



Prevalence of Nasopharyngeal Carriage and Antimicrobial Susceptibility Pattern of Streptococcus Pneumoniae among Preschool Children in Gondar Town, North West Ethiopia

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ABSTRACT

Introduction

Despite the availability of effective antimicrobials and vaccines, Streptococcus pneumoniae remains a major public health problem in children under five years old in the world. Pneumococcal carriage is believed to be an important source of the horizontal spread of this pathogen within the community, particularly in areas where there are crowded living conditions, like schools and daycare centers. The resistance of Streptococcus pneumoniae to antibiotics is gradually becoming a serious problem.

Objective

This study aimed to assess the prevalence of nasopharyngeal carriage and antimicrobial susceptibility pattern of Streptococcus pneumoniae among preschool children in Gondar town, North West Ethiopia.

Methods

A cross-sectional study was conducted from January to March 2018. A total of 401 children aged 1-6 years old attending daycare centers and kindergartens in Gondar town were selected using a multistage sampling technique. Nasopharyngeal swabs were collected and inoculated into appropriate media. Identification of Streptococcus pneumoniae was performed using Gram stain, optochin test, and bile solubility test. Antimicrobial sensitivity tests were done using a modified Kirby-Bauer disk diffusion method. Data were entered into the EpiData 3.1 software and analyzed using SPSS version 20. P-value < 0.05 at 95%CI was considered statistically significant.

Results

The overall carriage rate of Streptococcus pneumoniae was 23.2%. Among the tested antibiotics, 83.9% of the isolates were non-susceptible to trimethoprim-sulfamethoxazole, 33.3% to erythromycin, 27.9% to penicillin, and 24.7% to tetracycline. Multidrug resistance was detected in 17 (18.3 %) isolates. In multivariate logistic regression analysis, young age and passive smoking were associated with pneumococcal carriage.

Conclusions

This study revealed a substantially low prevalence of Streptococcus pneumoniae nasopharyngeal carriage. High antimicrobial resistance was observed for most antimicrobial drugs tested. The younger age group and passive smokers were at risk of Streptococcus pneumoniae nasopharyngeal carriage.

Keywords: Streptococcus Pneumoniae; Antimicrobial Susceptibility; Preschool Children; Nasopharyngeal Carriage; Ethiopia

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INTRODUCTION

Streptococcus pneumoniae is part of the normal flora of the upper respiratory tract and colonizes the mucosal surfaces in the nasopharynx of human beings from the first day of life through transmission by contact with respiratory secretions, especially in children due to poor mucosal immune response [1-2].

Asymptomatic carriage is regarded as a risk factor for the development of pneumococcal disease [3]. After primary colonization of the nasopharynx, pneumococci can migrate to other sites, such as the middle ear, sinus, lung, blood, or cerebrospinal fluid, and cause damage, from local manifestations such as otitis media and sinusitis to invasive diseases such as sepsis or meningitis. It is also the most common cause of pneumonia, which is a major global health problem and the leading cause of death in children under five years of age [4].

In addition, the pneumococcal carriage is believed to be an important source of the horizontal spread of this pathogen within the community, particularly in areas where there are crowded living conditions like schools and daycare centers [2,5-7]. Studies have reported that many factors, including age, immune status, socioeconomic factors, crowding, temporal association with respiratory viral diseases, and geographic diversity, influence the nasopharyngeal carriage and incidence of severe pneumococcal disease [8-12]. Nowadays, recent consideration has focused on the role of pneumococcal proteins, including the pneumococcal surface adhesion A (PsaA), used for the detection of invasive pneumococcal serotypes in carriers, as a virulence factor in the pathogenesis of infections with *Streptococcus pneumoniae* [13].

