scientific reports

Check for updates

OPEN

Streptococcus pyogenes carriage rate, associated factors and antimicrobial susceptibility profiles among urban and rural schoolchildren at Gondar city, Northwest Ethiopia

Yalewayker Gashaw[1]✉, Alem Getaneh[2], Desie Kasew[2], Mitkie Tigabie[2] & Baye Gelaw[2]

Streptococcus pyogenes remains one of the top ten causes of mortality from infectious diseases. Children in low-income nations have high carrier rates of _Streptococcus pyogenes_, which can serve as a source of infections, including simple superficial infections that may lead to invasive and post-streptococcal diseases, particularly among schoolchildren. This study aimed to assess the prevalence of _Streptococcus pyogenes_, associated factors, and antimicrobial susceptibility profiles among urban and rural public schoolchildren in Gondar City, Northwest Ethiopia. A school-based comparative cross-sectional study was conducted via a multistage sampling technique among elementary schoolchildren from April to June 2022 in Gondar City. Sociodemographic and clinical data were collected via a pretested structured questionnaire. Standard microbiological methods were used to collect and process throat swabs to isolate _Streptococcus pyogenes._ An antimicrobial susceptibility test was performed via the disk diffusion method. Epi-Info version 7.2.5 was used to enter the data, which were then exported to SPSS version 25 for analysis. Logistic regression analysis was used to determine the strength of associations between variables, and $p < 0.05$ was considered statistically significant. The overall prevalence of _Streptococcus pyogenes_ in 438 children was 11.2% ($n = 49$), with 71.43% (35/49) being urban and 28.57% (14/49) being rural. Low-income parents, hospital admission history, and cigarette smoking in the home were found to be substantially linked with _Streptococcus pyogenes_ carriage among students ($p < 0.05$). All the _Streptococcus pyogenes_ isolates were susceptible (100%) to penicillin and cefotaxime, but 18.8% and 12.25% of the isolates were resistant to amoxicillin and tetracycline, respectively. The prevalence of _Streptococcus pyogenes_ throat carriage among was intermediate. All the isolates were sensitive to penicillin and cefotaxime, but 18.8% and 12.25% of the isolates were resistant to amoxicillin and tetracycline, respectively. Thus, regular screening and surveillance of _Streptococcus pyogenes_ among schoolchildren should be conducted to minimize carriage or infections and maintain the rational use of antimicrobials. Health education about cigarette smoking in the house also needs to be provided to and the community.

Keywords *Streptococcus pyogenes*, *Schoolchildren*, *Antimicrobial susceptibility profile*

Abbreviations

ARF	Acute rheumatoid fever
CLSI	Clinical laboratory and standard institute
IQR	Interquartile range
IPA	Isopropanol
RHD	Rheumatoid heart disease

[1]Medical Microbiology, Department of Medical Laboratory Sciences, Woldia University, Woldia, Ethiopia. [2]Department of Medical Microbiology, School of Biomedical and Laboratory Sciences, University of Gondar, Gondar, Ethiopia. ✉email: yalewgashaw42@gmail.com

SPSS	Statistical package for the social sciences
S. pyogenes	Streptococcus pyogenes
STS	Streptococcal toxic shock syndrome

Streptococcus pyogenes (S. pyogenes) is a facultative anaerobe, gram-positive, β-hemolytic bacterium arranged in chains[1], and a major human-specific bacterial pathogen[2]. Poverty increases the risk of all *S. pyogenes* infections because living conditions facilitate the organism's spread and make prevention and control initiatives less likely to be effective[3].

The human skin and mucous membranes are the only reservoirs of *S. pyogenes* in nature and first colonize the oropharynx and skin, from which it can progress to severe disease[4]. It affects people of any age, but children are the most likely to be affected[5]. *S. pyogenes* is responsible for various infections, ranging from superficial to more serious disorders, such as streptococcal toxic shock syndrome (STSS) necrotizing fasciitis, and meningitis. In addition, it also causes autoimmune diseases such as acute rheumatic fever (ARF) that can lead to rheumatic heart disease (RHD)[6,7]. These serious disease is caused by a wide range of surface antigens, toxins, and enzymes[8].

Streptococcus pyogenes is the most common cause of pharyngitis in schoolchildren and is considered a "hazard" in school-aged children[9]. It is responsible for 616 and 111 million cases of pharyngitis and pyoderma in children, respectively[10]. Approximately 18 million people suffer from severe *S. pyogenes*-related diseases worldwide, with 1.78 million new cases and at least 517,000 deaths occurring each year[11]. More than 660,000 people suffer from invasive *S. pyogenes* infections, which result in more than 160,000 deaths annually across the globe. A more recent analysis of data concerning the worldwide burden of ARF and RHD in Africa revealed that 15.6–19.6 million people have RHD, of which 2.4 million are children between the ages of 5 and 14, with 1 million living in Sub-Saharan Africa[12]. Children from low- and middle-income nations are at risk for invasive and RHD infections[13]. Ethiopia is a country with a high prevalence of ARF and RHD, accounting for 50–64% of children's RHD[14].

The carriage rate of *S. pyogenes* in asymptomatic school-aged children is estimated to be 15–20% worldwide. On the other hand, its prevalence was reported to be 8.4–12.9% in children from high-income nations[15]. The carriage rate in Ethiopia was 9.7%[15], and a current study in Hawasa, Ethiopia, found 22.9% in urban and 8.8% in rural areas[16]. Untreated *S. pyogenes* infections can progress to ARF, and inappropriate antibiotic prescriptions based on inaccurate data can lead to the emergence and spread of antibiotic-resistant bacteria[17].

