

## Nasopharyngeal Carriage Rate and Antimicrobial Susceptibility Pattern of Potential Pathogenic Bacteria among Paediatrics Outpatients at Gondar University Teaching Hospital, Ethiopia

Abate Assefa<sup>1\*</sup>, Baye Gelaw<sup>1</sup>, Yitayal Shiferaw<sup>1</sup> and Zemene Tigabu<sup>2</sup>

<sup>1</sup>Department of Medical Microbiology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Ethiopia

<sup>2</sup>Department of Pediatrics and Child Health, School of Medicine, College of Medicine and Health Sciences, University of Gondar, Ethiopia

### Abstract

**Background:** *Neisseria meningitidis* and *Staphylococcus aureus* usually carried without symptoms in nasopharynx, but carriage of these pathogens can lead to serious infectious diseases in children. Meningitis beyond the neonatal period caused by *N. meningitidis* remains the main reason for morbidity and mortality among children in developing countries. The aim of the study was to assess nasopharyngeal carriage and antimicrobial susceptibility pattern of *N. meningitidis* and *S. aureus* among paediatrics outpatients at Gondar, Ethiopia.

**Method:** A cross-sectional study was conducted on children aged  $\leq 10$  years from February to May, 2012. Data on potential risk factors were gathered by interview-based questionnaire. Nasopharyngeal specimens were collected with a sterile plastic cotton tip swab. Bacteria were characterised by colony appearance, Gram staining and biochemical tests. Antimicrobial susceptibility test was performed using disc diffusion method. A logistic regression analysis was used to examine possible risk factors. All tests with P-value less than 0.05 were considered statistically significant.

**Result:** Of 234 samples screened, 37 (15.8%) had positive cultures, with 24 (10.3%) being *S. aureus* and 14 (6.0%) *N. meningitidis*. The higher carriage rate of *S. aureus* 9 (14.3%) and *N. meningitidis* 6 (9.6%) were found among 8-10 years old children. *S. aureus* were resistant to 24 (100%) ampicillin, 20 (83.3%) amoxicillin, 8 (33.3%) tetracycline, 6 (25%) erythromycin and 2 (8.2%) chloramphenicol. However, *N. meningitidis* isolates were resistant only to cotrimoxazole 14 (100%), ciprofloxacin 7 (50%) and ceftriaxone 3 (21.4%).

**Conclusion:** *N. meningitidis* nasopharyngeal carriage was considerably higher among older children. All isolates of *S. aureus* showed higher resistance rate for ampicillin and amoxicillin.

**Keywords:** Antimicrobial susceptibility; Children; Ethiopia; Nasopharyngeal carriage; *N. meningitidis*; *S. aureus*

### Introduction

*Neisseria meningitidis* (*N. meningitidis*) and *Staphylococcus aureus* (*S. aureus*) are potentially pathogenic bacteria found in the nasal cavity especially in children. Although *N. meningitidis* and *S. aureus* nasopharyngeal carriage is usually asymptomatic, it can lead to serious infectious diseases in children, including pneumonia, meningitis and bacteremia [1,2]. Asymptomatic carriage is a prerequisite first step in the pathogenic route of those potential nasopharyngeal pathogens towards developing invasive and non-invasive diseases, and is a major source of bacterial spread between individuals [3-5].

Meningococcal carriers can pass the bacteria *via* droplets out of the upper respiratory tract or saliva. When the bacteria gain access to the bloodstream, invasive disease may develop, this can lead to severe sepsis, meningitis or pneumonia. Household and other close contacts of persons with meningococcal disease have a higher risk for carriage, and therefore, invasive disease [2,3]. Bacterial meningitis is a serious infection that has high risk of sequelae, as well as mortality in African children. *N. meningitidis* is one of the main bacterial pathogen causing meningitis beyond the neonatal period. Meningococcal disease causes substantial morbidity and mortality, and approximately 10% of cases are fatal. Among those who survive, 10%-15% have long-term disability like deafness [3].

*S. aureus* is an invasive human pathogen, with increasing incidence and morbidity in hospitals and the community. Both healthy persons and those with underlying illness are at risk for diverse skin and soft tissue infections, endocarditis, osteomyelitis, meningitis, bacteremia, and pneumonia due to *S. aureus* [6], with mortality rates ranging from 6-40% [4,7]. *S. aureus* is an important cause of respiratory tract

infections, such as chronic recurrent otitis media and pneumonia, skin infections and community-acquired bloodstream infections, particularly in children [8]. Pneumonia is the leading cause of death among children in Ethiopia, an economic burden for families, and for the development of the country. Over 100,000 children die each year before their fifth birthday due to pneumonia, which accounts for 28% of under five mortality in Ethiopia [9]. *S. aureus* has remained as one of the major reasons for hospitalisation, and causes of death from pneumonia in children in developing countries [10].

*S. aureus* has become resistant to various antimicrobial agents, including the commonly used penicillin-related antibiotics. Methicillin resistance *S. aureus* (MRSA) exhibits varying resistance to many antibiotics [11]. Vancomycin, used as a last resort in the treatment of MRSA infections, which in the past it exhibited 100% susceptibility, is recently being witnessed with increased resistance from these strains [5]. Community acquired MRSA infection in children is an increasing problem worldwide [12]. Ciprofloxacin, a second-generation fluoroquinolone, is effective chemoprophylaxis agent for *N. meningitidis*, but currently there is evidence of resistant strains [3].

