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RESEARCH ARTICLE

Nasopharyngeal Carriage of *Streptococcus pneumoniae* and Associated Factors among Children in Southwest Ethiopia

Dejene Derseh Abateneh[1,2,*], Abera Kumalo Shano[2,3] and Teshale Worku Dedo[2]

[1]*Kotebe Metropolitan University, Menelik II College of Medicine and Health Sciences, Addis Ababa, Ethiopia*
[2]*Department of Medical Laboratory Sciences, College of Health Sciences, Mizan-Tepi University, Mizan Teferi, Ethiopia*
[3]*Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Wolaita Sodo University, Sodo, Ethiopia*

Abstract:

Background:

In Ethiopia, *Streptococcus pneumoniae* is the predominant causative agent of pneumonia. About, 95% of bacterial pneumonia cases in under five years of children are caused by *pneumococci*.

Objective:

To assess the nasopharyngeal carriage of *Streptococcus pneumoniae*, its antibiotic susceptibility pattern, and associated factors among children in Southwest Ethiopia.

Methods:

A cross-sectional study was conducted from October 01, 2018, to December 30, 2018. A total of 293 children aged ≤15 years were included in the study using a systematic random sampling technique. A nasopharyngeal swab was collected using a sterile cotton swab and cultured on blood agar supplemented with 5µg/ml gentamicin. The antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion technique.

Results:

The ages of participants ranged from 5 months to 14 years. The carriage rate of *Streptococcus pneumoniae* was 74/293 (25.3%). Being within the age group <3 years, the habit of sleeping with parent(s)/guardians and numbers of rooms per household were significantly associated with pneumococcal carriage. *Streptococcus pneumoniae* showed the highest resistance to Tetracycline, 36 (48.65%), and Trimethoprim/sulfamethoxazole, 29 (39.2%), and was found to be susceptible to Chloramphenicol, 54 (77%), and Erythromycin, 38 (51.4%).

Conclusion:

The nasopharyngeal carriage rate of *Streptococcus pneumoniae* is considerably high. High antimicrobial resistance of *Streptococcus pneumoniae* against Tetracycline and Trimethoprim/sulfamethoxazole was observed. Living in a house with a single room, children's habit of sleeping with parents/guardians and age are associated factors of high pneumococcal carriage. Strategies need to be designed to address the modifiable associated factors and the bacterium antibiotic resistance pattern should be monitored regularly.

Keywords: *Streptococcus pneumoniae*, Nasopharyngeal carriage, Antibiotics susceptibility, Children, Ethiopia, Trimethoprim/sulfamethoxazole.

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1. INTRODUCTION

Streptococcus pneumoniae or pneumococci is the etiology of bacterial pneumonia, bacteremia, meningitis, otitis media, and sinusitis mainly in children, the elderly, and immune-compromised patients [1]. The human nasopharynx is the primary reservoir of *Streptococcus pneumoniae* [2, 3]. Asymptomatic carriage is a precondition for pneumococcal diseases and serves as the main source of person-to-person transmission [4, 5]. Nasopharyngeal isolates reflect the epidemiological aspects of diseases caused by *Streptococcus pneumoniae* in the community [6, 7], and which indicates the infection-causing strains currently circulating within the

* Address correspondence to this author at the Kotebe Metropolitan University, Menelik II College of Medicine and Health Sciences, Department of Medical Laboratory Sciences, Addis Ababa, Ethiopia; P.O. Box: 3268. Ethiopia; Tel: +251920514158; E- mail: dejenieh@gmail.com

community [8]. The isolates can be used as an estimate of the incidence of resistance in systemic isolates [9].