The factors that promote resistance are misuse, overuse, and poor infection prevention practices and empirical treatment practices[18]. Diseases caused by *S. pyogenes* and their post-infection sequelae, which mostly affect children, have considerable impacts on public health and the economy[19]. The carriage rate of *S. pyogenes* is directly related to children's crowding, low socioeconomic status, limited medical access, and residence[9]. *S. pyogenes* is highly contagious, and school settings provide excellent conditions for its transmission due to close contact among children[20]. Individuals who have this organism on their pharynx but show no symptoms may contribute to infection in households and public areas, including offices, schools, and daycare centers[21]. Evidence showed that *S. pyogenes* carriers have the potential to cause reinfection and may develop life-threatening conditions[22].

Although *S. pyogenes* poses serious issues, there is limited data in Ethiopia[3,21] and only a few studies have been conducted in the past, specifically in Gondar City, northwest Ethiopia[23]. Data on *S. pyogenes* carriage rate-associated factors and antimicrobial susceptibility profiles among schoolchildren in Ethiopia are needed, particularly in the study area. Thus, this study aimed to evaluate and fill the knowledge gap concerning antibiotic susceptibility patterns, current throat carriage rates, and related risk factors for *S. pyogenes* among students attending elementary schools in Gondar City. It will also serve as preliminary data for further research and to highlight preventative and control strategies in the broader population.

Methods and materials

Study area and period

The study was conducted in six urban and six rural governmental elementary schools from April to June 2022 in Gondar City. The city is located in the Central Gondar Zone of the Amhara Regional State, 748 km northwest of Addis Ababa, Ethiopia's capital, and approximately 180 km from Bahir Dar, the Amhara Regional State's capital. Gondar is Ethiopia's fourth largest city, with an estimated population of 378,000 residents[24]. During the study period, the city had 44 elementary schools, of which 23 were urban (29,582 students) and 21 were rural (12,863 students), with a total of 42,445 students. Among these, 20,742 were males and 21,703 were females, according to the Gondar City education department.

Study design and population

A school-based comparative cross-sectional study was conducted. A lottery system was used to choose students at random from six urban and six rural government elementary schools. Participants' parents supplied written informed consent and oral assent (7–11 years), while children ≥ 12 years old provided written assent.

Study population

All who attended in the selected government elementary schools in Gondar City, during the study period.

Inclusion and exclusion criteria

Schoolchildren aged 7–15 years old selected from each classroom of the selected schools who attended the class during the study period were included.

However, children who have been on antibiotic treatment for the last 2 weeks and at the time of data collection, children with throat infections or any related signs and symptoms of pharyngitis, children who were unable to give sociodemographic information and nasal samples, and those who were involuntary to participate in the study due to different reasons were excluded.

Sample size determination and sampling technique
The double population proportion formula was used by considering the following statistical assumption: 95% CI, taking the prevalence of *S. pyogenes*, p1 = 22.9% in urban areas and p2 = 8.8% in rural areas from the previous study[21].

$$n = (Za/2 + Zb)^2 * (p_1(1 - p_1) + p2\ (1 - p2))/\ (p1 - p_2)^2$$

$$n = (1.96 + 0.84)^2 \times (0.229\,(1 - 0.229) + 0.088\,(1 - 0.088))/\ (0.229 - 0.088)^2 = 102$$

By considering a 10% non-response rate and design effect 2: 102 × 2 = 204, 204 × 0.1 = 20.4 ≈ 21, n_1 = 204 + 21 = 225.

Therefore, the final calculated sample size was **225** for each area and the total sample size was **450**.

Sampling procedure and sampling technique
Twelve[12] elementary schools were picked at random from a total of 44 using a lottery system. The selected schools were then assigned a proportionate percentage of the study's finalized sample size. Children were identified using a random sampling procedure. To include study participants, a multistage sampling procedure was used. Children whose parents agreed to participate were sampled until the sample size was attained at each chosen school.

Sample collection and transport methods
A predesigned structured questionnaire based on prior research[3,21,25] was used to collect information on the socio-demographic characteristics of the parents/guardians and children, as well as the children's clinical history. The interviews were administered by a qualified nurse, and two skilled laboratory workers collected a sample of the child's throat using cotton swabs. The throat swab samples were labeled, put in Amies transport media (Oxoid, England), and transferred via cold chain to the Microbiology Laboratory at the University of Gondar's College of Medicine and Health Sciences in less than two hours.

Laboratory investigation
The throat sample was cultured on 5% sheep blood agar plates (Blood Agar Base, Oxoid UK) by rolling the swab over a small area of the plate, streaking the sample with a sterile loop, and then incubating at 37 °C in a 5% CO_2 atmosphere for 24 h. All plates with beta-hemolytic colonies were microbiologically processed and *S. pyogenes* were identified by conventional methods (colony morphology, hemolysis pattern, catalase test, Gram stain, and morphological observation). All catalase-negative and gram-positive cocci were sub-cultured for 24 h. at 37 °C on 5% fresh blood agar plates with a Bacitracin disk in a 5% CO_2 condition to identify further colonies suspected of *S. pyogenes*[26].

Antimicrobial susceptibility testing
The antimicrobial susceptibility test was conducted via a disk diffusion technique utilizing Muller Hinton Agar (MHA) supplemented with 5% sheep blood. The colony suspension was prepared by mixing normal saline (0.85% NaCl) with the equivalent of 0.5% McFarland standard from grown overnight colonies (18–24 h.) on sheep blood agar plates. Then, the suspension was streaked on an MHA plate with 5% sheep blood, and the following antibiotic disks were dispensed.

