Carriage Rate of Neisseria meningitidis, Antibiotic Susceptibility Pattern and Associated Risk Factors among Primary School Children in Gondar town, Northwest Ethiopia

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Research article

Keywords: Neisseria meningitidis, meningococcal disease

Posted Date: July 23rd, 2019

DOI: https://doi.org/10.21203/rs.2.11842/v1

Version of Record: A version of this preprint was published at BMC Infectious Diseases on May 20th, 2020. See the published version at https://doi.org/10.1186/s12879-020-05080-w.

Abstract

Background Invasive meningococcal disease has 70–80% mortality. Globally, 1.2 million estimated cases were reported with ~135,000 deaths annually. In African meningitis belt bacterial meningitis remains a serious threat to health accounting an estimated 500,000 cases of invasive meningococcal disease annually.In Ethiopia, specifically in our study area, limited information is found on the oropharyngeal carriage, antimicrobial resistance pattern and associated risk factors for N. meningitidis especially in school children. So, the aim of this study was to assess oropharyngeal carriage rate of N. meningitidis, antibiotic susceptibility pattern and associated risk factors among primary school children in Gondar town, Northwest Ethiopia. Methods A cross sectional prospective study was conducted from January-April, 2019 among primary school children. Multi stage simple random sampling technique was used. A total of 524 oropharyngeal swabs were collected using sterile plastic cotton swabs. Modified Thayer Martin media was used for primary inoculation. Antimicrobial susceptibility pattern was done on Muller-Hinton agar supplemented with 5% sheep blood. Logistic regression model was used to see the association between dependent and independent variables. P ≤0.05 at 95% CI was considered as statistically significant. Results A total of 53(10.1%) (CI: 7.6-12.8) N. meningitidis isolates were identified. Serogroup A 13 (24.5%) was the most prevalent followed by Y/W135 11(20.7%) whereas serogroup B 4(7.6%) was the least identified. Meningococcal isolates were resistant to ciprofloxacin (45.3%) and trimethoprim-sulfamethoxazole (73.6%). Overall, most of meningococcal isolates showed high level of multidrug resistance with the rate of 32(60.4%). Meningococcal carriage rate was associated with family size, tonsillectomy, passive smoking, number of students per class, sharing utensils, history of visiting healthcare institutions and indoor kitchen. Conclusion Neisseria meningitidis prevalence in the present study was high. Serogroup A and W135/Y was the most prevalent isolate. High multidrug resistance pattern was observed.

Background

Neisseria meningitidis is an obligate human pathogen of the genus *Neisseria.* It is gram-negative, non-motile, capsulated, kidney-shaped intracellular diplococci with peculiar characteristics of glucose and maltose fermentation, moreover, the pathogen is catalase and oxidase-positive[1]. It is classified into 13 serogroups based on capsular polysaccharides, however, the most important serogroups associated with 90% of disease in humans are A, B, C, X, Y, and W135 [2].

Neisseria meningitidis can pass a multistep process to cause disease, as colonization starts with adhesion to the epithelial cell layer of nasopharynx, then invade the bloodstream, evade the immune system, adhere to the endothelial cell layer of the brain vessels, cross the blood-brain barrier and replicate in the cerebral spinal fluid (CSF) of the subarachnoid space with subsequent replication in human CSF[3]. The ability to bind to ligands on the surface of host cells, mediated mainly by the type IV pili allows to easily enter in contact with the endothelial cell layer of the brain vessels and to form micro colonies. *Neisseria meningitidis* is associated with generalized sepsis, thrombosis, coagulation, congestion, and vascular leak, leading to extensive necrosis of the skin and surrounding tissues [4].

Neisseria meningitidis presents in the nasopharynx and oropharynx in 5-10% of healthy people (carriers) and during epidemics, the carrier state raises to 70-80% [5]. Though there have been an improved and well-established diagnosis, treatment, prevention, and control mechanisms, Meningococcal disease still remains a major cause of child morbidity and mortality worldwide, the problem is much more evident in developing countries most notably sub-Saharan African countries [6]. Immunological susceptibility, travel, large population displacement, poor living condition, overcrowding, housing condition and climatic condition are among risk factors that led carriage and infection with *Neisseria meningitides* [7].

Meningococcal infection is a global problem occurring as sporadic, hyper-sporadic, and epidemic disease. There were an estimated 1.2 million cases of meningococcal infection per year, with ~135,000 deaths worldwide. Some countries rarely experience epidemics with high attack rates [8]; however, the occurrence varies widely over time and between geographical areas, age groups, and bacterial serogroups. In the United States of America (USA), the incidence rate was now less than one case per 100,000 per year whereas in Europe it accounts ≤2 per 100,000 per year.In Latin America, the overall incidence of meningococcal disease per year varies from less than 0.1 cases per 100,000 to 2 cases per 100,000 [9].