Despite the availability of effective antibiotics and vaccines, *S. pneumoniae* is the leading cause of lower respiratory tract infections worldwide, contributing to more deaths than all other etiologies of lower respiratory tract infection combined [14]. Around 14.5 million episodes of severe pneumococcal disease occur annually in the world, causing 1,612,000 deaths; of them, 825,000 occur among children < 5 years old, representing 11% of the total number of infant deaths, 70% are in developing countries [15, 16].

The resistance of *S. pneumoniae* to antibiotics is gradually becoming a serious problem for public health. Globally, 13.2-68.8% of *S. pneumoniae* isolates have been reported as multidrug-resistant (MDR) [2,17-22]. Initially, pneumococcal strains were universally susceptible to penicillin, which remained the drug of choice. Penicillin reduced susceptible *S. pneumoniae* strains, and multiple drugs emerged during the 1970s and 1980s and spread globally [2,11,17,18,22].

According to the reports by the Asian Network for Surveillance of Resistant Pathogens (ANSORP), the drug resistance rate of *S. pneumoniae* to β -lactam or macrolide antibiotics in recent years continued at a high level [23,24]. In addition, Biocides could act on multiple sites in microorganisms and cause resistance by non-specific means such as efflux pumps, cell wall changes to the reduction of permeability, genetic linkage with both biocide resistance genes and antibiotic resistance genes, the penetration/uptake changes in the envelope by passive

diffusion, effect on the integrity and morphology of membrane, and effects on diverse key steps of bacterial metabolism [25].

Antibiotic choices in Ethiopia are significantly governed by empirical therapy. Vaccination is an alternative way to control pneumococcal disease and reduce nasopharyngeal carriage. The major cause of under-five mortality is acute respiratory tract infection (ARI), 18%, based on 2014 WHO/CHERG (Child Health Epidemiology Reference Group) estimates. A ten-valent pneumococcal conjugate vaccine (PCV10), containing ten pneumococcal serotype-specific polysaccharides conjugated to DPT-HepB-Hib (Synflorix, GlaxoSmithKline, Rixensart, Belgium) have been licensed in Ethiopia for routine immunization of infants starting from November 2011 [26] in the national vaccination as a 3 dose primary series at 6, 10 and 14 weeks of age and no booster dose (3p + 0).

Studies in Ethiopia have reported nasopharyngeal carriage rates ranging from 18% to 43.8% among children [6,7,10,12,20]. However, to date, only a few studies have been conducted on the prevalence of *S. pneumoniae* nasopharyngeal carriage, antimicrobial susceptibility patterns, and associated risk factors in the community. Therefore, this study aimed to assess the prevalence of nasopharyngeal carriage, antimicrobial susceptibility pattern, and associated risk factors of *S. pneumoniae* among preschool children attending kindergartens and daycare centers in Gondar town, North West Ethiopia.

MATERIALS AND METHODS

Study setting and Population

A community-based cross-sectional study was conducted from January 2018 to March 2018 at selected kindergartens and daycare centers in Gondar town, North West Ethiopia. All children 1-6 years of age attending selected kindergartens and daycare centers were included in the study. However, Children treated with antibiotics for the last two weeks and with malformation or trauma of the nasopharynx were excluded from this study.

Study design

A multistage sampling technique was used to select kindergartens by using a simple random sampling technique (lottery method). Nine kindergartens were selected randomly with a 1:2 private to government kindergarten proportion, and the two day-care centers were included. After obtaining consent from each study participant's parent/guardian, a single nasopharyngeal swab was obtained from 401 children. Socio-demographic characteristics were collected using a pretested questionnaire-guided interview. The child's PCV10 status was confirmed by asking parents/guardians to obtain the vaccination card and checking whether the child is fully vaccinated, partially vaccinated, or non-vaccinated.

Isolation and identification of strains

Nasopharyngeal specimens were obtained by a trained person according to the Centers for Disease Control manual [27]. One nasopharyngeal specimen per child was obtained with a sterile flexible flocked swab (Copan Italia S.P.A). After collection, the swab was placed immediately into a skim-milk tryptone glucose

glycerol (STGG) transport medium and transported by cold box to the University of Gondar College of Medicine and Health Sciences Medical Microbiology Laboratory within 4 hours of collection.