Antibiotic disks containing penicillin (*P* = 10 U), ampicillin (AMP = 10 µg), clindamycin (DA = 2 µg), erythromycin (E = 15 µg), chloramphenicol (C = 30 µg), tetracycline (TE = 30 µg), vancomycin (VA = 30 µg), azithromycin (ATH = 15 µg), ceftriaxone (CRO = 30 µg), cefotaxime (CTX = 30 µg), and cefepime (FEP = 30 µg) were used. The preparation was incubated at 37 °C in a candle jar for over 18–24 h. Finally, the sensitivity pattern is read and interpreted.

These antibiotics were selected following the Ethiopian Drug Administration standard guidelines for health centers and control authorities[27] and the Clinical Laboratory and Standard Institute (CLSI) 2021 guidelines[28]. The antibiotic sensitivity pattern was determined by measuring the zone of inhibition via a ruler and interpreted as sensitive, intermediate, or resistant according to the diameter of inhibition established by the CLSI guidelines[28].

Data quality control
All methods were performed in accordance with the relevant guidelines and regulations. To ensure consistency, the questionnaire was initially written in English, translated into Amharic, and then back into English. The questionnaire was pretested before the start of the real data collection. To assess the sterility of every produced media batch in a laboratory setting, 5% of the culture media was incubated for 24 h. at 37 °C in an atmosphere enriched with 5% CO2. *Streptococcus pyogenes* (ATCC 19615) and *Streptococcus agalactiae* (ATCC 12386), were used for positive and negative controls of the Bacitracin disk test, respectively[28].

Data entry and analysis
The data were coded and entered into Epi Info version 7.2.5, and its completeness and clearness were checked to ensure the recorded data's validity. The data were subsequently exported to SPSS version 25 for analysis. Descriptive statistics and frequency tables were used to summarize the data. Logistic regression analysis determined the association between each independent variable and the outcome variable.

All variables with $P \leq 0.20$ in the bivariate analysis were included in the final model of the multivariate analysis to identify factors associated with the throat carriage of *S. pyogenes*. The direction and strength of the statistical associations were measured via odds ratios with a 95% confidence intervals. An adjusted odds ratio with a 95% CI was estimated, and a P value < 0.05 was considered statistically significant.

Results

Sociodemographic characteristics of the study participants

A total of 438 study participants were included in this study, with a response rate of 97.33%. The data revealed that 240 (54.8%) and 280 (63.93%) participants were females and urban dwellers, respectively. The study participants ranged in age from 7 to 15 years, with a median of 12 years (IQR ± 3). Two hundred eighty-nine (66%) study participants were 11 to 15 years of age. Furthermore, 232 (53%) of the study participants were from families with more than five members. Approximately 183 (41.8%) of the study participants had a history of sore throat before two weeks, and 83 (18.9%) had a history of sore throat in their families (Table 1).

Prevalence of streptococcus pyogenes

The overall throat carriage rate of *S. pyogenes* was 11.2% (*n* = 49, 95% CI 8.4–14.2) among SSchil438 study participants. Of the forty-nine S. pyogenes isolates, 35/49 (71.43%) were recovered from urban, and 28.57% (14/49) were from rural. The carriage rate in females was 11.67% (28/240). A higher carriage rate was observed within an age group of 7–10 year, 13.86% (Table 1).

In urban elementary schoolchildren, the carriage rate was 12.5% (35/280), whereas in rural schoolchildren, it was 8.86% (14/158) (Table 1). In this study, the distribution of *S. pyogenes* differed among schoolchildren. For example, the carriage rate of *S. pyogenes* at one urban elementary school, Edigetfeleg, was 22.22% (8/36) (Fig. 1), whereas at another rural school, Deresgie, the carriage rate was 45.5% (5/11) (Fig. 2). Children who lived with non-immediate families had the highest carriage rate, 42.86% (6/14), followed by children who lived with a cigarette smoker in the family, 38.46% (10/26) (Table 1).

Factors associated with streptococcus pyogenes colonization

Among the variables analyzed via bivariate logistic regression, those with a P value ≤ 0.20 were eligible for inclusion in the multivariable analysis. In the multivariable analysis, parents' income 2000EBR (*P* = 0.003; AOR = 5.56; CI: (1.76, 17.57), history of hospital admission (*p* = 0.000; AOR = 7.25; CI: (2.61, 20.17) and cigarette smokers in the house (*p* = 0.000; AOR = 6.9; CI: (2.49, 19.10) were significant predictors of the *S. pyogenes* carriage rate among schoolchildren (Table 2).

Antimicrobial susceptibility pattern of Streptococcus pyogenes

All 49 *S. pyogenes* isolates identified were susceptible to penicillin and cefotaxime. The majority of these strains were susceptible to azithromycin (97.96%), ampicillin and ceftriaxone (95.9%), chloramphenicol (93.87%), clindamycin (93.87%), ceftriaxone (95.9%), vancomycin (93.87%), and erythromycin (87.75%). Relatively low activity was observed for amoxicillin (79.6%) among isolates recovered from rural schoolchildren. Two (4.1%) of the 49 isolates were multidrug-resistant to amoxicillin, tetracycline, and erythromycin (Table 3).