The global distribution of children dying from pneumonia is uneven, in which the majority of the cases occur in developing countries. The bacterium is grouped as microorganism of serious public health threat [10]. Antibiotic-resistant pneumococci is an increasing global concern and it is the principal cause of treatment failures for acute respiratory illness (ARI) and meningitis in developing countries since ARI is often treated empirically with antibiotics [4]. *Streptococcus pneumoniae* has developed resistance to drugs in the penicillin and erythromycin groups. In recent years, the spread of *Streptococcus pneumoniae* strains that are resistant to more than one antibiotic has increased in many countries. The rapid emergence of resistance to multiple antimicrobial agents has further complicated the problem of the disease. The rapid increase in resistance to penicillin and other antimicrobial agents worldwide has made the choice of antimicrobial drugs for pneumococci infections more difficult [11, 12].

In sub-Saharan Africa, *Streptococcus pneumoniae* accounts for about 25% to 30% of meningitis cases and 30% to 50% pneumonia cases in children under 5 years [13 - 15]. Diseases caused by pneumococci are the leading causes of childhood mortality in low and middle-income countries [16].

Ethiopia is grouped in the top 10 countries in which two-third of deaths are reported due to pneumonia [17 - 19], which means that one in every four deaths among children under five years of age is caused by pneumonia. The mortality rate under five years of children due to *Streptococcus pneumoniae* is high although Pneumococcal Conjugate Vaccine 10 (PCV-10) was introduced in 2011 [20, 21]. *Streptococcus pneumoniae* is identified in most cases of pneumonia, which accounts for 95% of bacterial pneumonia cases, and it is therefore considered the biggest infectious killer of children aged below 5 years [14, 15].

Data on pneumococci is limited in the country studied and antimicrobial resistance pattern of *Streptococcus pneumoniae* is not regularly monitored. Therefore, this study aimed to assess the nasopharyngeal carriage, antibiotic susceptibility pattern, and associated factors of *Streptococcus pneumoniae* among children in Southwest Ethiopia.

2. MATERIALS AND METHODS

2.1. Study Site, Design, and Participants

A cross-sectional study was conducted in Mizan Tepi University teaching hospital, Mizan Teferi, Southwest Ethiopia. It is a public hospital located in the Bench Maji zone, Southwest region of Ethiopia, 561 km far from the capital city, Addis Ababa. The area has a latitude and longitude of 7°0'N 35°35'E/7.000°N35.583°E with an elevation of 1451 meters above sea level. The hospital provides medical services for people in Southwest Ethiopia and its surroundings. The study site is in a district where pastoral, semi-pastoral, and agrarian communities reside.

The study was conducted from October 01, 2018, to December 30, 2018. All children aged ≤ 15 years and visiting the pediatric outpatient department (OPD) of a hospital for seeking care were included as the study population. The study participants were selected systematically at regular intervals from their sequence of visits using a systematic random sampling technique. An average 15 pediatric visits occurred in the hospital every day based on the previous records of the pediatrics OPD. Considering the daily flow of pediatric visits along with the study period, sampling interval (k) three was calculated and used. The first participant to be included in the study was selected by the lottery method from the order of the first three. Thereafter, at every third interval, participants were included in the study until the total sample size was achieved.

The minimum sample size (n) was determined using single population proportion sample size formula [$n = (Z\ \alpha/2)^2\ P\ (1\text{-}P)\ /d^2$], where $Z\alpha/2$ = the value under the standard normal table at the 95% level of confidence which was 1.96, d= precision which was set at 5%, P= the prevalence of nasopharyngeal carriage rate of *Streptococcus pneumoniae, i.e.* 41.03% taken from a previous study conducted in Gondar University Hospital, Ethiopia [22]. Considering sample size correction and a 10% nonresponse rate, the final minimum sample size included 293 children.

2.2. Data Collection and Laboratory Methods

2.2.1. Socio-demographic and Clinical Characteristics

A semi-structured questionnaire adapted from peer-reviewed literature [22 - 24] was used to collect socio-demographic and other clinical characteristics by trained data collectors. Socio-demographic factors included age of the child, sex of the child, parents' or guardians' educational status, religion, occupation, socioeconomic living conditions such as family size, presence of <5 years siblings, presence of siblings ≥5 years, number of rooms in a house, the habit of sleeping with parents/guardians, previous antibiotic use, previous hospitalization, and respiratory tract infections.