The specimens were inoculated onto tryptone soya agar base (TM media, India) supplemented with 5% sheep blood and 5g/mL gentamycin plates by rolling the swab over a small area of the plate and streaking the sample using a sterile loop. The inoculated media were incubated in a 5% CO₂-enriched atmosphere using a candle jar at 37°C for 24-48 hours.

Streptococcus pneumoniae was identified based on the methods outlined and the recommendations given by the WHO manual [28]. Suspect colonies were isolated and identified according to Gram staining, α -hemolysis, susceptibility to optochin, and bile-solubility test (Oxoid Ltd.) [29].

Antimicrobial susceptibility testing

Resistance to penicillin (PEN), erythromycin (ERY), clindamycin (CD), tetracycline (TET), and trimethoprim-sulfamethoxazole (SXT) was determined by the disk diffusion (modified Kirby-Bauer) method (Biomérieux®) following the 2018 Clinical and Laboratory Standards Institute (CLSI) guidelines on Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, England) supplemented with 5% sheep blood [30].

The antimicrobial agents were selected based on the Ethiopian standard treatment guideline for the treatment of *S. pneumoniae* infection. Pure colonies were introduced to 5 mL of normal saline and compared to 0.5 McFarland turbidity standards. A sterile cotton swab was dipped into the adjusted suspension, and any excess was removed by gently rotating the swab against the inside wall of the tube. The swab was evenly applied to the whole surface of Mueller-Hinton agar supplemented with 5% sheep blood, and the plates were left to air dry for 15 minutes. The disks were placed aseptically on the plate with sterile forceps, and the plates were incubated for 24 hours at 37°C in a 5–10% CO₂ environment. Finally, the

diameter of the inhibitory zone was measured to the nearest millimeter from the disks using a ruler. A standard strain of *S. pneumoniae* ATCC 49619 was used as a positive control strain in each procedure. The results were interpreted by comparing them to cut-off points in the Clinical and Laboratory Standard Institute (CLSI) result interpretive standards [30]. Penicillin susceptibility testing was interpreted using oxacillin (1 μ g) disks, with findings comparing the results to the interpretive standards of CLSI [30].

Data organization, processing, and analysis

Data were coded and entered with the statistical package Epi-Data version 3.1 and analyzed using SPSS version 20 software. The results were presented through tables. Descriptive statistics were used to summarize the socio-demographic, carriage rate, and susceptibility patterns of isolates. Bivariate and multivariate logistic regression analyses were carried out to identify potential factors of pneumococcal NP carriage. Adjusted odds ratio at 95% confidence intervals (CI) was used to measure the association between potential risk factors and nasopharyngeal (NP) carriage. Those variables with a cut-off point p-value less than 0.25 in bivariate analysis were candidates for multivariate analysis. P-value < 0.05 at 95% CI was considered statistically significant.

RESULTS

Socio-demographic and clinical characteristics of the study participants

Four hundred and one study subjects aged 1-6 years old, attending the selected kindergartens and day-care centers between January and March 2018 were enrolled in the study. Their median age was 5 years old (interquartile range: 1-6 years old), 188 (46.9%) were male, and 213 (53.1%) were female. The majority of the children, 390 (97.3%), were fully vaccinated. One hundred fourteen (28.4%) children had taken antimicrobials within the past 3 months of enrolment (Table 1).