Discussion

S. pyogenes throat carriage is an important public health issue, as the infection leads to post-streptococcal sequelae and individuals colonized with *S. pyogenes* can serve as a source of infections to others[29]. Evidence showed that *S. pyogenes* carriers have the potential to cause reinfection and may develop life-threatening conditions[22]. *S. pyogenes* infections can lead to acute rheumatic fever (ARF) and, subsequently, rheumatic heart disease (RHD) through a process known as molecular mimicry. Initial infection usually starts with pharyngitis (strep throat) or skin infections caused by *S. pyogenes*. The body mounts an immune response to clear the bacteria. However, due to similarities between bacterial antigens and human tissue antigens, some antibodies produced during this response can mistakenly target the body's tissues, particularly the heart, joints, skin, and central nervous system[30]. Certain proteins on the surface of *S. pyogenes* resemble human proteins, especially those found in heart muscle and connective tissues. This cross-reactivity leads to an autoimmune response. Repeated episodes of ARF can cause chronic damage to the heart valves, leading to RHD[31].

In the current study, the overall throat carriage rate of *S. pyogenes* was 11.2% (95% CI; 8.4–14.2). This finding was in line with study reports from Nepal (10.8%)[32], Sana'a, Yemen (12.8%)[33], Argentina (14%)[34], the United Arab (10%)[35], Indonesia (13.9%)[25], Kenya (9.5%)[9], Pakistan (11.5%)[36] and Ethiopia, such as Hawasa (12.2%)[21] and Jijiga (10.6%)[3]. In contrast, the results of this study were higher than the *S. pyogenes* carriage rates reported in Gondar Ethiopia (8.3%)[23], Nepal (5.4%)[37], Iran (3–6%)[38], India (1.9%)[39], Côte d'Ivoire (4.6%)[40], and Nigeria (3.3%)[22]. On the other hand, the findings of this work revealed a lower prevalence of *S. pyogenes* throat carriage than reported in Yemen (39.03%)[41], Kerala, India (23.1%)[42], Uganda (16%)[43], and Egypt (16%)[44]. The difference might be due to variations in the control of respiratory infections, hygiene practices, geographical location, population density, and sample size[37,40].

In the present study, females had a slightly higher carriage rate (11.67%) than did males (10.6%), which is consistent with research conducted in Nepal[32,45] and Pakistan[36]. This may be due to social attitudes toward females or high contact with objects or others while supporting their mothers in daily tasks, which may contribute to upper respiratory infections. A higher prevalence of *S. pyogenes* was recorded in the age group of 7–10 years (13.42%), which is supported by studies in Argentina[34] and Jijiga Ethiopia[3]. This might be due to poor personal and hand hygiene practices and low immunity status.

Urban children had a higher *S. pyogenes* carriage rate (12.5%) than did rural children (8.86%), which is similar to the findings of studies conducted in Hawasa[21] and Uganda[43]. This could be due to overcrowding and the presence of several governmental and nongovernmental health care facilities, such as hospitals and health