2.2.2. Specimen Collection and Processing

After written informed consent was obtained from each participant's parent(s) or guardian, a single nasopharyngeal swab was collected from each participant using a sterile cotton swab inserted through the nostrils into the posterior according to the recommendation of Centers for Diseases Control and Prevention guidelines. The swab was rotated at 180°, withdrawn and inserted in Amies transport medium (Oxoid, Basingstoke, Hampshire, England). The nasopharyngeal swab with Amies transport medium was transported to the Mizan Tepi University biomedical laboratory within 4hrs at room temperature and vortexed for approximately 10-20 seconds for culture.

The nasopharyngeal swab was cultured on sheep blood agar supplemented with 5μg/ml gentamicin which was used as a selective medium to culture pneumococci, and incubated overnight at 37°C in a 5-10% CO_2 enriched atmosphere by using a candle jar; then finally, the identification of *Streptococcus pneumoniae* was done based on colony morphology, hemolysis pattern, sensitivity to optochin, and bile solubility test [25]. A typical colony of *Streptococcus*

pneumoniae was small, grayish, moist and watery surrounded by a greenish zone of alpha hemolysis of the medium around the colony. As a confirmatory test of *Streptococcus pneumoniae*, the alpha-hemolytic colony was sub-cultured on blood agar with 5 μg optochin disc and was read after overnight incubation in a candle jar at 37°C. Isolates with a zone of inhibition > 14 mm diameter were considered susceptible to optochin and identified as *Streptococcus pneumoniae*. Isolates with zones of inhibition ranging from 9 mm to 13 mm diameter were tested for bile solubility for confirmation of *Streptococcus pneumoniae* using 2% Sodium deoxycholate (bile salt) (Oxoid, Basingstoke, Hampshire, England) (Supplementary file S1).

2.2.3. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility test was done using the Kirby-Bauer disc diffusion technique on Mueller–Hinton agar (MHA) (Oxoid, Basingstoke, Hampshire, England) supplemented with 5% sheep blood [26]. Direct colony suspension, equivalent to a 0.5 McFarland standard, was prepared using colonies from an overnight incubated (18 to 20 hours) sheep blood agar plate. A sterile cotton swab was dipped into the standardized solution and used for evenly inoculating Mueller–Hinton plates. The plates were then allowed to dry. Antibiotic disks with the following concentrations were placed on the plates: Oxacillin (1μg), Erythromycin (15μg), Chloramphenicol (30μg), Tetracycline (30μg) and Trimethoprim/Sulfamethoxazole (TMP/SMX) (1.25/ 23.75μg).

The antimicrobial susceptibility patterns of *Streptococcus pneumoniae* isolates were classified as susceptible, intermediately resistant, or resistant to the selected antibiotics based on Clinical Laboratory Standard Institute guidelines (CLSI) 2014 recommendation [26]. As per the CLSI guidelines, for nonmeningitic isolates of *Streptococcus pneumoniae,* interpretation of penicillin susceptibility testing was performed using Oxacillin (1μg) disks (Supplementary file S2). Isolates of pneumococci with Oxacillin zone sizes of ≥20 mm were susceptible to Penicillin. However, penicillin's minimum inhibitory concentration should be determined for those isolates with Oxacillin inhibition zones ≤19mm to report penicillin resistance. The antimicrobial agents were selected based on the prescription practices in hospitals and the Ethiopian standard treatment guideline for the treatment of *Streptococcus pneumoniae* infection as well as the susceptibility test type affordability. The antibiotics were well spaced in order to prevent the overlapping of inhibition zones. The plates were incubated at 37°C for 24 hours. Quality control for susceptibility testing was performed daily using *S. pneumoniae* ATCC49619 control strain.