Asian countries reported the different magnitude of meningococcal disease. Research conducted in Bangladesh between 1999 and 2006 showed that *N. meningitidis* was detected in 24.8% of which, 97.7% were serogroup A. The overall prevalence in this continent ranges from 0.02 cases per 100 000 inhabitants in Thailand to 13 cases per 100 000 people in Mongolia [10].

The African meningitis belt is an area of increased risk of bacterial meningitis characterized by distinct seasonal patterns in disease incidence with peaks in the dry season. This region, which stretches from Ethiopia in the East to Senegal and the Gambia in the West, has suffered high morbidity and mortality due to bacterial meningitis for more than a century, though rates have declined in recent years [11].

In Ethiopia, a major epidemic was recorded in 2001with 6964 cases and 330 deaths followed by another epidemic during 2003-2004 with 3326 cases and 160 deaths from all regions of the country [12]. The study conducted in Addis Ababa, Ethiopia showed that the prevalence of *N. meningitidis* among elementary school children was 20.4% [13].

In the study area, limited information is found on the oropharyngeal carriage, antimicrobial resistance pattern and associated risk factors for *N. meningitidis* especially in school children. So, the aim of this study was to assess oropharyngeal carriage rate of *N. meningitidis,* antibiotic susceptibility pattern and associated risk factors among primary school children in Gondar town, Northwest Ethiopia.

Materials And Methods

Study setting, design, population, and sampling techniques

This study was conducted in six primary schools in Gondar town. The town is located at 12°36'N 37°28'E latitude and longitude with an elevation of 2133 meters above sea level. Gondar is the capital of the central Gondar administrative zone, in the Amhara region of Northwest Ethiopia. The town is found 737 km far away from Addis Ababa, the capital city of Ethiopia, and 180 km away from Bahir Dar, the capital of Amhara national regional state. The town has 6 sub-city and25 urban and eleven rural kebeles with a total projected population of 323 900 [14]. Gondar town and its surroundings have 44 elementary schools, 11 secondary schools and 30 kindergartens. A community-based cross-sectional study was conducted among primary school children in Gondar town, North West Ethiopia, from January to April 2019. The sample size (524) was determined by using a single population proportion formula by considering the prevalence of 20.4% [13], with a 95% confidence interval, and a 5% margin of error, with 10% no-response rate and design effect. The multistage sampling technique was used to select schools. Then schools were stratified to grades and sections. The total number of study participants were allocated proportionally to each school, grades and sections based on the school sampling frame and the study subjects were selected by simple random sampling technique (Table 1).

Table 1: List of selected elementary schools and number of selected students in Gondar town, Northwest Ethiopia, January to April, 2019

S.no	Elementary school	Students per school	Proportion	Proportionally allocated number of students per school	number of students taken (sample)
1	Abiwotfire	2010	23.4	128	120
2	Hubret	1090	12.7	70	66
3	AtseBekafa	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

Data collection and laboratory methods

Data collection procedures

A pre-tested questionnaire based on postulated or known risk factors was developed and modified to explore the objectives of the study. Then it was checked on school children who were not included in the study. It was prepared in English and translated to Amharic then translated back into English to check the accuracy of the translation. The questionnaire design included two parts; socio-demographic characteristics and associated risk factors. The questionnaire and assent/consent form were distributed to the selected students at school after informing the purpose of the study and the right of the study

participants. Questionnaire and assent/consent form were also distributed to guardians and emphasis was given to return the questionnaire and assent/consent form after twenty-four hours. Students living alone were considered as adults and informed to fill the questionnaire and sign the consent form at school. Socio-demographic characteristics and other relevant information filled by the parents/guardians and students were collected at school by trained laboratory technologists before sample collection.

Laboratory methods

Oropharyngeal sample collection

Oropharyngeal swabs were collected by a trained medical microbiologist using a plain cotton swab (Unison Narula, India) using tongue depressor (Unison Narula group, India) at the posterior pharyngeal wall behind the uvula and tonsils of each volunteer participant. After collection, samples were transported by using Amies transport media (Bio mark, India) to the University of Gondar teaching hospital laboratory within two hours of collection within a cold box.

Culture and identification

Once the specimens reached to Gondar University teaching laboratory, it was inoculated on Modified Thayer Martin (MTM) culture media (Oxoid, UK). The inoculated MTM plates were incubated at 37°C with 5-10% CO_2 for 24 to 48 hours. A presumptive diagnosis was done by gram stain and colony characteristics on the agar plate. Further confirmation was done by the oxidase test (Deben Diagnostics Ltd, UK). After confirmation, the presence of gram-negative diplococcus with oxidase-positive, isolates were sub-cultured on a blood agar plate (BAP) (oxoid, uk) at 5-10% CO_2 for 24 to 48 hours to guarantee the purity of colonies for the biochemical test. Plates were monitored every 24 hours for the growth of typical colonies.

