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Meningococcal carriage among a university student population – United States, 2015

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ABSTRACT

Objectives: Several outbreaks of serogroup B meningococcal disease have occurred among university students in recent years. In the setting of high coverage of the quadrivalent meningococcal conjugate vaccine and prior to widespread use of serogroup B meningococcal vaccines among adolescents, we conducted surveys to characterize the prevalence and molecular characteristics of meningococcal carriage among university students.

Methods: Two cross-sectional oropharyngeal carriage surveys were conducted among undergraduates at a Rhode Island university. Isolates were characterized using slide agglutination, real-time polymerase chain reaction (rt-PCR), and whole genome sequencing. Adjusted prevalence ratios and 95% confidence intervals were calculated using Poisson regression to determine risk factors for carriage.

Results: A total of 1837 oropharyngeal specimens were obtained from 1478 unique participants. Overall carriage prevalence was 12.7–14.6% during the two survey rounds, with 1.8–2.6% for capsular genotype B, 0.9–1.0% for capsular genotypes C, W, or Y, and 9.9–10.8% for nongroupable strains by rt-PCR. Meningococcal carriage was associated with being male, smoking, party or club attendance, recent antibiotic use (inverse correlation), and recent respiratory infections.

Conclusions: In this university setting, the majority of meningococcal carriage was due to nongroupable strains, followed by serogroup B. Further evaluation is needed to understand the dynamics of serogroup B carriage and disease among university students.

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1. Background

Meningococcal disease is a rare but serious illness resulting in high rates of morbidity and mortality. Transmission of the causative organism, *Neisseria meningitidis*, occurs through close contact with respiratory secretions, resulting primarily in asymptomatic nasopharyngeal carriage and rarely, invasive meningococcal disease. Adolescents and young adults in the United States are at increased risk of meningococcal carriage and disease due to increased social mixing, crowded living conditions, smoking, and other behaviors [1–4].

The Advisory Committee on Immunization Practices (ACIP) recommends routine vaccination of adolescents with a quadrivalent meningococcal conjugate vaccine (MenACWY) at age 11 or 12 years, with a booster dose at age 16 years to sustain protection through early adulthood [5]. Like other conjugate vaccines, MenACWY (MenACWY-D [Menactra®, Sanofi Pasteur [6]], MenACWY-CRM [Menveo®, Novartis [7]]) may reduce acquisition of nasopharyngeal carriage, and thus transmission, when high coverage is achieved, though data are limited [8–10]. Since implementation of the adolescent MenACWY program in 2005, coverage with at least one dose of MenACWY among 13–17 year olds reached 81.3% in 2015 [11]. Incidence of serogroup C and Y meningococcal disease among adolescents has subsequently declined, with serogroup B *Neisseria meningitidis* becoming the leading cause of meningococcal disease in this age group [12].

In 2014 and 2015, two serogroup B meningococcal (MenB) vaccines, MenB-4C (Bexsero®, GlaxoSmithKline) and MenB-FHbp

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(Trumenba®, Pfizer), were licensed for use in the United States [13,14]. In 2015, ACIP recommended that adolescents and young adults aged 16–23 years may be vaccinated with a MenB vaccine based on individual clinical discretion [15]. MenB vaccines are routinely recommended for certain persons aged ≥ 10 years at increased risk of serogroup B meningococcal disease, including during an outbreak of serogroup B meningococcal disease [16]. The impact of MenB vaccines on carriage remains under investigation [8,17,18].

In the context of high adolescent MenACWY coverage and low meningococcal disease incidence, with a predominance of outbreaks and sporadic disease now due to serogroup B, there is little recent data on the prevalence and serogroup distribution of carried *N. meningitidis* among U.S. university students. We conducted a carriage evaluation among undergraduate students at a Rhode Island university in a non-outbreak setting prior to widespread availability of MenB vaccines to determine prevalence and molecular characteristics of meningococcal carriage and identify risk factors for carriage in this population.

2. Materials and methods

2.1. Study design

Two cross-sectional oropharyngeal carriage surveys were conducted in March (round 1) and April (round 2) 2015 at a Rhode Island university ('University A'), with participants recruited by convenience sampling among an undergraduate student population of 6320. All undergraduate students aged 18 years or older were eligible for voluntary participation and were recruited through emails and printed materials. University A is in the same city as Providence college, which experienced an outbreak of serogroup B meningococcal disease, with two cases reported among an undergraduate population of 4500 students, in February 2015 [19]. Though not assessed formally, university officials speculate there is minimal interaction between students at the two universities, and no cases of meningococcal disease were reported at University A.