2.3. Statistical Analysis

Data were entered by using Epi info version 7.0 and transferred onto SPSS version 16 for cleaning, categorization, and analysis. Frequencies and proportions were calculated as univariate analysis to describe the study population in relation to relevant variables. Bivariate analysis was conducted to identify the association between each independent variable with the outcome variable. Multivariate analysis was employed to identify independent predictors associated with the outcome variable. In the multinomial model, the adjusted odds ratio (AOR) and corresponding 95% confidence interval (CI) were retrieved. Those variables with a p-value of less than 0.05 were considered as statistically significant.

2.4. Data Quality Control

The data collectors were trained, and supervision was made during data collection. All necessary materials were prepared and inspected before sample collection. A control organism was used for each test or a new set of testing conditions to guarantee the accuracy of the susceptibility test result. Standard strain, *Streptococcus pneumoniae* ATCC 49619 control strain, was used. Zone diameters obtained for the control strain were compared with CLSI 2014 guideline. The optochin disk was tested with positive and negative controls. As a positive control, *Streptococcus pneumoniae* ATCC 49619 was used, and *Streptococcus m*itis strain ATCC 49456 as a negative control. Then the results of the laboratory tests were recorded in a well-prepared format carefully and finally were attached to the questionnaire.

3. RESULTS

3.1. Socio-demographic Characteristics of the Participants

A total of 293 participants were enrolled in the study. Most of the participants were males, 170 (58%). Only 6.1% of participants had a history of hospitalization. Most of the study participants, (78.2%) were living in a family size of > 5 members per household. The ages of participants ranged from 5 months to 14 years with mean age, 5±3.8 years and most of the study participants were within the age group < 3 years, 123 (42%) (Table **1**).

Table 1. Sociodemographic characteristics and distribution of nasopharyngeal carriage rate of *Streptococcus pneumoniae* among children (N=293).

Characteristics		Frequency (%)	*Streptococcus pneumoniae* carriage	
			Carrier	Non-carrier
Sex	Male	170(58)	46(62.2)	124(56.6)
	Female	123 (42)	28(37.8)	95(43.4)
Age	<3	123(42)	37(50)	86(39.3)
	3-9	118(40.27)	31(41.9)	87(39.7)
	9-15	52(17.75)	6(8.1)	46(21)

(Table 1) cont.....