**\*Corresponding author:** Abate Assefa, Department of Medical Microbiology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Ethiopia, E-mail: [abezew@gmail.com](mailto:abezew@gmail.com)

**Received** April 24, 2013; **Accepted** June 17, 2013; **Published** June 21, 2013

**Citation:** Assefa A, Gelaw B, Shiferaw Y, Tigabu Z (2013) Nasopharyngeal Carriage Rate and Antimicrobial Susceptibility Pattern of Potential Pathogenic Bacteria among Paediatrics Outpatients at Gondar University Teaching Hospital, Ethiopia. J Infect Dis Ther 1: 109. doi:10.4172/2332-0877.1000109

**Copyright:** © 2013 Assefa A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Therefore, studies of the prevalence of *S. aureus* and *N. meningitidis*, their resistance patterns and possible risk factors can provide useful indications for more rational therapeutic and preventive strategies. In African countries, children are suffering from high burdens of *S. aureus* and *N. meningitidis* diseases, and therapy remains empiric because of the lack of rapid, sensitive, and specific diagnostic tests. In many African countries, and particularly in Ethiopia, there are no epidemiologic data on *N. meningitidis*. Therefore, the aim of this study was to determine nasopharyngeal carriage rate, antimicrobial susceptibility pattern and possible risk factors of *S. aureus* and *N. meningitidis* among paediatrics outpatients attending Gondar University Hospital, Ethiopia.

## Materials and Methods

### Study design and subjects

The study was conducted in children, who were attending Gondar University Hospital paediatrics outpatient department, from February to May, 2012. Based on figures from the Central Statistical Agency in 2008, Gondar has an estimated total population of 231,977. Gondar University Hospital is a referral hospital with more than 400 beds for North West Ethiopia, serving a population of about 5 million. All children who were aged ten years and below, and who were seen for either well-child care visits or sick care visits were eligible for the study. There were no exclusion criteria. Study subjects were selected by systematic random sampling method.

### Collection of demographic and clinical characteristics

The study was reviewed and approved by the Institutional Review Board (IRB) of the University of Gondar School of Biomedical and Laboratory Science. Written informed consent was obtained from each participant's parent or legal guardian. Individual records were coded and accessed only by research staff. After obtaining written informed consent, three trained interns from the paediatrics department gave questionnaire-based interviews of the parents or guardians of the children to collect necessary demographic information. Demographic information included in this study was age, sex, family size, siblings <5 years, siblings  $\geq$  5 years, number of rooms in the house and habits of sleeping with parents. Additionally, the clinical presentation of illness (upper and lower respiratory tract infection) was collected after clinical diagnosis by the same questionnaire. Each tuberculosis (TB) suspected child was sent to radiology department for chest X-ray, and with the same radiologist performing the procedure.

### Nasopharyngeal specimen collection and processing

Nasopharyngeal specimens were obtained by the same trained interns. A single nasopharyngeal specimen per child was obtained with a sterile synthetic cotton swabs on flexible aluminium wire (Fisher Scientific, Pittsburgh, USA). After sampling, swabs were placed immediately in to Amies media (Oxoid, Hampshire, England), and transported to microbiology laboratory. Within four hours of collection, the specimen were inoculated onto chocolate agar (HiMedia, Mumbai, India), and mannitol salt agar by rolling the swab over small area of the plates and streaking the sample using a sterile loop. Inoculated chocolate agar plates incubated under a 5% CO<sub>2</sub> enriched atmosphere and mannitol salt agar plates in aerobic conditions, at 37°C for up to 24 to 48 hours [13].

### Identification of bacterial isolates

All isolates were identified based on the method outlined by the Centers for Disease Control and prevention manual [13]. A Gram-positive small to large yellow colonies from mannitol salt agar was

taken and subcultured on to blood agar (HiMedia, Mumbai, India) plate. The blood agar plate then incubated at 37°C aerobically for 18 to 24 hours. A large, round, golden-yellow colony, often with  $\beta$ -hemolysis on the blood agar plates was taken. Using a sterile inoculating loop, a small amount of organism was picked up and catalase test was done. Moreover, coagulase test was performed to distinguish *S. aureus* from other *Staphylococcus* species. Gram-positive cocci that produced catalase and coagulase and ferment mannitol were identified as *S. aureus*. A Gram negative diplococci, greyish, non-hemolytic, glistening colonies on chocolate agar was taken and subcultured on to blood agar plate to ensure purity. After overnight incubation, the colony was tested by Gram staining. The isolate was further confirmed by Kovac's oxidase test for cytochrome oxidase production and carbohydrate utilization test using glucose, maltose, lactose and sucrose. Acid production from carbohydrates was done in a tube containing cystine trypticase acid medium. The inoculated tubes were incubated at 37°C in aerobic condition, and examined at 24 hours intervals until reactions are interpretable for up to 72 hours. Gram-negative diplococci that produced oxidase and oxidized glucose and maltose, but not lactose and sucrose, were identified as *N. meningitidis*.

### Antimicrobial susceptibility testing

Antimicrobial agents, erythromycin (15  $\mu$ g), chloramphenicol (30  $\mu$ g), ampicillin (10  $\mu$ g), ceftriaxone (10  $\mu$ g) and cotrimoxazole (25  $\mu$ g), tetracycline (30  $\mu$ g), vancomycin (30  $\mu$ g), methicillin (5  $\mu$ g), ciprofloxacin (5  $\mu$ g), gentamycin (10  $\mu$ g), amoxicillin (10  $\mu$ g) and augmentin (10  $\mu$ g) were tested using disc diffusion method (modified Kirby-Bauer). Susceptibility of *N. meningitidis* was done on Muller Hinton agar (Oxoid, Hampshire, England) supplemented with 5% sheep's blood were as Muller Hinton agar plate was used for *S. aureus*. To standardize the inoculum density for susceptibility test, a 0.5 McFarland standard was used. Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The dried surface plates were inoculated by streaking the swab over the entire sterile agar surface. The antimicrobial discs were placed on the lawn of bacterial isolates using sterile forceps. Inoculated Muller Hinton agar with 5% sheep blood and Muller Hinton agar were incubated in a 5% CO<sub>2</sub> atmosphere, and aerobically, respectively within 15 minutes after the discs were applied for 20-24 hours at 37°C. The results were interpreted by comparing to the standard zone sizes of clinical and laboratory standard institute (CLSI). *S. aureus* ATCC<sup>™</sup> 25923 and *S. pneumoniae* ATCC 49619 were used as the quality control strains for each run as recommended by CLSI [14].

