Original Article

Oropharyngeal Carriage and Penicillin Resistance of *Neisseria meningitidis* in Primary School Children in Manisa, Turkey

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Abstract

Introduction: To determine the oropharyngeal carriage rates and serogroups of *Neisseria meningitidis* in primary school children in Manisa, Turkey as well as the prevalence and penicillin resistance of *N. meningitidis*. Materials and Methods: Throat swabs obtained from 1128 children were cultured and recovered organisms were tested by disk diffusion method and the E-test for antimicrobial susceptibilities. Results: The carriage rate of *N. meningitidis* in our region was 6.2% (71 strains) and the serogroups identified were serogroups A (28.1%), B (22.5%), C (35.2%), D (2.8%) and W-135 (11.2%). Penicillin resistance was found in 16 strains (22.5%), while beta-lactamase activity was found in none. Conclusions: The carriage rate of *N. meningitidis* and serogroups are similar to the rates reported in other countries. Continued surveillance of meningococci for antimicrobial resistance will allow early detection of changes in susceptibility patterns that might affect recommendations for chemoprophylaxis as well as for treatment.

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Key words: Carrier state, Drug resistance, *Neisseria meningitidis*, Oropharynx

Introduction

Infections by *Neisseria meningitidis* are significant causes of mortality and morbidity in young children and adolescents. The epidemiology of serious meningococcal disease is an area of considerable interest, and many unanswered questions surround this organism and the types of diseases it causes.[1] Group A and group C meningococci are frequently the cause of major epidemic disease, particularly in underdeveloped countries and among the poorer segments of society, perhaps reflecting certain risk factors associated with transmission, such as crowding and poor sanitation. Humans are the only natural hosts for *N. meningitidis*, and the organisms spread by respiratory droplets. The organisms may be asymptomatically carried in the oropharynx and nasopharynx of a variable percentage of individuals, and the rate of carriage is related to several factors such as age, socioeconomic class, and the presence of actual disease in a community.[2]

Penicillin G has been considered the treatment of choice for infections caused by this organism. Over the past decade, *N. meningitidis* strains with decreased susceptibility to penicillin have been described in Europe. Resistance in these strains is related to altered forms of penicillin-binding protein (PBP). A few beta-lactamase-producing strains have also been described in South Africa, Spain and Canada. The National Committee for Clinical Laboratory Standards (NCCLS) has recommended microdilution (with cation-adjusted Mueller-Hinton broth or agar dilution with Mueller-Hinton agar for susceptibility testing of *N. meningitidis*.[3] There are no specific guidelines from NCCLS regarding the susceptibility of meningococci by disk diffusion method, but the use of 1 μg oxacillin and 2-U penicillin G disks has been proposed for the screening of *N. meningitidis* moderately susceptible to penicillin. A more recent possibility for the quantitative determination of antimicrobial activity is the E-test.[4]

This study aimed to determine the oropharyngeal carriage rates and serogroups of *N. meningitidis* in children in our region. Moreover, penicillin resistance and resistance mechanisms of the strains isolated from healthy school children, and the usefulness of the oxacillin screening and E-tests as methods of recognising *N. meningitidis* isolates relatively resistant to penicillin were also evaluated.

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Materials and Methods

Of the 32,444 students attending primary schools (2001 to 2002 academic year) in Manisa city centre, which is located in western Turkey, 1128 were assigned randomly but systematically for this study. Informed consent from the parents and the local ethics committee approval was obtained prior to the commencement of the study. Information about those included in the study was obtained using standardised questionnaires for sociodemographic variables, and all underwent a physical examination regarding upper respiratory tract infection. Children with no signs of respiratory infection and history of receiving antibiotic treatment within the last 2 weeks were included in the study.

