ORIGINAL ARTICLE

# Evaluation of pharyngeal carriage of *Neisseria meningitidis* in Tehran, Iran

**A. Fahimzad[1], M. Khatmi[2], F. Shiva[2], S. E. Mortazavi[2], F. Shirvani[1] and F. A. Gorji[3]**

1) *Pediatric Infections Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences,* 2) *Shahid Beheshti University of Medical Sciences, Department of Pediatrics and* 3) *Clinical Development Research Center, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

## Abstract

Meningitis and meningococcal septicemia are potentially life-threatening illnesses; young people in educational institutions have been repeatedly exposed to outbreaks of meningococcal infections. Since invasive meningococcal disease is preceded by pharyngeal carriage of *Neisseria meningitidis*, ascertaining the prevalence of meningococcal carriage in this population is of utmost importance. The aim of this study was to determine the rate of meningococcal carriage in students of Shahid Beheshti University of Medical Sciences. This cross-sectional study was conducted on pharyngeal swab specimens of 251 healthy asymptomatic students from November 2019 for one year. A questionnaire was used to find correlation between isolation of *Neisseria* spp. and the place of residence, number of roommates, antibiotic use in the last month, and smoking. One sample from each student was used for culture on general and selective culture media for *Neisseria* spp. Polymerase chain reaction was used for the final diagnosis of *Neisseria meningitidis*. Participants in the study included 222 medical students (88.4%), 23 nursing students (9.2%) and 6 radiology students (2.4%). Mean (IQR1) age of students was 23 years, 134 students were female, (53.4%); 234 students were single, (93.2%). 92 students (36.7%) lived in dormitories. *Neisseria* were isolated from 18 specimens (7.2%), of which 11 (4.4%) were pigmented bacteria. PCR assay did not detect *Neisseria meningitidis* in any of the samples. This study showed that meningococcal bacteria were not detected in any of the oropharyngeal specimens from students participating in the study during the one-year study period.


**Keywords:** Carriage, Iran, Medical student, *Neisseria meningitides*, Tehran
**Original Submission:** 7 July 2021; **Accepted:** 22 December 2021
**Article published online:** 10 January 2022

**Corresponding author:** F. Shirvani Pediatric Infections Research Center, Research Institute of children's Health, Shahid Beheshti University of Medical Sciences, Tehran, P.O. Box 15468-15514, Iran.
**E-mail:** Shirvani@sbmu.ac.ir

## Introduction

*Neisseria meningitidis* (meningococcus), is a gram-negative diplococcus, divided into thirteen serotypes, among which, serotypes A, B, C, Y, W135 are responsible for most human disease [1].The clinical spectrum of invasive meningococcal disease is very diverse and may range from moderate fever, meningitis, septicemia, to multiple organ damage and even death within a few hours of diagnosis [2]. According to the World Health Organization (WHO), outbreaks and epidemics continue to occur, mainly in poor resource countries, especially the African meningitis belt [3]. Complications of meningitis include hearing loss and long-lasting neurological disability [2] Up to 10% of the Human population may carry this bacterium in their nasopharynx without exhibiting any symptoms, (asymptomatic carriers); the carriage rate may rise to 25–80% during epidemics. Most people with meningitis, do not have a history of direct contact with symptomatic patients, thus, asymptomatic carriers remain as an important source of disease transmission. Therefore, it becomes important to identify meningococcal carriers in high-risk communities in order to intervene and prevent disease [4–6]. The disease occurs in the absence of specific antibodies [7].



https://doi.org/10.1016/j.nmni.2022.100953

People attending mass gatherings, (like pilgrimage trips), and those living in in crowded environments such as military barracks, mines, student dormitories, prisons, and sanatoriums are more exposed to this disease than others. Smokers or those who smoke hookah have a much higher risk of becoming meningococcal carriers [8].University students are at a higher risk for colonization because they tend to gather together in work places, living quarters, and various extra-curricular activities [9].The direct effects of this on our hospitalization rate and health economy prompted us to examine the rate of pharyngeal colonization of meningococcus in students of Shahid Beheshti Medical University.