### Data analysis

The data were analysed by SPSS package software version 20. A descriptive analysis was used to determine demographic characteristics and prevalence of each isolated organism. The association between the characteristics of the children and the nasopharyngeal carriage of *S. aureus* and *N. meningitidis* were first analysed by a series of bivariate analyses. Then, to control simultaneously for the possible confounding effects of the different variables, the risk of being an *S. aureus* and *N. meningitidis* carriers were estimated by multivariate analysis, with stepwise variable selection. In both analyses, the association was expressed in odds ratios (OR) and 95% confidence intervals (CI). All tests of multivariate analysis with P-value < 0.05 were considered statistically significant.

## Results

### Demographic and clinical characteristics

A total of 234 children were enrolled in the study, of whom 121

(51.7%) were female. The mean age of the children was 5.2 years, with a standard deviation of 3.0 years. One hundred and thirty-three (56.8%) lived in urban environments. The majority of the children 148 (63.2%) were living within a single room (house with only one room), and only 17 (7.3%) of the children had their own bed. Eighty-seven (37.2%) children had received antibiotic within the past two weeks of enrolment. During enrolment, 98 (41.9%) of the children were clinically diagnosed as having respiratory infection (Table 1).

### Nasopharyngeal carriage

Of the total 234 children screened, 24 (10.3%) carried *S. aureus* and 14 (6.0%) carried *N. meningitidis*. Concomitantly, the two pathogens were isolated from one (4.2%) child only. High carriage of *S. aureus* 9 (14.3%) and *N. meningitidis* 6 (9.6%) were observed in 8 to 10 years old children. The proportion of *S. aureus* carriages were 7 (6.2%) in males versus 17 (14%) in females. In children living with <5 years age siblings, the overall carriage rate was 11 (10.8%) for *S. aureus* and 6 (5.9%) for *N. meningitidis*. Of 156 children living with ≥ 5 years old siblings, 16 (10.3%) *S. aureus* and 9 (5.2%) *N. meningitidis* were observed (Table 2).

### Risk factors analysis for nasopharyngeal carriage

The highest and lowest *S. aureus* carriage rate were observed among the age groups of 8 to 10 years old 9 (14.3%) and the younger age (<3 years old) 3 (5.3%), respectively. Difference in the proportions of *S. aureus* carriage between genders was observed (6.2% males versus 14% females). However, we found no significant association between sex and age groups and carriage rate by *S. aureus*. Relatively

high *S. aureus* carriage 4 (25%) was found to be in children who have tonsilopharyngitis during enrolment. Moreover, multivariate analysis showed significant association between upper respiratory tract infection, tonsilopharyngitis and sinusitis, and *S. aureus* carriage rate (adjusted OR 9.49, 95% CI 2.16- 41.79, p=0.003 and 5.26, 95% CI 1.35- 20.50, P=0.017, respectively) (Table 3).

The carriage rate of *N. meningitidis* was higher (9.6%) in the relatively older age groups (8-10 years old), compared to the younger age groups (less than 3 years old) carriage rate (1.8%). However, logistic regression analysis showed no significant association with age and carriage rate by *N. meningitidis*. Furthermore, high *N. meningitidis* carriage rate was found among radiologically confirmed pulmonary tuberculosis patients (15.6%), children with sinusitis (9.1%), and children who had use antibiotics within the past two weeks enrolment (8.0%). Nonetheless, the only characteristic that was positively associated with *N. meningitidis* nasopharyngeal carriage rate was radiological confirmed pulmonary tuberculosis (adjusted OR 8.02, 95% CI 1.7-37.7, P=0.008) (Table 4).

### Antimicrobial susceptibility patterns of bacterial isolates

The antimicrobial susceptibility pattern of all isolates is shown in Table 5. All isolates of both *S. aureus* and *N. meningitidis* showed resistance to at least one antimicrobial agent. The isolated *S. aureus* were resistant 24 (100%) to ampicillin, 20 (83.3%) to amoxicillin, and 8 (33.3%) to tetracycline. *S. aureus* were also resistant (16.7%) and intermediate resistant (8.3%) to erythromycin. However, all isolates of *S. aureus* were susceptible to methicillin, vancomycin, ciprofloxacin, gentamycin and augmentin. Isolated *N. meningitidis* were resistant to cotrimoxazole 14 (100%), ceftriaxone 7(50%) and ciprofloxacin 3 (21.4%). Moreover, almost all isolated *S. aureus* 22 (91.7%) showed multidrug resistance (resistance to more than one antibiotic). Two isolates of *N. meningitidis* were resistant to cotrimoxazole, ciprofloxacin and ceftriaxone.

