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Brief Original Article

Prevalence of *Neisseria meningitidis* carriage: a small-scale survey in Istanbul, Turkey

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Abstract

Introduction: The human nasopharynx is the main reservoir of *Neisseria meningitidis*, and asymptomatic carriage is common. *N. meningitidis* one of the common causes of bacterial meningitis in Turkey, especially after the implementation of the national immunization program that includes conjugated pneumococcal and *Haemophilus influenzae* type b vaccines. The purpose of this study was to evaluate the prevalence of meningococcal carriage and determine the leading serogroup, which may help authorities to adapt appropriate meningococcal vaccine into the national immunization programme.

Methodology: The prevalence of oropharyngeal carriage of *N. meningitidis* in 1,000 healthy subjects, 0–79 years of age, was investigated. Oropharyngeal swabs were collected during an 18-month period. Samples obtained were inoculated onto Thayer-Martin agar. The API-NH test and VITEK-MS system were used for identification of colonies. Multiplex real-time polymerase chain reaction assay was used to determine serogroups with serogroup-specific genes.

Results: *N. meningitidis* was isolated from 6 of 1,000 subjects (0.6%). Meningoccocal carriers were between 21 and 40 years of age. All isolates were serogrouped as B, except one that did not survive on subculture. *N. lactamica* was isolated from 13 of 1,000 subjects (1.3%).

Conclusions: Carriage rate of meningococci in our study was relatively low. However, we detected that serogroup B was the leading strain in meningococcal carriage in Istanbul; choosing an appropriate meningococcal vaccine containing serogroup B should therefore be considered. High absolute humidity throughout the year in Istanbul may explain the low prevalence of carriage in our study. This should be verified with a multicenter national survey.

Key words: *Neisseria meningitidis*; meningococcal carriage; oropharyngeal carriage; healthy subjects; Istanbul; Turkey.

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Introduction

Neisseria meningitidis is an aerobic, Gram-negative coccus and may be encapsulated. There are 13 serogroups (A, B, C, D, X, Y, Z, E, W-135, H, I, K, L), and serogroups A, B, C, W-135, X, and Y are the most common causes of invasive meningococcal diseases [1]. Meningococci spread person to person by droplets. The acquisition of this microorganism may result in asypmtomatic nasopharyngeal carriage or invasive disease. Serogroup distribution of meningococci in asymptomatic carriers or in invasive diseases varies in different geographical areas [1-3].

National surveillance data on the etiological agents and their serogroups/serotypes causing invasive infections can help authorities to adapt appropriate vaccines into the national immunization program (NIP). In Turkey, the meningococcal vaccines are not implemented in the NIP but are recommended for high-risk groups including Hajj pilgrims and military recruits. Moreover, few surveillance data exist on meningococcal invasive diseases in Turkey [4,5]. During the years 2006 to 2009, we performed national bacterial meningitis surveillance in children and found that *N. meningitidis* was the second-most common pathogen, and after 2008, due to implementation of *Haemophilus influenzae* type b vaccine and pneumococcal conjugated vaccine in NIP, *N. meningitidis* became the leading pathogen in 2008 and 2009 [4]. During this surveillance, we detected 47 *N. meningitidis* cases; serogroup B (86%) was the leading serogroup [4].

Since the human nasopharynx is the main reservoir of *N. meningitidis*, transmission occurs person to person with droplets, and invasive disease usually occurs after

recent nasopharyngeal acquisition of microorganism; it is therefore crucial to know the prevalence of nasopharyngeal carriage of *N. meningitidis* and its serogroups.

The aim of this study was to define the prevalence of meningococcal carriage and serogroups in healthy subjects on a small scale that may be representative of Istanbul's population. The epidemiologic dynamics of Istanbul are important because of the city's location as a bridge between Europe and Asia and its value as an important destination for many tourists from all over the world.

Methodology

The study was performed in Marmara University Pendik Training and Research Hospital during an 18-month period from June 2012 to December 2013. This hospital is one of the largest hospitals in Istanbul and has a per-year capacity of 1.3 million outpatients. As such, its patient population may be accepted as a small representative of Istanbul's population. Marmara University's Medical Faculty Ethical Committee and Review Board, which serves as a central ethical committee, approved the study. The adult subjects were the healthy relatives of the patients who were admitted to the outpatient clinics. The subjects in infant and child age groups were incorporated from the well-child clinics. All subjects or their parents signed the informed consent forms prior to participation.

