



# Throat Carriage rate, Associated Factors, and Antimicrobial Susceptibility Pattern of Group a Streptococcus among Healthy School Children in Jigjiga City, Eastern Ethiopia

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## ABSTRACT

**Background:** Group a streptococcus has been recognized as an important human pathogen and it remains among the top ten causes of mortality from an infectious disease. Group a streptococcus throat carriage plays an important role in the development of infection and transmission to contacts. In Ethiopia, there is little information about screening of children for Group a streptococcus carriage.

**Objective:** This study was aimed to assess the magnitude of throat carriage, associated factors, and antimicrobial susceptibility pattern of Group a streptococcus among healthy school children in Jigjiga city, Eastern Ethiopia from 12 April to 27 May 2021.

**Method:** A cross-sectional study was conducted among 462 healthy school children in ages ranging from 7 to 14 years. The throat sample was collected using a sterile cotton swab. Identification of Group a streptococcus was done by colony characteristics, gram staining, catalase negativity, bacitracin sensitivity and Pyroglutamate acrylamides tests. Antibiotic susceptibility test was done on Muller-Hinton agar containing 5% sheep blood by modified Kirby-Bauer disc diffusion method. Data on socio-demographic and related characteristics were gathered through interviews using a questionnaire. The data were double-checked, coded, cleaned, and entered onto Epidemic Version 3.1 then exported to SPSS version 26.0 for analysis. Bivariate and multivariable logistic regression was used to determine the relationship between outcome and predictor variables. A p-value <0.05 was taken as statistically significant.

**Results:** The overall prevalence of Group a Streptococcus carriage was 10.6% (95% CI; 8.1%-13.7%). Children who live with a family member with a sore throat (AOR=2.51; 95% CI 1.09-5.73), children who live with a large family (AOR=4.64; 95% CI 1.53-14.1), and children who live with non-immediate families (AOR=3.65; 95% CI 1.39-9.61), showed significant association with Group a Streptococcus carriage. Tetracycline resistance was shown to be high, while antimicrobial drugs such as penicillin, amoxicillin, ceftriaxone, erythromycin, azithromycin, chloramphenicol, and vancomycin were found to be effective. Multi-drug resistance was found in 4.1% of the isolates.

**Conclusion:** The present study showed a significant throat carriage of Group A Streptococci/*S. pyogenes* in Jigjiga city school children. Family member with a sore throat, having a large family, and living with non-immediate families have all been identified as independent predictors of carriage prevalence. It is recommended that regular screening and surveillance should be conducted in schools.

**Keywords:** Throat carriage; Group a Streptococcus; Antimicrobial susceptibility; Children

## INTRODUCTION

Group A Streptococcus (GAS) has been an important human pathogen since the early days of modern microbiology, and it is still among the top ten causes of mortality from an infectious disease [1]. All Group A streptococcal diseases are most common in settings of poverty, where living conditions promote transmission of the organism, and prevention and treatment programs are

less likely to be present or effective [2]. Group a Streptococcus or *S. pyogenes* can infect people of any age, while children are more likely to be infected [3]. *Streptococcus pyogenes* is responsible for a wide range of clinical symptoms, including impetigo and pharyngitis, as well as more serious disorders such as Streptococcal Toxic Shock Syndrome (STSS), endocarditis, and necrotizing fasciitis. Furthermore, autoimmune diseases such as acute post-

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streptococcal glomerulonephritis and Acute Rheumatic Fever (ARF) can be triggered by recurrent episodes of GAS infection [4]. According to global disease burden figures, WHO ranked GAS as the ninth leading cause of human mortality, with the majority of deaths attributable to invasive GAS infections and RHD. The prevalence of GAS disease is estimated at >18.1 million cases with an incidence of >1.78 million cases per year [5]. The most common infection caused by GAS is pharyngitis in children between 5 and 15 years of age [6]. It is responsible for approximately 15%-30% of cases of pharyngitis in children [7]. Failure to eradicate Streptococci from the pharynx occurs in about one-third of non-treated cases, giving rise to the carrier status in those individuals [8]. Untreated GAS pharyngitis may trigger acute rheumatic fever and its sequela, rheumatic heart disease; remain important public health problems in low and middle-income countries [9]. The existence of the carrier state is reported to be as high as 15%-20% in previous studies [10]. Carriers of GAS may represent a potential source for the acquisition of infections for other children and adults [11,12]. According to a review article published by Jane Oliver and colleagues in 2018, reported 10.5% and 5.9% pooled prevalence of GAS carriage in children from high-income countries and children from low/middle-income countries, respectively [13]. A few studies were done in Africa on the GAS carriage which ranged around 9.0% [8]. And there is scarcity of information in Ethiopia as well [14,15]. Crowding, limited access to hygiene, inadequate medical care, housing quantity and quality, healthcare access and quality, education, or economic advantage are all risk factors for GAS infection or colonization (carriage).