Characteristics		Frequency (%)	*Streptococcus pneumoniae* carriage	
			Carrier	Non-carrier
Religion	Orthodox	64(21.8)	17(23)	47(21.5)
	Protestant	196(66.9)	49(66.2)	147(67.1)
	Muslim	32(10.9)	7(9.5)	25(11.4)
	Others	1(0.3)	1(1.4)	0(00)
Educational status of parents/guardians	Unable to read & write	46(15.7)	12(16.2)	34(15.5)
	Primary (1-8)	37(12.6)	39(52.7)	128(58.4)
	Secondary (9-12)	167(57.0)	8(10.8)	29(13.2)
	12 and above	43(14.7)	15(20.3)	28(12.8)
Marital status of parents/guardians	Single	6(2)	2(2.7)	4(1.8)
	Divorced	7(2.4)	1(1.4)	6(2.7)
	Widowed	1(0.3)	0(00)	1(0.5)
	Married	279(95.2)	71(95.9)	208(95)
Parents/guardians occupation	Housewife	77(26.3)	21(28.4)	56(25.6)
	Merchant	22(7.5)	4(5.1)	19(8.7)
	Student	10(3.4)	5(6.8)	5(2.3)
	Govt. employed	50(17.1)	19(25.7)	31(14.2)
	Private employed	134(45.7)	26(35.1)	108(49.3)
Monthly income (ETB) of parents/guardians	<250	6(2)	1(1.4)	5(2.3)
	250-500	28(9.6)	8(10.8)	20(9.1)
	500-1000	203(69.3)	51(68.9)	152(69.4)
	1000-2000	27(9.2)	5(6.8)	22(10)
	>20000	29(9.9)	9(12.2)	20(9.1)
Family size	<5 members	229(78.2)	59(79.7)	170(77.6)
	>=5 members	64(21.8)	15(20.3)	49(22.4)
Number of rooms per household	1	58(19.8)	18(24.3)	40(18.3)
	2+	235(80.2)	56(75.7)	179(81.7)
Previous antibiotic use	Yes	179(61.1)	51(68.9)	128(58.4)
	No	114(38.9)	23(31.1)	91(41.6)
Siblings <5 years	Yes	118 (40.3)	51(68.9)	128(58.4)
	No	175 (59.7)	23(31.1)	91(41.6)
Siblings >5 years	Yes	165(55.3)	40(54.1)	125(57.1)
	No	128 (44.7)	34(45.9)	94(42.9)
Habit of sleeping with parent(s)/guardians	Yes	286(97.6)	73(98.6)	213(97.3)
	No	7(2.4)	1(1.4)	6(2.7)
Previous hospitalization	Yes	18 (6.1)	7(9.5)	11(5)
	No	275(93.9)	67(90.5)	208(95)
Respiratory tract infections	Yes	137(46.8)	39(52.7)	98(44.1)
	No	156(53.2)	35(47.3)	121(55.3)
PCV-10 vaccination status	Yes	282(96.2)	71(95.9)	211(96.3)
	No	11(3.8)	3(4.1)	8(3.7)
Total		**293 (100%)**	**74**	**219**

Table 2. Bivariate and multivariate logistic regression analysis of nasopharyngeal carriage rate of *Streptococcus pneumoniae* with independent variables (N=293).

Characteristics		*COR (95% CI)*	*p-value*	*AOR (95% CI)*	*p-value*
Sex	Male	1.259(0.733-2.161)	0.404	-	-
	Female	1*	-	-	-
Age	<3	3.298(1.296-8.393)	0.012	2.9(1.042-8.27)	0.042
	3-9	2.732(1.062-7.024)	0.037	2.5(0.957-6.803)	0.061
	9-15	1*	-	1*	-

(Table 2) cont.....

Characteristics		*COR (95% CI)*	*p-value*	*AOR (95% CI)*	*p-value*
Religion	Orthodox	1.063(0.560-2.018)	0.851	-	-
	Protestant	0.823(0.336-2.020)	0.671	-	-
	Others	1*	-	-	-
Educational status	Unable to read & write	0.659 90.265-1.6350	0.368	-	-
	Primary (1-8)	0.515(0.189-1.404)	0.195	-	-
	Secondary (9-12)	0.569(0.276-1.171)	0.126	-	-
	12 and above	1*	-	-	-
Marital status of parents/guardians	Single	0.799(0.217-2.946)	0.736	-	-
	Married	1*	-	-	-
Parents/guardian's occupation	Non- employed	1.12(0.651-1.925)	0.682	-	-
	Employed	1*	-	-	-
Family size	<5	1*	-	1*	-
	>=5	0.882(0.461-1.689)	0.0705	0.99(0.501-1.99)	0.999
Number of rooms per household	1	2.438(1.764-2.762)	0.0260	2.36(1.68-2.717)	0.038
	2+	1*	-	1*	-
Previous antibiotic use	Yes	1.576(0.900-2.762)	0.112	1.367(0.71-2.64)	0.351
	No	1*	-	1*	-
Siblings <5 years	Yes	1.337(0.774-2.312)	0.298	-	-
	No	1*	-	-	-
Siblings >5 years	Yes	0.885(0.521-1.503)	0.650	-	-
	No	1*	-	-	-
Habit of sleeping with parents	Yes	2.056(1.243-17.367)	0.048	2.26(1.25-20.24)	0.031
	No	1*	-	1*	-
Previous hospitalization	Yes	0.976(0.736-5.3)	0.176	1.704(0.59-4.93)	0.326
	No	1*	-	1*	-
Respiratory tract infections	Yes	1.376(0.811-2.334)	0.237	1.14(0.62-2.08)	0.673
	No	1*	-	1*	-
PCV-10 vaccination status	Yes	1*	-	-	-
	No	1.114(0.288-4.316)	0.875	-	-