Oropharyngeal swabs were collected from 1,000 healthy subjects with transport medium containing throat culture strips. Five hundred samples were obtained in the spring/summer seasons, and 500 samples were obtained in the fall/winter seasons to make the seasonal distribution homogeneous. The obtained samples were inoculated onto Thayer-Martin agar and plates were incubated at 37°C in an incubator containing 5% CO2 for three days. Every plate was checked on the second and third day of inoculation. Small, smooth, round, moist, gray-colored colonies were selected for further analyses. In Gram staining, colonies appearing as Gram-negative diplococci with adjacent sides flattened were tested for catalase and oxidase. If the tested microorganisms were found to be positive for both catalase and oxidase, the API-NH (BioMérieux, Marcy l'Etoile, France) test and VITEK-MS (BioMérieux, France) system were used to identify the suspected colonies [6].

Serogroup identification

The isolated meningococcal strains were transferred to the Turkish Public Health Agency in Ankara, Turkey. Multiplex real-time polymerase chain reaction (PCR) assay was used to determine serogroups with serogroup-specific genes in the cap locus for *N. meningitides* serogroups A (sacB), W135 (synG), X (xcbB), B (synD), C (synE), and Y (synF) [7].

Results

The study involved 1,000 healthy subjects 0–79 years of age with a mean ± standard deviation (SD) age of 27.3 ± 1.44 years (range: 0-79; median: 30 years). Of the subjects, 184 (18%) were under 18 years of age, and 816 were adults whose mean age was 33 ± 8 years. A total of 131 subjects were under 1 year of age; 21 subjects were 1–5 years of age; 54 subjects were 5–18 years; 793 subjects were 18–65 years; and 1 subject was 65–79 years of age. With respect to gender, 667 patients were female and 333 were male.

N. meningitidis was isolated from 6 of 1,000 subjects (0.6%) (Table 1). Meningoccocal carriers were between 21 and 40 years of age, and their mean age was 33 ± 8 years. Four of them were male and two were female. All of the carriers resided in flats with a central heating system in Istanbul, and three of them were cigarette smokers. There were two household members for two carriers, three household members for three carriers, and four household members for one carrier. All isolates were serogrouped as B, except one that did not survive on subculture. *N. lactamica* were isolated from 13 of 1,000 subjects (1.3%) (Table 1). *N. lactamica* carriers were between 23 and 41 years of age and their mean age was 32 ± 6. *N. lactamica* carriage

Table 1. Summary of *Neisseria meningitidis* oropharyngeal carriage surveillance results

Microorganism detected in oropharyngeal swab	Number of subjects (n, %) N = 1,000	Gender and age	Mean age	Serogroups
N. meningitidis	**6 (0.06%)**	**M 21, M 36, M 37, M 40, F 26, F 40**	33 ± 8 years	**5 serogroup B(1 strain could not be serogrouped)**
N. lactamica	**13 (1.3%)**	**M 31, M 31, M 35, M 36, M 41, F 23, F 28, F 30, F 35, F 40, F 40, F 25, F 25**	32 ± 6 years	**-**

was not detected in subjects who were under 18 years of age.*N. gonorrhea* carriage was not detected.

Discussion

Meningococcal disease remains a major cause of mortality in children worldwide [3]. The relationship between carriage of *N. meningitidis* and progression to invasive disease is not fully understood. Although it is suggested that the overall rates of meningococcal carriage have not been especially useful in predicting outbreaks of meningococcal disease, pharyngeal carriage of the organism is the first step to invasive infection [8].

Identification of common serogroups in carriers and serogroups responsible for invasive meningococcal diseases may help to choose proper vaccine products for public immunization.

There are few studies that have investigated meningococcal carriage in Turkey. The results indicate that Istanbul has lower meningococcal carriage rates, with a predominance of serogroup B compared to other regions of the country (Table 2) [9-12]. Studies from the Aegean region reported the meningococcal carriage prevalence as 6%–28% and the predominant serogroups as A, B, and C (Table 2) [9,12]. A study from central Anatolia reported the prevalence rate as 10.4% and predominant serogroup as B [10]. A study from Istanbul reported the prevalence rate as 1.2% and predominant serogroups as Y and B (Table 2) [11].

