ORIGINAL ARTICLE

Prevalence of meningococcal carriage among male university students living in dormitories in Kerman, southeast of Iran

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ABSTRACT

Neisseria meningitidis is an important causative agent of bacterial meningitis. The nasopharynx is the only known reservoir of this organism. Although the relationship between carriage and invasive disease is not completely understood, asymptomatic meningococcal carriers are considered as the most important sources for causing strains of disease. Living in closed and overcrowded places such as university dormitories can increase the carriage rate and meningococcal disease. This cross-sectional study was conducted to determine the prevalence of *N. meningitidis* carriers among male students living in three dormitories affiliated with Kerman University of Medical Sciences (Kerman, Iran).

Nasopharyngeal swab was taken from all participants recruited in the study. Conventional microbiological tests were performed for isolation and detection of the organism. The amplification of *crgA* gene was used to confirm the identity of isolates. Molecular serogrouping was used to detect the six most frequent serotypes.

The overall carriage rate was 6.8% (23/335). The capsular type of these isolates was in determinate (56.5%) or of serogroup C (43.5%). Multivariate logistic regression analysis revealed that cigarette smoking was significantly associated with meningococcal carriage (OR = 5.02; $p = 0.01$). Additionally, using univariate regression analysis, a significant association was found between water pipe smoking and carriage ($p = 0.018$).

The rate of meningococcal carriage among male students in the studied population was lower as compared to other high-risk group (freshmen conscripts) in Iran. University students should be aware of the consequences of cigarette and water pipe smoking as risk factors in meningococcal carriage.

KEYWORDS

Neisseria meningitidis;
university dormitories;
meningococcal carriage

Introduction

Bacterial meningitis in young adults is commonly caused by *Neisseria meningitidis*. The incidence of meningococcal disease which appeared epidemically more frequently in developing countries is dependent on the geographical region [1,2]. Nasopharynx is the only known reservoir for this organism. Since most of the individuals with invasive meningococcal disease do not report any history for direct contact with patients, so asymptomatic carriers are considered as the most important natural sources for the development of disease [3,4]. Approximately, 5 to 30% of normal population may harbor meningococci in nasopharynx, but the rate of carriers may increase to 70 to 80% during epidemics [5]. In this study, some risk factors are found more in carriage and subsequently in infection, including male sex, cigarette smoking or passive exposure to smoke, living in closed or overcrowded communities such as dormitories, military barracks, homeless shelters, long term care facilities, prisons, etc [1,3,6].

To date, 13 serogroups of meningococci have been identified, of which serogroups A, B, C, X, Y and W are the most frequently associated with the disease. Since the epidemiology and serogroups distribution of *N. meningitidis* can change quickly, so having knowledge about carriage state in high-risk communities will be crucial for controlling the emergence of virulent strains and understanding the epidemiology and pathogenesis of related infections [1,5].

In 2000, Advisory Committee on Immunization Practices and the Committee on Infectious Diseases of the American Academy of Pediatrics recommended meningococcal vaccination for college students [7], but there is no vaccination program for this population in Iran. In addition, the prevalence of carriage among students of university dormitories is not clear. So, this study was conducted to determine the incidence rate of pharyngeal carriers of *N. meningitidis* and the most prevalent serogroups among male students living in dormitories in Kerman (Southeast of Iran).

Materials and methods

Study population

In this cross-sectional study, during a 6-month period (October 2017 to March 2018), 335 male students living in dormitories affiliated with Kerman University of Medical Sciences (Kerman, Iran) were recruited. In Iran, due to religious and ethical criteria, there are separate dormitories for male and female students. All subjects were given an information sheet and all volunteers enrolled in this study completed a questionnaire inquiring about age, duration of residency in university dormitory, active cigarette smoking/passive exposure to smoke, water pipe smoking, history of meningococcal vaccination, recent history of antibiotic consumption, and having knowledge about meningococcal disease.

Isolation and identification of *N. meningitidis*

Using sterile cotton swabs, posterior pharyngeal specimen was taken from all participants and was immediately inoculated onto chocolate agar containing vancomycin (3 mg/L), colistin (2 mg/L), and nystatin (3 mg/L) and was placed at 37°C in an incubator containing 5% CO₂. Following 48 to 72 h, isolates were identified based on conventional tests which include colony morphology, Gram staining, oxidase test, carbohydrate fermentation tests and lack of growth at 25°C. If necessary, subculture of suspicious colonies was repeated in order to obtain pure colonies. Then, all phenotypically identified isolates were confirmed by species-specific PCR for *crgA* gene, a conserved regulatory gene which is involved in the adhesion regulation of *N. meningitidis* to target cells [8,9].

