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ORIGINAL RESEARCH

Colonization Rate and Associated Factors of Non-Pathogenic *Neisseria* Species, and *Moraxella catarrhalis* Among Healthy School Children in Gondar, Northwest Ethiopia

Teshome Belachew[1], Muluneh Assefa[1], Zelalem Tefera[2], Andualem Fenta[3], Sirak Biset[1]

[1]Department of Medical Microbiology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia; [2]Kemissie General Hospital, Kemise, Ethiopia; [3]Tibebe Ghion Specialized Hospital, Bahir Dar, Ethiopia

Correspondence: Sirak Biset, Tel +251-911-598-568, Email serbis33@gmail.com

Background: Although commensal *Neisseria* species inhabiting mucosal surfaces in the upper respiratory tract (URT) are rarely associated with infections, their presence in the area has been linked to the development of immunity against *N. meningitidis* and the source of antibiotic resistance determinants in pathogenic species. *M. catarrhalis* in the oropharynx of children is also a predisposing factor for otitis media. As a result, determining the oropharyngeal carriage rate of these commensal species and associated factors among healthy schoolchildren is substantial.

Materials and Methods: This community-based cross-sectional study was conducted in Gondar, Northwest Ethiopia, from January to April 2019. A multi-stage and simple random sampling technique were used to select schools and participants, respectively. A total of 524 oropharyngeal swabs were collected using cotton swabs. Modified Thayer-Martin media was used for primary bacterial isolation, and battery of biochemical tests was performed to identify species. For frequencies, descriptive statistics were computed and the logistic regression model was used to see the relationship between dependent and independent variables.

Results: A total of 524 healthy schoolchildren with a mean age of 12.2 ± 2.74 years participated in this study. The overall oropharyngeal carriage rate was 21.8% (114/524). Of these, *N. meningitidis*, *N. lactamica*, *N. sicca*, and *M. catarrhalis* were identified in 53 (46.5%), 14 (12.3%), 11 (9.6%), and 36 (31.6%) children, respectively. The culture positivity rate was higher at a younger age, which was 8.1%, 11.3%, and 14.9% in ages between 15–18, 11–14, and 7–10, respectively. The oropharyngeal carriage was significantly associated with the number of students per class (>40).

Conclusion: There is a higher proportion of carriers of commensal *N. lactamica* and *M. catarrhalis* in Gondar town schoolchildren. The oropharyngeal carriage rate was associated with a crowded classroom. The characterization of non-pathogenic *Neisseria* species and *M. catarrhalis* in the study area can support the diagnosis of patients suspected of having *N. meningitis* infections.

Keywords: *N. lactamica*, *N. sicca*, *Moraxella catarrhalis*, schoolchildren, Gondar

Background

Bacterial species in *Neisseria* and *Moraxella* genera are Gram-negative and oxidase-positive diplococci that usually colonizes the upper respiratory tract (URT).[1] Pharyngeal carriage of these bacteria has been considered as a prerequisite for the development of invasive meningococcal disease and otitis media, respectively.[2] The members of the *Neisseria* genus are frequently carried asymptomatically in the nasopharynx, while gonococcal infection of the urogenital tract usually elicits a marked local inflammatory.[3] *N. lactamica* is one of the non-pathogenic *Neisseria* species, which dominantly colonizes the oropharynx of young children, and rarely, adults. However, the colonization rate decreases with age.[4–6]

Although, *N. lactamica* lacks a disease-causing capsule and the outer-membrane protein PorA, it can exhibit striking antigenic similarities with *N. meningitidis* that develops cross-protective immunity against *N. meningitidis* in carriers. Therefore, childhood colonization with *N. lactamica* will protect against *N. meningitidis* through natural immunity.[7,8]

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Moreover, the carriage and high level of genetic diversity in *N. lactamica* may facilitate the design of vaccines for meningococcus.[9] The carriage rate varies between 5.0% (in adolescents, 14–17 years old) and 20.0% (during childhood, particularly between 1 and 2 years after birth). Alternatively, the carriage of *N. meningitidis* increases from birth and peaks in 15–19 year-olds, and then drops with age.[4]