			Culture result ()					
Variables	**category**	**Frequency n (%)**	**Pos (*N*=49) n (%) n (%)**	**Neg (*N*=389)**	**Urban (*N*=280) Pos(*N*=35)**	**Neg(*N*=245)**	**Rural (*N*=158) Pos(*N*=14),**	**Neg (*N*=144)**
Age	7-10 years	149 (34)	20 (13.42)	129 (86.58)	14/101 (13.86)	87/101 (86.14)	6/48 (12.5)	42/48 (87.5)
	11-15 years	289 (66)	29 (10.03)	260 (89.97)	21/179 (11.73)	154/179(86.03)	8/110(72.27)	102/110(92.72
Sex	male	198 (45.2)	21(10.6)	177 (89.4)	13/120 (10.38)	107/120(89.16	8/78(10.26)	70/78 (89.74)
	Female	240 (54.8)	28 (11.67)	212 (88.33)	22/160(13.75)	138/160(86.25)	6/80 (7.5)	74/80 (92.5)
Students' educational status	1-4 grades	225 (51.37)	29 (12.9)	196 (88.1)	21/154(13.64)	133/154(86.36)	8/71(11.2)	63/71(88.8)
	5-8 grades	213 (48.63)	20 (9.40)	193 (90.1)	14/126(11.11)	112/126(88.89)	6/87(6.8)	81/87 (93.2)
Households (n)	2-5	232 (53)	23(9.9)	209 (90.1)	19/162(11.73)	102/162(88.27)	4/60(6.67)	56/60(93.33)
	Greater than 5	206 (47)	26 (14.44)	180 (85.56)	16/118(13.56)	102/118(85.44)	10/98(10.20)	88/98(89.8)
Children living with non-	Family	424 (96.8)	43 (10.14)	381 (89.86)	30/270 (11.11)	240/270 (88.89)	13/154(8.44)	121/154(91.56
	immediate family	14 (3.2)	6 (42.86)	8 (57.14)	5/10 (50)	5/10 (50)	1/4(25)	3/4(75)
Sharing sleeping bed	1-2	319 (72.8)	31(9.7)	288 (90.3)	20/215(9.3)	195/215(90.7)	11/104 (10.5)	97/104(89.5)
	above 2	119 (27.2)	18(15.13)	101(84.87)	15/65(23)	50/65(77)	3/54 (5.5)	52/54(94.5)
No. of students in a class	≤50	207 (47.3)	17(8.2)	190 (91.8)	15/158(9.5)	143/158(90.5)	2/49 (4.1)	47/49(96)
	above 50	231(52.7)	32 (14.5)	199 (85.5)	20/122(16.4)	102/122(83.6)	12/109 (11.0)	97/109(89)
Parent Education status	unable to read &write	93 (21.2)	13 (13.98)	80 (86.02)	8/56 (14.3)	48/56(85.7)	5/43(11.63)	37/43(88.37)
	primary school complete	196 (44.7)	23(11.73)	173(88.27)	19/160 (11.87)	139/160(88.13)	4/36(11.11)	32/36(88.89)
	secondary school complete	46(10.5)	7(15.2)	39(84.8)	5/29(17.24)	24/29(82.76)	2/17(11.76)	15/17(88.24)
	college/university	103(23.5)	6(5.82)	97 (94.18)	3/64 (4.7)	61/64(95.3)	3/39(7.7)	36/39(92.3)
Parents' occupation	Gov.t employment	119 (27.2)	7(5.9)	112 (94.1)	4/57(7.1)	53/57(92.9)	3/62 (4.5)	59/62(95.5)
	Private employment	101 (23.1)	9(8.91)	92 (91.09)	7/89(7.86)	82/89 (92.14)	2/12(16.67)	10/12(83.33)
	merchant	151(34.7)	24(15.89)	127 (84.11)	20/105(19.05)	85/105(80.95)	4/46 (8.7)	42/46(8/3)
	others	66(15.1)	9(13.63)	57 (86.37)	4/29 (13.8)	25/299(86.2)	5/37 (13.51)	32/37(86.49)
Family's monthly income	≤2000 EBR	95 (21.7)	23(19.5)	95 (80.5)	14/64(21.9)	50/64(62.5)	9/31(29.03)	22/31(70.97)
	2001-5000EBR	259 (59.1)	21(7.5)	259 (92.5	16/161 (9.94)	145/161(90.06)	4/98(4.1)	94/98(95.9)
	>5000 EBR	84 (19.2)	5 (5.62)	84 (94.38)	35/280(12.5)	1/29(3.4)	1/29(3.4)	28/29(96.6)
History of chronic disease	Yes	17 (3.9)	5 (29.41)	12 (70.59)	3/9 (33.33)	6/9(66.7)	2/8 (25)	6/8 (75)
	No	421(96.1)	44(10.45)	377(89.55)	32/271(11.8)	239/271(88.2)	12/150(8)	138/150(92)
History of health institution visits	Yes	44 (10)	5 (11.36)	39 (88.64)	4/35 (11.43)	31/35(88.57)	1/9 (11.11)	8/9(88.89)
	No	394(90)	44(11.1)	350(88.83)	31/245(12.65)	214/245(87.35)	13/149(8.72)	136/149(81.28
Hx. of antibiotic use without prescription	Yes	91 (20.8)	8 (8.79)	83 (91.21)	3/35(8.57)	33/35(91.43)	5/56(8.93)	51/56(91.17)
	No	347 (79.1)	41(11.82)	306 (88.18)	32/245(13.6)	213/245(86.4)	9/102(8.82)	93/102(91.18)
Hx. Of cigarette smoker in the house	Yes	26 (5.9)	10 (38.46)	16 (61.34)	7/18(38.88)	11/18(61.12)	3/8 (37.5)	5/8(62.5)
	No	412 (94.1)	39 (9.5)	373 (90.5)	28/262(10.68)	234/262(89.32)	11/150(7.3)	139/150(92.7)
Hx of hospitalization to any case	Yes	26 (5.9)	9 (34.6)	17 (96.54)	6/15(40)	9/15(60)	3/11(27.3)	8/11(72.7)
	No	412 (94.1)	40 (9.71)	372 (90.29)	29/265(11)	36/265(89)	11/1477.5)	136/147(92.5)
History of heart disease	Yes	120 (27.4)	15 (13.16)	99 (86.84)	11/85 (13)	74/85(87)	4/35 (11.43)	31/35(88.57)
	No	318 (72.6)	34 (10.5)	290 (89.5)	24/195(12.31)	171/195(87.69	10/123(8.13)	113/123(91.87)
Hx. of headache	Yes	114 (26)	15 (13.16)	99 (86.84)	9/66(13.64)	57/66(84.36)	6/48(12.5)	42/48(87.5)
	No	324 (74)	34 (10.5)	290 (89.5)	26/214(12)	188/214(88)	8/10(80)	2/10(20)
Repeated of URTI w/in six months in the past	Yes	136 (31.1)	21(15.44)	115 (84.56)	15/82(18.3)	67/82(81.7)	6/54(11.11)	49/54(88.89)
	No	302 (68.9)	28 (9.27)	274 (90.73)	20/198(10.10)	178/198(89.9)	8/104(7.7)	96/104(92.3)
Hx. of lymphadenopathy	Yes	113 (25.8)	18 (15.93)	95 (84.17)	11/54(20.37)	43/54(79.63)	7/59(11.86)	52/59(88.14)
	No	325 (74.2)	31(9.54)	294 (90.46)	24/226(10.62)	202/226(89.38	7/99(7.10)	92/99(92.9)
Hx. of body temperature	≥38	122 (27.9)	14 (11.48)	108 (88.52)	11/80(13.75)	69/80(86.25)	3/42(7.10)	39/42(92.9)
	<38	316 (72.1)	35 (10.07)	281 (89.89)	24/200(12.0)	176/200(88)	11/116(9.5)	105/116(90.5)
Continued								