Carbohydrate utilization test (glucose, maltose, lactose, and sucrose) was performed by cystine trypticase agar (CTA) (SRL, India) to further differentiate *Neisseria meningitides* from *Moraxella* species and other nonpathogenic *Neisseria* species. Isolates with gram-negative diplococci, oxidase-positive, glucose fermenter, maltose fermenter, lactose andsucrose none- fermenter were interpreted and confirmed as *N. meningitidis*. Once the species are known, the serogroup of isolates was determined with the slide agglutination method while using commercially prepared antiserum A, B, C, W135/Y, and X (Bio-Rad, France) and antiserum X (BD Difco, USA). Negative for these six serogroups was classified as none-serogroupable[13]

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out on isolates of *N. meningitidis* by using disc diffusion technique as per the standard Kirby-Bauer method on Mueller-Hinton agar (Bio mark, India) supplemented with 5% sheep blood at 37°c for 18-24 hours [15]. A suspension of the test organism was prepared equivalent to 0.5 McFarland. The surface of Mueller-Hinton agar supplemented with 5% sheep blood was

completely covered by rotating the swab. The plates were allowed to dry for 3-5 minutes; then discs were evenly distributed on the inoculated plate using sterile forceps and incubated in 5-10% CO_2 at 37ºc for 20-24 hours. The following routinely used antimicrobial agents were tested: cefotaxime (30µg), minocycline (30µg) meropenem (10µg), azithromycin (15µg), ciprofloxacin (5µg), trimethoprim-sulfamethoxazole (1.25/ 23.75 µg), chloramphenicol (30µg), and rifampin (5µg). Diameters of the zone of inhibition around the disc was measured to the nearest millimeter using a graduated caliper in millimeters and results were classified as sensitive, intermediate and resistant based on CLSI-2018 guideline[16]. Multidrug resistance was defined as resistance of an isolate to two or more antimicrobial classes tested [17].

Laboratory data quality assurance

The reagents and chemicals were checked by performing quality control test using known *N. meningitidis* ATCC strains (ATCC13090) as a positive control and *E. coli* (ATCC 25922) strain as a negative control. Sterility of the prepared media was checked by incubating 5% of the prepared media at 35-37^{o}C for 18 – 24 hours. The performance of prepared media was also checked by using known *N. meningitidis* ATCC strains (ATCC13090).

Data analysis and interpretation

All data was entered to EPI info version 7 for data clearance and consistency and exported to SPSS version 20.0 for analysis. Descriptive statistics was computed to calculate frequencies. The magnitude of the association between different variables and oropharyngeal meningococcal carriage was assessed using bivariate and multivariate analysis. Variables which had a P- value ≤0.20 for bivariate analysis was taken to multivariate analysis to check real association of meningococcal carriage rate with risk factors and expressed by adjusted odds ratio at 95% confidence interval. A P-value ≤ 0.05 was considered as statistically significant. Data was summarized using numbers, percentages and tables.

Ethical considerations

The study was conducted after obtaining institutional ethical clearance from the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences University of Gondar. The reference number for the ethical paper was “Ref No-SBMLS/2123/11”. A letter of support was also taken from the school of biomedical and laboratory science to Gondar town education department. The education department wrote a letter of support to all selected primary schools to cooperate on the study. Legal permission was obtained from directors of each elementary school prior to data collection. Written assent from the parents/guardians of youth students and assent/consent from the study participants was obtained. The participants recruited to the study were informed about the objectives of the study and their participation was on voluntarily basis and participants had the right to withdraw at any point from the study.

Results

Demographic characteristics of study participants

A total of 524 school children (283 males and 241 females) were included in this study. The mean ± SD age of the participants was 12.2 ± 2.74 years. About 49% of the study participants were within the age group of 11-14 years (Table 2).

Oropharyngeal carriage isolates

The overall prevalence of *Neisseria meningitidis* was 53(10.1%) (95% CI: 7.6, 12.8). Meningococcal carriage identified among male 30/53 (56.6%) was higher than females 23/53 (43.4%) (Table3).

Serogroup distribution of *N. meningitidis*

All types of invasive meningococcal serogroups were identified, of which, serogroup A was the leading isolate with the isolation rate of 13 (24.5%) followed by serogroup Y/W135, 11(20.7%). Serogroup B, 4 (7.5%) was the least isolate. Serogroup A dominates on male (15.1%) than female (9.4%) (Table4).