*reference category

3.2. The Nasopharyngeal Carriage Rate of *Streptococcus pneumoniae*

From a total of 293 nasopharyngeal swab specimens, *Streptococcus pneumoniae* was isolated from 74 of them yielding a carriage rate of 25.3%. The carriage rate was high in the age group <3 years, 37/74 (50%) and males, 46/74 (62.2%) (Table **1**).

3.3. Factors Associated with Nasopharyngeal Carriage Rate of *Streptococcus pneumoniae*

Bivariate logistic regression analysis of socio-demographic variables demonstrated that only age group <3 years, (COR 3.298, 95% CI ([1.296-8.393]), p= 0.012) and age group 3-9 years, (COR 2.732, 95% CI ([1.062-7.024]), p=0.037), were significantly associated with carriage rate of *Streptococcus pneumoniae*. In addition, bivariate logistic regression analysis of related factors with carriage rate showed number of rooms per household (COR 2.438, 95% CI ([1.764-2.762]), p=0.020) and habit of sleeping with parents/guardians (COR 2.056, 95% CI ([1.243-17.367]), p=0.048) to be significantly associated with nasopharyngeal carriage rate of *Streptococcus pneumoniae.* The other sociodemographic characteristics such as sex, religion, educational status of parents/guardians, marital status of parents, and parents/guardian's occupation were not significantly associated with pneumococcal carriage (Table **2**).

Variables with a p-value of ≤0.25 in the bivariate analysis were candidate variables for multivariate analysis. After adjusting the possible confounders, age group < 3 years, the habit of sleeping with parent(s)/guardians, and the number of rooms per household were independent predictors of carriage rate.

Children of younger age group <3 years were three times more likely to carry *Streptococcus pneumoniae* in their nasopharynx compared to the age group 9-15 years (AOR 2.9, 95% CI ([1.042-8.27]), p=0.042). Those who had a habit of sleeping with parent(s)/guardians were two times more likely to carry *Streptococcus pneumoniae* in their nasopharynx compared to those who did not (AOR 2.26, 95% CI ([1.25-20.24]) and those who lived in a house with a single room were two times more likely to carry *Streptococcus pneumoniae* in their nasopharynx compared to those living in a house with more than two rooms (AOR 2.36, 95% CI ([1.68-2.717]), p=0.038) (Table **2**).

3.4. Antimicrobial Susceptibility Pattern of *Streptococcus pneumoniae*

Susceptibility test for applied antibiotics was done on

MHA with a 5% sheep blood supplement following CLSI recommendation. Only 10 isolates showed susceptibility to all applied antibiotics. Twenty-one isolates (28.4%) were resistant to two antimicrobial agents and 14 (18.9%) isolates were resistant to three or more antibiotics. The isolates were susceptible to Erythromycin, 38 (51.4%), and Chloramphenicol, 57 (77%). Forty-nine (66.2%) isolates of pneumococci showed Oxacillin zone size > 20mm which were susceptible to penicillin. Whereas, 36 (48.65%) and 29 (39.2%) of *Streptococcus pneumoniae* isolates were resistant to Tetracycline and Trimethoprim/sulfamethoxazole, respectively. About 25 (33.8%) isolates of pneumococci showed Oxacillin zone of inhibition <19mm diameter, in which the minimum inhibitory concertation (MIC) of penicillin needs to be determined in order to report the penicillin resistance and intermediate resistance. However, the MIC of penicillin was not determined due to the scarcity of resources in the current study (Table **3**).