In recent researches, the commensal *Neisseria* species are thought to be reservoirs of antibiotic resistance and virulence genes for pathogenic *Neisseria* species.[10] Evidences showed drug-resistant *N. meningitidis* can acquire mutated *gyrA* alleles and *penA* genes from a commensal *N. lactamica* through horizontal gene transfer.[11,12] The presence of capsule genes in nonpathogenic *Neisseria* species and the acquisition of these genes by some genotypes of *N. meningitidis* has increased their pathogenicity.[13] The nonpathogenic commensal *Neisseria* can also cause opportunistic invasive disease in individuals with immunosuppression or indwelling prosthetic material.[14]

M. catarrhalis is also part of the commensal species in the URT of approximately 7.0% to 36.0% of healthy children but decreases substantially in adulthood. Otitis media is an infection of the middle ear in children that is closely related to the colonization of *M. catarrhalis*. Therefore, colonization of *M. catarrhalis* in healthy carriers may be a predisposing factor for otitis media.[15,16] In the study area and Ethiopia at large, information about the frequency of nonpathogenic *Neisseria* species and *M. catarrhalis* among Schoolchildren is limited. Therefore, this study aimed to determine the carriage rate of *N. lactamica*, *N. sicca*, and *M. catarrhalis* among healthy schoolchildren in Gondar, Northwest Ethiopia.

Materials and Methods

Study Area, Design, and Population

A community-based cross-sectional study was conducted among healthy schoolchildren in Gondar, Northwest Ethiopia, from January to March 2019. The study included healthy primary school children from six different schools in Gondar (Table 1). Gondar is in the central Gondar administrative zone, Amhara Region, northwest Ethiopia. The town has a total projected population of 323,900.[17] Gondar and its surroundings have 44 elementary and 11 secondary schools, including preparatory governmental schools. At the time of enrolment, subjects aged 7–18 years were stratified by age into three groups, such as 7–10 years, 11–14 years, and 15–18 years.

Operational Definitions

Primary school was defined as typically the first stage of formal education, coming after preschool and before secondary education. The first two grades of primary school, grades 1 and 2, are also part of early childhood education. Primary education usually takes place in a primary school or elementary school.[18]

Sample Size and Sampling Technique

A multi-stage sampling technique was used to select six schools among forty-four elementary schools in Gondar town and its surroundings. A total of 524 study participants were recruited from selected schools using a random sampling technique. To accomplish this, we first proportionally distributed the number of participants among the schools based on

Table 1 List of Selected Elementary Schools and Number of Selected Students in Gondar Town

S. No	Elementary School	Students per School	Proportion	Proportionally Allocated Number of Students per School	Actual Number of Students That Sample Was Taken
1	Abiwot fire	2010	23.4	128	120
2	Hibret	1090	12.7	70	66
3	Atse Bekafa	1146	13.2	73	64
4	TsadikuYohanis	1450	16.8	92	92
5	Meseret	1512	17.6	97	94
6	Chechela	1400	16.3	90	88
Total		8608	100	550	524

the total number of students in each school. To make the sampling process more representative, we stratified each school into grades (Grade 1–Grade 8) and their sections, and then proportionally assigned the number of participants in each school to these sub sections. Finally, we selected the study participants from each section by using a simple random sampling technique (Figure 1).

Data Collection and Laboratory Methods

Data were collected using a semi-structured questionnaire. A face-to-face interview was carried out to collect socio-demographic characteristics and other relevant information by a trained laboratory technologist either at school or at the children's parents' home.

Specimen Collection and Transportation

Plain cotton swabs (Unison Narula, India) were used to collect oropharyngeal swabs. This was done by rolling the moistened swab at the posterior pharyngeal wall behind the uvula and tonsils. After collection, samples were immediately transported to the University of Gondar Comprehensive Specialized Referral Hospital Microbiology Laboratory using Amies transport media (Bio Mark, India) with an ice box within two hours of collection.