Oropharyngeal swab samples taken with synthetic tipped swabs were placed into a Stuart transport medium (Oxoid Limited, Hampshire, England) and sent to the Bacteriology Laboratory of the Medical Faculty of Celal Bayar University. The specimens were cultured in GC agar-enriched vitox (Oxoid Limited, Hampshire, England) and Thayer Martin medium (VCNT antibiotic supplement SR91; Oxoid Limited, Hampshire, England) for the growth of *Neisseria* species. The media were incubated for 48 h at 37ºC in 5% CO_2 and then examined. Potential meningococcal colonies were stained and tested with oxidase reagent (Oxoid Limited, Hampshire, England). Gram-negative cocci which were oxidase-positive were subcultured for biochemical identification with commercial kits (BBL Crystal N/H ID, Becton Dickenson and Co, Sparks, MD, USA). The capsular antigenic typing of *N. meningitidis* was done by slide coagglutination with commercial antisera (Difco Laboratories, Detroit, Michigan, USA).

Penicillin resistance of strains was investigated by using oxacillin (Oxoid Limited, Hampshire, England) disk diffusion and E-test (AB Biodisk, Solna, Sweden) methods. Beta-lactamase activity was assayed with chromogenic cephalosporin nitrocefin strips (Oxoid Limited, Hampshire, England).

A statistical package SPSS 10.0 (SPSS incorporated, Chicago) was used for all statistical analysis.

Results

The results of this study showed that the carriage rate of *N. meningitidis* in our region was 6.2% (71 strains) and the serogroups identified were serogroups C (35.2%), A (28.1%), B (22.5%), W-135 (11.2%) and D (2.8%). In this study, socioeconomic status of the children was assessed by using a questionnaire. No statistically significant difference was found between the socioeconomic status and the carriage prevalence. Penicillin resistance was found in 16 strains (22.5%), while beta-lactamase activity was found in none. Distribution of oropharyngeal carriage of *N. meningitidis* according to age, gender, serogroup and the results of antimicrobial susceptibility testing were summarised in Tables 1 to 4.

Discussion

In our country, the number of comprehensive epidemiological studies on the carriage rate of *N. meningitidis* and its serogroups in prepubertal ages is not sufficient. To the best of our knowledge, the vast majority of the studies in the literature deals either with serogroups of the strains isolated from asymptomatic carriers or with the susceptibility to antibiotics. However, having better knowledge of the epidemiology of *N. meningitidis* and identifying the serogroup of the strains isolated from asymptomatic carriers are important with respect to public health and controlling the infection, because *N. meningitidis* may lead to the outbreak of an epidemic disease or invasive epidemics in public places such as schools and dormitories.

In this study, the carriage prevalence of *N. meningitidis* among school-age children in Manisa was investigated and the regional epidemiological profile of the isolated bacteria was determined based on the serogroup. The cohort

Table 1. Distribution of Oropharyngeal Carriage of *N. meningitidis* According to Age

Age (y)	Positive No. (%)	Negative No. (%)	Total No. (%)
7-8	22 (8.0)	252 (92.0)	274 (100.0)
9-10	15 (4.8)	295 (95.2)	310 (100.0)
11-12	16 (5.4)	276 (94.6)	292 (100.0)
13-14	18 (7.1)	234 (92.9)	252 (100.0)
Total	71 (6.2)	1057 (93.8)	1128 (100.0)

$P = 0.369$

Table 2. Distribution of Oropharyngeal Carriage of *N. meningitidis* According to Gender

Gender	Positive No. (%)	Negative No.(%)	Total No. (%)
Male	43 (7.3)	545 (92.5)	588 (100.0)
Female	28 (5.1)	512 (94.9)	540 (100.0)
Total	71 (6.2)	1057 (93.8)	1128 (100.0)

$P = 0.142$

Table 3. Distribution of *N. meningitidis* Serogroups

Serogroup	No. (%)
A	20 (28.1)
B	16 (22.5)
C	25 (35.2)
D	2 (2.8)
W-135	8 (11.2)
Total	71 (100.0)

Table 4. Rates of Penicillin Susceptibility in *N. meningitidis* Serogroups (%)

