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Streptococcus pyogenes: Antimicrobial Resistance Pattern and Associated Factors among Children with Pharyngitis in Southern Ethiopia

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Abstract

Streptococcus pyogenes accounts for hundreds of thousands of deaths each year in developing countries, yet data on its prevalence among children in Southern Ethiopia is scarce. This study evaluated the prevalence, patterns of antibiotic resistance, and associated factors of S. pyogenes among children with pharyngitis at government health facilities in Dilla town. A pre-tested semi-structured questionnaire was administered using a systematic random sampling technique. Standard microbiological methods were employed to process throat swabs, and the Kirby-Bauer disc diffusion method was used to assess antimicrobial susceptibility, following CLSI recommendations for data interpretation. Statistical analyses were performed using SPSS version 25. The overall prevalence of S. pyogenes was found to be 34 out of 259 (13.1%) [CI: 9.0-17.0%]. None of the S. pyogenes isolates were resistant to penicillin G or vancomycin, though variable resistance to other antibiotics was observed. Multi-drug resistant (MDR) S. pyogenes accounted for 13 out of 34 isolates (38.2%). Low family income [<1500 ETB (approximately $29); p = 0.015] was significantly associated with the presence of S. pyogenes. These findings are consistent with other studies conducted in Ethiopia, highlighting the continued effectiveness of penicillin G and vancomycin against S. pyogenes, while also revealing a higher percentage of MDR strains. Additionally, low socio-economic status was identified as a risk factor, indicating the need for health education initiatives to reduce the burden of pharyngitis.

Keywords: : Antibiotic Susceptibility, Children, Pharyngitis, *Streptococcus pyogenes*

1 Introduction

Streptococcus pyogenes is a Gram-positive, spherical, aerotolerant bacterium belonging to the genus Streptococcus, characteristically forming pairs or chains during growth [1]. It is known to induce various illnesses, including acute rheumatic fever, scarlet fever, necrotizing fasciitis, glomerulonephritis, meningitis, sepsis, impetigo, streptococcal toxic shock syndrome, and acute pharyngitis [2, 3].

Pharyngitis is the inflammation of the mucous membranes of the oropharynx or posterior pharynx and tonsils, leading to a reddened pharynx with exudates. Clinical signs of pharyngitis include a red pharynx, sore throat, enlarged tonsils, sudden onset of fever, yellowish or blood-tinged exudates, and petechiae on the posterior pharynx and soft palate [4, 5]. Group A Streptococcus (GAS) is the most common bacterial cause of pharyngitis in children aged 5 to 15 years, though it can infect individuals of any age [6, 7].

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Transmission of the pathogen occurs from an infected person to a healthy individual via large respiratory droplets produced during coughing, sneezing, or talking. Airborne droplet transmission typically occurs over minimal distances (0.6 to 1.5 m) during social interactions, particularly in families and schools, especially in the fall and winter months. While GAS can survive for some time in dry droplets, materials from the environment and fomites are not essential modes of transmission [8].

The World Health Organization (WHO) reported in 2005 that *S. pyogenes* is the ninth leading infectious cause of human death, primarily affecting non-industrialized countries [9, [10]. Globally, the prevalence of *S. pyogenes* disease is high, with an estimated incidence of 663,000 cases annually. Furthermore, there are approximately 616 million cases of pharyngitis and 500,000 fatalities, predominantly due to acute rheumatic fever, invasive infections, and rheumatic heart disease [11, 12]. *S. pyogenes* accounts for 15–30% of estimated cases in children and 4–10% in adults in the Global North, while the rates are 5 to 10 times higher in developing countries [13].

Several factors contributing to cases of Streptococcal pharyngitis can be categorized into host and environmental factors, with the latter explaining most of the risk across different populations [11, 14]. Many of these risk factors overlap and are commonly associated with poverty, overcrowding, and low socioeconomic status [14.

Prevention of GAS infections and their suppurative consequences largely depends on the accurate diagnosis of bacterial pharyngitis followed by appropriate antibiotic treatment. Although current treatment guidelines recommend against administering antibiotics without definitive microbiological data, most *S. pyogenes* infections are treated empirically with penicillin [11]. Additionally, studies have highlighted the failure of penicillin in treating *S. pyogenes* infections, possibly due to emerging penicillin resistance [15]. Resistance to macrolides, often prescribed as alternative antibiotics for patients with penicillin allergies, is also common [16].