2.2. Data and specimen collection

Enrollment and specimen collection were performed in common areas of the university, such as the student center and adjacent to a large cafeteria. Upon enrollment, all participants provided written informed consent and completed a self-administered questionnaire consisting of demographic information and potential factors associated with meningococcal carriage, including university year, living arrangements, history of tobacco and marijuana use, attendance at parties and bars, upper respiratory infection in the past 14 days, and history of antibiotic use in the past 30 days. MenACWY and MenB vaccination status was obtained through abstraction of student medical records at the university health center, where vaccination records are submitted upon university matriculation and maintained thereafter. As no MenB vaccine doses were documented in participant medical records and the carriage evaluation took place within five months of U.S. licensure of the first MenB vaccine and prior to ACIP recommendations for use of MenB vaccines among healthy adolescents and young adults, we assumed all participants to be unvaccinated with MenB.

Oropharyngeal swabs from each tonsillar pillar and the posterior pharynx were collected by trained personnel using bifurcated swabs. One swab was directly inoculated onto Modified Thayer–Martin (MTM) media (BD BBL, Sparks, MD) for culture. Inoculated media were stored in CO_2-enriched Mitsubishi boxes at room temperature along with a positive control plate inoculated with *N. lactamica*. Inoculated plates and specimens were transported to the Rhode Island State Health Department Laboratory for primary testing with a maximum delay of four hours.

2.3. Laboratory methods

Inoculated culture plates were streaked, incubated at 37 °C (5% CO_2), and examined for bacterial growth after 24, 48, and 72 h. Colonies with typical Neisserial morphology underwent Gram staining and were sub-cultured onto blood and/or chocolate agar plates. A single colony per participant per round was selected for further characterization. At a species level, *N. meningitidis* was identified using Gram stain, oxidase test (Hardy Diagnostics; Santa Maria, CA), API NH strip (bioMerieux; Durham, NC), and *sodC* PCR assay [20,21]. Once confirmed as *N. meningitidis*, serogroup was determined by slide agglutination and capsular genotype by real-time PCR (rt-PCR). Expression of the capsular polysaccharide was determined by slide agglutination for serogroups A, B, C, E, W, X, Y, and Z (BD DIFCO; Franklin Lakes, NJ; and Thermo Scientific Remel; Waltham, MA). rt-PCR was used to detect the capsule biosynthesis genes specific for serogroups A, B, C, W, X, and Y [22]. An isolate was defined as nongroupable by slide agglutination when it autoagglutinated or did not agglutinate. As previously described, carriage isolates are commonly nongroupable phenotypically due to a low level or absence of capsule gene expression, while these isolates may still contain the capsule biosynthesis genes as detected by rt-PCR [23,24]; because of this, nongroupable genotype was independently defined by rt-PCR as no amplification in any serogroup-specific PCR assay.

For further characterization of serogroup B isolates and other isolates with discrepant results (e.g., discrepant NH strip and *sodC* results), genomic DNA was prepared for whole genome sequencing (WGS) on Illumina MiSeq platform (250 × 250 cycle paired-end sequencing kit; San Diego, CA) using 5 Prime ArchivePure DNA Purification kit (Gaithersburg, MD), Ampure (Beckman Coulter Inc.; Indianapolis, IN), and dual-index NEBNext Ultra sequencing libraries (New England Biolabs Inc.; Ipswich, MA). Published primer sequences were used to extract the seven meningococcal MLST house-keeping genes, *fetA*, and *porA* from CLC (v8.5.1; Qiagen; Waltham, MA) assembled WGS data [25]. After running extracted sequences through BLAST against the *Neisseria* PubMLST database, MLST sequencing type (ST) and clonal complex (CC), outer membrane (FetA and PorA) types and vaccine antigens (FHbp, NhbA, and NadA) were determined [26,27]. As previously described, a combination of the three nomenclature systems was used for FHbp type (Oxford numeric identifier followed by Pfizer subfamily and Novartis variant) [28].

2.4. Data analysis

Descriptive analyses were conducted for demographic and risk factor information. Significant differences ($P < .05$) by round were assessed using a chi-square test for categorical variables and the Wilcoxon-Mann-Whitney test for continuous variables. The prevalence of meningococcal carriage overall and by serogroup and capsular genotype was determined for each survey round. To determine factors associated with overall carriage, prevalence ratios were calculated with bivariate Poisson regression analyses using generalized estimating equations and an exchangeable correlation structure to account for correlation of observations from individual participants across rounds. Collinearity and interactions were assessed, given the potential relatedness of several variables (e.g., smoking and partying, upper respiratory tract infection and use of antibiotics). Factors found to be significantly associated with carriage at $P < .1$ were included in a multivariable model. Factors