Variables	category	Frequency n (%)	Culture result ()					
			Pos (*N*=49) n (%) n (%)	Neg (*N*=389)	Urban (*N*=280) Pos(*N*=35)	Neg(*N*=245)	Rural (*N*=158) Pos(*N*=14),	Neg (*N*=144)
Hx. of respiratory instrument use while admission	Yes	12 (2.74)	2 (16.67)	10 (83.33)	2/9(22.22)	7/9(77.78)	0/3(0)	3/3(100)
	No	426 (97.26)	47 (11.03)	379 (88.87)	33/271(12.17)	238/271(87.83	14/155(9)	141/155(91)
Hx. of sore throat before two weeks	Yes	183 (41.8)	28 (15.3)	155 (84.7)	19/113(16.8)	94/113(83.2)	9/70(12.86)	61/70 (87.14)
	No	255 (58.2)	21(8.24)	234 (91.76)	35/280(12.5)	245/280(87.5)	5/88(5.7)	144/158 (91.14)
Hx. of sore throat in family members	Yes	83 (18.9)	13(15.66)	70 (84.34)	9/49(18.4)	40/49(81.6)	4/34(11.76)	30/34(88.24)
	No	355 (81.1)	36 (10.14)	319 (89.86)	26/231(11.26)	205/231(88.74)	10/124(8)	114/124(92)

Table 1. Frequency (%) of the *S. pyogenes* concerning the sociodemographic and clinical variables among urban and rural elementary schoolchildren in Gondar City, Northwest Ethiopia, 2022. Key: *URTI* upper respiratory tract infection, *Hx* history, *Pos* positive, *Neg* Negative, *EBR* Ethiopian birr.

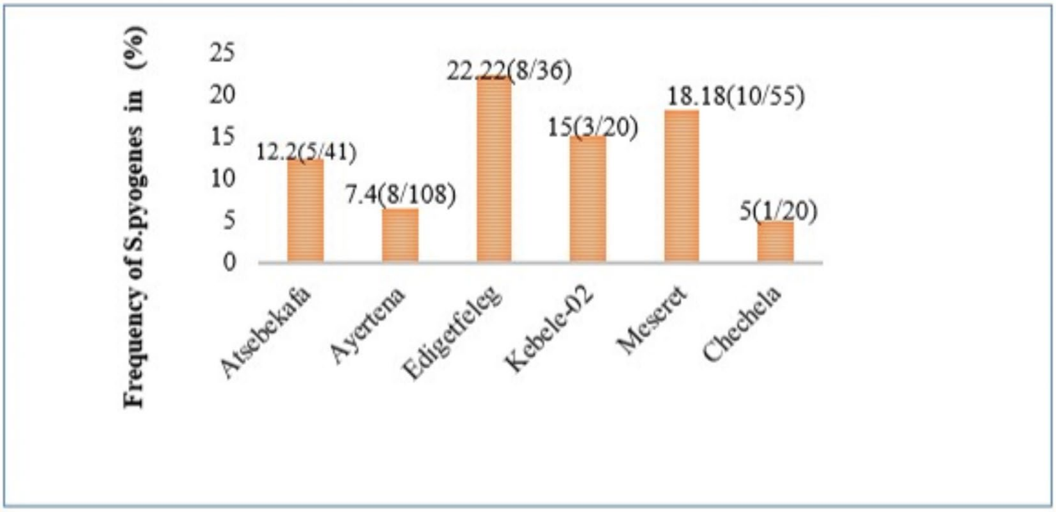

Fig. 1. Frequency of *S. pyogenes* among study participants in urban elementary schoolchildren (*N* = 280), Gondar, Northwest Ethiopia, 2022.

centers/clinics, which attribute overcrowding and a higher level of social contact in these settings to the urban community rather than the rural community[46].

Several studies have shown that sociodemographic characteristics and risk factors contribute greatly to the throat carriage rate of *S. pyogenes*[3,21,22,33,43,47]. In the present study, we assessed different factors that could increase the colonization rate of *S. pyogenes.* There was no significant connection of S. pyogenes carriage rate between urban and rural areas, or between females and males. Children living with parents earning less than 2000 EBR were 5.56 times more likely to get *S. pyogenes* colonization than children earning more than 5000 EBR. This finding was consistent with studies in Hawasa Ethiopia[21], Indonesia[47], and Kenya[46]. Possible factors could be inadequate health care and housing conditions, low household income, poor personal hygiene, and inadequate nutrition[46]. Children who had a history of hospitalization were 7.25 times more likely to have a carriage rate of *S. pyogenes* than those who had no history of hospitalization. This result was in line with the findings of a study from Hawasa[21]. Since hospitals are common areas that are overcrowded and exposed to *S. pyogenes*, they increase the spread of bacteria.

Children living with cigarette smokers in the house had a 6.9 times increased likelihood of carrying *S. pyogenes* than children living in houses without cigarette smokers. This result was in agreement with reports from Ethiopia such as Bahir Dar[48] and Adama[49]. Smoking may disrupt and reduce the commensal population of normal flora, providing opportunities for pathogenic microorganisms and affecting both innate and adaptive immunity by intensifying the pathogenic immune response or attenuating defensive immunity[50].

All the *S. pyogenes* isolates were sensitive to penicillin and cefotaxime, which is in line with studies reported in many nations, such as Ethiopia[3,21], Nepal[32,37], India[42], Egypt[44], southern Nigeria[22], Indonesia[25], Pakistan[36], Argentina[34], and Kerala, India[42]. Ampicillin demonstrated 95.9% activity against *S. pyogenes*, which was in line with the findings of research conducted in Nepal[32].

Amoxicillin and tetracycline slightly reduced activity (71.43% and 78.57%, respectively) among rural schoolchildren compared with urban children, which was comparable to the findings of a study in Argentina[34].