The patterns of antibiotic resistance among various microorganisms differ based on geographical conditions, temporal fluctuations, and community drug practices [17]. This underscores the need for ongoing monitoring of antimicrobial resistance in different regions.

Overall, there is limited data regarding the prevalence, associated factors, and antibiotic resistance patterns of *S. pyogenes* in Southern Ethiopia. Therefore, this study aimed to evaluate the prevalence and risk factors of *S. pyogenes* and to characterize the antimicrobial resistance patterns of isolated *S. pyogenes* among young children suffering from streptococcal pharyngitis at government health facilities in Dilla town.

2 Methods

2.1 Study design, period, and setting

A cross-sectional study was conducted in governmental health facilities in Dilla town from September to November 2022. Dilla is the capital city of the Gedeo zone in Southern Ethiopia, located 359 km south of Addis Ababa and 100 km from Hawassa. The total population of the town is estimated to be 954,120 [18].

In Dilla town, there are two health centers (Dilla and Odaya) and one government hospital (Dilla University General Hospital), which provide health services to residents of Gedeo and Amaro Kele Zones in the SNNPR region, as well as neighboring regions like Sidama and Oromia. The hospital delivers clinical and community services to over 2 million people, while the health centers offer preventive and curative services, including outpatient care, pharmacy, and laboratory services, to approximately 48,880 people in the catchment area.

All children aged 5 to 15 years who visited governmental health institutions in Dilla town for pharyngitis during the study period were included, provided they had parental or guardian consent. Children were classified as having pharyngitis if they presented with the following clinical manifestations: sore throat, temperature $\geq$38.3°C, tonsillar or pharyngeal exudates, and cervical lymphadenopathy at the index visit [19].

Exclusion criteria included children who had received antibiotics within the week prior to visiting the selected health institutions and those who were critically ill.

2.2 Sample size determination and sampling technique

The sample size was calculated using the single population proportion formula to estimate the prevalence of *S. pyogenes*. The following assumptions were made: a prevalence rate of 11.3% based on data from Jimma, Ethiopia [20], a 95% confidence level, and a maximum allowable error of 4% between the sample size and the population. After accounting for a 10% non-response rate, the final sample size was determined to be 265.

2.3 Sampling technique

Participants in the study were recruited using systematic random sampling, which required calculating a sampling interval (k) from the total population. The k-th value was determined by substituting a sample size (n) of 265 and an estimated population size (N) of 879, based on average data obtained from Dilla University General Hospital (398), Dilla Health Center (213), and Odaya Health Center (268) over three consecutive months (September to November) in 2021. This calculation yielded a k value of 3, meaning every third study participant was selected.

The first child was randomly chosen using a lottery method from the first three patients and determined to be the 3rd patient. Subsequently, every third patient who came to the facilities during the study period was included in the study. A proportional allocation formula was used to distribute study participants among the selected health institutions (Dilla University General Hospital = 120, Dilla Health Center = 64, and Odaya Health Center = 81).

2.4 Data collection and laboratory processing

Written informed consent was obtained from the parent or legal guardian, along with age-appropriate assent from the child where applicable. Each participant underwent a standardized examination conducted by a trained healthcare provider. Socio-demographic and clinical data were collected using a pre-tested semi-structured questionnaire administered through face-to-face interviews, after translating the questionnaire into Amharic. Due to the participants' ages, clinical history and symptoms were obtained from the accompanying parent or legal guardian during these interviews.

2.5 Sample collection and transportation

Trained health professionals used sterile cotton swabs to collect samples from the posterior pharynx and tonsils. A tongue depressor was employed to gently push down the tongue during the throat swab collection. The swabs were placed in brain heart infusion broth and promptly transported to the Microbiology Laboratory at Dilla University College of Health Sciences and Medicine. Within one hour of collection, the swabs were inoculated onto 5% sheep blood agar plates. Each sample was labeled with the date, time, and a code number.

2.6 *S. pyogenes* isolation and identification

Following the collection of throat swabs, the sample was streaked with a sterile loop and rolled over one-quarter of the area of a 5% sheep blood agar plate for direct inoculation. The plate was then incubated for 24 to 48 hours at 35 to 37 °C in an incubator providing 5% CO_2, and examined for beta-hemolytic colonies.

Cultural identification of the isolates was performed based on characteristics such as fine, shiny, dry, translucent, or mucoid colony appearance, as well as beta-hemolysis on the 5% sheep blood agar plate. The hemolysis zone was larger than the size of the colonies. Colonies exhibiting a β-hemolytic reaction that were catalase-negative and gram-positive cocci in chains were sub-cultured onto 5% sheep blood agar plates with 0.04 U bacitracin and cotrimoxazole disks, incubating them under the same conditions as before.