Seasonal variation in invasive meningococcal disease (IMD) and meningococcal carriage incidence has long been recognized, but related enviromental risk factors responsible for this phenomenon are poorly understood. Kinlin *et al*. defined an inverse relationship between ultraviolet B radiation index and IMD risk but found a correlation with high humidity in Philadelphia [13]. Lindsay *et al*. found that IMD increased with high humidity and cooler temperatures but decreased with prolonged periods of heavy rain in Auckland [14]. Contrary to these studies, Tikhomirov *et al*. defined low humidity and drought as predisposing factors for IMD epidemics, and Cheesbrough *et al*. mentioned that continuous high absolute humidity appears to reduce the transmission of meningococci [15,16]. Moore discussed the presence of coincident respiratory tract infections and low humidity as enviromental risk factors for meningococcal epidemics [17]. High absolute humidity throughout the year in Istanbul may explain the low prevalence of carriage in our study. Because of the study design, it was not possible to define any meteorological variable as an exact reason for the low meningococcal carriage rate.

Previous studies investigating invasive meningococcal diseases reported serogroup B as the leading serogroup, especially in European countries and Turkey, in recent years [4,18]. Another study from Turkey reported that serogroup B was the most common cause of meningococcal meningitis in the Marmara region, including the city of Istanbul [5]. The European Centre for Disease Prevention and Control reported that 73.6% of invasive meningococcal diseases were caused by serogroup B in Europe [18].

Carriage of *N. lactamica* may assist in the development of natural immunity by inducing cross-reactive antibodies. Furthermore, nasal secretions of volunteers colonized by *N. lactamica* were shown to impair the attachment of meningococci to oroepithelial cells [19]. In our study, *N. lactamica* carriage was twofold higher than *N. meningitidis* carriage.

Meningococcal carriage is relatively more common in late adolescents and young adults [10,20]. In our study, all six meningoccal carriers were older than 18 years of age.

Risk factors associated with meningococcal diseases are close contact with an index case, recent upper respiratory tract infection, exposure to cigarette smoke and weather pollution, being a freshman in a high school dormitory, being a military recruit, and crowded living conditions [3]. Additionally, previous Turkish studies reported the following as risk factors associated with meningoccal carriage: heating with a stove, living in shanty houses, three or more household members, primary school attendance, and *S. pneumonia* and *H. influenzae* colonization [10]. In our study, risk

Table 2. Summary of previous studies investigating meningococcal carriage in Turkey

Study	Years	Number of subjects	Region/city	Carriage rate	Predominantserogroups
Coskun *et al*. [9]	1990	N/A	Aegean	28%	Serogroup C
Ercis *et al*. [10]	1995–1996	1,155	Ankara (Middle Anatolia)	10.4%	Serogroup B
Bakır *et al*. [11]	2000	1,382	Istanbul	1.2%	Serogroup Y and B
Gazi *et al*. [12]	2001–2002	N/A	Aegean (Manisa)	6.2%	Serogroup A, B, and C
Present study	2013	1,000	Istanbul	0.6%	Serogroup B

factors associated with meningoccal carriage could not be defined because of the lower rates of carriage.

The most important prevention method for meningoccal diseases is vaccination. Meningococcal vaccine recommendations differ depending upon the region of the world and the dynamic epidemiology of meningococcal diseases. Infants may reproduce immune response to conjugated vaccines. To date, quadrivalent meningococcal polysaccaride vaccine (Menomune, MPSV4); quadrivalent meningococcal conjugate vaccine (Menactra, MenACWY, Menveo, Nimenrix); serogrup C, Y, and *H. influenzae* combined (MenHibrix); serogroup C vaccine (MenC); and serogroup B vaccine (Bexsero) have been licensed, and some countries recommend meningococcal vaccination in routine childhood vaccination schedules. The European Medicines Agency approved a meningococcal serogroup B vaccine (Bexsero) for use in individuals older than two months of age in the European Union, in 2012 [21]. In Turkey, quadrivalent polysaccharide vaccine (A, C, Y, W135) is mandatory for Hajj pilgrims before visiting Mecca and for military recruits. Quadrivalent conjugated meningococcal vaccines (A, C, Y, W135) have been licensed in the last three years for children but are not free of charge as are other vaccines in our NIP. According to meningoccoccal disease and carriage data in Turkey, we believe that a meningococcal vaccine implemented in the NIP should contain serogroup B also. This prediction should be improved with further large-scale multicenter studies.

Disadvantages of study included its not being systematic, that risk factors associated with meningoccal carriage could not be defined because of the low prevalence of the carriage, that meteorological variables were not evaluated during the study in Istanbul, and that the study was not a nationwide survey.

Conclusions

The carriage rate of meningococci in our study was relatively low. The accurate incidence of meningococcal carriage and disease in Istanbul is not yet known. This should be verified with a multicenter national survey, and results may be helpful in the selection of meningococcal vaccine type for the NIP.

According to meningococcal disease epidemiology data in Turkey, we believe a meningococcal vaccine implemented in the NIP should contain serogroup B.

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