Molecular serogrouping

For serogroup prediction, amplification of *sia D* gene was performed to detect serogroups B, C, X, Y, and W. Also, PCR experiment was performed using specific primer, *orf-2*, which is a gene cassette required for the capsule biosynthesis of serogroup A [9].

Ethics standards

The study was approved by Ethics Committee at Kerman University of Medical Sciences (Ethic approval code: IR.KMU.REC.1395.979). Written informed consent was obtained from each individual included in the study.

Statistical analysis

Sample size calculation was made to ensure that the enrolled population is a representative of studied population. So, 335 subjects were selected to be representative of 650 boy students who reside in dormitories affiliated with Kerman University of Medical Sciences. For continuous variables, means and standard deviations were determined. Absolute and relative frequencies were calculated for descriptive variables. To find any significant correlation between the risk factors and meningococcal carrier status, Fischer's exact test was used. A *p*-value < 0.05 was considered statistically significant. Risk factor analysis was performed using logistic regression and odd ratios (ORs) were determined with 95% confidence intervals (CIs).

Results

A total of 335 male students aged 18 to 34 (mean: 21.4 ± 2.7) years old living in three dormitories affiliated with Kerman University of Medical Sciences were enrolled in this study. Duration of residency in dormitory was not less than one term for each participant and in average it was 3.69 ± 2.8 terms. Some information of enrolled subjects are shown in Table 1.

In total, 23 meningococcal carriers (23/335, 6.86%) were identified. The highest rate of carriage was seen at 22 years old (5/23, 21.7%). Also, about 70% of carriers were of ages 19 to 22.

Carriage rate in each dormitory ranged from 4.3 to 8.3% (Table 2). However, no statistically significant difference was found between three dormitories. Analysis of the studied risk factors showed no correlation between carriage and exposure to smoke (passive smoking), having respiratory infection, antibiotic consumption, and even duration of residency in dormitory. However, univariate analysis showed that active cigarette smokers had significantly higher carriage rate than non-smokers (21.9% vs. 5.3%; *p* = 0.003). In addition, significant association was detected between water pipe smoking and meningococcal carriage (*p* = 0.018). Similarly, statistically significant association was found between lack of vaccination and carriage (*p* = 0.007). So, none of the carriers had history of meningococcal vaccination.

Logistic regression analysis (multivariate analysis) showed that among different risk factors investigated in this study, only cigarette smoking was significantly

Table 1. Characteristics of the studied population.

Carrier status	Active smoker	Exposure to smoke	Respiratory infection	Antibiotic use	History of vaccination	Duration of stay in dormitory
Carriers (N = 23)	7 (30.4)	6 (26.1)	1 (4.3)	7 (30.4)	0 (0)	3.74 ± 2.7
Non-carriers (N = 312)	25 (8.0)	53 (17.0)	20 (6.4)	83 (26.6)	47 (15.1)	3.69 ± 2.8
Total (N = 335)	32 (9.5)	59 (17.6)	21 (6.3)	90 (26.9)	47 (14)	3.69 ± 2.8

Table 2. Distribution of subjects, carriers and related serogroups in different dormitories.

Dormitory	No. of subjects	Mean age	Carriage rate (%)	Serogroup distribution	
				C	Indeterminate
A	168	22.2 ± 2.6	11 (6.5)	5 (45.4)	6 (54.5)
B	120	20 ± 2.0	10 (8.3)	4 (40)	6 (60)
C	47	22.3 ± 3.6	2 (4.3)	1 (50)	1 (50)
Total	335	21.4 ± 2.8	23 (6.86)	10 (43.5)	13 (56.5)

associated with meningococcal carriage. So, the risk for meningococcal carriage in smokers was 5.02 times as high as non-smokers (OR = 5.02, CI: 1.89–13.35). On the contrary, water pipe smoking and vaccination were not effective when compared with other variables. However, the lack of an effect may be due to small sample size or student's reluctance to tell the truth about water pipe smoking.

In addition, of 23 meningococcal isolates studied by molecular typing, serotypes of 13 (56.5%) isolates were indeterminate. Capsular group C was detected for the remaining isolates.

Discussion

There is complex relationship between carriage and invasive disease. Although meningococcal carriage may rarely lead to systemic infection, being carrier is considered as a prerequisite for development of infection. Dormitories as closed and crowded places are of importance, because carriage rate may increase to 40 and 50% in such locations [3]. This study provides information on the carriage rate of meningococcus among boy students living in dormitories in Kerman. The prevalence of meningococcal carriage and serogroup distribution is not only different between countries but also within countries and even it may change over time [10].