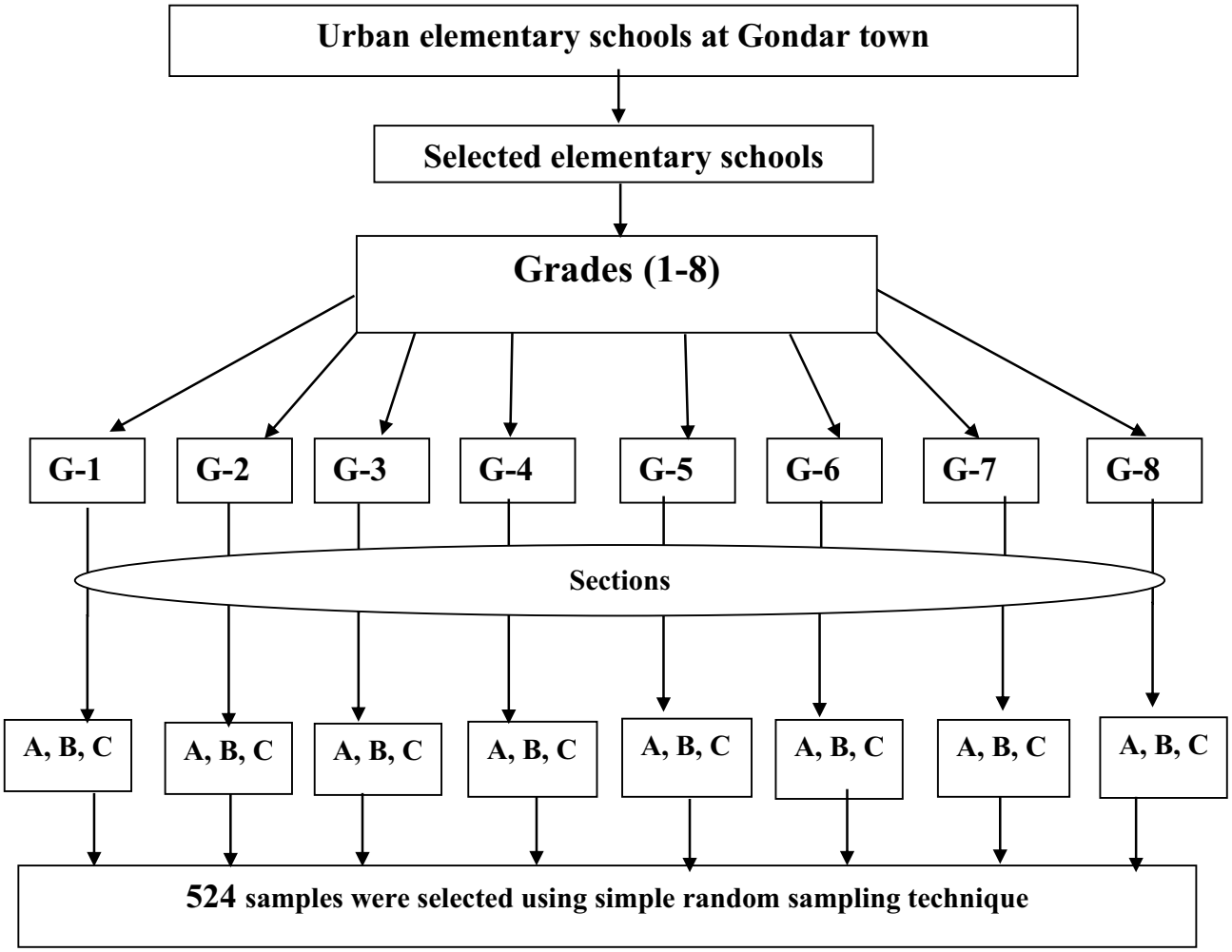

Figure 1 Sampling technique.

Bacterial Identification

Oropharyngeal swabs were inoculated on Modified Thayer-Martin culture media (Oxoid Ltd., Basingstoke, UK). The inoculated plates were incubated at 37°C with 5–10% carbon dioxide for 24 to 48 hours. A primary inspection was performed by gram stain and colony characterization on culture. Then, the isolates were confirmed by the oxidase test (Deben Diagnostics Ltd., UK) and fermentation of carbohydrates such as glucose, maltose, lactose, and sucrose using cystine trypticase agar (SRL, India). The bacterial species identification was illustrated as the following: *N. meningitidis*, if gram-negative diplococci, oxidase-positive, glucose fermenter, maltose fermenter, and lactose and sucrose non-fermenter; *N. lactamica*, if glucose fermenter, lactose fermenter, maltose fermenter, and sucrose non-fermenter; *N. sicca*, if glucose fermenter, maltose fermenter, lactose fermenter, and sucrose non-fermenter; *M. catarrhalis*, if non-fermenter to all four tested carbohydrates.