Antimicrobial susceptibility patterns of *N. meningitidis*

Neisseria. meningitidis isolates were tested for different routinely used antimicrobial agents. In this study, most of the meningococcu*s* isolates showed a high level of resistance to trimethoprim/ sulfamethoxazole (73.6%), ciprofloxacin (45.3%) and cefotaxime (35.8%). However, the majority of the isolates were susceptible to azithromycin (96.2%), chloramphenicol (92.5%) and minocycline (88.7%) (Table5).

Multidrug resistance pattern of *N. meningitidis*

Multidrug resistance pattern of *N. meningitis* isolates was also determined. Overall, most of **the** meningococcal isolates showed **a** high level of multidrug resistance with the rate of 32(60.4%). On the serogroup level, serogroup B was 100% MDR followed by serogroup X, 80% and serogroup Y/W-135, 72.7%. Only 9 (16.9%) of **the** meningococcal isolates had no resistance for all class of antimicrobials tested. Similarly, about 50% of none-serogroupable (NG) isolates had no resistance to **the** tested class of antimicrobials (Table6).

Associated risk factors of study participants

In this study, the average family size of students was 5.3 people per household and the average number of rooms per household was 2.3. From all study participants, 21% had a history of hospitalization at least for one day at different health institutions. Of the total participants, the majority (60.5%) had a history of tonsillectomy. among the study participants who had a history of treatment before two weeks of the study period, 19.3% had poor treatment adherence.

About 10.1% of the family of the study participants smokes cigarettes while 29.2% of the family had a history of living in crowded area (Table7).

Risk factors analysis for oropharyngeal carriage of *N. meningitidis*

Factors associated with oropharyngeal carriage were explored. The prevalence of *N. meningitidis* carriage rate was observed with no significant difference among the age groups and sex. Bivariate and multivariate statistical analysis showed that *N. meningitides* oropharyngeal carriage had a significant association with family size (AOR; 2.71 95% CI 1.41-5.18, P= 0.003), sharing utensils (AOR; 4.15, 95% CI 1.49-11.58, P=0.007), attending healthcare institutions (AOR;2.76, 95% CI 1.098-6.94, P=0.031),history of tonsillectomy (AOR;2.84, 95% CI 1.36-5.93, P=0.006), indoor kitchen (AOR;5.55, 95% CI, 1.53-20.17 P=0.009), parental cigarette smoking (AOR;4.62, 95% CI,1.65-12.89, P=0.004) and number of students per classroom (AOR;7.81, 95% CI, 1.02 59.78,P=0.048) (Tables 8).

Discussion

Meningococcal infection is a global problem occurring as sporadic, hyper-sporadic, and epidemic disease [8]. The problem has mainly occurred in the developing world especially in the African meningitis belt where there has been an increased risk of bacterial meningitis characterized by distinct seasonal patterns in disease incidence with peaks in the dry season [11, 18].

In Ethiopia, meningitis outbreaks have been occurred over several years being responsible for morbidity and mortality [12]. Therefore, this study was intended to show the gap and fill the limited information on the oropharyngeal carriage, antimicrobial resistance pattern and associated risk factors for *N. meningitidis* especially in school children in our study area.

The overall *N. meningitidis* oropharyngeal carriage rate in this study was 10.1%. The predominant serogroup in our study was serogroup A (24.5%) and W135/Y (20.6%) while the least was serogroup B (7.6%). This overall carriage prevalence was markedly higher than a study conducted at Gondar University teaching hospital in 2012 (234 oropharyngeal swabs) among <10 years OPD patients with 6% carriage [19]. The reason for this variation might be due to the difference in the target population, time of investigation and sample size. Our study also had a high prevalence of carriage than the studies conducted in Arba Minch, Southern Ethiopia among 7479 oropharyngeal samples (PCR method) with 6.6% prevalence [20] and in Gurage Zone, Southern Ethiopia with 4.6% carriage rate [21]. On those mentioned studies, no serogroup A was identified and serogroup B was the least identified. The overall difference might be due to the difference in the target population, sample size, type of sample, the method used and an area of investigation done.

In contrast, our study had less carriage rate compared to three local studies conducted at Gondar university hospital (2019 CSF samples) in the year 2011 to 2013 with 18.4% prevalence [22], Addis Ababa (240 nasal swabs) with 20.4% carriage rate [13] and bacterial meningitis surveillance in Ethiopia, 2012–2013 (139 CSF samples with PCR method) with 19.4% prevalence rate [23]. The predominant serogroups

in our study had shown variation with findings in Addis Ababa W-135 [13] and Gondar W-135[23], but in line with the study conducted in Gondar[22]. The variation of the prevalence compared to these three studies might be due to the difference in the type of sample investigated, a period of the investigation, local area, sample size, method used and target population.

