Figure 1. Frequency distribution of clonal complexes (CC's) for 325 meningococcal carriage isolates. Numbers following the CC designation represent the number of isolates.

Demographic characteristics of 3,311 high school meningococcal carriage study participants, by initial vaccination assignment group of schools – Georgia and Maryland, 2006–2007.

Table 1

Group	Vaccination Schools		Control Schools		Chi-square p value
	N	%	N	%	
Gender					
Male	777	44.3	718	46.2	0.27
Race					
White	1,036	59.0	1,003	64.5	0.002
Black	503	28.7	410	26.4	
Black	503	28.7	410	26.4	
Other/Unknown	216	12.3	143	9.2	
Hispanic	133	7.6	104	6.7	0.32
Current smoker	141	8.0	170	10.9	0.004
Age	16	(13-19)	15	(13-21)	0.34
Grade					
9 th	455	26.0	452	29.1	0.07
10 th	399	22.8	360	23.2	
11 th	487	27.8	377	24.2	
12 th	412	23.5	366	23.5	
Paternal Education	178	10.1	173	11.1	0.03
Less than high school					
High school graduate	432	24.6	438	28.2	
Some post high school	306	17.4	264	17.0	
Post-high school degree	511	29.1	383	24.6	
Unknown	328	18.7	298	19.2	
Maternal Education	160	9.1	151	9.7	0.55
Less than high school					
High school graduate	462	26.3	444	28.5	
Some post high school	373	21.3	307	19.7	
Post-high school degree	573	32.7	490	31.5	
Unknown	187	10.7	164	10.5	

Table 2

Percentage of high school students with meningococcal carriage by phenotypic serogroup, state, vaccination status of schools, and survey round – Georgia and Maryland, 2006–2007. The percentages in parentheses for serogroups B, C, and Y, represent students with isolates that were genotypically groupable by serogroup specific (SGS)-PCR.

For the non-groupable (NG) columns, the top numbers represent the percentage of students with isolates that were negative by phenotypic serogrouping and are therefore presumed to not express capsule of the serogroups included in the assay (serogroups A, B, C, E, W, X, Y, Z). The bottom numbers in parentheses represent the percentage of students with isolates that were negative by the SGS-PCR assay (which includes groups A, B, C, W, X, and Y); this group likely represents a mix of isolates that are capsule null, belong to groups not included in the SGS-PCR assay, or belong to groups included in the assay but are falsely negative because of polymorphisms in the SGS-PCR primer binding sites.

State	School Group	No.	Round 1 Serogroup			Round 2 Serogroup			Round 3 Serogroup			Total				
			B	C	Y	B	C	Y	B	C	Y					
All	Vaccination	1,731	0.35 (1.04)	0 (0)	0.35 (0.58)	1,644	0.12 (0.61)	0 (0.12)	0.36 (0.79)	3,28 (2.25)	1,549	0 (0.58)	0 (0.19)	0.32 (0.84)	3.87 (2.58)	4.20
	Control	1,543	0.19 (1.10)	0 (0)	0.19 (0.39)	1,469	0 (0.61)	0 (0)	0.20 (0.75)	2.52 (1.36)	1,343	0.15 (0.74)	0 (0)	0.22 (0.89)	3.43 (2.16)	3.80
	Total	3,274	0.27 (1.07)	0 (0)	0.27 (0.49)	3,113	0.10 (0.61)	0 (0.06)	0.29 (0.77)	2.89 (1.83)	2,892	0.07 (0.66)	0 (0.10)	0.28 (0.86)	3.67 (2.39)	4.01
MD	Vaccination	795	0 (1.13)	0 (0)	0.75 (1.26)	758	0 (0.66)	0 (0.13)	0.66 (1.45)	4.49 (2.90)	709	0 (0.99)	0 (0.28)	0.56 (1.55)	5.22 (2.96)	5.78
	Control	611	0 (1.15)	0 (0)	0.33 (0.65)	576	0 (0.87)	0 (0)	0.17 (0.52)	3.13 (1.91)	510	0 (0.98)	0 (0)	0.39 (1.37)	5.69 (3.73)	6.08
	Total	1,406	0 (1.14)	0 (0)	0.57 (1.00)	1,334	0 (0.75)	0 (0.07)	0.45 (1.05)	3.90 (2.47)	1,219	0 (0.98)	0 (0.16)	0.49 (1.48)	5.41 (3.28)	5.91
GA	Vaccination	936	0.64 (0.96)	0 (0)	0 (0)	886	0.23 (0.56)	0 (0.11)	0.11 (0.23)	2.26 (1.69)	840	0 (0.24)	0 (0.12)	0.12 (0.24)	2.74 (2.26)	2.86
	Control	932	0.32 (1.07)	0 (0)	0.11 (0.21)	893	0 (0.45)	0 (0)	0.22 (0.90)	2.13 (1.01)	833	0.24 (0.60)	0 (0)	0.12 (0.60)	2.04 (1.20)	2.40
	Total	1,868	0.48 (1.02)	0 (0)	0.05 (0.11)	1,779	0.17 (0.51)	0 (0.06)	0.17 (0.56)	2.14 (1.35)	1,673	0.12 (0.42)	0 (0.06)	0.12 (0.42)	2.39 (1.73)	2.63