### Discussion

In this study, the prevalence of *S. aureus* and *N. meningitidis* were 24 (10.3%) and 14 (6.0%), respectively. A consistent nasopharyngeal carriage rate of *S. aureus* was recorded in Israel (10%) [15]. A relatively higher carriage rate of *S. aureus* was found, in Czech city (16%) [16], in Japan (17.9%) [17], and in Taiwan (23.2%) [18]. However, comparatively lower nasopharyngeal carriage of *N. meningitidis* was reported in healthy Dutch children (1.5%) [19], and in Greece children (4.0%) [20]. Higher carriage of *N. meningitidis* was reported from another study in Greece (7.2%) [21], and in India (10.4%) [22]. Possible explanation for the discrepancies between the data from previous studies and our study could be the geographic site and methodological differences. Our results showed higher nasopharyngeal carriage rate of *S. aureus* and *N. meningitidis* among older children. The high prevalence of *S. aureus* and *N. meningitidis* could be due to the fact that many children may be immune debilitated due to the diseases, which were the reason for children to visit the hospital. This may, therefore, suggest that large numbers of meningococcal carriers are at high risk of developing invasive meningococcal diseases and the family members, and their peers with whom they interact in the community are at risk of acquiring the pathogens.

The significant association of some risk factors, particular demographic characteristics, with nasopharyngeal carriage of *S. aureus* in children was previously reported. For instance, in Taiwan, sleeping with parents and use of antibiotics within the previous two weeks was associated with decreased carriage rate of *S. aureus* [18]. In addition, in

| Characteristic of children (n=234) |           | n   | %    |
|------------------------------------|-----------|-----|------|
| Sex                                | Male      | 113 | 48.3 |
|                                    | Female    | 121 | 51.7 |
| Residence                          | Urban     | 133 | 56.8 |
|                                    | Rural     | 101 | 43.2 |
| Age (in year)                      | <3        | 57  | 24.4 |
|                                    | 3 ≤ 5     | 54  | 23.4 |
|                                    | 5 ≤ 8     | 60  | 25.6 |
|                                    | 8-10      | 63  | 26.9 |
| Family size                        | <5        | 90  | 38.5 |
|                                    | 5+        | 144 | 61.5 |
| Siblings <5 years                  | Yes       | 102 | 43.6 |
|                                    | No        | 132 | 56.4 |
| Siblings ≥ 5 years                 | Yes       | 156 | 66.7 |
|                                    | No        | 78  | 33.3 |
| Sleeping with parents              | Yes       | 217 | 92.7 |
|                                    | No        | 17  | 7.3  |
| Number of room                     | 1         | 148 | 63.2 |
|                                    | 2+        | 86  | 36.8 |
|                                    | Yes       | 87  | 37.2 |
| Recent use of antibiotic           | No        | 147 | 62.8 |
|                                    | No        | 195 | 83.3 |
| URTI                               | TP        | 16  | 6.8  |
|                                    | Sinusitis | 23  | 9.8  |
|                                    | No        | 175 | 74.8 |
| LRTI                               | Pn        | 27  | 11.5 |
|                                    | TB        | 32  | 13.7 |
|                                    | Yes       | 12  | 5.1  |
| Otitis media                       | No        | 222 | 94.9 |

Pn: Pneumonia; TP: Tonsilopharyngitis; TB: Pulmonary Tuberculosis; URTI: Upper Respiratory Tract Infection; LRTI: Lower Respiratory Tract infection

**Table 1:** Socio-demographic and clinical characteristics of paediatrics outpatient children at University of Gondar Hospital.

| Variables                |        | n=234 | S. aureus   |              | N. meningitidis |              |
|--------------------------|--------|-------|-------------|--------------|-----------------|--------------|
|                          |        |       | No<br>N (%) | Yes<br>n (%) | No<br>N (%)     | Yes<br>N (%) |
| Sex                      | Male   | 113   | 106 (93.8)  | 7 (6.2)      | 107 (94.7)      | 6 (5.3)      |
|                          | Female | 121   | 104 (86.0)  | 17 (14.0)    | 113 (93.4)      | 8 (6.6)      |
| Residence                | Urban  | 133   | 119 (89.5)  | 14 (10.5)    | 128 (96.2)      | 5 (3.8)      |
|                          | Rural  | 101   | 91 (90.1)   | 10 (9.9)     | 92 (91.1)       | 9 (8.9)      |
| Age (in year)            | <3     | 57    | 54 (94.7)   | 3 (5.3)      | 56 (98.2)       | 1 (1.8)      |
|                          | 3 ≤ 5  | 54    | 47 (87.0)   | 7 (13.0)     | 49 (90.7)       | 5 (9.3)      |
|                          | 5 ≤ 8  | 60    | 55 (60.0)   | 5 (8.3)      | 58 (96.7)       | 2 (3.3)      |
|                          | 8-10   | 63    | 54 (85.7)   | 9 (14.3)     | 57 (90.4)       | 6 (9.6)      |
| Family size              | <5     | 90    | 83 (92.2)   | 7 (7.8)      | 84 (93.3)       | 6 (6.7)      |
|                          | 5+     | 144   | 127 (88.2)  | 17 (11.8)    | 136 (94.4)      | 8 (5.6)      |
| Siblings <5 years        | Yes    | 102   | 91 (89.2)   | 11 (10.8)    | 96 (94.1)       | 6 (5.9)      |
|                          | No     | 132   | 119 (90.2)  | 13 (9.8)     | 124 (90.2)      | 8 (94.0)     |
| Siblings ≥ 5 years       | Yes    | 156   | 140 (89.7)  | 16 (10.3)    | 147 (94.2)      | 9 (5.2)      |
|                          | No     | 78    | 70 (89.7)   | 8 (10.3)     | 73 (93.6)       | 5 (6.4)      |
| Sleeping with parents    | Yes    | 217   | 196 (90.3)  | 21 (9.7)     | 204 (94.0)      | 13 (6.0)     |
|                          | No     | 17    | 14 (82.4)   | 3 (17.6)     | 16 (94.1)       | 1 (5.9)      |
| Number of room           | 1      | 148   | 134 (90.5)  | 14 (9.5)     | 139 (93.9)      | 9 (6.1)      |
|                          | 2+     | 86    | 76 (88.4)   | 10 (11.6)    | 81 (94.2)       | 5 (5.8)      |
| Recent use of antibiotic | Yes    | 87    | 80 (92.0)   | 7 (8.0)      | 80 (92.0)       | 7 (8.0)      |
|                          | No     | 147   | 130 (88.4)  | 17 (11.6)    | 140 (95.2)      | 7 (4.8)      |