Compared to the studies conducted in the African countries, our study had more prevalent isolates than the study conducted in Kaya, Burkina Faso in 2009 (6686 throat swabs) with 6.27% [24]. The variation for this difference might be due to geographic differences, sample size and time of investigation. In contrast, our finding had a less prevalent carriage rate than the study conducted among students in Kano Nigeria, (150 nasal swabs) with 15.3% meningococcal carriage rate [25]. Compared to this study, the predominant serogroup in our study was serogroup A. The overall variation for these differences might be due to sample size and geographic differences.

The prevalence of *N. meningitidis* in our study had a high carriage rate than three studies conducted in Mali, with oropharyngeal carriage of 5%, 7.7%, and 6.9% [11,26, 27] respectively. The predominant serogroup was none-serogroupabable and serogroup A was not identified. The possible reason for this variation might be the geographic difference.

The present study showed a lower carriage rate compared to meningococcal carriage in Dutch adolescents and young adult (1715 oropharyngeal swabs detected by rt-PCR) with 15.6% [28], besides, most common serogroup identified was B. The difference might be due to geographic location, sample size and method used. In contrast; our finding was six times higher prevalence than a study conducted on healthy Dutch children aged 1–19 years (3098 nasal swabs) which had a 1.5% meningococcal prevalence with serogroup B and C were predominant [29]. This variation might be due to the difference in the geographic area and time of investigation.

In this study, meningococcal carriage rate had lower prevalence than the study conducted in the 11-18 year age group with 13.9% prevalence in the United Kingdom [30]. The variation might be due to geographic area differences and method used for identification (ELISA).

The meningococcal carriage rate in our study was higher than the study conducted in Chile in 2013 (4217 throat swabs) from 10-19 years children & adolescents with a 6.5% carriage rate with serogroup B was the predominant [31], this variation might be due to geographic difference, time investigation and sample size. In the present study, meningococcus prevalence was higher than the study conducted in Turkey in 2002 in the age 7-14 years of 1128 primary school children with 6.2% prevalence, with serogroup C more prevalent than others [7]. The possible reason for this variation might be geographic area difference sample size and time of the investigation.

In our study, the prevalence of *N .meningitidis* was in line with the study conducted in Brazil with 9% [32] and 9.9% meningococcal prevalence [33] and, a study conducted in Kashan Iran, with 8.9% carriage [34]. However, our study had two times higher prevalence rate than the study conducted among students aged 11-19 years with a 4.9% meningococcal prevalence in Brazil [35]. The serogroup findings in our study

were different from these study findings. The possible reason for the variation might be due to geographic area differences and methods used.

The antimicrobial susceptibility pattern of *N. meningitis* was determined. In the present study, higher resistance was reported for cefotaxime (35.8%), ciprofloxacin (45.3%) and trimethoprim-sulfamethoxazole (73.6%). The possible reason for increasing resistance was due to the easy accessibility of drugs, the simplicity of taking drugs (oral route of administration) and the use of these antibiotics for a long period of time in the country especially ciprofloxacin and trimethoprim-sulfamethoxazole and on top of that, irrational drug use. The study conducted in Gondar reported the highest resistance rates of *N. meningitidis* against cotrimoxazole (100%) and ceftriaxone (50.0%) [19]. In contrary to our study, in Addis Ababa [13] and Gondar [19], *N. meningitides* was susceptible to ciprofloxacin with the rate of 83.7% and 78.6% respectively. This discrepancy might be due to the difference in the period of investigation.

However, most bacterial isolates were sensitive to azithromycin (96.2%), chloramphenicol (92.5%), minocycline (88.7%) and meropenem (84.9%). This was inconsistent with the study conducted at Gurage zone with minocycline (92%), meropenem (89%) and azithromycin (95%) [21]. The highest sensitivity to azithromycin in the present study might be due to the expensiveness of the cost of the drug that every individual could not access easily, while chloramphenicol might not be routinely administered due to bone marrow depression effect and minocycline is currently not in use in Ethiopia. In the present study, the multidrug resistance pattern of *N. meningitidis* was higher compared to 14.3% in Addis Ababa [13] and 54.5% in Gurage zone Ethiopia [21]. This result noted that *N. meningitides* is developing a resistance to the antibiotics through time.

In the present study, independent variables like living in large family size, sharing drinking and eating utensils, history of tonsillectomy, history of visiting healthcare institutions, indoor cooking, parental cigarette smoking and the number of students per classroom were significantly associated with *N. meningitidis* oropharyngeal carriage.