Even though GAS causes significant problems, there is a lack of data on the epidemiology of GAS carriage in our country. Only a few investigations on GAS have been conducted in previous years [14], particularly it was untouched in Jigjiga, Eastern Ethiopia. Therefore, this study was designed to assess throat carriage prevalence, associated factors, and antimicrobial susceptibility pattern of Group A Streptococci among healthy school children in Jigjiga city, Eastern Ethiopia.

## MATERIALS AND METHODS

### Study area and period

The study was conducted in Jigjiga, a city in the Somali Region of Ethiopia from 12 April to 27 May 2021. Located in the Fafan Zone with 60 km west of the border with Somalia. Jigjiga is 630 km east far from the capital city, Addis Ababa. There are currently ninety-nine (99) primary schools, seventy-four (74) private schools, and twenty-five (25) government schools, with a total of 36,507 students, 20,056 males and 16,451 females [16].

### Study design and population

A cross-sectional study was conducted. School children from governmental and private primary schools who were randomly selected from twenty schools using a lottery system.

### Inclusion and exclusion criteria

All students in selected schools aged 7-14 years old who attend the class during the study period were included in the study. All children on antibiotics for the previous two weeks, as well as those with any signs or symptoms of respiratory diseases such as fever, sore throat, cough, or watery nasal discharge, were excluded from

the study.

### Sample size determination and sampling technique

A single proportion formula was used to determine the sample size for this quantitative study, considering the following assumptions: A 95% confidence level, margin of error (0.05) carriage rate among school children, prevalence study of asymptomatic pharyngeal carriage prevalence of *S. pyogenes*, antimicrobial pattern and related risk factors among school children in Hawassa, southern Ethiopia 12.2% [15]. To compute for non-response rate, 10% of the total sample=16 was added. Thus, a total of 178 study subjects were included.

Sample size was determined by considering different factor-associated GAS or *S. pyogenes* colonization using double population proportion formula with the assumption of a two-sided confidence level of 95%, the margin of error 5%, and power of 80%. Finally, 231 study participants were calculated. So, the sample size for a single population proportion was smaller than the sample size calculated for the second calculated double population proportion, which is a sample size of 231 was used. Because we used a multi-stage sampling method, we introduced a design effect. As a result, 231 were multiplied by 2, resulting in a sample size of 462.

### Sampling procedure

In order to include study participants in the study, a multistage sampling technique was used. There were a total of 99 primary schools (74 private and 25 governments). Through a lottery system, twenty (20) schools were chosen at random from both government and private schools. Then the calculated sample size for the study was proportionally allocated to each selected school. A simple random sampling method was used to enroll children. Samples were taken from children whose parents agree to participate until the sample size attends from each selected school.

### Sample collection and transportation methods

Data on the socio-demographic characteristics of the parents/guardians and children, as well as the children's clinical history, were collected using a structured questionnaire adopted from previous studies [15-17]. Two professional nurses administered the questionnaires, and two trained laboratory personnel used cotton swabs to collect a throat sample from a selected child. The throat swab samples were placed in Amies transport media. Within two hours, the sample was sent in a cold chain to Jigjiga University Sultan Sheik Hassan referral hospital laboratory for investigation.

### Laboratory investigation

The throat sample was cultured on 5% sheep blood agar plates (Himedia, India) by rolling the swab over a small area of the plate and streaking the sample with a sterile loop, then incubated at 37°C with 5% CO<sub>2</sub> atmosphere for 24 hours. A catalase test and gram staining were performed on colonies having β-hemolysis. All catalase-negative and gram-positive cocci were subcultured for 24 hours at 37°C on 5% fresh blood agar plates with a Bacitracin disk in a 5% CO<sub>2</sub> atmosphere to differentiate colonies suspected to be *S. pyogenes*. Any zone of inhibition around the bacitracin disk was a candidate for Pyrrolidonyl arylamidase tests, change of color to red/purple was confirmed positive for *S. pyogenes*. [18,19].