**Table 2:** Nasopharyngeal carriage rate of *S. aureus* and *N. meningitidis* among paediatrics outpatients at University of Gondar Hospital.

| Factors                        |           | S.aureus    |              | Odds Ratio with 95% CI |                        |      |
|--------------------------------|-----------|-------------|--------------|------------------------|------------------------|------|
|                                |           | No<br>n (%) | Yes<br>n (%) | Crude OR (95% C.I.)    | Adjusted OR (95% C.I.) | P    |
| Sex                            | Female    | 104 (86.0)  | 17 (14.0)    | 2.48 (.99-6.22)        | 2.63 (.89-7.686)       | .078 |
|                                | Male      | 106 (93.8)  | 7 (6.2)      | 1                      | 1                      |      |
| Age ( in year)                 | <3        | 54 (94.7)   | 3 (5.3)      | 1                      | 1                      |      |
|                                | 3 ≤ 5     | 47 (87.0)   | 7 (13.0)     | 2.68 (.66-10.96)       | 1.8 (.39-8.3)          | .451 |
|                                | 5 ≤ 8     | 55 (60.0)   | 5 (8.3)      | 1.64 (.37-7.199)       | 1.1 (.22-5.62)         | .910 |
|                                | 8-10      | 54 (85.7)   | 9 (14.3)     | 3.00 (.77-11.68)       | 1.9 (.38-9.66)         | .422 |
| Residence                      | Rural     | 119 (89.5)  | 14 (10.5)    | 1.07 (.46-2.52)        | .79 (.23-2.68)         | .700 |
|                                | Urban     | 91 (90.1)   | 10 (9.9)     | 1                      | 1                      |      |
| Family size                    | <5        | 83 (92.2)   | 7 (7.8)      | 1.59 (.63-3.99)        | 2.16 (.64-7.32)        | .216 |
|                                | 5+        | 127 (88.2)  | 17 (11.8)    | 1                      | 1                      |      |
| Siblings <5 years              | Yes       | 91 (89.2)   | 11 (10.8)    | 1.11 (.47-2.58)        | 1.23 (.42-3.63)        | .707 |
|                                | No        | 119 (90.2)  | 13 (9.8)     | 1                      | 1                      |      |
| Siblings ≥5 years              | Yes       | 140 (89.7)  | 16 (10.3)    | 1.00 (.41-2.45)        | .51 (.14-1.80)         | .293 |
|                                | No        | 70 (89.7)   | 8 (10.3)     |                        |                        |      |
| Sleeping with parents          | Yes       | 140 (89.7)  | 16 (10.3)    | .50 (.13-1.88)         | 2.1 (.47-9.33)         | .335 |
|                                | No        | 70 (89.7)   | 8 (10.3)     | 1                      | 1                      |      |
| No. of room                    | 1         | 196 (90.3)  | 21 (9.7)     | .79 (.34-1.87)         | .82 (.28-2.45)         | .728 |
|                                | 2+        | 14 (82.4)   | 3 (17.6)     | 1                      |                        |      |
| Recent antibiotic use          | Yes       | 134 (90.5)  | 14 (9.5)     | .67 (.27-1.68)         | .69 (.24-2.03)         | .504 |
|                                | No        | 76 (88.4)   | 10 (11.6)    | 1                      | 1                      |      |
| URTI                           | TP        | 12 (75.0)   | 4 (25.0)     | 5.49 (1.67-18.0)       | 9.49 (2.16-41.8)       | .003 |
|                                | Sinusitis | 20 (90.9)   | 2 (9.1)      | 3.35 (1.08-10.4)       | 5.26 (1.35-20.5)       | .017 |
| LRTI                           | No        | 166 (90.2)  | 18 (9.8)     | 1                      | 1                      |      |
|                                | Pn        | 24 (88.9)   | 3 (11.1)     | 1.15 (.31-4.19)        | 2.06 (.47-9.06)        | .339 |
|                                | TB        | 30 (93.8)   | 2 (6.2)      | .91 (.25-3.28)         | 1.52 (.34-6.86)        | .589 |
| <i>N. meningitidis</i> carrier | No        | 156 (89.1)  | 19 (10.9)    | 1                      | 1                      |      |
|                                | Yes       | 13 (92.9)   | 1 (7.1)      | 1.52 (.19-12.14)       | 1.94 (.19-19.51)       | .573 |
|                                | No        | 197 (89.5)  | 23 (10.5)    | 1                      | 1                      |      |

URTI=Upper Respiratory Tract Infection; LRTI=Lower Respiratory Tract Infection; Pn=Pneumonia; TP= Tonsilopharyngitis; Tb= Tuberculosis; OR=Odds Ratio

**Table 3:** Bivariate and multivariate analysis of risk factors for *S.aureus* carriage among paediatrics outpatients at University of Gondar Hospital.