Bacterial isolates were identified as *S. pyogenes* if they were gram-positive, catalase-negative cocci that showed an inhibition zone around the bacitracin disk but no inhibition zone around the co-trimoxazole disk [21].

2.7 Antimicrobial Susceptibility Testing

The Kirby-Bauer disk diffusion method was employed to assess the antibiotic susceptibility profiles of the bacterial isolates, following the criteria set by CLSI M100 | Performance Standards for Antimicrobial Susceptibility Testing, 30th Edition (2021) [22]. A bacterial suspension was prepared by emulsifying pure colonies, selected using a sterile wire loop, in sterile normal saline. The inoculum was adjusted to a turbidity equivalent to the 0.5 McFarland standard. A sterile cotton swab was used to apply the standardized inoculum, ensuring a semi-dry, saturated swab by firmly pressing and rotating it against the inner wall of the tube.

A swab was streaked in three directions to inoculate the surface of each Mueller-Hinton agar (MHA) supplemented with 5% defibrinated sheep blood agar plate [23]. This technique ensured uniform growth of a confluent bacterial lawn. The plate was left at room temperature for 3 to 5 minutes to dry. Subsequently, antibiotic discs of various classes and concentrations were applied: Penicillins (Penicillin G [10 U]), Glycopeptides (Vancomycin [30 μg]), Cephalosporins (Ceftriaxone [30 μg]), Phenicols (Chloramphenicol [30 μg]), Macrolides (Erythromycin [15 μg]), Tetracyclines (Tetracycline [30 μg]), and Lincosamides (Clindamycin [10 μg]).

After incubation in an environment containing 5% CO_2, isolates were categorized as susceptible, intermediate, or resistant based on their inhibition zone diameters [22]. Notably, the interpretive standards for the Kirby-Bauer zone diameter were specifically designated for beta-hemolytic streptococcal breakpoints, as outlined in Table 2C of the CLSI M100 Performance Standards for Antimicrobial Susceptibility Testing, 30^{th} Edition (2021) [22]. Furthermore, isolates categorized as intermediate (I) by CLSI criteria were reported as resistant (R) for reporting purposes, following a conservative clinical interpretation. The selection of antimicrobial agents for susceptibility testing was based on their clinical relevance in the local context, practical considerations such as cost and availability, and adherence to the testing guidelines established by CLSI.

2.8 Data Quality Assurance

To ensure data quality, a pretest involving 5% of the sample (n = 14) was conducted. The research team reviewed all collected information daily to maintain data integrity, checking for completeness, accuracy, clarity, and consistency. Any discrepancies, including errors, ambiguities, or incomplete data, were immediately rectified. All experimental protocols were carried out in strict accordance with established standard operating procedures (SOPs).

The expiry date of each reagent was verified, and all solutions were prepared in line with the manufacturer's guidelines. To confirm sterility, one uninoculated plate from each batch of prepared media was incubated at 37°C for 24 hours to ensure no microbial growth. The performance of the Mueller-Hinton agar and the integrity of the antibiotic disks were evaluated using American Type Culture Collection (ATCC) reference strains. *Streptococcus pyogenes* (ATCC 19615) served as the positive control for bacitracin susceptibility, while Streptococcus agalactiae (ATCC 13813) was used as the negative control for bacitracin resistance.

2.9 Statistical analysis

After data collection, all records were entered into EpiData version 4.4.3.1, where they were reviewed for consistency and completeness. The cleaned and coded dataset was then exported to IBM SPSS Statistics version 25 for analysis. Descriptive statistics were calculated to summarize sample characteristics. A logistic regression model was employed to identify the associations between independent variables and the outcome variable.

Initially, a bivariate analysis was performed for each independent variable, utilizing Chi-square tests at a significance level of $p < 0.05$ to determine statistical significance. Variables that demonstrated an association with a p-value < 0.25 during the bivariate screening were selected as candidates for the multivariable model. Subsequently, a final multivariable logistic regression model was constructed. The results are presented as adjusted odds ratios (AOR) with 95% confidence intervals (CI), with a p-value < 0.05 considered statistically significant.

The model's calibration was evaluated using the Hosmer-Lemeshow goodness-of-fit test, where a p-value > 0.05 indicated an acceptable fit. The study results are presented using a combination of descriptive text, tables, and figures.