4. DISCUSSION

This study provides insights into the nasopharyngeal carriage rate of *Streptococcus pneumoniae* and its antimicrobial susceptibility pattern among children. The nasopharyngeal carriage rate of 25.3% in the current study is higher than a study conducted in Japan, 22% [27]. Whereas, the result is in harmony with a study carried out in India, 27.9% [28] and far lower than studies done in Brazil, 55% [29], Gambia, 58.6% [30], Uganda, 33% [31], Ghana, 48.9% [32], Nigeria, 52.5% [33], and Kenya, 51% [34]. Compared to previous studies in Ethiopia, the result is lower as compared to studies conducted in Gondar, 41.03% [22], and Jimma, 43.8% [23]. The difference might be because most of the above studies were conducted among children under 5 years old. *Streptococcus pneumoniae* carriage rate has a downward proportion with age and the prevalence of colonization is higher in young children and reaches a peak at two and three years old. Whereas, the current study included children aged < 15 years. Therefore, an age difference of the target population might contribute to the difference. The difference might be also due to differences in the study settings, in which some of the above studies were carried out in daycare centers of preschool-age children only. Daycare centers such as kindergartens have been identified as risk factors for the nasopharyngeal colonization of pneumococci. In addition, the difference might be due to socioeconomic differences in the study population.

Age was found as a significantly associated factor for the nasopharyngeal carriage rate of *Streptococcus pneumoniae*. A similar study from Brazil documented children under 2 years of age group to be associated with pneumococcal colonization [29]. In addition, a study from India indicated the progressive decrease in the carriage rate with the increasing age of the children [28]. Being within the young age group and living with younger children were identified as risk factors of pneumococcal carriage in a study conducted at Gondar University Hospital, Ethiopia [22]. Carriage of *Streptococcus pneumoniae* was significantly higher in children living with siblings < 5 years old. The previous study conducted in Ethiopia also indicated that age (being 5 and 6 years) was significantly associated with *S. pneumoniae* carriage rate [23]. The decline in the *Streptococcus pneumoniae* carriage rate with increasing age may reflect the gradual acquisition of mucosal immunity and reduction of exposure.

In the current study, the habit of sleeping with parents (AOR 2.258, 95% C.I (1.252-20.239), p=0.031), and the number of rooms per household (AOR 2.359, 95% C.I (1.680-2.717), p=0.038) were significantly associated with carriage rate of *Streptococcus pneumoniae*. Those children who live in a house with a single room and those who have the habit of sleeping with parent(s)/guardians are two times more likely to carry *Streptococcus pneumoniae*. A similar finding was reported from the previous study in Northwest Ethiopia [22] and Southwest Ethiopia [23] in which the carriage rate of *Streptococcus pneumoniae* was significantly associated with the number of rooms per household. These factors are related to overcrowded conditions that favor the high transmission and acquisition of *Streptococcus pneumoniae*.

In the current study, only 10 (13.5%) *Streptococcus pneumoniae* isolates were susceptible to all applied antibiotics. About a quarter (28.4%) of isolates were resistant to two antimicrobial agents and 14 (18.9%) isolates were resistant to three or more antibiotics. Similar findings were reported from studies in India, Gambia and Gondar, Ethiopia in which 19% [28], 26.3% [30], and 19.8% [22] of isolates, respectively, were resistant to three or more antimicrobials.

The high number of *Streptococcus pneumoniae* isolates showed resistance against Tetracycline, 36 (48.65%), and TMP/SMX, 29 (39.2%). A similar finding was observed in a study from India, in which 36% of isolates were resistant to Tetracycline [28]. In addition, 73.8% and 82.6% of resistance to TMP/SMX were reported from studies conducted in Brazil [29] and Tanzania [35], respectively. Related findings were reported from studies in other African countries, Uganda, 97% resistance to TMP/SMX [31], Gambia, 39% resistance to

Table 3. Antimicrobial susceptibility pattern of *Streptococcus pneumoniae* isolates (n =74).