Netherlands, presence of siblings and increasing age were all negatively associated with *S. aureus* carriage [23]. In our study, we found no significant association between demographic factors with *S. aureus*

carriage rate. For instance, although age is an important determinant of *S. aureus* carriage in children, we found no statistical significant association between age distribution and *S. aureus* carriage. This

| Variable                 | <i>N. meningitidis</i> |            | Odds ratio with 95% CI |                        |                  |      |
|--------------------------|------------------------|------------|------------------------|------------------------|------------------|------|
|                          | No n (%)               | Yes n (%)  | Crude OR (95% C.I.)    | Adjusted OR (95% C.I.) | P                |      |
| Sex                      | Female                 | 113 (93.4) | 8 (6.6)                | 1.26 (.42-3.76)        | 1.78 (.47-6.79)  | .401 |
|                          | Male                   | 107 (94.7) | 6 (5.3)                | 1                      | 1                |      |
| Age ( in year)           | <3                     | 56 (98.2)  | 1 (1.8)                | .35 (.07-1.79)         | .26 (.03-2.38)   | .226 |
|                          | 3 ≤ 5                  | 49 (90.7)  | 5 (9.3)                | .76 (.20-2.85)         | .75 (.15-3.79)   | .729 |
|                          | 5 ≤ 8                  | 58 (96.7)  | 2 (3.3)                | .33 (.06-1.69)         | .66 (.096-4.50)  | .670 |
|                          | 8-10                   | 57 (90.4)  | 6 (9.6)                | 1                      | 1                |      |
| Residence                | Rural                  | 128 (96.2) | 5 (3.8)                | 1.82 (.61-5.43)        | 3.32 (.71-15.49) | .127 |
|                          | Urban                  | 92 (91.1)  | 9 (8.9)                | 1                      | 1                |      |
| Family size              | <5                     | 84 (93.3)  | 6 (6.7)                | .78 (.24-2.55)         | .58 (.12-2.88)   | .507 |
|                          | 5+                     | 136 (94.4) | 8 (5.6)                | 1                      | 1                |      |
| Siblings ≥ 5years        | Yes                    | 147 (94.2) | 9 (5.2)                | .89 (.29-2.76)         | 1.08 (.21-5.56)  | .928 |
|                          | No                     | 73 (93.6)  | 5 (6.4)                | 1                      | 1                |      |
| Siblings <5 years        | Yes                    | 96 (94.1)  | 6 (5.9)                | 1.32 (.45-3.88)        | 1.21 (.32-4.60)  | .784 |
|                          | No                     | 124 (90.2) | 8 (94.0)               | 1                      | 1                |      |
| Sleeping with parents    | Yes                    | 204 (94.0) | 13 (6.0)               | 1.02 (.13-8.30)        | .65 (.05-8.49)   | .743 |
|                          | No                     | 16 (94.1)  | 1 (5.9)                | 1                      | 1                |      |
| No. of room              | 1                      | 139 (93.9) | 9 (6.1)                | 1.05 (.34-3.24)        | .49 (.11-2.13)   | .338 |
|                          | 2+                     | 81 (94.2)  | 5 (5.8)                | 1                      | 1                |      |
| Recent antibiotic use    | Yes                    | 80 (92.0)  | 7 (8.00)               | 2.38 (.80-7.11)        | 1.99 (.54-7.42)  | .304 |
|                          | No                     | 140 (95.2) | 7 (4.8)                | 1                      | 1                |      |
| Otitis media             | Yes                    | 10 (83.3)  | 6 (16.7)               | 3.5 (.69-17.79)        | 6.28 (.83-47.44) | .070 |
|                          | No                     | 210 (94.6) | 12 (5.4)               | 1                      | 1                |      |
| URTI                     | Sinusitis              | 20 (90.9)  | 2 (9.1)                | 1.65 (.34-8.03)        | 2.15 (.31-14.94) | .440 |
|                          | No                     | 173 (94.0) | 11 (6.0)               | 1                      | 1                |      |
| LRTI                     | Pn                     | 25 (92.6)  | 2 (7.4)                | .97 (.11-8.18)         | .85 (.08-9.52)   | .892 |
|                          | TB                     | 27 (84.4)  | 5 (15.6)               | 5.6 (1.7-17.9)         | 8.02 (1.71-37.7) | .008 |
|                          | No                     | 168 (96.0) | 7 (4.0)                | 1                      | 1                |      |
| <i>S. aureus</i> carrier | Yes                    | 23 (95.8)  | 1 (4.2)                | 1.52 (.19-12.14)       | .98 (.097-9.96)  | .989 |
|                          | No                     | 197 (93.8) | 13 (6.2)               | 1                      | 1                |      |

URTI=Upper Respiratory Tract Infection; LRTI=Lower Respiratory Tract Infection; Pn=Pneumonia; TP=Tonsilopharyngitis; Tb=Tuberculosis; OR=Odds Ratio

**Table 4:** Bivariate and multivariate analysis of risk factors for *N. meningitidis* carriage among paediatrics outpatients at University of Gondar Hospital.

| Antimicrobials  | <i>S. aureus</i> (n=24) |         |           | <i>N. meningitidis</i> (n=14) |         |           |
|-----------------|-------------------------|---------|-----------|-------------------------------|---------|-----------|
|                 | R n (%)                 | I n (%) | S n (%)   | R n (%)                       | I n (%) | S n (%)   |
| Cotrimoxazole   | 0 (0)                   | 0 (0)   | 24 (100)  | 24 (100)                      | 0 (0)   | 0 (00)    |
| Chloramphenicol | 2 (8.3)                 | 0 (0)   | 22 (91.7) | 0 (0)                         | 0 (0)   | 14 (100)  |
| Erythromycin    | 4 (16.7)                | 2 (8.3) | 18 (75)   | 0 (0)                         | 0 (0)   | 14 (100)  |
| Tetracycline    | 8 (33.3)                | 0 (0)   | 16 (66.7) | 0 (0)                         | 0 (0)   | 14 (100)  |
| Ceftriaxone     | 0 (00)                  | 0 (00)  | 24 (100)  | 7 (50.0)                      | 0 (0)   | 7 (50)    |
| Ciprofloxacin   | 0 (0)                   | 0 (0)   | 24 (100)  | 3 (21.4)                      | 0 (0)   | 12 (78.6) |
| Vancomycin      | 0 (0)                   | 0 (0)   | 24 (100)  | *                             | *       | *         |
| Methicillin     | 0 (0)                   | 0 (0)   | 24 (100)  | *                             | *       | *         |
| Ampicillin      | 24 (100)                | 0 (0)   | 0 (0)     | *                             | *       | *         |
| Amoxicillin     | 20 (83.3)               | 0 (0)   | 4 (16.7)  | *                             | *       | *         |
| Gentamycin      | 0 (0)                   | 0 (0)   | 24 (100)  | *                             | *       | *         |
| Augmentin       | 0 (0)                   | 0 (0)   | 24 (100)  | *                             | *       | *         |