In our study, history of students with tonsillectomy is significantly associated with meningococcal carriage (P = 0.006). Because of removal of uvula decreases the effectiveness of innate immunity around the oropharynx. Uvula helping to recognize mucosal surface antigens, like bacteria, virus and fungal elements and is the immune response to clear them from the body. Another risk factor that significantly associated with meningococcal carriage in this study was large family size living together (P = 0.003). In different studies, the number of family members [34], crowded living condition [13, 36], the number of children living in the house [31], the number of positive household members [33], lower socioeconomic status [30] and overcrowding in the house [35] were significantly associated risk factors in which coincided with our study finding. As family size increase per house, it leads to low socioeconomic condition that results in low immunity and overcrowding by itself promotes a high rate of respiratory tract infection transmission.

In the present study, parental cigarette smoking was identified as a risk factor for the colonization of *N. meningitidis* (P = 0.004). A study conducted in Chile among children and adolescents aged 10-19 identified the number of smoking cohabitants and smoking were independent risk factors for oropharyngeal colonization of *N. meningitides* [31]. In another study, participants mothers or siblings smoked cigarettes [36] and passive smoking [33] were identified as significant risk factors for colonization. Active and passive smoking damages the upper layer of the mucosal surface of the respiratory tract, which favors the colonization of bacteria.

In our study, another identified risk factor for meningococcal colonization was; the study participants history of visiting health care institutions (P = 0.031) and number of students per class greater than 40 (overcrowding) (P = 0.048). In different studies, attending in areas that people visited crowded area was stated as risk factor for meningococcal carriage. A study conducted in Dutch describe youth-club visits for 13 hours per week and discotheque visits for 13 hours per week [29] and a study from Brazil, attendance of night clubs and learning at public school [33], a study from Wales, social gatherings and number of students per class [37] were significant risk factors for the colonization of *N. meningitidis*. Attending in areas that people gathering is an important factor for the transmission of respiratory transmitted infections. It might be due to a large number of students in one class room makes them more frequent contact among each other, overcrowding and greater sharing of aerosol droplets which cause the more spread of the bacteria.

Indoor kitchen location (P = 0.009) was another independently associated risk factor for the colonization of meningococcus in our study which in line with the study conducted in the African meningitis belt countries, kitchen location (indoor) was significantly associated risk factor [36]. Family member share utensils for drinking and eating (P = 0.007) were statistically significant to the meningococcal oropharyngeal carriage in our study, even though it not stated in another study. The possible reason might be utensils carry oral droplets and transfer from carrier individual to another that fasten meningococcal colonization.

Conclusion

Neisseria meningitidis prevalence in the present study had a high carriage rate among males than females. Serogroup A and Y/W135 were predominantly circulating meningococcal isolates in the community. Meningococcal carriage rate among primary school students was significantly associated with larger family size, students with tonsillectomy, parental cigarette smoking, students with greater than 40 per class, sharing utensils, history of visiting healthcare institutions and indoor kitchen. The antibiotics markedly resisted by meningococcal isolates were trimethoprim-sulfamethoxazole, ciprofloxacin, and cefotaxime. The effective antibiotics identified in this study were minocycline, azithromycin, meropenem and chloramphenicol. Most of the meningococcal isolates were identified as multidrug resistance, with serogroup B and serogroup X had markedly higher resistance.

List Of Abbreviations

ATCC: American Type Culture Collection, BAP: Blood Agar Plate, CLSI: Clinical Laboratory Standard Institute, CSF: Cerebro Spinal Fluid, CTA: Cysteine Trypticase Agar, ELISA: Enzyme Linked Immuno-Sorbent Assay, MTM: Modified Thayer-Martín Media, OPD: Out-Patient Department, PCR: Polymerase Chain Reaction, SBML: School of Biomedical and Laboratory Science

Declarations

Ethics approval and consent to participate

An ethical clearance letter was obtained from the Departmental Research and Ethics Review Committee of school of biomedical laboratory science. The reference number of the ethical letter was “Ref no-SBMLS/2123/11”. This ethical letter was obtained from Mr. Mekonnen Girma (mekonnen2302@cmail.com), Markos Negash (markosnegash@yahoo.com) and Bamilaku Enawgaw (bamlak21@gmail.com). All eligible subjects were informed as their participation was voluntary. Study participants were informed about the purpose of the study. Confidentiality was maintained at all levels of the study. In addition, study participants involvement was based on a voluntary basis and participants who were unwilling to take part in the study and those who need to quit their participation at any stage were informed to do so without any restriction.

Consent for publication

All authors read the manuscript and have provided their consent to publish.

Availability of data and material

Data and supporting materials associated with this study will be shared upon request

Competing interests

The authors declare that they have no competing interest.

Funding

This study was not funded.

Authors’ contribution

ZT did conceptualization, analyzing the data, methodology designing, investigation during the laboratory work, writing original draft and review the final manuscript.