Characteristics of study participants (n=401)		Frequency	Percent (%)
Sex	Male	188	46.9
	Female	213	53.1
Age	01-Feb	7	1.7
	03-Apr	87	21.7
	05-Jun	307	76.6
Mothers' education level	Illiterate	79	19.7
	Primary school	131	32.7
	Secondary school	111	27.7
	College/university	80	20
Fathers' education level	Illiterate	46	9
	Primary school	145	36.2
	Secondary school	121	30.2
	College/university	89	22.2

Mothers' occupation	Merchant	53	13.2
	Civil servant	83	20.7
	Housewife	196	48.9
	Labor workers	51	12.7
	Others	18	4.5
Fathers' occupation	Merchant	106	26.4
	Civil servant	110	27.4
	Farmers	17	4.2
	Labor workers	152	37.9
	Others	16	3.9
Parents income	<250	1	2.2
	251-500	30	7.5
	501-1000	76	19
	1001-2000	73	18.2
	>2000	221	55.1
Family size	< 5	213	53.1
	> 5	188	46.9
Siblings < 5 years	Yes	185	46.1
	No	216	53.9
Siblings > 5 years	Yes	196	48.9
	No	205	51.1
Number of rooms in the house	1	223	55.6
	>2	178	44.4
Bed-sharing with parents	Yes	325	81
	No	76	19
PCV10 immunization status	Fully	390	97.3
	Partially	7	1.7
	None	4	1
Receive recent antibiotics	Yes	114	28.4
	No	287	71.6
Recent hospitalization	Yes	15	3.7
	No	386	96.3
Respiratory infections in the previous 3 months	Yes	46	11.5
	No	355	88.5
Main fuel	Electric	175	43.6
	Charcoal	221	55.1
	Gas	5	1.2
Passive smoking	Yes	13	3.2
	No	388	96.8

Number of children in one class	<21	10	2.5
	21-40	103	25.7
	41-60	218	54.4
	>60	70	17.5
Waiting time in kindergartens	Full	279	69.9
	Half	122	30.4

Table 1: Socio-demographic and clinical characteristics of study participants among preschool children in kindergartens and daycare centers in Gondar Town, North West Ethiopia, 2018.

Prevalence and associated risk factors for nasopharyngeal carriage of *S. pneumoniae* isolates

The overall prevalence of nasopharyngeal carriage of *S. pneumoniae* was 23.2% (93/401) (95% CI: 19-27). The carriage was common in a younger age group of 1 to 4 years old, with a

prevalence of 29.8% (28 of 94) and passive smoking-exposed children (6/13). Children aged 1-4 years old were 1.8 times more likely to have increased pneumococcal nasopharyngeal carriage than children whose ages were 5 to 6 years old (AOR= 1.771; 95% CI, 1.011-3.103, p=0.046). In addition, children who were exposed to passive smoking were 3.504 times more likely to have increased carriage than children who were not exposed to passive smoking (AOR = 3.504; 95% CI, 1.056-11.630, p=0.040) (Table 2).

Characteristics (n=401)		No	Yes	COR (95%CI)	p-Value	AOR (95%CI)	p-Value
		n (%)	n (%)				
Sex	Male	146 (77.7)	42 (22.3)	1.1 (0.574-1.456)	0.704		
	Female	162 (76.1)	51 (23.9)	1			
Age	01-Apr	66 (70.2)	28 (29.8)	1.579 (0.939-2.654)	0.085	1.771 (1.011-3.103)	0.046*
	05-Jun	242 (78.8)	65 (21.2)	1		1	
Family size	> 5	147(78.2)	41 (21.8)	1.9(0.542-1.377)	0.538		
	< 5	161 (75.6)	52 (24.4)	1			
Siblings < 5	Yes	140 (75.7)	45 (24.3)	0.9 (0.707-1.790)	0.619		
	No	168 (77.8)	48 (22.2)	1			
Siblings > 5	Yes	152 (77.6)	44 (22.4)	1.1 (0.579-1.466)	0.73		
	No	156 (76.1)	49 (23.9)	1			
Number of rooms in the house	1	166 (74.4)	57 (25.6)	0.74 (0.843-2.175)	0.209	1.610 (0.953-2.720)	0.075
	>2	142 (79.8)	36 (20.2)	1		1	
Bed-sharing with parents	Yes	247 (76)	78 (24)	0.8 (0.691-2.386)	0.429		
	No	61(80.3)	15 (19.7)	1			
PCV10 immunization	None	2 (50)	2 (50)	3.333 (0.463-24.00)	0.232	5.080 (0.649-39.775)	0.122
	Partially	6(85.7)	1 (14.3)	0.556 (0.066-4.675)	0.489	0.608 (0.070-5.308)	0.652
	Fully	300(76.9)	90(23.1)	1		1	