## Materials and methods

This is a cross sectional study conducted on medical and paramedical students in Shahid Beheshti University of Medical Sciences and Health Services (2019–2020). The sample size was calculated to 250 people using RAO (Raosoft 2004) sample size calculator software with confidence interval of 95% and error level of 5%. The sampling method was non-randomized. Based on the necessary coordination with the Deputy Minister of Education of Mofid Children's Hospital, list of medical and paramedical students taking their course were recruited for pharyngeal sampling to provide the required number of samples. The objectives of the study were told to the students and written consent was obtained from them. If satisfied, demographic data including, age, sex, weight, place of residence, (whether living in student hostel or not), marital status and tobacco use, travel to Karbala and antibiotic use in the past 30 days were entered in the information form. Each student was sampled once during the study period from November 2019 till October 2020; specimens were collected during all seasons. Samples were obtained from posterior part of the oropharynx and tonsil crypts using standard sterile swabs passed orally to the posterior oropharynx and deeply rotated in place [10].The swab was placed on Trypticase soy broth supplemented with 15% glycerol at −80 °C for follow up and subsequent operations. The samples were initially cultured on Blood agar, an unselective medium, and GC agar selective medium enriched with V.C.N.T. Supplement (Vancomycin 600 µg, colistin 1500 micrograms and trimethoprim 1000 micrograms per 200 ml of culture medium, Quelab, Canada) and sheep blood (7%). The grown colonies were assessed for similarity to morphology of *Neisseria*. Suspected colonies were analyzed for positive oxidase reaction and gram staining was done to confirm cellular morphology as well. Isolation of *Neisseria* species and their pigmented or non-pigmented types were recorded for each sample and PCR method was used for the final diagnosis of *Neisseria meningitidis*. In this method, DNA of suspected colonies was separated by a DNA extraction kit (Nucleic Acid Extraction Kit, DNAbiotech, China). A total of 3 µl of DNA, 1 µl of primers targeting *ctrA* gene (F: GGCTTCAGCTTATCGCTTTCTGAAGCC and R: TCTGCCTCACTGCCATAACC), in 12.5 µl of Ampliqon mastermix was used. PCR conditions for this reaction were included 1 cycle at 50 °C for 2 min; 95 °C for 2 min; 35 cycles at 95°C for 5 s, followed by 50° C for 10 s followed by one cycle at 60°C for 20 s that was done in a thermal cycler. The obtained data were analyzed using SPSS (Ver 25) software and calculated using descriptive and analytical statistics. Normal quantitative variables as mean, standard deviation and range and Nonparametric quantitative variables as median, quadratic range and qualitative variables as number and percentage Are displayed. Fisher exact and Mann–Whitney and T-test and logistic analysis were used. Significance level in these tests was $P<0.05\%$

## Results

Two-hundred twenty-two Medical students, (88.4%), 23 nursing students, (9.2%) and 6 radiology students, (2.4%) participated in this study. 134, (53.4%) were female. Mean age of the participants was 23 years, (age range 19–34years). 234, (93.2%) were single and 92 (36.7%) lived in student hostels. Number of persons living together had the range of (1–5 persons) the median, (IQR1) was 3 (2). Forty-one participants, (16.3%) had used antibiotics during the previous month; 22 (8.8%) were smokers and 27 (10.8%) were passive smokers. Twelve individuals, (4.8%) had been exposed to crowded places during a recent travel to Karbala in the Arbaeen Walk. *Neisseria* grew in pharynx of 18 (7.2%) cases, 11 (4.4%) were pigmented. In one case there was both pigmented and non-pigmented *Neisseria* so Non-pigmented *Neisseria* were isolated from 8 persons (3.2%). Polymerase Chain Reaction, (PCR) was negative for *Neisseria meningitidis* in all of the studied samples irrespective of the season of sample collection (Table 1).