Laboratory Quality Control

Quality assurance was maintained while undertaking the laboratory procedures. Standard Operating Procedures (SOPs) were strictly followed during collection, transportation and processing of samples, and all stages of the laboratory work. The reagents and chemicals were checked by performing a quality control test using known *N. meningitidis* ATCC strains (ATCC13090) as a positive control. The manufacturer's instructions and microbiological standard procedures involved during culture media preparation, bacterial identification, and reading and reporting of results were strictly followed. The sterility and performance of prepared culture media were checked by incubating 5% of the batch at 35–37°C overnight and inoculating standard strains, respectively.

Statistical Analysis

Data were entered and analyzed using EPI info version 7 and Statistical Package for Social Science (SPSS) version 20.0 (IBM-SPSS Inc., Chicago, IL, USA), respectively. Descriptive statistics were tabulated to calculate frequencies. Tables and figures were used to present the findings. The association between risk factors and colonization of nonpathogenic *Neisseria* species and *M. catarrhalis* was assessed using bivariate and multivariate analysis. A p-value <0.05 at a 95% confidence interval was considered statistically significant.

Ethical Consideration

Ethical clearance was obtained from the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences University of Gondar ethical review committee (Ref No-SBMLS/2123/11). Assent and consent were obtained from students and their parents/guardians, respectively. The participants were well informed about the objectives of the study and had full right to withdraw at any time from the study. The collected data and specimen were used for the research purpose only. We conducted the study following the Declaration of Helsinki.[19]

Results

Socio-Demographic Characteristics of the Study Participants

Overall, 524 school children (283 males and 241 females) were enrolled in this study. Their mean age was 12.2 ± 2.74 years. Nearly half of them, 257/524 (49%), were within the age group of 11–14 years, whereas 119/524 (22.7%) were in the age group of 15–18 years. Most of the subjects (514/524) (98.1%) lived in urban areas, and 271/524 (51.7%) were 5–8 grade students (Table 2).

Oropharyngeal Carriage Rate

The overall carriage rate in this study was 21.8% (114/524). Of these, 53/114 (46.5%) were *N. meningitidis* and 61/114 (53.5%) were nonpathogenic *Neisseria* species and *M. catarrhalis* species. Among nonpathogenic *Neisseria* species, *N. lactamica* accounted for the largest proportion, 14/114 (12.3%), whereas *N. sicca* was accounted for 11/114 (9.6%) of the isolates. About 36 of 114 (31.6%) isolates were *M. catarrhalis* (Figure 2). The culture positivity rate in oropharyngeal

Table 2 Socio-Demographic Characteristics of Primary School Children in Gondar Town

Variables		Frequency (%)	Culture Result		Chi-Square
			Positive (%)	Negative (%)	
Age	7–10	148 (28.2)	22 (14.9)	126 (85.1)	0.254
	11–14	257 (49.0)	29 (11.3)	228 (88.7)	
	15–18	119 (22.7)	10 (8.1)	109 (91.6)	
Sex	Male	283 (54.0)	31 (11.0)	252 (89.0)	0.682
	Female	241 (46.0)	30 (12.4)	211 (87.6)	
Residence	Urban	514 (98.1)	60 (11.7)	454 (88.3)	1.000
	Rural	10 (1.9)	1 (10.0)	9 (90.0)	
School	Abiwot fire	120 (22.9)	12 (10.0)	108 (90.0)	0.016
	Atse Bekafa	64 (12.2)	13 (20.3)	51 (79.7)	
	Chechela	88 (16.8)	3 (3.4)	85 (96.6)	
	Hibret	66 (12.6)	7 (10.6)	59 (89.4)	
	Meseret	94 (17.9)	10 (10.6)	84 (89.4)	
	Tsadiku Yohannes	92 (17.6)	16 (17.4)	76 (82.6)	
Grade level	1–4	253 (48.3)	30 (11.9)	223 (88.1)	0.892
	5–8	271 (51.7)	31 (11.4)	240 (88.6)	

swabs was higher at a younger age, which was 8.1%, 11.3%, and 14.9% in ages between 15 and 18, 11 and 14, and 7 and 10 years, respectively (Table 2).

A crowded classroom (p = 0.008), being less than 5-years-old (p = 0.040), and students sleeping with their parents (p = 0.014) were associated with positive culture (Table 3). There were no significant differences detected in the carriage rate of *N. lactamica*, N. *sicca*, and *M. catarrhalis* with sex, school type, and grade level of students. However, carriage rate was increased with younger age (Table 4).