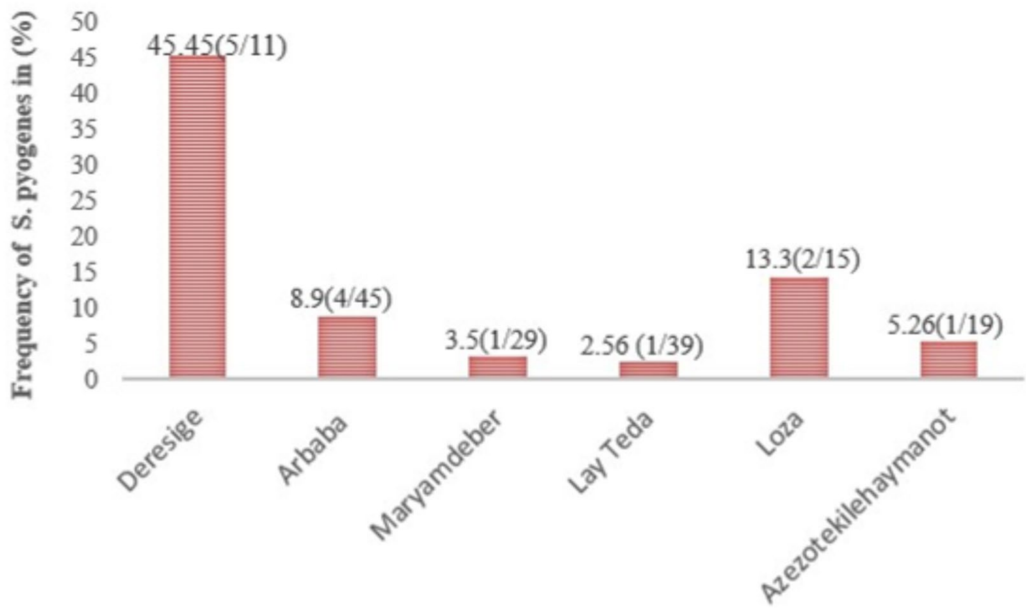

Fig. 2. Frequency of S. pyogenes among study participants in rural elementary schoolchildren ($N = 158$), Gondar, Northwest Ethiopia, 2022.

The possible reason might be that children from rural areas have weak experience using health institution services, and they may even misuse antibiotics. The isolates presented the same level of sensitivity to clindamycin, chloramphenicol, and vancomycin (93.87%). This finding was similar to the report found in Pakistan[36]. In the present study, the isolates were sensitive to tetracycline and erythromycin (87.75%), azithromycin (97.96%), and ceftriaxone (95.9%). These findings were almost identical to earlier studies in Ethiopia, such as Hawassa[21] and Jijiga[3], as well as other studies carried out in Pakistan[36].

Limitations of the study

Some of the children who were categorized as colonized might have been ill or experienced recall bias. The pyrrolidonyl aminopeptidase (PYR) test is more specific (100%) than bacitracin (87.5%), but it was not performed because of its unavailability. Confirming the use of antibiotics in the two weeks preceding the study was often challenging for some of the younger children.

Conclusion

The prevalence of *S. pyogenes* throat carriage among schoolchildren was intermediate in this study. However, it still poses a threat to children and the community. Parents who had low income, a history of admission to a hospital, and cigarette smokers in the house were significant predictors of throat carriage *of S. pyogenes* among schoolchildren. Penicillin and cefotaxime were fully active against all the isolates, but erythromycin and amoxicillin had relatively reduced activity in rural schoolchildren. Large community-based studies and continuous surveillance in antimicrobial resistance of *S. pyogenes* are paramount in elementary schools, and carriers shouldn't be underestimated to decrease the spread and burden of *S. pyogenes* infection and its sequelae among children.

Variable	Category	*S. pyogenes*		COR(95%CI) P-value	AOR (95% CI)	P-value
		Yes (*n*=49)	No (*n*=389)			
Residence	Urban	35	245	1.47(0.66,2.82) 0.248	1.38(0.65-2.94)	0.406
	Rural	14	144	1(ref.)	1	
Households(n)	2-5	23	209	1	1	
	Greater than 5	26	180	0.76 (0.42-1.38) 0.371	1.06 (0.51-2.45)	0.25
Parent income/month (EBR)	≤2000	23	72	5.05(1.82-13.98) 0.009	5.56(1.76-17.57)	0.003*
	2001-5000	21	238	1.4(0.05-3.82) 0.820	1.26 (0.35-4.58)	0.722
	>5000	5	79	1	1	
Children living condition with	Family	42	381	1	1	
	Non immediate family	6	9	6.05 (2.20-20.05) 0.001	3.37(0.74-15.29)	0.115
Number of person per bed	1-2	31	288	1	1	
	>2	18	101	1.66(0.9-3.1) 0.113	1.15(0.54-2.45)	0.722
Number of students per class	≤50	17	190	1	1	
	>50	32	199	1.8(0.97-3.34)	1.60 (0.77-3.33)	0.21
History of hospital admission	Yes	9	17	4.92(2.06-11.77) 0.001	7.25(2.25-20.17)	0.001*
	No	40	372	1		
History of sore throat before two week	Yes	28	155	2.01(1.10-3.67) 0.023	1.02(0.36-2.88)	0.975
	No	21	234	1	1	
Repeated URTI in the past six month	Yes	21	115	1.79(0.98-3.28) 0.061	1.56(0.56-4.38)	0.394
	No	28	274	1	1	
History of tender cervical lymphadenopathy	Yes	18	95	1.797(0.96-3.36) 0.066	0.702(0.24-2.02)	0.511
	No	31	294	1	1	
History of chronic disease	Yes	5	12	3.57(1.20-10.61) 0.022	3.03(0.86-10.69)	0.085
	No	44	377	1	1	
Family member with sore throat	Yes	13	70	1.65(0.83-3.264) 0.154	1.22(0.54-2.75)	0.635
	No	36	319	1	1	
Cigarette smoker in the house	Yes	10	16	5.98(2.54-14.1) 0.001	6.9 (2.49-20.73)	0.001*
	No	39	373	1	1	
Cleanness of the class	Yes	9	112	1	1	
	No	40	277	1.80 (0.44-3.83) 0.128	2.01 (0.86-4.71)	0.107