FM did conceptualization, methodology designing, writing original draft and review the final manuscript.

MT did conceptualization, analyzing the data, methodology designing, writing original draft and review the final manuscript.

TB did conceptualization, methodology designing, investigation during the laboratory work, writing original draft and review the final manuscript.

All authors have read and approved the manuscript.

Acknowledgments

The authors would like to thank all the study participants and parents for their good collaboration. The authors would like to thank the department of Medical Microbiology, School of Biomedical and Laboratory Science, College of Medicine and Health Sciences, University of Gondar for logistic and material supports. Finally, the authors would like to thank Ethiopian Public Health Institute for reagent support.

References

1. Trivedi K, Tang CM, Exley RM. Mechanisms of meningococcal colonization. *Trends in microbiology* 2011; **19**(9):456-463.
2. Caugant DA, Maiden MC. Meningococcal carriage and disease—population biology and evolution. *Vaccine* 2009; **27**:B64-B70.
3. Schoen C, Kischkies L, Elias J, Ampattu BJ. Metabolism and virulence in Neisseria meningitidis. *Frontiers in cellular and infection microbiology* 2014; **4**:114.
4. Soriani M. Unraveling *Neisseria meningitidis* pathogenesis: from functional genomics to experimental models. *F1000Research* 2017; **6**.
5. Montero-Martin M, Inwald DP, Carrol ED, Martinon-Torres F. Prognostic markers of meningococcal disease in children: recent advances and future challenges. *Expert review of anti-infective therapy* 2014; **12**(11):1357-1369.
6. Stoof SP. Invasive meningococcal disease and prevention through vaccination: Towards an optimal meningococcal serogroup C vaccination schedule. 2015.
7. Gazi H, Surucuoglu S, Ozbakkaloglu B, Akcali S, Ozkutuk N, Degerli K, et al. Oropharyngeal carriage and penicillin resistance of *Neisseria meningitidis* in primary school children in Manisa, Turkey. *Ann Acad Med Singapore* 2004; **33**(6):758-762.
8. Jafri RZ, Ali A, Messonnier NE, Tevi-Benissan C, Durrheim D, Eskola J*, et al*: Global epidemiology of invasive meningococcal disease. *Population health metrics* 2013; **11**(1):17.
9. Rouphael NG, Stephens DS. Neisseria meningitidis: biology, microbiology, and epidemiology. In *Neisseria meningitidis. Springer* 2012; 799: 1-20.
10. Vyse A, Wolter J, Chen J, Ng T, Soriano-Gabarro M. Meningococcal disease in Asia: an under-recognized public health burden. *Epidemiology & Infection* 2011; **139** (7):967-985.
11. 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. *Journal of Infection* 2018;

76(2):140-148.

12. Ethiopian Health and Nutrition Research Institute Federal Democratic Republic of Ethiopia. National guideline on meningococcal meningitis surveillance and outbreak management. Addis Ababa Ethiopia; 2013.
13. Alemayehu T, Mekasha A, Abebe T. Nasal carriage rate and antibiotic susceptibility pattern of Neisseria meningitidis in healthy Ethiopian children and adolescents: A cross-sectional study. PloS one 2017; **12**(10): e0187207.
14. CSA: Summary and statistical report of the 2007 population and housing census. *Federal democratic republic of Ethiopia population census commission* 2008:1-10.
15. Winn WC: Koneman's color atlas and textbook of diagnostic microbiology: *Lippincott williams & wilkins* 2006.
16. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. *CLSI supplement M100 Wayne, PA: Clinical and Laboratory Standards Institute;* 2018, **28th ed.**
17. Exner M, Bhattacharya S, Christiansen B, Gebel J, Goroncy-Bermes P, Hartemann P*, et al*: Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria? *GMS hygiene and infection control* 2017; **12**.
18. Harrison LH, Pelton SI, Wilder-Smith A, Holst J, Safadi MA, Vazquez JA, et al. The Global Meningococcal Initiative: recommendations for reducing the global burden of meningococcal disease. *Vaccine* 2011; **29**(18):3363-3371.
19. Assefa A, Gelaw B, Shiferaw Y, Tigabu Z. Nasopharyngeal carriage and antimicrobial susceptibility pattern of streptococcus pneumoniae among pediatric outpatients at gondar university hospital, north west ethiopia. *Pediatrics & Neonatology* 2013; **54**(5):315-321.
20. Bårnes GK, Kristiansen PA, Beyene D, Workalemahu B, Fissiha P, Merdekios B*, et al*: Prevalence and epidemiology of meningococcal carriage in Southern Ethiopia prior to implementation of MenAfriVac, a conjugate vaccine. *BMC infectious diseases* 2016; **16**(1):639.
21. Fikerte M, Zelalem M, Biruk Y, Hiwot T, Melaku Y, Marechign Y. Antibiotic Susceptibility Pattern of *Neisseria meningitides* Isolates from Asymptomatic Carriers in Gurage zone, Southern Ethiopia. *American Journal of Health Research* 2019; 7(1): 12-18.
22. Tegene B, Kassahun Denekew GM. Phenotypic Characterization and Serotypes Identification of CSF isolates in Acute Bacterial Meningitis. *American Journal of Infectious Diseases* 2017; **5**(3):100-105.
23. Mihret W, Lema T, Merid Y, Kassu A, Abebe W, Moges B, et al. Surveillance of bacterial meningitis, Ethiopia, 2012–2013. *Emerging infectious diseases* 2016; **22**(1):75.
24. Ba AK, Sanou I, Kristiansen PA, Sangaré L, Ouédraogo R, Ouattara K, et al. Evolution of meningococcal carriage in serogroups X and Y before introduction of MenAfriVac in the health district of Kaya, Burkina Faso. *BMC infectious diseases* 2014; **14**(1):546.
25. Aminu A, Yahaya S. Carriage rate of Neisseria meningitides among pupils of islamic boarding schools (Tsangaya Almajirai) in Kano, Nigeria. *Bayero Journal of Pure and Applied Sciences* 2017; **10**(1):239-242.