Association of Risk Factors and Oropharyngeal Carriage

There was no significant relationship between oropharyngeal carriage and children younger than 5 years old (AOR: 0.617, 95% CI: 0.344–1.107, p = 0.106), or student sleep with parents (AOR: 0.057, 95% CI: 0.248–1.019, p = 0.057), according to the multivariable logistic statistical analysis. Oropharyngeal carriage had a significant association with the number of students per classroom (AOR: 0.099, 95% CI: 0.013–0.733, p = 0.024) (Tables 5).

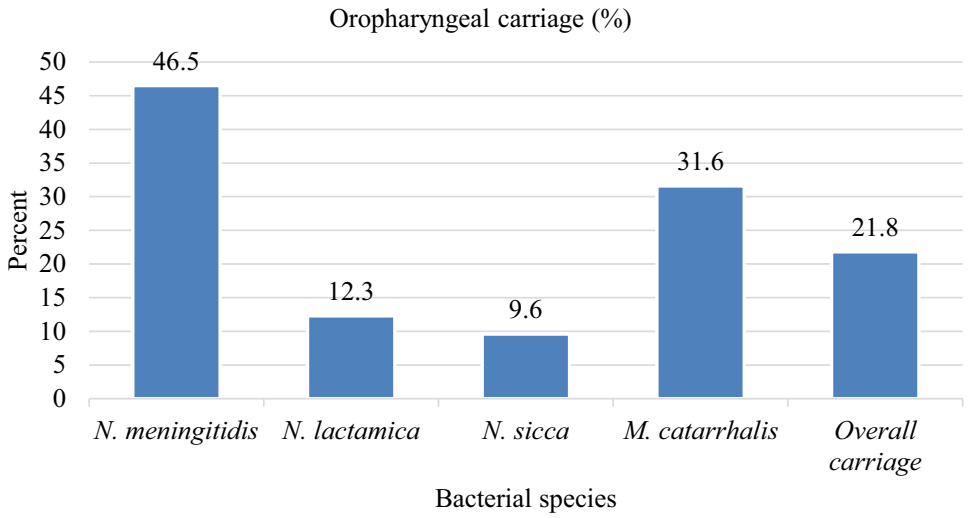

Figure 2 The distribution of bacteria isolated from oropharyngeal swab.

Table 3 Family and Other Clinical Characteristics

Variables		Frequency (%)	Culture Result		Chi-Square
			Positive (%)	Negative (%)	
No of student per class	≤40	58 (11.1)	1 (1.7)	57 (98.35)	0.008
	>40	466 (88.9)	60 (12.9)	406 (87.1)	
Family occupation	Employee	206 (39.3)	18 (8.7)	188 (91.3)	0.269
	Merchant	200 (38.2)	30 (15.0)	170 (85.0)	
	Daily laborer	83 (15.8)	9 (10.8)	74 (89.2)	
	Farmer	35 (6.7)	4 (11.4)	31 (88.6)	
Family education	Not read and write	69 (13.2)	6 (8.7)	63 (91.3)	0.264
	Read and write	145 (27.7)	19 (13.1)	126 (86.9)	
	Primary school	130 (24.8)	20 (15.4)	110 (84.6)	
	≥ Secondary school	180 (34.4)	16 (8.9)	164 (91.1)	
Family size	≤5	315 (60.1)	35 (11.1)	280 (88.9)	0.677
	>5	209 (39.9)	26 (12.4)	283 (87.6)	
< 5 years old child	Yes	241 (46.0)	36 (14.9)	205 (85.1)	0.040
	No	283 (54.0)	25 (8.8)	258 (91.2)	
Student sleep with parents	Yes	346 (66.0)	49 (14.2)	297 (85.8)	0.014
	No	178 (34.0)	12 (6.7)	166 (93.3)	
Living in crowded areas	Yes	153 (29.2)	19 (12.4)	134 (87.6)	0.765
	No	371 (70.8)	42 (11.3)	329 (88.7)	
Tonsillectomy	Yes	317 (60.5)	33 (10.4)	284 (89.6)	0.329
	No	207 (39.5)	28 (13.5)	179 (86.5)	
Hospitalization	Yes	110 (21)	15 (13.6)	95 (86.4)	0.503
	No	414 (79)	46 (11.1)	368 (88.4)	
Sharing Utensils	Yes	397 (75.8)	48 (12.1)	349 (87.9)	0.636
	No	127 (24.2)	13 (10.2)	114 (89.8)	