2.10 Ethical Consideration

The study protocol was reviewed and approved by the Institutional Review Board (IRB) of the College of Health Sciences and Medicine at Dilla University, with the unique protocol number duirb/045/23-01. All research methods were conducted in accordance with relevant guidelines and regulations, in compliance with the Helsinki Declaration. Permission for participant recruitment and data collection was granted by the administrative leadership of the participating health institutions.

Written informed consent was obtained from the parent or legal guardian, along with age-appropriate assent from the child where applicable. The consent form provided a comprehensive overview of the study's purpose, procedures, potential risks and benefits, confidentiality measures, and the right to withdraw at any time.

To ensure participant confidentiality, all personal identifiers were removed from the dataset and replaced with unique study codes. Similarly, all biological specimens were anonymized prior to analysis and were used exclusively for the research objectives outlined in the approved study protocol.

3 Results

3.1 Socio-demographic characteristics of participants

A total of 259 children were included in the study, resulting in a response rate of 97.7%. Among the participants, 119 (45.9%) were female. The mean age of the children was 6.34 years (SD ± 3.3), with 145 (56.0%) aged between 5 and 9 years. Urban residents comprised 237 (91.5%) of the study participants, while 206 (79.5%) were students.

More than half of the children came from families consisting of three to five members (132, or 51.0%). Likewise, more than half of the children slept on a single bed with fewer than three individuals (135, or 52.1%). On the other hand, over one-third of the families had an income of ≤1500 Ethiopian Birr (ETB) (93, or 35.9%) (Table 1).

The chi-square test of independence was employed in the bivariate analysis to determine association between dependent and independent variables.

*The Ethiopian Birr (ETB) to the USD equivalent conversion was using the mid-2022 exchange rate (1 USD was about 52.5 ETB) (National Bank of Ethiopia).

3.2 Clinical Characteristics of the Study Participants

In this study, the frequency of participants with a history of sore throat was 51 out of 259 (19.7%), and more than half of these children (34 out of 51, or 66.7%) experienced one episode of sore throat in the past six months.

3.3 Prevalence of *S. pyogenes*

The overall prevalence of *S. pyogenes* was found to be 34 out of 259 (13.1%) [CI: 9.0-17.0]. Males had a higher prevalence of *S. pyogenes* (23 out of 34, or 67.6%) compared to females. Children aged 5 to 9 years also exhibited a higher prevalence (22 out of 34, or 64.7%). Among students, the prevalence was 26 out of 34 (76.5%), while it was 14 out of 30 (46.7%) among children from households with more than five members.

Additionally, the study revealed the prevalence of streptococcal pharyngitis based on the type of governmental health institution in Dilla town. Specifically, Dilla General Hospital reported a prevalence of 17 out of 117 (14.5%), Odaya Health Center had a prevalence of 10 out of 79 (12.6%), and Dilla Health Center showed a prevalence of 7 out of 63 (11.1%).

Table 1 Frequency, prevalence of *S. pyogenes* and bivariate analysis of socio-demographic characteristics and clinical factors among children with pharyngitis at Dilla town governmental health institutions, Southern Ethiopia from September to November 2022

Variables		Frequency (%)	*S. pyogenes*		P-value	COR (95% CI)
			Positive	Negative		
Sex	Female	140(54.1)	11	108	1	
	Male	119(45.9)	23	117	0.092*	0.518 (0.241-1.113)
Age in years	5-9	145(56.0)	22	123	0.274	1.520 (0.718-3.221)
	10-15	114(44.0)	12	102	1	
Residence	Rural	237(91.5)	3	19	0.941	0.953(0.266-3.410)
	Urban	22(8.5)	31	206	1	
Schooling status of children	Yes	206(79.5)	26	180	0.635	1.231(0.522-2.900)
	No	53(20.5)	8	45	1	
Educational status of fathers	Unable to read and write	14(5.4)	3	11	0.71	0.837(0.329-2.131)
	Primary	42(16.2)	7	35	0.35	1.925(0.487-7.606)
	Secondary	137(52.9)	17	120	0.48	1.412(0.542-3.677)
	College and above	66(25.5)	7	59	1	
Educational status of mothers	Unable to read and write	26(10.0)	5	21	0.688	0.788(0.265-2.345)
	Primary	102(39.4)	9	93	0.709	1.238(0.404-3.798)
	Secondary	93(35.9)	15	78	0.126*	0.503(0.209-1.213)
	College and above	38(14.7)	5	33	1	
Father's occupations	Government employee	77(29.7)	7	54	1	
	Farmer	108(41.7)	9	68	0.716	1.500(0.169-13.312)
	Merchant	61(23.6)	14	94	0.8	1.324(0.151-11.592)
	Other	13(5.0)	4	9	0.789	1.340(0.158-11.403)
Mother's occupations	Government employee	36(13.9)	6	28	1	
	Farmer	66(25.5)	4	32	0.567	1.929(0.204-18.230)
	Housewife	113(43.6)	12	101	0.92	1.125(0.111-11.365)
	Merchant	34(13.1)	11	55	0.951	1.069(0.124-9.188)
	Other	10(3.9)	1	9	0.595	1.800(0.207-15.687)