Receive recent antibiotics	Yes	81 (71.1)	33 (28.9)	1.541(0.940-2.528)	0.087	1.380 (0.805-2.364)	0.242
	No	227 (79.1)	60 (20.9)	1		1	
Recent hospitalization	Yes	11 (73.3)	4 (26.7)	1.213 (0.377-3.904)	0.746		
	No	297 (76.9)	89 (23.1)	1			
Respiratory tract infections in the past 3 months	Yes	32 (69.6)	14 (30.4)	1.528 (0.777-3.005)	0.219	1.376 (0.658-2.876)	0.397
	No	276 (77.7)	79 (22.3)	1		1	
Main fuel	Electric	132 (75.4)	43 (24.6)	1			
	Charcoal	173 (78.3)	48 (21.7)	0.852(0.532-1.362)	0.503		
	Gas	3 (60)	2 (40)	2.047 (0.331-12.656)	0.441		
Passive smoking	Yes	7 (53.8)	6 (46.2)	2.966 (0.971-9.055)	0.056	3.504 (1.056-11.630)	0.040*
	No	301 (77.2)	87 (22.8)	1		1	
Number of children in one class	< 21	9 (90)	1 (10)	1			
	21-40	76 (73.8)	27 (26.2)	3.197 (0.387-26.429)	0.281		
	41-60	164 (75.2)	54 (24.8)	2.963 (0.367-23.929)	0.308		
	> 60	59 (84.3)	11(15.7)	1.678 (0.193-14.607)	0.639		
Waiting time in kindergarten or daycare	Full	208 (74.6)	71 (25.4)	1.552 (0.909-2.648)	0.107	1.427 (0.636-3.202)	0.389
	Half	100 (81.9)	22 (18.1)	1			
Mothers' education level	Illiterate	60 (75.9)	19 (24.1)	0.835 (0.410-1.702)	0.739		
	Primary school	103 (78.6)	28 (21.4)	0.717 (0.376-1.365)	0.619		
	Secondary school	87 (78.4)	24 (21.6)	0.727 (0.373-1.417)	0.311		
	College/ University	58(72.5)	22 (27.5)	1			
Fathers' education level	Illiterate	34 (73.9)	12 (26.1)	0.904 (0.404-2.019)	0.805	0.908 (0.319-2.587)	0.857
	Primary school	115 (79.3)	30 (20.7)	0.668(0.362-1.232)	0.196	0.611 (0.279-1.336)	0.217
	Secondary school	95 (78.5)	26 (21.5)	0.701(0.372-1.321)	0.271	0.690 (0.313-1.520)	0.69
	College/ University	64 (71.9)	25 (28.1)	1		1	