## Discussion

The prevalence of meningococcal carriers varies from country to country and even in different geographic locations of a country and changes during time [11]. In the present study, we studied the incidence of meningococcal carriers in students of Shahid Beheshti University of Medical Sciences in Tehran, aged 19 to 35 years of age, during their internship period. The results showed that none of the subjects were carriers of meningococcus. In the study by Sadeghi et al. in 2018, prevalence of

**TABLE 1. Comparison of risk factors by result of throat culture of university students Shahid Beheshti Medical Sciences**

| Variables | Negative n = 233 | Neisseria spp n = 18 | *P-value*[a] |
|---|---|---|---|
| Age (yrs) (median (IQR)) | 22.7 ± 1.02 | 23.4 ± 1.15 | *0.008* |
| Weight (kg) (mean ± SD) | 69.4 ± 16.4 | 67.7 ± 21.2 | *0.681* |
| Number of people residing together in one place (no.) (median (IQR)) | 3 (2) | 3 (3) | *0.623* |
| Sex | | | |
| • Female | 126 (54.1%) | 8 (44.4%) | *0.470* |
| • Male | 107 (45.9%) | 10 (55.6%) | |
| Field of study | 204 (87.6%) | 18 (100%) | |
| • General practitioner | 212 (91%) | 17 (94.4%) | |
| • Others | 217 (93.1%) | 17 (94.4%) | |
| Marital status | | | |
| • Single | 16 (6.9%) | 1 (5.6%) | *0.461* |
| • Married | 87 (37.3%) | 5 (27.8%) | |
| Housing | 146 (62.7%) | 13 (72.2%) | |
| • Dormitory | 212 (91%) | 17 (94.4%) | |
| • Nondormitory | 207 (88.8%) | | |
| Smoking | 21 (9%) | 1 (5.6%) | |
| • Negative | 207 (88.8%) | 17 (94.4%) | |
| • Positive | 26 (11.2%) | 1 (5.6%) | |
| Exposure to second-hand smoke | | | |
| • Negative | 222 (95.3%) | 17 (94.4%) | |
| • Positive | 11 (4.7%) | 1 (5.6%) | |
| Recent travel to Karbala in the Arbaeen | | | |
| • Negative | 193 (82.8%) | 17 (94.4%) | |
| • Positive | 40 (17.2%) | 1 (5.6%) | |
| History of antibiotic use during the previous month | | | |
| • Negative | | | |
| • Positive | | | |

[a]Mann–Whitney test.

meningococcal carriers among male students of Kerman University of Medical Sciences living in dormitories was about 6.8% [12] and Islaminejad (2006), studied the prevalence of meningococcal pharyngo-tonsillar carriage on 750 samples from New entrants to Kerman University of Medical Sciences who were examined before and after accommodation in the dormitory. Seventy-seven samples (10.3%) were colonized with meningococcus; 28 cases (7.2%) were detected in the first sampling, at admission to the university and 49 cases (13.6%) were identified in the second sampling two months later; difference in the percentage was statistically significant (P = 0.008) [13]. In a study performed in Turkey between October 2013 and March 2015, *Neisseria meningitidis* was detected in 3 of the 475 nasopharyngeal swabs from asymptomatic medical students, revealing a prevalence of 0.6% [14]. Nasopharyngeal samples from 437 health care professionals at a tertiary university pediatric hospital in Australia were studied in 2020; colonization rate of meningococcus was 1.14 % [15]. The prevalence of meningococcal carriers in dormitory students in South Korea was reported as 11.8% and 14% four weeks apart in 2012 [16]. Oropharyngeal carriage of *Neisseria meningitidis* was found to be 12.7–14.6% in 1837 samples collected from 1487 students of Rhode Island University during two survey rounds [6]. In December 2014, after nasopharyngeal swabs of 2 students from a dormitory in Tokyo had tested positive for meningococcus, nasopharyngeal swabs from all students in that dormitory were collected; pharyngeal colonization was found to be 33.7% in students residing in the same dormitory, [17]. Globally, the incidence of meningococcal disease ranges widely from 1 to 1000 cases per 100,000 population [18].

In our study, cases of meningococcal colonization in students' throats were zero. Transmission of *Neisseria meningitidis* is from person to person through dispersion of respiratory droplets. The reason for the low rate of colonization with meningococcus in our study may be the use of masks and other preventive measures, including less communication and social distancing due to the COVID19 pandemic. In the 2015 Breakwell study, meningococcal throat colonization rates were higher in male students [6].In the review of the literature, the relationship between different variables and colonization was not studied in other *Neisseria Species* and this was done in our study. The relationship between age and pharyngeal colonization varies in different studies. In one study, colonization increased with age and in another, it decreased [10,19]. There is no study that has assessed the effect of body weight on meningococcal colonization. In a German study (2019) of asymptomatic meningococcal throat colonization in adults, married people were more likely to be colonized [20]. Living in a dormitory and having at least three roommates increases the rate of colonization [14]. In a study of pharyngeal carriage of meningococcus in Brazil (2014) the overall pharyngeal carriage of meningococcus in 11–19 years old children was 4.9% and it was higher when mother was the only smoker in the home [21].Tobacco use and passive smoking in the last 30 days had a significant relationship with pharyngeal colonization in students, as reported from a study done in the United States in 2015 [6] The rate of pharyngeal colonization with meningococcus in Hajj pilgrims has decreased due to vaccination and ciprofloxacin consumption before leaving for the trip to Mecca [22]. We could not find studies about pharyngeal colonization with meningococcus in Karbala pilgrims and in our study also