Variables		Frequency (%)	*S. pyogenes*		P-value	COR (95% CI)
			Positive	Negative		
*Family income per month (ETB/USD)	≤1500 (≤$29)	93(35.9)	20	73	0.017*	4.804(1.330-17.346)
	1501-3000($29–57)	72(27.8)	5	67	0.953	1.045(0.246-4.431)
	3001-6000	76(29.3)	4	72	0.584	0.648(0.138-3.055)
	($57–114)	18(7.0)				
	≥6001	25(9.6)	5	13	1	
	(≤ $114)	132(51.0)				
Family size	<3	102(39.4)	2	23	1	
	3-5	135(52.1)	16	116	0.333	0.467 (0.100-2.181)
		21(8.1)				
	>5	103(39.8)	16	86	0.432	0.741 (0.351-1.565)
Number of people slept on a single bedroom	<3	140(54.1)	14	121	1	
	3-5	119(45.9)	5	16	0.329	0.679(0.312-1.478)
	>5	145(56.0)	15	88	0.299	1.833(0.584-5.574)
History of sore throat in the last six months	Yes	51(19.7)	9	42	1	
	No	208(80.3)	25	183	0.289	0.638(0.277-1.466)
Frequency of recurrent sore throat episodes (n=51)	One times	34(66.7)	5(30.8)	29(85.3)		
	Two times	10(19.6)	1(10.0)	9(90.0)		
	More than two times	7(13.7)	3(42.9)	4(57.1)		

3.4 Factors Associated with *S. pyogenes*

Factors such as age, sex, residence, schooling status, family income, parental occupation, parental educational level, family size, number of individuals sleeping in a single bedroom, and a history of sore throat were analyzed using bivariate analysis. The results indicated that a family income of ≤1500 ETB was statistically significantly associated with *S. pyogenes* infection (Crude Odds Ratio [COR] = 4.804, 95% CI: 1.330-17.346). Additionally, the mother's secondary educational status showed a potential protective trend, along with the variable of sex. To explore potential confounders among these variables, an exploratory multivariable logistic regression model was constructed using variables with a p-value < 0.25 from the bivariate analysis. In the multivariable analysis, only a family income of ≤1500 ETB remained significantly associated with streptococcal pharyngitis (Adjusted Odds Ratio [AOR] = 5.037, 95% CI: 1.367-18.563, P = 0.015) (Table 3).

3.5 Antimicrobial Resistance Profile of Isolated *S. pyogenes*

The antibiotic susceptibility of the isolates (n = 34) was assessed through antimicrobial susceptibility tests. None of the *S. pyogenes* isolates showed resistance to Penicillin G or Vancomycin; however, varying percentages of resistance were observed for the other tested antibiotics: Clindamycin (13 out of 34, or 38.2%), Ceftriaxone (5 out of 34, or 14.7%), Erythromycin (13 out of 34, or 38.2%), and Chloramphenicol (11 out of 34, or 32.4%). Notably, 28 out of 34 isolates (82.4%) were resistant to Tetracycline (Figure 1).