R=Resistance, I=Intermediate Resistance, S=Susceptible, \*=Not Tested

**Table 5:** Socio-demographic and clinical characteristics of paediatrics outpatient children at University of Gondar Hospital.

suggests that unknown host or environmental factors are more likely to be prime determinants of the carriage distribution in children [24]. The only clinical characteristic positively associated with *S. aureus* carriage was upper respiratory tract infection, particularly tonsilopharyngitis and sinusitis. Probably, these relatively significantly higher *S. aureus* carriage among children with upper respiratory tract infections may be the prime factor for the difference in *S. aureus* carriage distribution in current study subjects.

Although nasopharyngeal carriage of *N. meningitidis* was found to

be closely associated with living conditions like overcrowding [22], the only characteristic positively associated with *N. meningitidis* carriage was radiological confirmed pulmonary tuberculosis. An earlier study had revealed that individuals with respiratory tract viral or bacterial infections may be at high risk for meningococcal carriage [25]. However, tuberculosis was not associated with carriage of *S. aureus*. This may be due to our relatively small population of those with underlying respiratory conditions. For instance, pneumonia patients were very small (n=27, three *S. aureus* and two *N. meningitidis*). Even

though previous antibiotic use expected to reduce the carriage of most sensitive organisms like meningococci, we found no association between carriage of *S. aureus* and *N. meningitidis* and previous antibiotic use.

In this study, the antimicrobial susceptibility tests against *S. aureus* revealed that amoxicillin and ampicillin were the least effective agents with 100% and 83.3% resistance, respectively. This might be due to the effect of beta-lactamases produced by *S. aureus*. We also observed moderately high resistance to tetracycline (33.3%) and erythromycin (25%). Higher MRSA were observed in different countries of the world from healthy children. For instance, MRSA were reported in Japan (42.9%) [17], in western Nepal (52.9%) [26], and in Indian (19%) [27]. However, we found no MRSA strains in this study. Furthermore, all the isolated *S. aureus* were susceptible to vancomycin, ciprofloxacin, gentamycin and augmentin. This low resistance rates of isolated *S. aureus* for vancomycin, ciprofloxacin, gentamycin, and augmentin, while higher resistance against ampicillin and amoxicillin may be explained by the restricted use of some antibiotics in the community. The susceptibility of all *S. aureus* to augmentin may be due to the presence of clavulanic acid (a competitive inhibitor of betalactamases), that restores the antimicrobial activity of the beta-lactam antibiotic (amoxicillin) against *S. aureus*. Methicillin is also resistant the enzyme betalactamases. Moreover, methicillin and vancomycin are very costly, and only rarely prescribed antibiotics that showed lower resistance rate. However, ampicillin and amoxicillin are the cheap and readily available drugs to the local population, thus heralding the emergence of resistant strains to these drugs. Therefore, the uncontrolled availability of some antimicrobial agents, leading to frequent use and misuse exerts greater selection pressure for the resistant strains, and thereby makes these agents almost ineffective.

In the present study, all isolated *N. meningitidis* were susceptible to chloramphenicol, erythromycin and tetracycline. Our results demonstrated the highest resistance rates of *N. meningitidis* against cotrimoxazole 14 (100%), ceftriaxone 7 (50.0%) and ciprofloxacin 3 (21.4%), which were among the effective antimicrobial against *S. aureus*. Contrary to the present results, a study of meningococcal carriage in Greece showed that no strains were resistant to ceftriaxone or ciprofloxacin [21]. Therefore, this result noted that *N. meningitidis* is developing a resistance to the antibiotics, ceftriaxone and ciprofloxacin, appropriate for management of meningococcal meningitis epidemic response. However, the overall finding of our result showed relatively chloramphenicol, ceftriaxone and ciprofloxacin, as most effective antibiotics in the currently included community.

## Conclusion

*N. meningitidis* nasopharyngeal carriage was considerably higher among older children. These high numbers of carrier children could serve as reservoirs for the transmission of *N. meningitidis* to the community, which could lead to serious meningococcal disease. All isolates of *S. aureus* showed higher resistance rate for ampicillin and amoxicillin. Although the number of isolated *N. meningitidis* may be too small to draw meaningful conclusions, there is indication of higher cotrimoxazole and ciprofloxacin resistances too. The effective antimicrobial agents were methicillin, vancomycin, ciprofloxacin, gentamycin and augmentin for *S. aureus* and chloramphenicol, erythromycin and tetracycline for *N. meningitidis*. Therefore, the study results highlight the pressing need to consider large-scale meningococci study for implementation of the meningococci vaccination in the community.

## Acknowledgements

We gratefully acknowledge all participants, staff of the Paediatrics Outpatient Department and Microbiology Laboratory of Gondar University Hospital for participating in and facilitation of this study. Our great thanks go to University of Gondar for providing the grant of this research.