The present study showed that the overall carriage prevalence was reported to 6.86% which is lower than the prevalence in Japan (32.2%) [11], Korea (11.8–14%) [1], and Europe and Africa (the average of 10 to 35%) [12,13]. However, it was higher than as reports in a similar study conducted in Chile (4%) [14]. It should be stated that there is limitation in comparing the results with many other studies in this field because the studied population are different in term of age, sex, and residential status.

In the present study, for a proportion of carriers, no serogroup was detected, which may reflect the presence of serogroups other than A, B, C, Y, X and W, or non-capsulated isolates. The former assumption is more unlikely because the six mentioned serogroups are the most frequent. Previously, non-capsulated isolates were thought to be non-pathogenic, but later it was shown that capsular expression is phase variable and loss of polysaccharide capsule enhances nasopharyngeal colonization. Lack of capsule may be the result of the deletion of capsule locus or temporary/permanent down-regulation of capsule expression [10,15].

Among groupable isolates, only serogroup C was detected, which is similar to report from students in a university dormitory in Korea [1]. Regardless of the studied population, serogroup C is the most common serogroup reported from the country of study, e.g. among military recruits before vaccination and among 7–19 year-old students [4,5,16]. Also, this serogroup has been reported frequently in many European countries [3].

Different factors have been proposed in asymptomatic pharyngeal colonization of *N. meningitidis*. According to a meta-analysis study, carriage increases from infants (4.5%) to 19-years old (23.7%) and then decreases in older than 50-years old (7.8%) [17]. In the present study, 70% of the carriers were 19 to 22 year-old students. According to the results, active smoking was identified as a risk factor which is similar to other studies such as those from United Kingdom [18] and Korea [1]. However, in some studies no association has been reported [3,14]. For the other studied parameters such as duration in dormitory, passive smoking, and having respiratory infection, no statistically significant difference was found between carriers and non-carriers.

Water pipe smoking has become a global youth trend particularly in the Middle East countries and Iran. To the best of our knowledge, this is the first study which assesses the role of water pipe smoking in meningococcal carriage. Univariate analysis showed that this variable is effective alone, while in the presence of other variables, it is not effective. As earlier discussed, the results might be influenced, since some students might be cautious in telling the truth.

In Iran, there is mandatory meningococcal vaccination program for military recruits and Hajj pilgrims [19]. Thus, vaccines of purified polysaccharide capsules (bivalent A/C for soldiers and polyvalent A, C, Y, and W for pilgrims) are administered since they are cheaper than conjugate ones. However, despite the mandatory vaccination in military aged-male subjects in the country of study, most of the reported cases of disease (5 of 6) were caused by serogroup C [20]. Also, in the other study, serogroup C was the most second frequent (4 of 16) cause of meningococcal meningitis (following serogroup B) [21]. This may emphasize on the use of conjugate vaccines in high-risk subjects.

According to Harrison et al. [22], the occurrence of meningococcal infection in college students is not different from the population of the same age. But

the rate of the disease in students living in dormitories is higher than those living outside campus. Also, an investigation in Japan showed that the carriage rate increases upon longer period of residency [11]. Similarly, in a longitudinal study in England, the rate of meningococcal carriage among university students was shown to increase rapidly throughout the term (13.9% at the first week vs. 31% after one month) [23]. Fortunately, the rate of carriage in the present study has not reached the critical rate of 20%, so, no definite recommendation can be made for routine meningococcal vaccination in our region. Although, some limitations exist in this study: firstly, though the sampling was done in the cold seasons, the weather was warmer than in previous years. This can affect the results since the accumulation of people in the closed areas decreases. So, to improve our understanding of the epidemiology of carriage ratio, conducting researches under different climate condition is recommended. Secondly, all participants in this study were students of health and medical sciences and this may introduce selection bias. So, investigations on students from different fields of study will be useful. Recently, the emergence of strains with intermediate resistance to penicillin has been described in several countries, so surveillance of antibiotic susceptibility to meningococcal strains is recommended [24]. Furthermore, molecular characterization of meningococci isolated from the pharynx is also essential towards understanding the dynamics and identifying the emergence of hyper virulent strains [25]. Unfortunately, despite the tendency to try the aforementioned techniques, the environment and resources did not allow them and this can be the third limitation of this study.

In conclusion, the data showed that the rate of meningococcal carriage among male students in university dormitories was lower than the other high-risk group (freshmen conscripts) in Iran [4,5]. There is need for follow-up studies and large scale longitudinal studies at other regions to determine the exact carriage rates in this population. Also, beside cigarette which was shown to be associated with carriage, water pipe smoking was also proposed to be important. Since in recent years, most of the young people including university students are accustomed to the use of water pipe, so more studies on this subject are recommended.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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