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Nasopharyngeal carriage of neisseria meningitidis among medical school students in Turkey.

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Abstract

Objective: Neisseria meningitidis can colonise healthy human nasopharynx and is one of the most important etiologic agent of epidemic meningitis. The purpose of this study is to investigate the carriage rate, serogroup distribution and antibiotic resistance of Neisseria meningitidis in medical faculty students.

Methods: Nasopharyngeal swab cultures were evaluated. Identification is made by convensional and semi-otomatised system. Serogroup analysis was performed with slide agglutination method. Minimum inhibitor concentration results were evaluated by gradient test. Risk factors for carriage were investigated.

Results: Neisseria meningitidis was isolated from 3 out of 475 (0.6%) students. Two of them were serogroup A and one was serogroup C. All strains were susceptible to penicillin and ceftriaxone. Living in a dormitory and sharing the room with three other students and smoking were found as risk factors. None of the students reported upper respiratory tract infection or antibiotic usage. Other potential pathogenic microorganisms colonising nasopharynx were also identified and most detected of them is methicillin susceptible *Staphylococcus aureus* (n: 87).

Conclusion: Neisseria meningitidis is one of the leading pathogen in our country as it's in the world. Since serogroups of isolated strains differ from region to region, it's important that every country should conduct regular surveillance in order to utilize an effective vaccine for prevention and outbreak management against this infection.

Keywords: Neisseria meningitidis, Nasopharyngeal carriage, Serogroup distribution.

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Introduction

Neisseria meningitidis (*N. meningitidis*) is a Gram-negative diplococcus bacteria and it is still identified as a major cause of infection around the world due to the infections and pandemics it can cause. Nasopharyngeal carriage in healthy individuals has been known since 1890. It causes pandemics in closed and semi-closed communities such as childcare centers, military units, universities, schools and houses. It is classified into 13 serogroups as A, B, C, D, X, Y, Z, E, W-135, H, I, K, L [1,2]. The major agents of the disease are the serogroups A, B, C, Y, W-135 [2]. Rate of colonization at nasopharynx is approximately 10%. Carriage mostly depends on the age, and the incidence is 30% in adolescents and young individuals [3]. *N. meningitidis* can cause many diseases such as septic arthritis, pneumonia, pericarditis alongside meningitis and septicemia [2,4].

Nasopharyngeal carriage can be alleviated by chemoprophylaxis. While initially sulphonamides were used in treatment of the infection, penicillin and chloramphenicol began to be used in 1950s and 1960s. After 1983, beta-lactamase producing strains were reported, and afterwards, beta-lactamase negative strains with reduced penicillin sensitivity were reported from many countries such as Spain, United Kingdom and United States. Ceftriaxone is recommended for the treatment of infections caused by penicillin-resistant strains. In addition, in treatment and in order to reduce the rate of carriage, rifampin, ciprofloxacin, and ceftriaxone are recommended [4-7].

The follow-up of serogroup distribution by regions will be helpful in pandemic management and epidemiologic studies which follow the efficiency of the vaccine in the prevention of infections. The aim of our study is to investigate the meningitis agent *N. meningitidis* carriage, serogroup distribution, and antibitotic resistance using the nasopharyngeal swab samples from medical school students and contribute to the epidemiologic studies performed in Turkey.

Materials and Methods

Study design

Nasopharyngeal carriage of medical school students were evaluated between October 2013 and March 2015. Specimens

were taken especially during the months of October-January and March-May. One sample was taken from each student in the study. All medical school students accepted in the study were asymptomatic, healthy individuals. The place of residence (i.e., dormitory), number of cohabitants, number of people who sleep in the same room, presence of any upper respiratory tract infections in the last month, antibiotic use, smoking were questioned as the risk factors of carriage. Also microorganisms other than *N. meningitidis* were identified and recorded.

Sample size

Five hundred students were targeted to take a sample of nasopharyngeal swabs, but 475 students became volunteers.

