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Original article

Meningococcal carriage in Dutch adolescents and young adults; a cross-sectional and longitudinal cohort study

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ABSTRACT

Objectives: Current information on rates and dynamics of meningococcal carriage is essential for public health policy. This study aimed to determine meningococcal carriage prevalence, its risk factors and duration in the Netherlands, where meningococcal C vaccine coverage is >90%. Several methods to identify serogroups of meningococcal carriage isolates among adolescent and young adults were compared.
Methods: Oropharyngeal swabs were collected from 1715 participants 13–23 years of age in 2013–2014; 300 were prospectively followed over 8 months. Cultured isolates were characterized by Ouchterlony, real-time (rt-) PCR or whole-genome sequencing (WGS). Direct swabs were assessed by rt-PCR. Questionnaires on environmental factors and behaviour were also obtained.
Results: A meningococcal isolate was identified in 270/1715 (16%) participants by culture. Of MenB isolates identified by whole genome sequencing, 37/72 (51%) were correctly serogrouped by Ouchterlony, 46/51 (90%) by rt-PCR of cultured isolates, and 39/51 (76%) by rt-PCR directly on swabs. A sharp increase in carriage was observed before the age of 15 years. The age-related association disappeared after correction for smoking, level of education, frequent attendance to crowded social venues, kissing in the previous week and alcohol consumption. Three participants carried the same strain identified at three consecutive visits in an 8-month period. In these isolates, progressively acquired mutations were observed.
Conclusions: Whole genome sequencing of culture isolates was the most sensitive method for serogroup identification. Based upon results of this study and risk of meningococcal disease, an adolescent meningococcal vaccination might include children before the age of 15 years to confer individual protection and potentially to establish herd protection. **M.B. van Ravenhorst, Clin Microbiol Infect 2017;23:573.e1–573.e7**

Introduction

Neisseria meningitidis is a common colonizer of the upper respiratory tract in asymptomatic carriers, but may occasionally cause invasive meningococcal disease (IMD) [1]. Of the 12 *N. meningitidis* serogroups, six (A, B, C, X, W and Y) cause the majority of IMD worldwide. Whereas the overall carriage prevalence in the population is estimated to be ~10%, the incidence rate of IMD per 100 000 population varies between 0.5 in North America up to 1000 in epidemic settings [2,3]. Although IMD incidence is highest in infants under 5 years of age, meningococcal carriage prevalence is low at this early age. Carriage increases during childhood and peaks at around 24% among young adults and declines to around 8% in older adults [4].

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Experience with serogroup C (MenC) polysaccharide conjugate vaccines demonstrated herd protection through reduced colonization and transmission of MenC after vaccination of children and adolescents [2,5]. In the Netherlands, a single MenC conjugate vaccine was offered to all children aged 1–18 years in 2002. Routine vaccination at 14 months was subsequently introduced [6]. The vaccine coverage of the population was estimated at 94% in 2002 [7]. At present, meningococcal serogroup B (MenB) is the predominant cause of IMD in Europe [8,9]. Only recently, MenB vaccines have been made available through the licensure of two protein-based multicomponent vaccines: bivalent rLP2086, Trumenba® (Pfizer, Philadelphia, PA, USA) and 4CMenB, Bexsero® (GlaxoSmithKline Biologicals SA, Rixensart, Belgium) [10,11]. A recent study among university students showed a reduction in overall carriage after a second dose of 4CMenB, but no impact was demonstrated for MenB carriage [12]. Currently, European carriage data are mostly based on studies from the UK and France [13]; however, carriage characteristics change over time and may differ by country.

In this study, we compared different methods to identify meningococcal serogroups by culture and Ouchterlony, real-time (rt-) PCR or whole-genome sequencing (WGS) of meningococcal carriage isolates among Dutch adolescents and young adults. Furthermore, we determined the prevalence of meningococcal carriage, its risk factors and duration.