## References

1. Gluck U, Gebbers JO (2000) The nose as bacterial reservoir: important differences between the vestibule and cavity. *Laryngoscope* 110: 426-428.
2. Stephens DS (2009) Biology and pathogenesis of the evolutionarily successful, obligate human bacterium *Neisseria meningitidis*. *Vaccine* 27: B71-B77.
3. Centers for Disease Control and Prevention (CDC) (2008) Emergence of fluoroquinolone-resistant *Neisseria meningitidis*-Minnesota and North Dakota, 2007-2008. *MMWR Morb Mortal Wkly Rep* 57: 173-175.
4. Nickerson EK, West TE, Day NP, Peacock SJ (2009) *Staphylococcus aureus* disease and drug resistance in resource-limited countries in south and East Asia. *Lancet Infect Dis* 9: 130-135.
5. Onanuga A, Temedie TC (2011) Nasal carriage of multi-drug resistant *Staphylococcus aureus* in healthy inhabitants of Amassoma in Niger delta region of Nigeria. *Afr Health Sci* 11: 176-181.
6. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, et al. (2007) Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* 298: 1763-1771.
7. Noskin GA, Rubin RJ, Schentag JJ, Kluytmans J, Hedblom EC, et al. (2007) National trends in *Staphylococcus aureus* infection rates: Impact on economic burden and mortality over a 6-year period (1998-2003). *Clin Infect Dis* 45: 1132-1140.
8. Reddy EA, Shaw AV, Crump JA (2010) Community-acquired bloodstream infections in Africa: A systematic review and meta-analysis. *Lancet Infect Dis* 10: 417-432.
9. Federal Ministry of Health (2005) National Strategy for Child Survival in Ethiopia. Family Health Department, Federal Ministry of Health, Addis Ababa, Ethiopia.
10. Zar HJ, Madhi SA (2006) Childhood pneumonia -Progress and challenges. *S Afr Med J* 96: 890-900.
11. Moellering RC Jr (1998) Problems with antimicrobial resistance in Gram-positive cocci. *Clin Infect Dis* 26: 1177-1178.
12. Kaplan SL, Hulten KG, Gonzalez BE, Hammerman WA, Lamberth L, et al. (2005) Three-year surveillance of community-acquired *S. aureus* infections in children. *Clin Infect Dis* 40: 1785-1791.
13. Centers for Disease Control and Prevention (2003) Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. Centers for Disease Control and Prevention, Atlanta, Georgia, USA.
14. Clinical and Laboratory Standard Institute (2011) Performance standards for antimicrobial susceptibility testing; Twenty-first informational supplement. CLSI document M100-S21.
15. Regev-Yochay G, Dagan R, Raz M, Carmeli Y, Shainberg B, et al. (2004) Association between carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in Children. *JAMA* 292: 716-720.
16. Zemlicková H, Urbásková P, Adámková V, Motlová J, Lebedová V, et al. (2006) Characteristics of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus* isolated from the nasopharynx of healthy children attending day-care centres in the Czech Republic. *Epidemiol Infect* 134: 1179-1187.
17. Masuda K, Masuda R, Nishi J, Tokuda K, Yoshinaga M, et al. (2002) Incidences of nasopharyngeal colonization of respiratory bacterial pathogens in Japanese children attending day-care centers. *Pediatr Int* 44: 376-380.
18. Chen CJ, Hsu KH, Lin TY, Hwang KP, Chen PY, et al. (2011) Factors associated with nasal colonization of methicillin-resistant *Staphylococcus aureus* among healthy children in Taiwan. *J Clin Microbiol* 49: 131-137.
19. Bogaert D, Hermans PWM, Boelens H, Sluiter M, Luijckendijk A, et al. (2005) Epidemiology of nasopharyngeal carriage of *Neisseria meningitidis* in healthy dutch children. *Clin Infect Dis* 40: 899-902.
20. Pavlopoulou ID, Daikos GL, Alexandrou H, Petridou E, Pangalis A, et al. (2004)

- Carriage of *Neisseria meningitidis* by Greek children: Risk factors and strain characteristics. Clin Microbiol Infect 10: 137-142.
21. Kremastinou J, Tzanakaki G, Levidiotou S, Markou F, Themeli E, et al. (2003) Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in northern Greece. FEMS Immunol Med Microbiol 39: 23-29.
22. Ercis S, Köseoğlu O, Salmazadeh-Ahrabi S, Ercis M, Akin L, et al. (2005) Prevalence of nasopharyngeal *Neisseria meningitidis* carriage, serogroup distribution, and antibiotic resistance among healthy children in Cankaya municipality schools of Ankara province. Mikrobiyol Bul 39: 411-420.
23. van Gils EJM, Hak E, Veenhoven RH, Rodenburg GD, Bogaert D, et al. (2011) Effect of seven-valent pneumococcal conjugate vaccine on *Staphylococcus aureus* colonisation in a randomised controlled trial. PLoS One 6: e20229.
24. Bogaert D, van Belkum A, Sluifjter M, Luijendijk A, de Groot R, et al. (2004) Colonisation by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. The Lancet 363: 1871-1872.
25. Stephens DS (1999) Uncloaking the *Meningococcus*: Dynamics of carriage and disease. The Lancet 353: 941-942.
26. Rijal KR, Pahari N, Shrestha BK, Nepal AK, Paudel B, et al. (2008) Prevalence of methicillin resistant *Staphylococcus aureus* in school children of Pokhara. Nepal Med Coll J 10: 192-195.
27. Ramana KV, Mohanty SK, Wilson CG (2009) *Staphylococcus aureus* colonization of anterior nares of school going children. Indian J Pediatr 76: 813-816.