MD, Maryland; GA, Georgia; NG, nongroupable

Univariate analysis of risk factors for carriage among 189 students found to be carriers on one or more occasion and 3,122 students found to be non-carriers on all swab surveys, stratified by school to adjust for clustering within school. For continuous variables, the odds ratio (OR) is for each unit increase in the variable. Only variables that were statistically significant are shown.

Table 3

Group	Carriers		Non-carriers		Chi-square p value	OR (95% CI)	
	N	%	N	%			
Race	White	162	85.7	1,877	60.1	<0.0001	Baseline
	Black	16	8.5	897	28.7		0.25 (0.15, 0.42)
	Other/unknown	11	5.8	348	11.2		0.37 (0.20, 0.69)
Current smoker	38	20.3	275	8.8	<0.0001		2.32 (1.58, 3.41)
Age (median, range)	16	(13–18)	15	(13–21)	<0.0001		1.40 (1.24, 1.59)
Student grade	9 th	27	14.4	880	28.2	<0.0001	Baseline
	10 th	30	16.0	729	23.4		1.27 (0.75, 2.16)
	11 th	60	31.9	804	25.8		2.36 (1.48, 3.77)
	12 th	71	37.8	707	22.7		3.16 (2.00, 4.99)
Any smoker in the home, excluding student	97	51.3	1,220	39.1	0.012		1.48 (1.09, 2.00)
Number of smokers in home (median, range)	1	(0–5)	0	(0–5)	0.001		1.27 (1.10, 1.47)

* The referent group is a person without the symptom being analyzed

Table 4

Serogroup (SG), sequence type (ST), clonal complex (CC) and outer membrane protein genotypes (*porA*, *porB*, *fetA*) for 5 and 2 students, respectively with strain evolution or strain replacement (see text for definitions). For each student, only changes from the previous isolate are shown (i.e., if only *porA* changed between rounds 1 and 2, only the *porA* result is shown for round 2). Serogroup data are based on slide agglutination.

Study ID	Survey 1				Survey 2				Survey 3			
	SG;ST;CC	<i>porA</i>	<i>porB</i>	<i>fetA</i>	SG;ST;CC	<i>porA</i>	<i>porB</i>	<i>fetA</i>	SG;ST;CC	<i>porA</i>	<i>porB</i>	<i>fetA</i>
Strain evolution during carriage												
0640	NG; 1117; 1117	18-1,30	3-64	F3-7		18-1,30-3			No change from survey 2			
1145		ND			Y;6145;167	5-1,10-4	2-55	F3-4	NG;6145;167	5-1,10-1		
1216	NG;60;60	5,2	2-37	F1-7			2-65					ND
4843	NG;53;53	7,30-3	3-64	F1-2		7,30						ND
5478		ND			NG;53;53	7-2,30	3-84	NT				F4-1
Strain replacement during carriage												
1012	B;44;41/44	5-2,10-1	3-45	F1-7	NG;865;865	7-1,1	3-1	F1-6	No change from survey 2			
4588	NG;33;32	19,15	3-8	F5-1	No change from survey 1				NG;823;198	17,9	3-84	F5-5

NG, nongroupable

ND, not done

NT, nontypeable