Materials and methods

Design and participants

The study was conducted in the Netherlands between January 2013 and March 2014. Healthy participants aged 13–23 years were recruited randomly and solely based on age from 15 educational institutions. Exclusion criteria included previous MenB vaccination, antibiotic use during the month before enrolment, or participation in any other clinical trial with an investigational drug. Written informed consent was obtained from both parents/guardians of participants aged <18 years and from all participants. Among all participants, all participants in the last year of secondary school who were included in the year 2013 were enrolled in the longitudinal study cohort and had study visits three times within 8 months after enrolment, twice before and once after starting tertiary school or universities. The study was conducted in compliance with ethical principles originating from the Helsinki Declaration, within the guidelines of Good Clinical Practice and the study was registered at the Dutch Trial register (www.trialregister.nl; NTR3785).

Clinical procedures

Trained research nurses visited schools and universities to collect swabs and demographic data, and provided questionnaires on environmental factors and behaviour based on previously identified risk factors [14–16]. Two oropharyngeal swabs, each of the tonsils/the tonsillar fossa and the posterior pharynx, were simultaneously collected to compare different methods for identification of meningococcal serogroups. One was inoculated on a Thayer–Martin agar and incubated at 37°C with 5% CO_2 immediately after swabbing and the second ('Direct swab', which was only collected at baseline from participants who were included in the year 2013) was placed directly into storage transport media (STM, DIGENE, Biomérieux, Marcy l'Etoile, France), and was kept at 4°C on study location. Within 5 h of collection, the samples arrived at the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM, Amsterdam, the Netherlands) for microbiological processing.

Bacterial identification

Meningococcal isolates were isolated at the NRLBM as previously described [17]. Up to a maximum of five colonies of isolates identified as *N. meningitidis* were frozen in Microbank tubes and stored at −80°C. Direct swabs were also stored at this temperature. Direct swabs and frozen colonies were shipped on dry ice to Pfizer Vaccine Research and Development for further identification by rt-PCR (both swabs) and WGS (cultured swabs only) (Pearl River, NY, USA).

Phenotyping cultured isolates. Serogrouping of cultured isolates was performed by means of microprecipitation in a modified Ouchterlony assay for serogroups A, B, C, E, W, X, Y and Z as previously described [18].

Real-time PCR. This was performed on cultured isolates in the subset of participants from whom two swabs were collected as previously described [19]. Isolates negative for group-specific assays but positive for *por*A or *ctr*A were defined as non-groupable meningococcal isolates (NG). Direct rt-PCR assays were performed on swab transport medium (without culture) of the second swab to detect *por*A, *ctr*A and genetic targets diagnostic for MenB [19].

WGS of cultured isolates and sequence analysis. All meningococcal isolates were analysed by WGS as previously described [20], and genogroup was identified using capsule locus genes [21]. Isolates without the capsule locus but positive for *por*A were identified as NG meningococcal isolates. Phylogenomic analysis using core genome sequences was conducted in Harvest Suite [22]. Genomes of carriage isolates were compared and maximum likelihood phylogenetic trees were built through Parsnp, a fast core-genome multi-aligner. Then the alignment and trees were visualized in Gingr and iTOL [23].

Statistical analyses

This study was a descriptive epidemiological *N. meningitidis* carriage study. On the basis of confidence interval (CI) estimates, an overall sample size of 1700 participants was chosen. Using the Clopper–Pearson exact method, a conservative estimate for MenB carriage prevalence of 2% would result in a 95% CI between 1.4% and 2.8% within a sample size of 1700 individuals [24]. Continuous variables were presented as means with standard deviation (SD), and categorical variables as numbers and percentages. Differences in proportion were tested with the Fisher's exact test. Statistical tests were two-sided and a p-value <0.05 was considered statistically significant. Data were analysed using SPSS statistics 22 (IBM, Armonk, NY, USA). Serogroup-specific meningococcal carriage was defined by WGS of cultured isolates. For laboratory methods comparison analysis, WGS of cultured isolates was used as reference. Possible risk factors for meningococcal carriage were determined using results of the first visit based on previous publications [14,15,25]. The association of age and carriage was evaluated with logistic regression analysis. We estimated the univariate association of age with carriage as well as an estimate corrected for all identified confounders. A confounder was defined as a variable that had a significant association with both age and carriage, and that altered the correlation coefficient of age by at least 10% if added to the model. A multivariate logistic regression model that included all evaluated risk factors for carriage simultaneously was also developed. Model stability was evaluated by performing stepwise backward model selection based on likelihood ratio testing with a p-in of 0.05 and p-out of 0.1. Acquisition rate was defined by the number of new carriers for the 8-month study period divided by