26. Basta N, Sow S, Berthe A, Tamboura B, Onwuchekwa U, Haidara FC, et al. Age-specific prevalence estimates and risk factors for asymptomatic Neisseria meningitidis carriage in Bamako, Mali. *International Journal of Infectious Diseases* 2012; **16**: e211.
27. Basta NE, Stuart JM, Nascimento MC, Manigart O, Trotter C, Hassan-King M, et al. Methods for identifying Neisseria meningitidis carriers: a multi-center study in the African meningitis belt. *PloS one* 2013; **8**(10):e78336.
28. Van Ravenhorst M, Bijlsma M, van Houten MA, Struben V, Anderson AS, Eiden J, et al. Meningococcal carriage in Dutch adolescents and young adults; a cross-sectional and longitudinal cohort study. *Clinical Microbiology and Infection* 2017; **23**(8):573. e571-573. e577.
29. Bogaert D, Hermans P, Boelens H, Sluijter M, Luijendijk A, Rümke H, et al. Epidemiology of nasopharyngeal carriage of Neisseria meningitidis in healthy Dutch children. *Clinical infectious diseases* 2005; **40**(6):899-902.
30. Cleary P, Calvert N, Gee S, Graham C, Gray S, Kaczmarski E, et al. Variations in Neisseria meningitidis carriage by socioeconomic status: a cross-sectional study. *Journal of Public Health* 2015; **38**(1):61-70.
31. 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. *Journal of infection and public health* 2016; **9**(4):506-515.
32. Weckx LY, Puccini RF, Machado A, Gonçalves MG, Tuboi S, Barros Ed, et al. A cross-sectional study assessing the pharyngeal carriage of Neisseria meningitidis in subjects aged 1-24 years in the city of Embu das Artes, São Paulo, Brazil. *Brazilian Journal of Infectious Diseases* 2017; **21**(6):587-595.
33. De Moraes JC, Kemp B, De Lemos APS, Gorla MCO, Marques EGL, do Carmo Ferreira M, et al. Prevalence, risk factors and molecular characteristics of meningococcal carriage among Brazilian adolescents. *The Pediatric infectious disease journal* 2015; **34**(11):1197-1202.
34. Valipour M, Piroozmand A, Khorshidi A, Akbari H, Mirzaee H: Identification of serological groups A, B, C, W135, Y, X Neisseria meningitidis carriers by multiplex PCR in the nasopharynx of students in Kashan during 2011-2012. *Feyz Journal of Kashan University of Medical Sciences* 2013; **17**(2).
35. Nunes AMPB. Colonização por *Neisseria meningitidis* entre adolescentes após introdução da vacina meningocócica C conjugada em Salvador, Brasil. 2017.
36. Diallo K, Trotter C, Timbine Y, Tamboura B, Sow SO, Issaka B, et al. Pharyngeal carriage of Neisseria species in the African meningitis belt. *Journal of Infection* 2016; **72**(6):667-677.
37. Fitzpatrick PE, Salmon RL, Hunter PR, Roberts RJ, Palmer SR. Risk factors for carriage of Neisseria meningitidis during an outbreak in Wales. *Emerging Infectious Diseases* 2000; **6**(1):65.

Tables

Due to technical limitations, tables 2 - 8 only available as a download in the supplemental files section.

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