Table 4 Distribution of Bacterial Isolates by Age, Sex, School, and Grade Level Among Primary School Children in Gondar Town

Variables		Isolated Bacteria			Total (%)
		N. lactamica (%)	*N. sicca* (%)	*M. catarrhalis* (%)	
Age	7–10 (n = 148)	7 (4.7)	5 (3.4)	10 (6.8)	22 (14.9)
	11–14 (n = 257)	6 (2.3)	3 (1.2)	20 (7.8)	29 (11.3)
	15–18 (n = 119)	1 (0.8)	3 (2.5)	6 (5.0)	10 (8.1)
Sex	Male (n = 283)	7 (2.5)	4 (1.4)	20 (7.1)	31 (11.0)
	Female (n = 241)	7 (2.9)	7 (2.9)	16 (6.6)	30 (12.4)
School	Abiwot fire (n = 120)	3 (2.5)	2 (1.7)	7 (5.8)	12 (10.0)
	Atse Bekafa (n = 64)	6 (9.4)	1 (1.6)	6 (9.4)	13 (20.3)
	Chechela (n = 88)	0	0	3 (3.4)	3 (3.4)
	Hibret (n = 66)	1 (1.5)	1 (1.5)	5 (7.6)	7 (10.6)
	Meseret (n = 94)	2 (2.1)	3 (3.2	5 (5.3)	10 (10.6)
	Tsadiku Yohannes (n = 92)	2 (2.2)	4 (4.3)	10 (10.9)	16 (17.4)
Grade level	1–4 (n = 253)	8 (3.2)	7 (2.8)	15 (5.9)	30 (11.9)
	5–8 (n = 271)	6 (2.2)	4 (1.5)	21 (7.7)	31 (11.4)
Total (n = 524)		14 (2.67)	11 (2.1)	36 (6.9)	61 (11.6)

Table 5 Multivariate Analysis of Factors Associated with Oropharyngeal Carriage

Variables		Culture Result		COR (95% CI)	AOR (95% CI)	*p*-value
		Positive (%)	Negative (%)			
No of student per class	≤40	1 (1.7)	57 (98.35)	1	1	0.024*
	>40	60 (12.9)	406 (87.1)	0.199 (0.016–0.873)	0.099 (0.013–0.733)	
Children < 5 years	Yes	36 (14.9)	205 (85.1)	0.552 (0.321–0.949)	0.617 (0.344–1.107)	0.106
	No	25 (8.8)	258 (91.2)	1	1	
Student sleep with parents	Yes	49 (14.2)	297 (85.8)	0.437 (0.227–0.847)	1	0.057
	No	12 (6.7)	166 (93.3)	1	0.057 (0.248–1.019)	

Note: *Indicates the association is statistically significant.
Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; COR, crude odds ratio.

Discussion

Neisseria lactamica is a true commensal bacterium occupying the same habitat as the pathogenic *N. meningitidis*, which causes various opportunistic infections such as ear infection, arthritis, meningitis, and septicemia, especially in sub-Saharan Africa.[14,20–22] Similarly, *N. sicca* causes fatal infective endocarditis in intravenous drug users and patients with underlying heart disease, resulting in prolonged fever, concurrent embolism, and destruction of the valve.[23,24] In case reports, *N. sicca* caused conjunctivitis and corneal involvement in immunocompetent hosts with no previous ocular history.[25] *M. catarrhalis* is normally found in the nasopharynx of children but can cause diseases of the upper respiratory tract, including sinusitis, laryngitis, and acute otitis media in children.[26] This study was part of a large meningococcal carriage study, aimed to determine *N. meningitidis*, N. *lactamica*, N. *sicca*, and *M. catarrhalis* in healthy primary school children.