## Antimicrobial susceptibility testing

The drug susceptibility test was done by a disk diffusion method by using Muller Hinton Agar (MHA) supplemented with 5% sheep's blood. Colony suspension was made using normal saline (0.85% NaCl) equivalent to 0.5% McFarland standard from grown overnight colonies (18-24 hours) on sheep blood agar plate.

The suspension was inoculated to an MHA plate with 5% sheep's blood using a culture swab and incubated at 5% CO<sub>2</sub> for 18 to 24 hours. Drug disks containing penicillin (10 IU), erythromycin (15 g), azythromycin (15 g), amoxicillin (10 g), chloramphenicol (30 g), ceftriaxone (30 g), vancomycin (30 g), and tetracycline (10 g) were utilized. The drugs are selected in accordance with the Ethiopian Drug Administration standard treatment guidelines for health centers and Control Authority's and the Clinical Laboratory Standards Institute's (CLSI). The zone of inhibition was measured with a ruler, then recorded and compared to the Clinical and Laboratory Standards Institute [20].

## Data quality control

The questionnaire was written in English, then translated into Amharic and Somali, and finally back to English to ensure uniformity. The questionnaire was pre-tested before actual data collection begins. For laboratory testing, the sterility of each batch of produced media was determined by incubating 5% of the culture media in a 5% CO<sub>2</sub> enriched atmosphere at 37°C for 24 hours before using it. *Streptococcus pyogenes* (ATCC 12696) and *Streptococcus agalactiae* (ATCC 13813) were used as a positive and negative control respectively. Quality assurance in antimicrobial susceptibility was done by repeating the selected tests on the same day as the original.

## Method of data analysis

The data was entered and coded into the Epi-Data version 3.1 upon creating the questionnaire template. The entered data was cleaned to ensure the validity of all recorded data. The analysis was then carried out using SPSS version 26.0. Descriptive statistics and frequency tables were used to summarize the data. The magnitude of the association between the different variables with the outcome variable was measured by the adjusted odds ratio with a 95% confidence interval. Bivariate and multivariable logistic regression analysis was made to obtain the odds ratio and the confidence interval of statistical associations. All variables that are significant at p-value <0.25 in the bivariate analysis were considered for multivariable analysis. The strength of statistical association was measured by adjusted odds ratios and 95% confidence intervals. Statistical significance was declared at p<0.05.

## RESULTS

### Sociodemographic characteristics of study participants

A total of 462 schoolchildren participated in the study, with 244 (52.8%) of them being female. Two hundred seven students (57.8%) were from governmental primary schools, while 195 (42.2%) were from a private primary school in Jigjiga city administration. The study participants' ages range from 7 to 14, with a mean age was of 10.9 years (SD ± 1.89). The majority (89.4%) of the participants lives in city, and 90.9% of the students attend school for half-days. Two hundred twenty-nine (49.6%) of research participants lived with 6 to 10 family members, while 214 (46.3%) shared a room with 1 to 3 people. large percentage of (73.5%) the parents/guardians of the children were married. Mothers were the primary caregivers in 320 (69.3%) of the cases, and 331 (71.6%) of the respondents had acquired formal education. One hundred forty-three (30.9%) of the respondents/caregivers were merchants and 108 (23.4%) were government employees (Table 1).

**Clinical history of the study participants:** The majority (76%) of the study participants had no history of hospitalization in the previous 5 years and 87.9% of family member had no history sore throat. Nearly, half of the parents 47.6% claimed that they had never given antibiotics to their children.

**Prevalence of *S. pyogenes*:** Among 462 school children, 49 (10.6%) (95% CI; 8.1%-13.7%) were confirmed to have *S. pyogenes* in their throats.

**Analysis for factors associated with GAS colonization:** Children who lived with a family member who had a sore throat were twice likely to have *S. pyogenes* in their throat compared with those who lived with no family member who had a sore throat (AOR=2.51; 95% CI 1.09-5.73;). Children living in families with more than 11 members were four times more likely carrying *S. pyogenes* compared to children living in families with less members (AOR=4.64; 95% CI 1.53-14.1). Children living with non-immediate families were more than three times more likely to have *S. pyogenes* compare to children living with their mothers (AOR=3.65;95% CI 1.39-9.61 (Table 2).