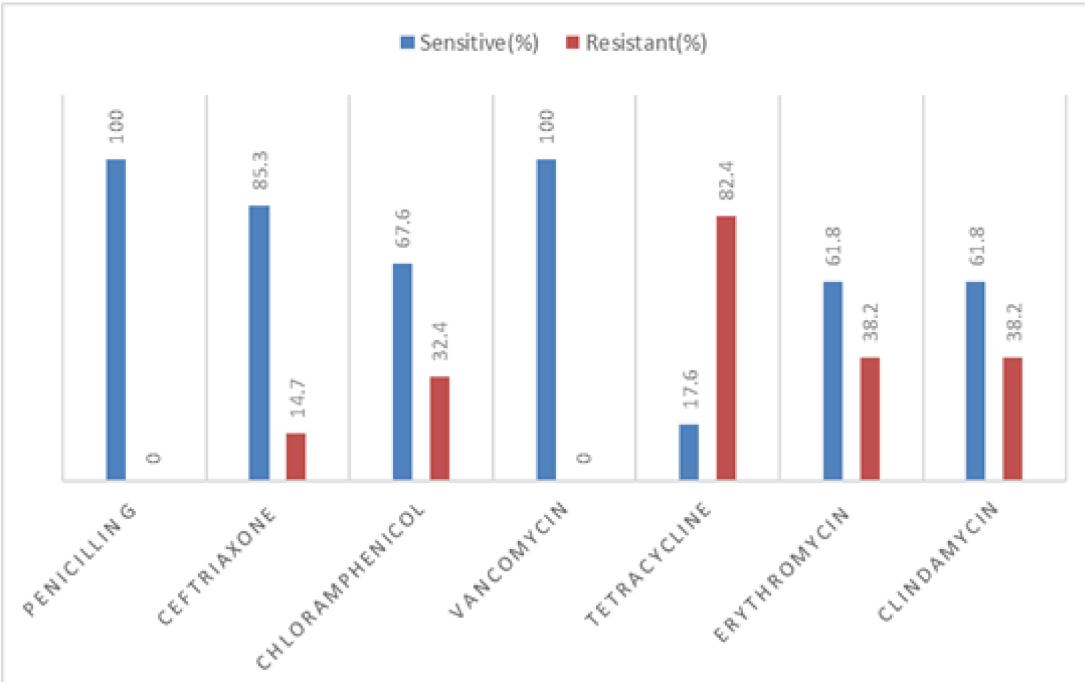

Figure 1 Antimicrobial resistance patterns of isolated *S. pyogenes* (n=34) from throat culture among Children aged 5 to 15 years Visiting Government Health Institutions in Dilla Town, Southern Ethiopia, 2022.

3.6 Multi-Drug Resistant (MDR) *S. pyogenes*

The overall prevalence of multidrug-resistant (MDR) *Streptococcus pyogenes* in this study was 13 out of 34 isolates (38.2%). The resistance rates to various antibiotic classes were as follows: 4 out of 34 isolates (11.8%) were resistant to three classes, 7 out of 34 isolates (20.5%) were resistant to four classes, and 2 out of 34 isolates (5.9%) were resistant to five classes (see Table 2).

Table 2 Multi-drug resistance patterns of *S. pyogenes* from children with pharyngitis at Dilla town governmental health institutions, Southern Ethiopia, from September to November 2022

MDR patterns of *S. pyogenes*	Bacterial number and percentage N (%)	Antibiotic Classes for which *S. pyogenes* is/ are resistant
R_3 N (%)	4/34 (11.8)	4 for tetracycline, macrolide, and lincosamide.
R_4 N (%)	6/34 (17.6)	5 for amphenicol, tetracycline, macrolide and lincosamide; and 1 for cephalosporins, amphenicol, tetracycline, and lincosamide
R_5 N (%)	3/34 (8.8)	3 for cephalosporins, amphenicol, tetracycline, macrolide, and lincosamide
*MDR N (%) = $R_3 + R_4 + R_5$	13/34 (38.2)	

Note: MDR stands for multi-drug resistance, which is defined as the ability of bacteria to resist the effects of multiple antimicrobial medications. It is characterized by non-susceptibility to at least one agent in three or more antimicrobial categories [24].

R_0 = *S. pyogenes* sensitive to all antibiotics, R_1 = *S. pyogenes* resistant to one antibiotic, R_2 = *S. pyogenes* resistant to two antibiotics of different classes, R_3 = *S. pyogenes* resistant to three antibiotics of different classes, R_4 = *S. pyogenes* resistant to four antibiotics of different classes, and R_5 = *S. pyogenes* resistant to five antibiotics. * Note that according to the current definition, the MDR percentage (%) value is the summation of R3 + R4 + R5 and is 13/34 (38.2).