Antimicrobial susceptibility patterns			
Antimicrobial agents	**Resistant N (%)**	**Intermediate N (%)**	**Susceptible N (%)**
Tetracycline	36 (48.65)	24 (32.4)	14 (18.9)
Trimethoprim/sulfamethoxazole	29 (39.2)	17 (39.2)	28 (37.8)
Chloramphenicol	17 (23%)	-	57 (77%)
Erythromycin	20 (27%)	16 (21.6)	38 (51.4)
Oxacillin	-	-	49 (66.2)

TMP/SMX, and 32.3% resistance to Tetracycline [30], Ethiopia, 43.9% resistance to TMP/SMX [23]. Resistance to Tetracycline in the current study was higher compared to a previous study done in Gondar, Ethiopia, *i.e*. 22.9% [22].

In the current study, a relatively low number of isolates showed resistance to Chloramphenicol, 17 (23%), and Erythromycin, 20 (27%). A similar result was reported from a study in Tanzania with 3.5% resistance to Chloramphenicol, and 6% resistance to Erythromycin [24]. In addition, a study from Gambia reported that 6.3% isolates were resistant to Chloramphenicol [30]. A previous study in Ethiopia reported 8.4% isolates to be resistant to Chloramphenicol and 15.9% to Erythromycin [23]. This difference could be attributed to the difference in bacterial serotype distribution, the difference in the general consumption of antimicrobial agents, and socioeconomic difference. Indiscriminate use of antibiotics and managing bacterial infections without having determined local antimicrobial sensitivity result contribute to the emergence of antimicrobial-resistant strains.

As a limitation of the current study, serotyping of the pneumococci was not performed which could have been significant to assess the vaccine and nonvaccine serotype distribution of pneumococci. In addition, the minimum inhibitory concentration of penicillin was not performed, and as a result, we could not able to report penicillin-resistant isolates of pneumococci.

CONCLUSION

The nasopharyngeal carriage of *Streptococcus pneumoniae* among children was considerably high. High antimicrobial resistance against Tetracycline and susceptibility to Chloramphenicol were observed. The findings indicated children's habit of sleeping with parent(s)/guardians, being within a young age group, and living in a house with a single room as associated with the high rate of *Streptococcus pneumoniae* carriage. Strategies should be designed to prevent pneumococcal nasopharyngeal carriage with a focus on modifiable associated factors and the bacterium antibiotic resistance pattern should be monitored regularly. Further researches on the distribution of pneumococci serotypes and drivers for antibiotic resistance are recommended.

LIST OF ABBREVIATIONS

ARI	=	Acute respiratory illness
CLSI	=	Clinical Laboratory Standard Institute
MHA	=	Mueller Hinton agar
OPD	=	Outpatient's department
PCV-10	=	Pneumococcal Conjugate Vaccine 10
TMP/SMX	=	Trimethoprim/sulfamethoxazole

AUTHORS' CONTRIBUTIONS

DDA was involved in proposal writing and design, data collection, analysis, interpretation, drafting of the manuscript, supervised data collection and analysis, TWD was involved in the study design, reviewing of the proposal and manuscript, supervised data collection. AKS was involved in the study design, reviewing of the proposal and manuscript, supervised data collection. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical clearance was obtained from the Institute of research and community service of Mizan Tepi University (Ref No: MTU/14/13/3/44/09) and official permission was obtained from the hospital.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

After explaining the objectives of the study, written consent was obtained from each study participant's parent(s)/legal guardian(s). For the participant's parent(s)/legal guardian(s) who were not able to read and write, a fingerprint was used as a signature.

AVAILABILITY OF DATA AND MATERIALS

All relevant data are within the paper, but any additional data required by the journal can be available anytime from the primary author.

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CONFLICTS OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers website along with the published article.

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