Epidemiology of Nasopharyngeal Carriage of *Neisseria meningitidis* in Healthy Dutch Children

D. Bogaert,† P. W. M. Hermans,† H. Boelen,‡ M. Sluijter,‡ A. Luijendijk,‡ H. C. Rümke,‡ S. Koppen,‡ A. van Belkum,‡ R. de Groot,† and H. A. Verbrugh‡

†Department of Pediatrics, Sophia Children’s Hospital of Erasmus Medical Center, ‡Department of Medical Microbiology and Infectious Diseases, Erasmus Medical Center, and ‡Vaxinostics, Erasmus University Rotterdam, Rotterdam, The Netherlands

We investigated the prevalence and determinants of nasopharyngeal carriage of *Neisseria meningitidis* in 3200 healthy children aged 1–19 years. The incidence of meningococcal carriage was, on average, 1.5%. Peak incidences were seen at age 1 year and after age 15 years. The independent determinants of meningococcal carriage included age, regular visits to youth clubs (odds ratio [OR], 2.2) and discotheques (OR, 4.3), and pneumococcal carriage (OR, 4.1).

Because of a nationwide increase in the number of cases of invasive meningococcal disease caused by serogroup C meningococci during the first few months of 2002 in The Netherlands [1], all Dutch children from 12 months to 19 years of age were offered immunization during a national vaccination campaign in the summer of 2002. This opportunity was used to screen >3000 healthy children in Rotterdam for nasopharyngeal carriage of *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Staphylococcus aureus* and to obtain information regarding age distribution and the social and environmental factors related to nasopharyngeal carriage of the 3 pathogens. This study was approved by the medical ethics review board of the Erasmus Medical Centre (Rotterdam, The Netherlands).

The prevalences and determinants of *S. pneumoniae* and *S. aureus* colonization have been described elsewhere [2]. In the present study, we focused on the prevalence and determinants of *N. meningitidis* carriage for a Dutch cohort of healthy children.

In total, 3198 children from 12 months to 19 years of age were enrolled. All children were residents of Rotterdam who were vaccinated in July 2002 (those aged 12 months to 5 years or 15–19 years) or September 2002 (those aged 6–14 years). Written informed consent was obtained from each child or a parent. When children and their parents agreed to participate in this study, demographic data were recorded for each child through a standardized questionnaire. Nasopharyngeal samples were obtained from the children by use of rayon-tipped dacron pernasal swabs (Copan Italia) and were transported in Amies transport medium to the medical microbiology laboratory of the Erasmus Medical Centre, where the samples were plated within 6 h on Thayer-Martin medium, for isolation of *N. meningitidis*. Bacteriological analysis was performed in accordance with standard procedures [3]. In case morphologically different colonies were observed within 1 sample, multiple colonies were stored for further analysis. Statistical analysis was performed using SPSS, version 11.0 for Windows (SPSS). To evaluate the determinants of colonization, we performed univariate regression analysis on the variables of age; family size; a minimum of 3 h/week of sport activities, attendance of youth or sport clubs, or visits to discotheques; a minimum of 3 days/week of day-care visits; passive or active smoking; recent antibiotic use (within the past 7 days); and cocolonization with *S. pneumoniae* and *S. aureus*. Colonization with *N. meningitidis* was used as a dependent variable. The variables were coded 1 for present and 0 for absent, except for family size, which was coded 0 for <5 persons and 1 for ≥5 persons, and age, which was considered a continual variable. All variables with univariate associations (*P* < .10) were considered to be potential determinants of colonization. To identify independent markers, the variables were further analyzed by multivariate logistic-regression analysis using the stepwise backward Wald method. Because data for patients with missing values were excluded from the multivariate analyses, the analysis was based on the data from 2850 (89.1%) of 3198 children participating in the study.

The 3198 children formed a balanced representation of all age groups, varying in size from 121 to 199 children per year of age. Fifty-two percent of all children were male. The average family size was 4.3 persons, with an average of 2.4 children per family. In 45% of the households, at least 1 person smoked regularly. Of all children aged >12 years, 21% were active smokers. Overall, 42% of all children aged >4 years were actively involved in physical sports (>3 h/week), and 29% attended a youth society or sports club for >3 h/week. Of all children aged >10 years, 21% visited discotheques for >3 h/week. Of all
children aged <4 years, 56% visited a day care center for at least 3 days/week.

Nasopharyngeal carriage of *N. meningitidis* was observed in 46 (1.5%) of 3098 children. Meningococci serogroup B and serogroup C were carried by 8 and 9 children, respectively. Twenty-four children were carriers of serogroup X, Y, Z, or W135 meningococci. Eight children carried other serogroups of meningococci. Only 1 child was a carrier of 2 meningococcal strains (serogroups B and C). Another child was a carrier of a serogroup B strain and a nongroupable strain; however, the second strain may have been the uncapsulated phenotype of the first one.