meningococcal pharyngeal colonization was not identified in any of the 12 students who had joined the Arbaeen Walk in Karbala. According to Breakwell et al. antibiotic use in the last 30 days is associated with increased meningococcal pharyngeal colonization [6].The PCR sensitivity in the diagnosis of meningococcus in the pharyngeal swab is 56% and the possibility that this technique could indicate the absence of meningococcal carriers is 89.9% (Negative Predictive Value) [23].We used this method to detect meningococcal pharyngeal colonization. The sensitivity of the PCR method is about 60% and the probability of false negatives is not very low. But because NPV is high in this study, the value of correctness of negative cases increases. It should be noted that PCR is currently the most accurate method for detecting the presence of meningococcus. Several researchers including Sadeghi et al., Diaz et al., and Breakwell et al. have used the PCR method to identify meningococcal pharyngeal colonization [6,12,19].

## Conclusion

Meningococcal pharyngeal colonization is not expected to be zero in students due to their lifestyle and dormitory life. Our findings indicate that the possibility of pharyngeal colonization with *Neisseria meningitidis* in individuals in the second and third decades of life in our society is low. The reason may be due to good hygiene practice, especially during the Covid pandemic, or the low prevalence of meningococcal colonization in our society. However, we must be wary of the possibility of meningococcal infection in children with symptoms or risk factors.

## Consent for publication

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data. They played an active role in drafting the article or revising it critically to achieve important intellectual content, gave the final approval of the version to be published, and agreed to be accountable for all aspects of the work.

## Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Vice Chancellor for Research of the Faculty of Medicine, Shahid Beheshti University of Medical Sciences with reference number IR.SBMU.MSP.REC.1399.129.

## Transparency declaration

The authors declare that they have no competing interests. Funding: This research did not receive any specific grant from funding agencies in public, commercial, or not-for-profit sectors.

## Data availability statement

All data generated or analyzed during this study are included in this published article.

## References

[1] González E, Reyes F, Otero O, Camacho F, Cuello M, Ramírez F, et al. Monoclonal antibodies against the capsular polysaccharides A, C, Y, W, and X of Neisseria meningitidis: a platform for the quality control of meningococcal vaccines. Methods Mol Biol 2019;1969:181–203.
[2] Parikh SR, Campbell H, Gray SJ, Beebeejaun K, Ribeiro S, Borrow R, et al. Epidemiology, clinical presentation, risk factors, intensive care admission and outcomes of invasive meningococcal disease in England, 2010-2015. Vaccine 2018;36(26):3876–81.
[3] Meningococcal meningitis [internet]. World Health Organisation. Available from: https://www.who.int/news-room/fact-sheets/detail/meningococcal-meningitis, ; 19 Feb 2018.
[4] Basta NE, Berthe A, Keita M, Onwuchekwa U, Tamboura B, Traore A, et al. Meningococcal carriage within households in the African meningitis belt: a longitudinal pilot study. J Infect 2018;76(2):140–8.
[5] Peterson ME, Li Y, Mile R, Shanks H, Nair H, Kyaw M. Meningococcal carriage in the European Union from 200W 2016: a systematic review and meta-analysis, vol. 66. Revue D Epidemiologie Et De Sante Publique; 2018.
[6] Breakwell L, Whaley M, Khan UI, Bandy U, Alexander-Scott N, Dupont L, et al. Meningococcal carriage among a university student population - United States, 2015. Vaccine 2018;36(1):29–35.
[7] Peschiera I, Giuliani M, Giusti F, Melero R, Paccagnini E, Donnarumma D, et al. Structural basis for cooperativity of human monoclonal antibodies to meningococcal factor H-binding protein. Commun Biol 2019;2:241.
[8] Cooper LV, Robson A, Trotter CL, Aseffa A, Collard JM, Daugla DM, et al. Risk factors for acquisition of meningococcal carriage in the African meningitis belt. Trop Med Int Health 2019;24(4):392–400.
[9] Oldfield NJ, Cayrou C, AlJannat MAK, Al-Rubaiawi AAA, Green LR, Dada S, et al. Rise in group W meningococcal carriage in university students, United Kingdom. Emerg Infect Dis 2017;23(6):1009–11.
[10] Watt JP, O'Brien KL, Katz S, Bronsdon MA, Elliott J, Dallas J, et al. Nasopharyngeal versus oropharyngeal sampling for detection of pneumococcal carriage in adults. J Clin Microbiol 2004;42(11):4974–6.
[11] Moreno J, Hidalgo M, Duarte C, Sanabria O, Gabastou JM, Ibarz-Pavon AB. Characterization of carriage isolates of Neisseria meningitides in the adolescents and young adults population of Bogota (Colombia). PLoS One 2015;10(8):e0135497.
[12] Sadeghi M, Ahmadrajabi R, Dehesh T, Saffari F. Prevalence of meningococcal carriage among male university students living in dormitories in Kerman, southeast of Iran. Pathog Glob Health 2018;112(6):329–33.