Mothers' occupation	Merchant	42 (79.2)	11(20.8)	0.917 (0.251-3.345)	0.895		
	Civil servant	63 (75.9)	20 (24.1)	1.013 (0.317-3.234)	0.982		
	Housewife	152 (77.6)	44 (22.4)	1.111 (0.328-3.763)	0.866		
	Labor workers	37 (72.5)	14 (27.5)	1.324 (0.372-4.716)	0.665		
	Others	14 (77.8)	4 (22.2)	1			
Fathers' occupation	Merchant	84 (79.2)	22 (20.8)	0.786 (0.231-2.675)	0.7		
	Civil servant	84 (76.4)	26 (23.6)	0.400 (0.062-2.568)	0.334		
	Farmer	15 (88.2)	2 (11.8)	0.929 (0.276-3.127)	0.905		
	Labor workers	113 (74.3)	39 (25.7)	1.035 (0.315-3.399)	0.954		
	Others	12 (75)	4 (25)	1			
Parent income	< 250	8 (80)	2 (20)	0.714 (0.147-3.463)	0.675		
	251-500	25 (83.3)	5 (16.7)	0.571 (0.208-1.565)	0.276		
	501-1000	60 (78.9)	16 (21.1)	0.761 (0.405-1.431)	0.397		
	1001-2000	58 (79.5)	15 (20.5)	0.738(0.387-1.408)	0.357		
	>2000	157 (74.1)	55 (25.9)	1			

Table 2: Bivariate and multivariate analyses of risk factors associated with *S. pneumoniae* among preschool children in kindergartens and daycare centers in Gondar town, North West Ethiopia, 2018.

Note: COR = Crude Odds Ratio, AOR = Adjusted Odds Ratio, PCV = Pneumococcal Conjugate Vaccine

Antimicrobial susceptibility of *S. pneumoniae* isolates

The resistance patterns of 93 pneumococcal isolates were tested

against five antimicrobial agents. In this study, the highest degree of non-susceptibility among the five antimicrobials was observed for trimethoprim-sulfamethoxazole (83.9%), followed by erythromycin (33.3%) and penicillin (27.9%). Twenty-three (24.8%) isolates were non-susceptible to tetracycline, and 5 (5.4%) isolates were non-susceptible to clindamycin. All penicillin (26/26) resistant strains were resistant to TMP-SMX (100%) and susceptible to clindamycin (96.2%). Only 8 isolates were susceptible to all the antibiotics tested (Table 3).

Antimicrobial Agents	Antimicrobial susceptibility pattern (n= 93)		
	Resistance: n (%)	Intermediate: n (%)	Susceptible: n (%)
Penicillin (PEN)	26 (27.9)	0 (0)	67 (72.1)
Trimethoprim-sulfamethoxazole (SXT)	65 (69.9)	13 (13.9)	15 (16.1)
Clindamycin (CLI)	5 (5.4)	0 (0)	88 (94.6)
Erythromycin (ERY)	20 (21.5)	11 (11.8)	62 (66.7)
Tetracycline (TET)	17 (18.3)	6 (6.5)	70 (75.3)

Table 3: Antimicrobial susceptibility pattern of *S. pneumoniae* among preschool children in kindergarten and daycare centres in Gondar town, North West Ethiopia, 2018.

Bacteria being resistant to three or more classes (penicillin and two or more non-beta-lactam antimicrobials) of antibiotics are multidrug resistant (MDR). Multidrug resistance was detected in

17 (18.3%) isolates. The predominant profile of MDR was observed in PEN, TET, and SXT, with 8 (47.1%), followed by PEN, TET, ERT, and SXT, with 4 (23.5%). One isolate was resistant to all antimicrobials, and four isolates were resistant to all antimicrobials except clindamycin (Table 4).

Antimicrobial Agents	Frequency	Percent (%)
PEN, TET, ERY	3	17.6
PEN, TET, SXT	8	47.1
PEN, ERY, SXT	1	5.9
PEN, TET, ERY, SXT	4	23.5
PEN, TET, ERY, SXT, CLI	1	5.9
Total	17	100

Table 4: Multi-drug resistance pattern of *S. pneumoniae* among preschool children in kindergarten and daycare centers in Gondar town, North West Ethiopia, 2018.