**Antibiotic susceptibility testing:** To determine the antimicrobial susceptibility patterns of isolates, those 49 bacterial isolates were tested for eight different antimicrobials. Majority of them were susceptible to Amoxicillin 44 (89.8%), Azithromycin 45 (91.8%), Ceftriaxone 47 (95.9%), Chloramphenicol 46 (93.9%), Erythromycin 46 (93.9%), Penicillin 49 (100%), and Vancomycin 45 (91.8%). At the same time, more than half of the bacterial isolates were resistant to Tetracycline 26 (53.1%). Moreover, two (4.1%) of the 49 isolates were drug-resistant to three drugs, particularly Tetracycline, Amoxicillin, and Eryomycin/Vancomycin (Table 3).

**Table 1:** Sociodemographic characteristics of study participants who participate in the throat carriage prevalence, associated factors, and antimicrobial susceptibility pattern of Group A Streptococcus among healthy school children in Jigjiga city, Eastern Ethiopia from 12 April to 27 May 2021.

Variables	Frequency	Percentage (%)
<b>Socio-demographic characteristics of the child</b>		
Age of the child	10-Jul	43.1
	14-Nov	56.9
Sex of the child	Male	47.2
	Female	52.8
Residence	In the town	89.4
	Outskirt	10.6

Duration of child stay in school	Half-day	420	90.9
	Full day	42	9.1
Family Size	5-Jan	142	30.7
	10-Jun	229	49.6
	15-Nov	91	19.7
Room Sharing	3-Jan	214	46.3
	6-Apr	199	43.1
	9-Jul	49	10.6
<b>The social condition of the parent/guardian of the child</b>			
Marital status	Married	340	73.5
	Divorced	35	7.6
	Widowed	17	3.7
	Single	70	15.2
Care givers	Mother	320	69.3
	Father	109	23.6
	Non-immediate family 1	33	7.1
Educational status	Unable to read and write	131	28.4
	Received formal education 2	331	71.6
Occupation	Searching for Job	25	5.4
	Stay home parent	106	22.9
	Merchant	143	31
	Private employee	80	17.3
	Government employee	108	23.4
	250-1000	108	23.4
Monthly income (ETB)	1000-3000	56	28.6
	>3000	222	48.1
	<b>Grandparent, aunt, and/or uncle.</b>		
Primary (1-8), secondary (9-12), tertiary (diploma and above), and/or degree and above.			

**Table 2:** Bivariate and multivariable analysis of participating on the throat carriage prevalence, associated factors, and antimicrobial susceptibility pattern of Group A Streptococci among healthy school children in Jigjiga city, Eastern Ethiopia from 12 April to 27 May 2021.

Variables	Culture result						
	GAS negative n=413 (89.4%)	GAS positive n=49 (10.6%)	COR (95% C. I)	p-value (a)	AOR (95% C.I)	p-value(b)	
<b>Socio-demographic characteristics</b>							
Age of the child	10-Jul	181(91.0)	18(9.0)	0.74 (0.40 -1.37)		0.35	
	11/14/2022	232 (88.2)	31 (11.8)	1			
Sex of the child	Male	197(90.4)	21 (9.6)	1		0.52	
	Female	216 (88.5)	28 (11.5)	1.22 (0.67-2.21)			
<b>Parental/Guardian social condition</b>							
Marital Status	Single	63 (90)	7 (10)	0.97 (0.41-2.28)		0.94	
	Widowed	13 (76.4)	4 (23.5)	2.68 (0.83-8.67)		0.1	
	Divorced	32 (91.4)	3 (8.6)	0.82 (0.24-2.80)		0.75	
	Married	305 (89.7)	35 (10.3)	1			
Caregivers	Non-immediate family 1	24 (72.7)	9 (27.3)	3.15 (1.36-7.34)	0.008	3.65 (1.39 -9.61)	0.009
	Father	104 (95.4)	5 (4.6)	0.49 (0.20-1.20)	0.12	0.50 (0.18-1.36)	0.174
	Mother	285 (89.1)	35 (10.9)	1		1	
Education status	Unable to read and write	111 (84.7)	20 (15.3)	1.88 (1.03-3.45)	0.043	1.11 (0.53-2.30)	0.78
	Received formal education 2	302 (91.2)	29 (8.8)	1			