Laboratory methods

Nasopharyngeal swab samples were collected using a dacron tipped swab (FLOQSwabs, Copan, Italy) and were plated on 5% sheep blood chocolate agar and Thayer Martin (BD, USA) agar, and were incubated for 24-48 h at 35-37°C with 5-10% CO_2. Gram staining was performed on suspected colonies growing on the agar and oxidase and catalase tests were performed on those with an appearance of Gram-negative diplococci, and the strains with a positive result from these tests were diagnosed as *N. meningitidis* using API NH (bioMérieux, France) half-automated system. Serogroups of the strains were identified by slide agglutination method using polyvalent antisera (DifcoTM *N. meningitidis* antiserum group A, B, C, Y, W/135, Becton Dickinson, USA). Gradient test (E test, bioMeriéux, France) was used to identify the sensitivity of these strains to penicillin and ceftriaxone, and results of the minimum inhibitor concentration (MIC) were evaluated based on the recommendations of Clinical Laboratory Standards Institute (CLSI) [8]. Microorganisms other than *N. meningitidis* were identified using conventional methods (Gram staining, sensitivity to catalase, oxidase, DNAse, cefoxitin, optochin, bile solubility, esculin hydrolysis, glucose-lactose-sucrose fermentation, mobility, urease, citrate use, etc.).

Ethical consideration

The study was approved by National Clinical Research Ethics Committee of İstanbul University Istanbul Medical Faculty (Approval number is 24-2013/12, dated 07.01.2013). The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000. Written informed consent was obtained from all participants. This work was supported by Scientific Research Projects Coordination Unit of Istanbul University. Project number 30147.

Results

Nasopharyngeal swab samples of a total of 475 students (181 females and 294 males) aged between 18 and 20 were evaluated. Growth of normal flora bacteria was detected in 309 (65%) of these students, and 3 (0.6%) *N. meningitidis* growth was detected. Of these strains, two (0.4%) were in serogroup A and 1 (0.2%) was is serogroup C. All these strains were found to be sensitive to penicillin and ceftriaxone (Table 1). One of the carriers was a female, and the others were male student. In terms of the risk factors, one lived in the dormitory and shared a room with three other individuals, and the other one was a smoker. They have not reported antibiotic use and upper respiratory tract infections in the last month. Growth statuses of any colonizing microorganisms other than *N. meningitidis* of 163 students are presented in the Table 2.

Table 1. *Distribution of serogroups and antibiotic sensitivity of Neisseria meningitides.*

Student	Serogroup distribution	Antibiotic sensitivity (MIC[1])							
		Penicillin				Ceftriaxone			
		MIC Interpretive Criteria[2]			Isolate	MIC Interpretive Criteria[2]			Isolate
		S[3]	I[4]	R[5]	S	I	R		
1	Serogroup A	≤ 0.06	-	≥ 0.12	0.094 (S)	≤ 0.5	1	≥ 2	<0.016 (S)
2	Serogroup A	≤ 0.06	-	≥ 0.12	0.008 (S)	≤ 0.5	1	≥ 2	0.016 (S)
3	Serogroup C	≤ 0.06	-	≥ 0.12	0.012 (S)	≤ 0.5	1	≥ 2	0.016 (S)

[1]Minimal inhibitor concentration, [2]MIC interpretive criteria for meningitidis, [3]S: Sensitive, [4]I: Intermediate, [5]R: Resistant

Discussion

Infections caused by *N. meningitidis* have an important place among the invasive bacterial infections worldwide. While the infections can occur in singular cases, there can be pandemics or even epidemics. This depends on the geographical region and time [9]. Meningococci can be transmitted from asymptomatic carriers or from individuals with upper respiratory tract infection through droplets. Transmission increases in close contact conditions or in crowded places [10]. Similar to age group of our study, carriage rate among children aged 15-19 living in social rehabilitation centers in Mexico is 1.9%, whereas it is 2.9% in children under 5 years old living in care centers; and no meningococcus was detected in college

students [11]. In Netherlands, 3,200 healthy children aged 1-19 were 1.15% carriers [12].

Table 2. *Distribution of the microorganisms detected in Nasopharyngeal swab samples other than Neisseria meningitides.*

Microorganism	Number (n)
MSSA[1]	87
Moraxella catarrhalis	18
Streptococcus pneumoniae	15
Haemophilus influenzae	15
Haemophilus parainfluenzae	14
MRSA[2]	6
Enterobacter spp.	6
Non-GAS[3]	5
Klebsiella pneumoniae	4
Klebsiella oxytoca	2
Klebsiella ozaneae	1
Serratia spp.	1
Citrobacter spp.	1
Pseudomonas aeruginosa	1
NFGNR[4]	1
Enterococcus spp.	1

[1]MSSA: Methicillin Sensitive *S. aureus*; [2]MRSA: Methicillin Resistant *S. aureus*; [3]Non-GAS: Non-Group A *Streptococci*; [4]NFGNR: Non-Fermentative Gram-Negative Rod