Table 3 Multivariable logistic regression analysis of socio-demographic characteristics and clinical factors among children with pharyngitis at Dilla town governmental health institutions, Southern Ethiopia from September to November 2022

Variables		*S. pyogenes*		P-value	AOR (95% CI)
		Positive	Negative		
Sex	Female	11	108		
	Male	23	117	0.098	0.504(0.224-1.135)
Age in years	9-May	22	123	-	-
	15-Oct	12	102		
Residence	Rural	3	19	-	-
	Urban	31	206		
Schooling status of children	Yes	26	180	-	-
	No	8	45		
Educational status of fathers	Unable to read and write	3	11	-	-
	Primary	7	35	-	-
	Secondary	17	120	-	-
	College and above	7	59		
Educational status of mothers	Unable to read and write	5	21	0.815	0.866(0.259-2.896)
	Primary	9	93	0.943	0.957(0.288-3.176)
	Secondary	15	78	0.094	0.453(0.179-1.145)
	College and above	5	33		
Father's occupations	Government employee	7	54		
	Farmer	9	68	-	-
	Merchant	14	94	-	-
	Other	4	9	-	-
Mother's occupations	Government employee	6	28		
	Farmer	4	32	-	-
	Housewife	12	101	-	-
	Merchant	11	55	-	-
	Other	1	9	-	-
Family income (ETB)	≤1500	20	73	0.015**	5.037(1.367-18.563)
	1501-3000	5	67	0.883	1.117(0.256-4.873)
	3001-6000	4	72	0.642	0.685(0.139-3.383)
	≥6001	5	13		
Family size	<3	2	23		
	5-Mar	16	116	-	-
	>5	16	86	-	-
Number of people slept on a single bedroom	<3	14	121		
	5-Mar	5	16	-	-
	>5	15	88	-	-
History of sore throat in the last six months	Yes	9	42		
	No	25	183	-	-

3.7 Discussion

Streptococcus pyogenes is a significant bacterial cause of acute pharyngitis, accounting for 20–30% of sore throats in children [20]. The overall prevalence of *S. pyogenes* in the study area was 13.1% (95% CI; 9.0-17.0), which aligns with findings from Jimma (11.3%) [20] and Felegehiwot Comprehensive Specialized Hospital (9.1%) [2], as well as reports from Brazil (12%) [15]. In contrast, this prevalence is lower than reports from Mali (25.5%) [25], Iran (30%) [26], Egypt (42.2%) [9], and Nigeria (66.7%) [27]. Conversely, it is higher than studies conducted in Arbaminch, Ethiopia (8.8%) [7], India (5.5%) [28], and Iran (2.5%) [29]. The discrepancies in findings may be attributed to variations in geographic regions (cold, hot, and humid climates), the season of data collection, and sample size.

In the present study, a monthly income of less than 1500 ETB showed a statistically significant association with the presence of *S. pyogenes* (AOR = 5.037, 95% CI (1.367-18.563), P=0.015). Children from families with low monthly incomes were nearly five times more likely to develop Streptococcal pharyngitis than those from higher-income families. This finding is consistent with studies conducted in Arbaminch [7] and Jimma, Ethiopia [20]. The underlying reason may be that lower socioeconomic status contributes to an imbalanced diet, poor sanitation, and overcrowding, thereby increasing transmission opportunities [30–32].

In this study, none of the *S. pyogenes* isolates were resistant to penicillin G and vancomycin, consistent with findings from studies conducted in Tunisia [33], Egypt [9], Iran [29], and Arbaminch, Ethiopia [7], where all isolated bacteria were susceptible. This universal sensitivity to penicillin and its derivatives is well-documented. However, this finding contrasts with a study conducted in India, which reported that 3 out of 25 isolates (12%) were resistant to penicillin [28].On the other hand, for Clindamycin the current study revealed resistance of 13/34(38.2%) which is tended higher than studies done in Iran 1/25 (4.0%) [29], India 5/25 (20%) [28] Arbaminch (0%) and Jimma (0%), Ethiopia [7]; but, tended lower than the reports from Beijing, Shanghai (97.2%) [34], and Felegehiwot Comprehensive Specialized Hospital, Ethiopia 7/14 (50.0%) [2].

The present study revealed that 11 out of 34 isolates (32.4%) were resistant to chloramphenicol. This rate is higher than that reported in Iran (4 out of 25, 16%) [29] and at Felegehiwot Comprehensive Specialized Hospital, Ethiopia (1 out of 14, 7.0%) [2], but lower than reports from India (19 out of 25, 76%) (28), Nigeria (64.7%) [27], and Arbaminch, Ethiopia (7 out of 15, 46.7%).