In this study, the overall oropharyngeal carriage rate among schoolchildren was 21.8%, with the most prevalent being *N. meningitidis* (46.5%), followed by *M. catarrhalis* (31.6%), *N. lactamica* (12.3%) and *N. sicca* (9.6%). Similarly, a study from Iran reported that *M. catarrhalis* (42.7%), *N. lactamica* (21.9%), and *N. sicca* (7.8%), as the most common bacteria isolated.[27] A study from Paraguay reported a 16.6% of carriage rate of commensal *Neisseria* species obtained from 334 samples, with *N. sicca* (0.6%) and *N. lactamica* (2.2%).[6] Another study conducted on Iranian children also showed lower colonization with *M. catarrhalis* (13.5%) in the oropharynx of 296 healthy children.[28] The high colonization rate of *M. catarrhalis* in the oropharynx is associated with an increased risk of otitis media.[29]

In contrast to our finding, Pourmand et al reported a 3.0% *N. lactamica* carriage among 364 healthy children in Tehran.[8] The most frequent *Neisseria* species in African study was *N. lactamica* (5.6%).[30] A study in Turkey indicated that *N. lactamica* carriage was 1.3%, which was twofold higher than *N. meningitidis* carriage.[31] A study conducted in Chile reported that *N. lactamica* and *N. sicca* were isolated in 65.2% and 5.6%, respectively.[32] The carrier state of nonpathogenic *Neisseria* species can impair the attachment of *N. meningitidis* to oroepithelial cells or competing for the same ecological niche and induce cross-protection by stimulating the immune response of the host.[4,33,34] But they can transfer virulence and drug-resistant genes to pathogenic *Neisseria* species and cause invasive opportunistic infections.[14] The variation in the oropharyngeal carriage rate among studies is related to the age, site of colonization and sampling, physiology of bacteria, and host immune system. Low prevalence in commensal *Neisseria* species is due to their susceptibility to environmental changes that may be lost in the process of diagnosis.[27]

In our study, the carriage of *N. lactamica*, N. *sicca*, and *M. catarrhalis* was increased with younger age. *N. lactamica* was isolated in 0.8% at 15–18 years, 2.3% at 11–14 years, and 4.7% at 7–10 years of age. This finding is consistent with a research group in Burkina Faso that detected *N. lactamica* in 18.2% of oropharyngeal samples and the carriage prevalence was highest among the 2-year-old (40.1%) and decreased with age.[35] Similarly, a study in Danbury evaluated carriage rates of two *Neisseria* species in healthy infants and children and the prevalence of *N. lactamica* carriage was 3.8% at 3 months of age, peaked at 21.0% at 18 months of age, and declined to 1.8% by early adolescence.[36] A study from Iran also reported *N. lactamica* (21.9%) in the age

group of one to nine years.[27] Another study reported 4.5% carriage prevalence of *N. lactamica* in 11–19 years old students in Salvador, Brazil.[37] A 3.0% *N. lactamica* carriage was reported among healthy children aged 10–12 years old in Tehran.[8] Moreover, another study demonstrated that a 13.5% *M. catarrhalis* was isolated in healthy children aged 2–6 years old.[28] This showed that the studied bacterial species are colonized commonly in the oropharynx of young children and the colonization rate decreases with age increase.

Several studies described factors related to the carriage rate of *Neisseria* species and *M. catarrhalis*, such as age, sex, season, socioeconomic status, living in crowded places, smoking, and drinking alcohol regularly have a higher carriage rate.[6,38–41] Although there was no significant association between the oropharyngeal carriage and most factors in our study, a higher number of students in the class (greater than 40) was significantly associated with the carriage rate of *N. lactamica*, N. *sicca*, and *M. catarrhalis*. In the present study, strain identification and molecular-based analysis to show the genetic diversity and mutations in commensals were not performed due to the limited resource. We did not perform drug susceptibility of commensals whose drug-resistance genes may transfer to *N. meningitidis*.

Conclusion

In this study, there was a higher proportion of oropharyngeal carriers of *N. lactamica*, and *M. catarrhalis* among school children. The carriage of *N. lactamica*, N. *sicca*, and *M. catarrhalis* was increased with younger age. The increase in carriage rate was associated with the high number of students or crowded classroom. A detailed molecular study for characterization of the genomic exchange between commensal and pathogenic *Neisseria* species might be helpful for further understanding of the association of commensal *Neisseria* species and *N. meningitidis* drug resistance and future vaccine development against meningococcal disease while using potential antigens of commensal *Neisseria* species.

Abbreviations

ATCC, American type culture collection; SPSS, Statistical Package for Social Science; URT, Upper respiratory tract.

Data Sharing Statement

All relevant data are within the manuscript.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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