[13] Honaarvar SH, Eslaminezhad Z. Study of alteration of meningococcal oropharyngeal carrier rate among students before and after lodging in dormitory and first serogrouping of some of isolated strains in Kerman. Med J Tabriz Univ Med Sci 2006;28(2):119–23.
[14] Karapınar BA, Yürüyen C, Gürler N, Kayacan Ç. Nasopharyngeal carriage of neisseria meningitidis among medical school students in Turkey. Biomed Res 2019;30(1).
[15] Steurer LM, Hetzmannseder M, Willinger B, Starzengruber P, Mikula C, Kormann-Klement A, et al. Pharyngeal carriage rates of Neisseria meningitidis in health care professionals at a tertiary university pediatric hospital. Eur J Clin Microbiol Infect Dis 2020;39(9):1703–9.
[16] Durey A, Bae SM, Lee HJ, Nah SY, Kim M, Baek JH, et al. Carriage rates and serogroups of Neisseria meningitidis among freshmen in a University dormitory in Korea. Yonsei Med J 2012;53(4):742–7.
[17] Kamiya H, Takahashi H, Sunagawa T, Oishi K, Ohnishi M. Unexpected high carriage rate of Neisseria meningitidis among dormitory residences in Tokyo, Japan, vol. 2. Open Forum Infectious Diseases; 2015. suppl. 1.
[18] Caugant DA, Maiden MCJ. Meningococcal carriage and disease–population biology and evolution. Vaccine 2009;27(Suppl. 2):B64–70 (4).
[19] Díaz J, Cárcamo M, Seoane M, Pidal P, Cavada G, Puentes R, et al. Prevalence of meningococcal carriage in children and adolescents aged 10-19 years in Chile in 2013. J Infect Publ Health 2016;9(4):506–15.
[20] Drayß M, Claus H, Hubert K, Thiel K, Berger A, Sing A, et al. Asymptomatic carriage of Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumoniae, Group A Streptococcus and Staphylococcus aureus among adults aged 65 years and older. PLoS One 2019;14(2).
[21] Nunes AMPB, Ribeiro GS, Ferreira IE, Moura ARSS, Felzemburgh RDM, De Lemos APS, et al. Meningococcal carriage among adolescents after mass meningococcal c conjugate vaccination campaigns in Salvador, Brazil. PLoS One 2016;11(11).
[22] Husain EH, Dashti AA, Electricwala QY, Abdulsamad AM, Al-Sayegh S. Absence of neisseria meningitidis from throat swabs of Kuwaiti pilgrims after returning from the Hajj. Med Principles Pract 2010;19(4):321–3.
[23] Jordens JZ, Williams JN, Jones GR, Heckels JE. Detection of meningococcal carriage by culture and PCR of throat swabs and mouth gargles. J Clin Microbiol 2002;40(1):75–9.