DISCUSSION

The overall prevalence of *S. pneumoniae* nasopharyngeal carriage in this study was 23.2% (95% CI: 19-27), which lies between the low carriage rate of 0.4% and the high rate of 56.3% in different areas of the world [17, 31]. The carriage rate was comparable to a study done in India at 27% [32], Hawassa, Ethiopia, at 21.5% [6], Arsi zone, Southeast Ethiopia, at 18.4% [7], and Southwest Ethiopia at 25.3% [12]. However, it was lower than studies done in Indonesia up to 56.3% [17], Nigeria 42.2% [33], South Ethiopia 43.8% [20], Colombia 41.8% [11], Uganda 58.6% [34], Jimma 43.8% [22] and Gondar 41% [8] and higher than studies done in Turkey 0.4% [31], Iran 15.7% [35] and Jig-Jiga, Ethiopia 18% [10]. These differences might be related to sampling, characteristics of the studied population (i.e., age, residence, having siblings, presence of respiratory tract infection, vaccination status), and laboratory methods.

In this study, a substantially lower prevalence was detected as compared to the previous studies. This might be because most of the children, 307 (76.6%), recruited were 5 to 6 years old. The decrease in *S. pneumoniae* carriage rate associated with increasing age may reflect the gradual development of mucosal immunity and progressive increase in clearance rates. Moreover, 97.3% of the children included in the present study were fully vaccinated and healthy.

Antimicrobial resistance has been recognized as an emerging problem worldwide, both in developed and developing countries. The increased use of antimicrobial agents is a significant factor in the emergence of antibiotic-resistant bacteria [34]. Treatment of infections due to *S. pneumoniae* has become a complicated global problem due to antibiotic resistance [24]. In this study, the majority, 69.9% (65/93) of the isolates tested were insensitive to trimethoprim-sulfamethoxazole, which is in line with studies done in Ethiopia [6,22]. However, the

insensitivity level of trimethoprim-sulfamethoxazole was lower in developed countries as compared to the current study [2,5,11,19]. The high proportion of insensitivity found in TMP-SMX could be because it has broad antimicrobial coverage in Ethiopia, is the least expensive orally administered antibiotic, and is readily available over the country in many settings. The pneumococcal nasopharyngeal isolates also demonstrated resistance to other antimicrobial agents like erythromycin, penicillin, tetracycline, and clindamycin. In this study, 27.9% of isolates were resistant to penicillin. This finding was in line with a study conducted in the Ecuadorian Andes at 27.7% [19]. However, higher proportions of *S. pneumoniae* were reported in Indonesia at 49.7% [17], Colombia at 57.7% [11], Iran at 73% [35], North Showa, Ethiopia at 47.2%, and Jimma at 36.1% [18]. This difference might be due to methodology differences; the majority of the studies used dilution techniques, and the low resistance rates of isolated *S. pneumoniae* for clindamycin might be due to the restricted use of some antibiotics in the community.

Multi-drug-resistant *S. pneumoniae* is increasingly being reported from many parts of the globe [2, 17-22]. Penicillin susceptibility is an important marker for the presence or absence of a multidrug-resistant phenotype. Strains with reduced susceptibility to penicillin are usually cross-resistant to other antibiotics. Prolonged carriage and rapid reacquisition provide an increased chance of exposure to antibiotics and thus may be an important selective factor in predisposing to antibiotic resistance [5, 24]. In our study, 18.3% of isolates were multidrug-resistant. The majority of MDR isolates consisted of resistance to penicillin, TMP-SMX, and tetracycline. The occurrence of MDR in this study was consistent with reports in India at 19% [36], Indonesia at 18% [2], and Jimma, Ethiopia, at 17.7% [18] but lower than studies done in the Ecuadorian Andes at 20.3% and Southern Ethiopia up to 42.2% [20, 21]. However, it was higher than the finding of another study, which was conducted in Gondar, Ethiopia, 6.3% [8], Indonesia 13.2% [17], Jig-Jiga, Ethiopia 4.4% [10], South Africa 9% [5], and Hawassa, Southern Ethiopia 2.9% [6]. The high MDR observed in this

study might be due to the administration of multiple antimicrobials for infections and the indiscriminate use of antimicrobials. This might be due to *S. pneumoniae* developing a beta-lactam resistance pathway, which includes genetic changes that alter the structure of penicillin-binding proteins, resulting in a lower affinity for all beta-lactam medicines.