For erythromycin, 13 out of 34 isolates (38.2%) exhibited resistance, which is higher than studies conducted in Iran (1 out of 25, 4.0%), India (8 out of 25, 32%) [28], Arbaminch (4 out of 15, 26.7%) [7], Felegehiwot Comprehensive Specialized Hospital (3 out of 14, 21.4%) [2], and Tunisia (5%) [33]. Conversely, it is lower than reports from Nigeria (76.4%) [27], Egypt (70%) (9), and Beijing, Shanghai (97.6%) [34]. Furthermore, the present study found that *S. pyogenes* isolates showed the highest resistance to tetracycline, with 28 out of 34 isolates (82.3%) resistant. This finding is higher than results from studies in Egypt (40 out of 60, 66.7%) [9], Tunisia (70.0%) [33], Arbaminch (9 out of 15, 60.0%) [7], and Jimma (52%) [20], as well as Felegehiwot Comprehensive Specialized Hospital (2 out of 14, 14.3%) [2], but lower than findings from Beijing, Shanghai (94.0%) [34]. The variation in tetracycline resistance may be attributed to the over-prescription and use of this antibiotic across different geographical locations [35].

Additionally, the study revealed that the overall prevalence of multidrug-resistant (MDR) *S. pyogenes* isolates was 13 out of 34 (38.2%). This finding is higher than that reported from Felegehiwot Comprehensive Specialized Hospital, Ethiopia, where the rate was 3 out of 14 (21.3%) [2], and from Gondar, North West Ethiopia, where none of the isolates (0 out of 23) were MDR [36].

The discrepancies in the antimicrobial resistance patterns of *S. pyogenes* isolates may be attributed to several factors, including the geographical distribution of bacterial strains, trends and practices of healthcare providers regarding antibiotic prescriptions, community drug usage habits, and the techniques employed for antimicrobial sensitivity testing [37, 38]. Specifically, variations in the reported MDR rates may be influenced by geographic differences in circulating *S. pyogenes* strains [39], local prescribing habits [40], and differences in antimicrobial susceptibility testing (AST) techniques and interpretive criteria [41].

Limitations of the study

Due to resource constraints, the determination of minimum inhibitory concentration (MIC) via E-test was not conducted. While our disk diffusion results categorize resistance, they do not provide quantitative data on the degree of susceptibility for the *S. pyogenes* isolates. A conventional method for identifying *S. pyogenes* was employed, which lacks the specificity of serological Lancefield grouping or molecular confirmation due to these limitations.

The study was conducted over a three-month period, which means it does not reflect the year-round burden of *S. pyogenes* in this population. Furthermore, the study was powered to estimate prevalence; thus, the multivariable analysis should be considered exploratory due to the limited number of events. Although the identified associations are significant, they require validation through larger, adequately powered studies.

4 Conclusion

The prevalence of *S. pyogenes* in the study area aligns with findings from other regions in Ethiopia. Notably, none of the isolated *S. pyogenes* showed resistance to penicillin G and vancomycin, which is encouraging for clinical practice. However, the isolates demonstrated variable resistance percentages to the tested antibiotics, and a higher percentage of multidrug-resistant (MDR) *S. pyogenes* was observed. This underscores the importance of ongoing antimicrobial surveillance to ensure the rational use of antibiotics in healthcare institutions and the community at large.

Additionally, low socioeconomic status among families and guardians was significantly associated with pharyngitis, highlighting the need for targeted health education about the transmission and burden of pharyngitis, especially for families with limited resources. To more accurately capture the seasonal epidemiology and actual annual incidence of *S. pyogenes* pharyngitis in this area, longitudinal studies spanning at least one year are necessary.

Abbreviations

ARF	Acute Rheumatic Fever
ATCC	American Type Culture Collection
AST	Antibiotic Susceptibility Testing
BHS	Beta hemolytic streptococci
CLSI	Clinical Laboratory Standard Institute
DUCHM	Dilla University College of Health Sciences
GAS	Group A Streptococcus
MDR	Multi-drug resistant
PSGN	Post-streptococcal Glomerulonephritis
RHD	Rheumatic Heart Disease
SBAP	Sheep Blood Agar Plate
SOPs	Standard Operating Procedure

Declarations

Competing interests

The authors declare no competing interests.

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Availability of data and materials

To protect patient confidentiality, the data supporting the conclusions of this study are not available in a public repository. Interested parties may request access to the data by contacting the corresponding author. Any data sharing will be subject to approval from the Ethics Committee.

Authors' contributions

Z.S. carried out proposal development, data collection, and data analysis, and drafted the manuscript. B.R.D., A.M., E.A., and M.A. were involved during data collection and data analysis. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Competing interests

The authors assert that they have no hostile interests.

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