	A	B	C	D	W-135	Total
No. sensitive (%)	18 (90.0)	12 (75.0)	15 (60.0)	2 (100.0)	8 (100.0)	55 (77.5)
No. resistant (%)	2 (10.0)	4 (25.0)	10 (40.0)	–	–	16 (22.5)
Total no. (%)	20 (100.0)	16 (100.0)	25 (100.0)	2 (100.0)	8 (100.0)	71 (100.0)

$P = 0.058$

comprised subjects of the same ethnic background. The study was not carried out during or immediately after the haj season which might contribute to the carriage rate. During this period, no bacterial meningitidis case was reported in our region. In the present study, the carriage rate was 6.2%. This is similar to the rates reported during non-epidemic situations in other countries. According to the data obtained from the studies on school-age children in other countries, nasopharyngeal carriage rate was found to be 2.3% in Spain,[5] 2.8% in Sweden,[6] 5.8% in Greece,[7] and 6.2% in Nigeria.[8]

While serogroup A epidemics have been observed over intervals of as long as 20 to 30 years throughout the world, it affects 1% of the population in the African "meningitidis belt" which is on the south of the Great Sahara and has been observed over intervals as long as 8 to 12 years.[9] In a study conducted in the USA between 1989 and 1991, the rates of serogroup B and serogroup C were found to be 46% and 45%, respectively.[10] While the rate of serogroup Y was 22% in 1992, it was observed to increase to 35% in 1997. In recent years, an increase has been observed in serogroup Y infections.[11]

In England and Spain, where outbreaks of epidemic diseases are common, epidemics and invasive infections are mostly caused by serogroups B and C, while serogroups X, Y and W-135 are rarely observed. Serogroup C was found to be 1.8% in 1996 in Spain, while this rate increased to 57.1% by 1991.[12,13] Recently, serogroup W-153 epidemics were identified among pilgrims visiting Saudi Arabia.[14] In the 2000 haj season, 40 cases of serogroup W-135 meningococcal disease were reported by Centers for Disease Control. Thirty out of 199 cases of meningococcal meningitidis observed in Saudi Arabia within the same period were shown to have occurred due to serogroup W-135.[14]

In a field study regarding the epidemiological characteristics of epidemical meningitidis disease observed in the Aegean region of our country, Coskun et al[15] reported that the carriage rate of *N. meningitidis* was 28% in nasopharyngeal swap samples taken prior to chemoprophylaxis. The strains isolated from the carriers were identified as serogroups A (12%), B (9%), C (63%), D (%0.4), Y (0.6%), X-Y (0.4%) and W-135 (0.1%). It was observed that there was no difference regarding gender, and serogroup C was dominant among the isolates. In our study, 28.1% of the strains isolated from asymptomatic carriers was serogroup A, 22.5% was serogroup B, 35.2% was serogroup C, 11.2% was serogroup W-135 and finally 2.8% was serogroup D. Regarding serogroup distributions, no significant difference was observed between this study and that of Coskun et al,[15] both conducted in the same region, and this leads to the consideration that A, B and C are the dominant serogroups in our country.

Even though the factors that play a role in the progression from the state of carrier to invasive disease are not fully known, socioeconomic status of the patient has been shown to be a very important factor with respect to catching meningococcal disease. Various studies have reported that low economical status, poor sanitation, crowded living conditions, insufficient air per person and too many people sleeping in a single room increase the meningococcal colonisation.[16,17]

In a study conducted on immigrants with low socioeconomic levels in Greece, carriage rate of *N. meningitidis* was found to be 13.1% among school-age children having poor living conditions and in Athens and this rate was 5.8% among school-age children having high socioeconomic status.[7] After an epidemic of serogroup B, De Wals et al[18] reported that carriage rate was 10% in school-age children having high socioeconomic status and 33% in those having low economic status. In this study, socioeconomic status of the children was assessed by using a questionnaire. However, no statistically significant difference was found between the socioeconomic status of the students and the carrier prevalence. It is thought that the students not being able to answer the items of the questionnaire correctly and the similarity in the socioeconomic status of the families of the students attending schools located within the centre of Manisa may have led to these results.