Long term carriage was investigated in 2,453 college students in United Kingdom at different times and carriage rate was changed from 44.1% to 57.1%. Of those who were initially carriers but stopped being carriers 16% were recolonized by these bacteria within 6 months. Students, who were not carriers initially, were re-evaluated again 13.7%-22.1% were carriers in another time periods [13]. Worldwide epidemiologic and surveillance studies are performed regularly, and studies on this subject have been performed in the recent years in Turkey *N. meningitidis* carriage prevalence among 1.155 students aged 7-19 was 10.4% and the most frequently detected serogroup was serogroup B (47.5%). In the same study, living in crowded homes and in slums, using stove for heating purposes were identified as risk factors for carriage; tonsillectomy, tonsil hypertrophy, smoking, and being exposed to cigarette smoke were not identified as risk factors [14]. In a study conducted among 1.995 newcomer soldiers, carriage rate was 4.2% and the most frequently observed serogroups were Y and W-135, and tetra vaccination was performed instead of bivalan vaccination [15]. In another study, *N. meningitidis* carriage was identified in 0.6% and all the strains were serogroup B [16].

The incidence of invasive meningococcus is 10-1.000/100.000 in Africa, whereas it is 0.92/100.000 in Europe and 0.28/100.000 in USA [17]. Serogroup distribution is important for vaccine studies and epidemiologic data. Distribution changes according to time and geographical region. Serogroups B, C, and Y are frequently observed in USA and Canada, serogroups B and C are frequently observed in Europe, whereas in Asia, serogroup A is observed in high rates, and the rates of serogroups C, Y and W-135 are increasing. In the region termed "meningitis belt" in Africa, serogroup A frequently causes pandemics [9,17,18]. After the pilgrimage season in 2000, there were 90 cases in 9 countries and this raised the importance of W-135 once more [19]. Considering the multicenter study, between 2006-2009, *N. meningitidis* detected in 19% of the meningitis patients (86% serogroup B, 6% serogroup C, 3% serogroup A-X-W135) and between 2005-2012, *N. meningitidis* was detected in 52.4% of the meningitis cases (38% serogroup W135, 26.1% serogroup B, and 8.4% serogroup A) [20,21]. In our study, consistent with the data of the other studies performed in Turkey, serogroups A and C were detected, but serogroups W-135 and B, which were observed more frequently as invasive infection agents, were not detected. Penicillin and ceftriaxone take the lead in *N. meningitidis* treatment. However, beta lactamase can be rarely detected and therefore sensitivity tests must be performed and MIC value should be determined [4]. In a surveillance study performed in USA between 2004-2011, it was found that penicillin sensitivity decreased from 90.8% to 88.5%, and strains with intermediate level sensitivity increased from 8.5% to 11.5%, and the resistance decreased from 0.7% to 0%. Ceftriaxone sensitivity was 100% [22]. National surveillance data in-between 1960-2012 were analyzed in Netherlands and penicillin resistance was not detected in 15.412 cases [23]. While the number of strains detected in our study is less, all strains were sensitive to penicillin and ceftriaxone.

While investigating nasopharyngeal *N. meningitidis* carriage in our study, other potential pathogenic microorganisms colonized in nasopharynx were detected in the meantime. In nasopharyngeal carriage studies, Farida et al. found 15% *K. pneumoniae*, 4% *Pseudomonas* spp., 4% *Enterobacter* spp., 1% *A. baumannii* in adults; and Hassaun et al. investigated 100 asymptomatic medical staff and 51.4%-55.9% MRSA, 17.6%-29.7% MSSA, 24.9% *M. catarrhalis* and 21.6% *K. pneumoniae* were investigated [24,25]. *S. pneumoniae* and *H. influenzae* were detected in lower rates. MSSA, MRSA, *M. catarrhalis* and *K. pneumoniae* were the most frequently observed microorganisms in school of medicine students [25]. In our study the most frequently encountered microorganism was MSSA (18.3%), contrary to the MRSA which was frequently observed in other studies. Consistent with the previous studies, Gram-negative rods except *M. catarrhalis* (3.7%), *S. pneumoniae* (3.1%), *H. influenzae* (3.1%) and *Enterobacter* spp. and *Klebsiella* spp. were observed less frequently in our study. *Neisseria meningitidis* is among the primary pathogens in Turkey as it is in the world. We believe that our study results would contribute to the epidemiologic data of our country.

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