Occupation	Searching for Job	23(92.0)	2 (8.0)	0.69 (0.15-3.33)		0.65	
	Stay home parent	88 (83.0)	18 (17.0)	1.64 (0.75-3.59)		0.22	
	Merchant	129 (90.2)	14 (9.8)	0.87 (0.38-1.96)		0.73	
	Private employee	77 (96.3)	3 (3.8)	0.31 (0.08-1.14)		0.08	
	Government employee	96 (88.9)	12 (11.1)	1	1	1	0
Monthly income (ETB)	250 - 1000	86 (79.6)	22 (20.4)	3.08 (1.56-6.09)		0.001	
	1000 - 3000	122 (92.4)	10 (7.6)	0.98 (0.44-2.23)		0.99	
	>3000	205 (92.3)	17 (7.7)	1	0	1	0
<b>Child living condition</b>							
Family size	1-5	134 (94.4)	8 (5.6)	1	0	1	0
	10-Jun	211 (92.1)	18 (7.9)	1.43 (0.60- 3.38)		0.42	
	11-15	68 (74.7)	23 (25.3)	5.67 (2.40- 13.3)		0	
Room sharing	1-3	201 (93.9)	13 (6.1)	1	0	1	0
	4-6	174 (87.4)	25 (12.6)	2.22 (1.10- 4.48)		0.025	
	7-9	38 (87.8)	11(22.4)	4.48 (1.87-10.7)		0.01	
Residence	In the town	372 (90.1)	41 (9.9)			1	
	Outskirt	41 (83.7)	8 (16.3)	1.77 (0.78 - 4.03)		0.174	
Duration of child stay in school	Half-day	375 (89.3)	45 (10.7)			1	
	Full day	38 (90.5)	4 (9.5)	1.14 (0.39 - 3.34)		0.811	
<b>Clinical history of the child</b>							
Earlier antibiotic use	1-6 months	61 (89.7)	7 (10.3)			1	
	6-12 months	73 (90.1)	8 (9.9)	0.84 (0.37-1.87)		0.662	
	>12 month	84 (90.3)	9 (9.7)	0.86 (0.37-1.98)		0.714	
Hospitalization history (<5 years)	No	195 (88.6)	25 (11.4)	0.89 (0.3-2.17)		0.806	
	Yes	96 (86.5)	15 (13.5)	1.46 (0.76-2.79)		0.256	
	No	317 (90.3)	34 (9.7)	1	1	1	1
Family member with a sore throat	Yes	45 (80.4)	11 (19.6)	2.37 (1.13-4.95)		0.022	
	No	368(90.6)	38 (9.4)	1		1	
Sore throat (within the last 30 days)	Yes	79 (83.2)	16 (16.8)	2.05 (1.08-3.91)		0.03	
	No	334 (91.1)	33 (8.9)			1	

**Table 3:** : Antimicrobial susceptibility pattern of isolates from children who participate in the study for throat carriage prevalence, associated factors, and antimicrobial susceptibility pattern of Group A Streptococcus among healthy school children in Jigjiga city, Eastern Ethiopia from 12 April to 27 May 2021.

Antibiotics	Total isolates	Susceptible		Intermediate		Resistant	
		Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)
Amoxicillin	49	44 (89.8%)		3(6.1%)		2(4.1)	
Azithromycin	49	45 (91.8%)		4 (8.2%)		-	
Ceftriaxone	49	47 (95.9%)		2 (4.1%)		-	
Chloramphenicol	49	46 (93.9%)		3(6.1%)		-	
Erythromycin	49	46 (93.9%)		2 (4.1%)		1(2.0%)	
Penicillin	49	49 (100%)		-		-	
Tetracycline	49	18 (36.7%)		5(10.2%)		26(53.1%)	
Vancomycin	49	45 (91.8%)		4(8.2%)		-	

## DISCUSSION

Group a streptococcus has been a major human pathogen, since the dawn of modern microbiology and it is still one of the top ten causes of death from infectious diseases. In the present study the overall pharyngeal carriage rate of *S. pyogenes* was 10.6% (95% CI; 8.1%, 13.7%). A carrier rate comparable to ours has been recorded in Ethiopia (9.7% and 12.2%, respectively) [14, 15], United Arab Emirates (10%) [21], Nepal (10.8%) [22], and Yemen (12.8%) [23]. However, the prevalence was lower than those reported in Uganda (16.0%) [24], Egypt (16%) [25], India (23.1%), Argentina (14.2%) [26] and Brazil (14.0%) [27]. Our results showed a higher carriage rate than Gabon's 5.8% (27) and Nepal's 5.0% [28]. The possible reasons for the discrepancy include hygiene, awareness of the route of microbial disease transmission, sample size, seasonal change, and geography, and socio-demographic diversity [23-25].