In recent years, widespread penicillin resistance in those with meningococcal disease is one of the greatest challenges with respect to meningococcal infections. Resistance to penicillin in those with meningococcal disease may be related to the *pen*A gene and the penicillin-binding protein (PBP2), which is encoded by the *penA* gene, whereas it can be rarely related to the production of beta-lactamase.[19] *N. meningitidis* strains with reduced susceptibility to penicillin

due to the alteration in penicillin-binding proteins were first described in a strain isolated from a carrier in 1970 and subsequently in a strain isolated from a patient having meningitis, in 1985. Reduced susceptibility to penicillin has been reported from a large number of countries in many continents.[20,21] Nearly half of the strains isolated in Spain display reduced susceptibility to penicillin.[13] The rate of strains having reduced susceptibility to penicillin is 7% in Canada, 4% in USA, and 3% in England.[22]

In a study conducted by Arreaza et al,[21] penicillin resistance was tested by agar dilution method in 904 *N. meningitidis* strains isolated from asymptomatic carriers and patients with meningococcal disease. They reported that the rate of resistance was 55.3% in clinical isolates and 3.9% in the strains isolated from the carriers. In 1999, Liassine et al[6] investigated the antimicrobial susceptibility of the bacterial pathogen isolated from the pharyngeal swabs taken from 1765 healthy children whose ages ranged between 2 and 14 years. Minimum inhibitory concentrations (MIC) assessment of the strains found to be resistant via disk diffusion method was performed using E-test and the ratios of resistance to penicillin were found to be 2.5% for *Streptococcus pneumoniae*, 0% for *Streptococcus pyogenes* and 12.5 % for *N. meningitidis*. In a study by Tzanakaki et al,[23] which was performed using agar dilution method, the rate of the reduced susceptibility to penicillin was reported as 48% in clinical isolates and as 19% in school-aged carrier children in Greece.

In a study performed in our country, the rate of penicillin-resistant strains was shown to be significantly high (43%) in primary school students with asymptomatic *N. meningitidis*.[22] However, there exists no large epidemiological studies showing the penicillin resistance rate in clinical isolates and carrier strains in Turkey. In our study, it was determined that 16 *N. meningitidis* strains out of 71 strains isolated from the carriers had reduced penicillin susceptibility (MIC values were 0.1 to 1.0 μg/mL). Beta-lactam activity was observed in none of the strains that were resistant to penicillin. For this reason, it was thought that the mechanism of resistance to penicillin in isolated strains occurs due to the alteration of PBP. Even though test results obtained by oxacillin disk are not considered to be reliable in determining penicillin resistance, the results of disk diffusion were found to be consistent with the results of the E-test. However, studies concerning large number of isolates are needed in this aspect. In this study, we concluded that, for a successful treatment outcome, meningococci found to be resistant to penicillin by oxacillin disk diffusion method should be considered as penicillin-resistant until MIC values are determined.

As a result, the *N. meningitidis* carriage rate was found as 6.2% in school-age children in Manisa and dominant serogroups were C, A and B, respectively. Data obtained from this study are similar to those obtained from studies performed during non-epidemic situations in other countries. The fact that *N. meningitidis* strains (22.5%) display reduced susceptibility to penicillin and do not produce beta-lactamase leads to a consideration that these strains are common in our country. One has to consider that in order to prevent the increase of resistant strains, using antibiotic medications is important in the treatment of *N. meningitidis* infections, just like in the eradication of other pathogens. For this reason, determining the regional resistance prevalence by starting surveillance programmes in every country, even in every centre, and informing the clinician are necessary. Even though the epidemiological data based on serogroups do not represent the real tendency of meningococcal infections, identifying the serogroups will provide a better understanding of temporal and geographical variations and provide practical benefits with regard to vaccination strategies.

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