Various demographic characteristics in different studies have been described to be associated with an increase in *S. pneumoniae* carriage including age, the number of rooms in the house, PCV10 vaccination status, antibiotic use in the previous 3 months, passive smoking, waiting time in kindergarten, and others [6-8, 10, 12, 17, 20, 22 and 32]. In our study, young age [1-4 years] [AOR = 1.771, 95% CI: 1.011-3.103, P = 0.046] was associated with nasopharyngeal *S. pneumoniae* carriage. This finding was consistent with studies performed in Gondar North West, Ethiopia [8], North Showa, Ethiopia [22], India [32], and Indonesia [17]. This can be explained by the fact that younger children are immunologically immature and carry *S. pneumoniae* in their nasopharynx in high magnitude. We also found an increased carriage of *S. pneumoniae* in children who were exposed to passive smoking [AOR = 3.504, 95% CI: 1.056-11.630, P = 0.040]. This was also in agreement with reports in North Showa, Ethiopia [22], and Indonesia [17]. This might be because smoke damages and inflames the nasopharyngeal mucosa, increasing susceptibility to *S. pneumoniae* colonization. The differences observed in this study might be because of changes in the epidemiologic characteristics of pneumococcal carriage in Ethiopia.

CONCLUSIONS

The findings in this study indicate a significantly low prevalence of pneumococcal carriage among children and reinforce previous observations indicating that asymptomatic colonization by these microorganisms is common among children, particularly among children. Passive smoking was associated with a significant increase in *S. pneumoniae* nasopharyngeal carriage rate. However, high antimicrobial resistance and multiple drug resistance of *S. pneumoniae* were observed in most antimicrobial drugs tested. Since data on isolates from nasopharyngeal colonization provides relevant information on the potential burden of pneumococcal diseases, antimicrobial resistance, and awareness should be created among community members to prevent risk factors associated with *S. pneumoniae* nasopharyngeal colonization. This is essentially important now in Ethiopia, where PCV10 has been introduced for routine immunization starting from November 2011.

LIMITATIONS

Due to a lack of resources for MIC, penicillin resistance was assessed using a modified disk diffusion approach. We did not serotype the pneumococcus in this investigation, which would have been useful to examine the distribution of serotypes. Similarly, there was no link between the genotypic and phenotypic MDR.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in this study were reviewed and approved by the Ethical Review Board of the University of Gondar in accordance with the 1964 Helsinki Declaration. Permission was obtained from each hospital laboratory for collecting the isolates. Written informed consent was obtained from each legal guardian after explaining the purpose and objective of the study.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included within the document.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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AUTHORS' CONTRIBUTIONS

YS: Conception; acquisition, analysis; Data collection, interpretation of data; drafted the work and revised the manuscript, **FM:** acquisition, analysis, and interpretation of data, manuscript review and editing **GY:** revised the manuscript, **AA:** acquisition, analysis, and interpretation of data, manuscript review and editing, **WA:** analyzed, and revised the manuscript, **NM:** Software, analysis, and interpretation of data, **AB:** analysis, and revised the manuscript, **BS:** Conception, data collection, software, review and editing.

ABBREVIATIONS

IPD: Invasive pneumococcal diseases; MDR: Multidrug resistance; MRSP: Macrolide-resistant *Streptococcus pneumoniae*; ANSORP: Asian Network for Surveillance of Resistant Pathogens; ARI: Acute Respiratory Infection; CHERG: Child Health Epidemiology Reference Group; CLSI: Clinical and Laboratory Standards Institute; NP: Nasopharyngeal; PCV: Pneumococcal conjugate vaccines; STGG: Skim milk tryptone-glucose-glycerin; CI: Confidence Interval.

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