Children who lived with a family member who had a sore throat were twice as likely to have Group a streptococcus than children who did not have a primary case or sore throat in their household. In Melbourne, Australia, researchers discovered that when a primary case is present, the likelihood of subsequent infection within a family increases by 1.8 times [29]. In addition, according to a study conducted in Nepal, one out of every five children is affected when a family member has already been infected.

Children from families with more than eleven members had a four-fold greater chance of carrying GAS than children from families with fewer members. Similarly, research in Hawassa found that children living in families with more than five members were more than 10 times more likely to be carriers of *S. pyogenes* than children living in families with fewer members. According to another study from Iraq, the carrier rate among children living in homes with more than six individuals is two times higher than among children living in families with fewer members [30]. According to the findings of these investigations, there is a substantial link between the carrier rate and the number of family members. The explanation for this could be that an increase in the number of family members increases the danger of infection, including GAS.

Unlike a study by Asrat Anja, in this study Children living with non-immediate families were more than three times more likely to have Group A Streptococci/*S. pyogenes* than children living with their immediate family. Crowding and poor hygiene, therefore, increases the chance of the transmission of *S. pyogenes* [31]. As reported in Ethiopia and other regions of the world, there was no significant statistical connection between GAS colonization rate and gender or age in this study.

All GAS isolates were sensitive to penicillin in our study. The same high activity of penicillin had been reported in many countries, namely Ethiopia [15-32], Uganda [24], India [17], Nepal [22-28] and Argentina [26]. Our findings reveal that amoxicillin (89.8%) and ceftriaxone (95.9%) have slightly reduced sensitivity against *S. pyogenes* when compared to recently published publications in Senegal and Ethiopia [33].

For patients allergic to penicillin, erythromycin and other macrolides were suggested as first-line alternatives. The isolates displayed the same level of sensitivity to erythromycin and azithromycin (93.9%). The isolates also showed 93.9% and 91.8% sensitivity to chloramphenicol and vancomycin, respectively. A comparable result was reported from Ethiopia and other parts of the world [33,34].

Tetracycline resistance was found to be relatively high (53.1%) in our study. Tetracycline resistance at high levels in GAS isolates may reflect the country's widespread usage of the antibiotic. This finding was comparable with high resistance rates (40%-100%) reported in Ethiopia [14,15], Uganda [24], Egypt [25], Senegal [33], and Argentina [26]. About (24.1%) of isolated GAS isolates were showed multiple drug resistant. A comparable result was reported from in Hawassa, Ethiopia.

## LIMITATION OF THE STUDY

Since the study was a cross-sectional study conducted over a short period, the impact of environmental or seasonal factors on variations in prevalence could not be determined. In addition, this study was unable to performed ASO titer due to a lack of resources.

## CONCLUSION

The present study showed a significant throat carriage of GAS in the Jigjiga city school children population. Children with a sore throat in the family, children from a large family, and children from non-immediate families have all been identified as independent predictors of carriage prevalence. Penicillin, amoxicillin, ceftriaxone, chloramphenicol, erythromycin, azithromycin, and vancomycin remain very sensitive against *S. pyogenes* Isolates. The current research highlights the importance of conducting school surveys regularly. In order to reduce the streptococcal disease burden in the community, well-designed longitudinal research and the adoption of effective public health strategies are also required.

## DECLARATIONS

### Ethical approval and consent

The study was conducted out after receiving ethical approval from Haramaya University College of Health Science and Medical Sciences Institutional Health Research Ethics Review Committee (IHRERC). This study was conducted in accordance with the Declaration of Helsinki. Each child's parent/guardian has been sufficiently informed of the study's purpose and the importance of their participation by the data collectors and/or the investigator. Written, informed, voluntary, and signed consent were taken from all parents/guardians and assents were taken from the child before commencing the study. All parents/guardians were consulted about the benefit of the treatment by a pediatrician who works at Jigjiga University Sultan Shiek Hassen Referral Hospital and the drugs were given free of charge.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The data sets generated during and/or analyzed during the current study are available from the corresponding authors on reasonable request.

## CONFLICTS OF INTERESTS

There were no conflicts of interest.

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university postgraduate directorate.

## AUTHORS' CONTRIBUTIONS

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

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