In light of the recent increase in the incidence of meningococcal disease, a higher incidence among the subjects was expected, something comparable to previously described incidences of up to 26% [4, 5]. The relatively low incidence might be the result of differences in the method of sampling—that is, nasopharyngeal instead of oropharyngeal cultures—although there is still uncertainty about the optimal method of meningococcal sampling [6–10]. In addition, the use of transport medium instead of direct plating might have decreased the sensitivity of meningococcal detection, as reported elsewhere [11, 12]. On the other hand, the high colonization rates of >20% have been reported mostly for certain risk groups, including students and military recruits. These risk groups were not included in our study. The time of sampling (i.e., the summer) might also explain the relatively low incidence in carriage. Most often, a peak in the rate of meningococcal colonization and infection is observed during autumn and winter, when the crowding factor increases [13]. This seasonal variation is also seen for invasive meningococcal diseases in Dutch children, in whom the highest incidence of invasive diseases is always found from January until April. This variation was also seen during 2002 [1].

Meningococci were most frequently isolated during the second year of a subject’s life, with a peak incidence of 3.2%. A typical age-related distribution of meningococcal carriage in general and of specific serogroups in particular was observed, with peak incidences in the first year of life and after age 15 years (figure 1). These data are in line with the findings of the Netherlands Reference Laboratory for Bacterial Meningitis [14]. Both meningococcal carriage and sepsis/meningitis seem to follow a similar pattern, with peak incidences in the second year of life and after age 14 years [14].

To evaluate potential determinants of nasopharyngeal carriage of *N. meningitidis*, we calculated the ORs by means of univariate logistic regression followed by multivariate logistic regression analysis. The initial correlation of active smoking and *N. meningitidis* carriage was demonstrated to be an indirect effect of age in the multivariate logistic regression model and, therefore, was excluded from the model. We identified age, pneumococcal carriage (OR, 4.1), youth-club visits for >3 h/week (OR, 2.3), and discotheque visits for >3 h/week (OR, 4.4) as significant determinants of meningococcal carriage (table 1). Our results are in agreement with Dominguez et al. [10], who identified the same determinants of meningococcal carriage after multivariate logistic-regression analysis.

We also investigated cocolonization of *S. pneumoniae* and *S.*
S. pneumoniae as potential determinants of meningococcal colonization. Although we did not find a correlation between *S. aureus* and meningococcal carriage, we did observe a positive correlation between *S. pneumoniae* and *N. meningitidis* colonization. This finding is in accordance with a previous clinical study performed by Bakir et al. [6]. Moreover, these data are supported by the findings of Pericone et al. [15], who have shown an enhanced growth of *S. pneumoniae* in coculture with *N. meningitidis* mediated by catalase produced by the meningococcus. In a previous analysis of nasopharyngeal carriage with *S. pneumoniae* and *S. aureus* that used the same cohort of children, we observed a negative correlation between *S. aureus* and *S. pneumoniae* carriage, but for vaccine serotype pneumococci only [2]. Therefore, we reanalyzed the data for meningococci and conjugate vaccine serotype pneumococci only [2]. Therefore, we reanalyzed the data for meningococci and conjugate vaccine serotype (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) and for meningococci and nonvaccine serotype carriage. A positive correlation was found for both groups of pneumococci (ORs, 3.5 and 4.4, respectively), indicating that this correlation is independent from the pneumococcal serotype. Meningococcal serogroup–specific analysis did not show specific correlations with pneumococcal carriage. This is presumably a result of the low number of meningococcal isolates observed in our study. Our data support recent reports about the existence of complex patterns of interactions between the different species colonizing the nasopharynx. Thus far, negative interactions between *S. pneumoniae* and *Haemophilus influenzae*, between *S. pneumoniae* and *S. aureus*, and between *Viridans streptococci* and several potential pathogenic microorganisms have been reported [16]. Our data demonstrate, to our knowledge, the first positive finding of interaction between 2 nasopharyngeal pathogens. Whether these phenomena are based on direct interspecies interactions or on the interference by the host immune system is currently not known. However, whether these interactions affect vaccination against these species is of major interest. For mass vaccination with the meningococcal serogroup C conjugate vaccine, the first efficacy data are available. Although these studies show a reduction in serogroup C meningococcal disease and no significant increase in the prevalence of other meningococcal serogroups 1 year after vaccination, the effect of the vaccine on colonization with other species needs to be investigated [17]. It is interesting that studies of population-based vaccination with the 7-valent pneumococcal conjugate vaccine have shown the replacement of colonization and infection with vaccine-serotype pneumococci with the colonization of nonvaccine serotype pneumococci, *S. aureus* and *H. influenzae* [18, 19], suggesting that large-scale surveillance is necessary to evaluate possible shifts in disease after vaccination.

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**References**