Table 2. Bivariate and multivariate logistic regression analyses of sociodemographic and clinical variables associated with *S. pyogenes* among urban and rural schoolchildren in Gondar city. *COR* crude odds ratio, *AOR* Adjusted odds ratio, *URTI* Upper respiratory tract infection. * Associations between independent variables and the *S. pyogenes* carriage rate.

Antimicrobial Agents	*Urban schools*			*Rural schools*			
	n =35 ***S*** ***n (%)***	***I*** ***n (%)***	***R*** ***n (%)***	***n=14*** ***S*** ***n (%)***	***I*** ***n (%)***	***R*** ***n (%)***	***Total***
Penicillin 10 µg	35 (100)	-	-	14 (100)	-	-	49 (100)
Ampicillin 10 µg	34 (97.14)	-	1(2.86)	13 (92.86)	-	1(7.14)	47 (95.9)
Amoxicillin 10 µg	29 (82.85)	-	6 (17.14)	10(71.43)	1(7.14)	3(21.43)	39 (79.6)
Cefotaxime 30 µg	35 (100)	-	-	14 (100)	-	-	49 (100)
Ceftriaxone 30 µg	34 (97.14)	-	1 (2.86)	13 (92.86)	-	1 (7.14)	47 (95.9)
Chloramphenicol 30	33 (94.3)	-	2 (5.7)	13(92.86)	-	1(7.14)	46(93.87)
Clindamycin 2 µg	34 (97.14)	-	1 (2.86)	13(92.86)	-	1(7.14)	46(93.87)
Azithromycin 15µg	35 (100)	-	-	13(92.86)	-	1(7.14)	48 (97.96)
Vancomycin 30 µg	33 (94.3)	-	2 (5.7)	13(92.86)	-	1(7.14)	46 (93.87)
Erythromycin 15µg	30 (85.71)	1(2.86)	4 (11.42)	12(85.71)	-	2(14.28)	43 (87.75)
Tetracycline 30 µg	32 (91.43)	2 (5.71)	2 (5.7)	11(78.57)	-	3(21.43)	43 (87.75)

Table 3. Antimicrobial susceptibility patterns of *S. pyogenes* isolates among urban and rural elementary schoolchildren in Gondar City, Northwest Ethiopia, 2022. Key: *S* Sensitive, *I* Intermediate, *R* Resistance.

Data availability

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

Received: 10 April 2024; Accepted: 2 December 2024
Published online: 15 January 2025

References

1. Efstratiou, A. Group A *Streptococci* in the 1990s. *J. Antimicrob. Chemother.* **45** (suppl_1), 3–12 (2000).
2. Siemens, N. & Lütticken, R. *Streptococcus pyogenes* (Group A StreptococcuhighlyHadapteddapted Human Patpotentialeimplicationsatioitsovirulenceuregulationlation for Epidemiology and Disease Management. *Pathogens* **10** (6), 776 (2021).
3. Barsenga, S., Mitiku, H., Tesfa, T. & Shume, T. Throat carriage rate, associated factors, and antimicrobial susceptibility pattern of group a Streptococcus among healthy schoolchildren in Jigjiga City, Eastern Ethiopia. *BMC Pediatr.* **22** (1), 1–8 (2022).
4. Ozberk, V. et al. Prime-pull immunization with a bivalent M-protein and spy-CEP peptide vaccine adjuvanted with CAF® 01 liposomes induces both mucosal and peripheral protection from covR/S mutant *streptococcus pyogenes*. *Mbio***12** (1), 10–128 (2021).
5. DeMuri, G. P. & Wald, E. R. The Group A streptococcal carrier state reviewed: still an Enigma. *J. Pediatr. Infect. Dis. Soc.* **3** (4), 336–342 (2014).
6. Khademi, F., Vaez, H., Sahebkar, A. & Taheri, R. A. Group a streptococcus antibiotic resistance in Iranian children: a meta-analysis. *Oman Med. J.* **36** (1), e222 (2021).
7. Hand, R. M., Snelling, T. L. & Carapetis, J. R. *Group A Streptococcus. Hunter's Trop Med Emerg Infect Dis*p. 429–438 (Elsevier, 2020).
8. Noori, A. Z., Naimi, H. M. & Yousufi, H. The rate of asymptomatic throat carriage of Streptococcus pyogenes and its associated risk factors among Kabul University students. *Int. J. Innov. Res. Sci. Stud.* **3** (4), 142–145 (2020).
9. Murugami, M. W. *Prevalence and Factors Associated with Group a Streptococcal Pharyngeal Carriage among Primary Schoolchildren in Bomet County* (Uon, 2021).
10. Sanyahumbi, A. S., Colquhoun, S., Wyber, R. & Carapetis, J. R. Global disease burden of group A *Streptococcus*. *Streptococcus pyogenes: basic biology to clinical manifestations [Internet]*. (2016).
11. Barth, D. D., Moloi, A., Mayosi, B. M. & Engel, M. E. Prevalence of group a streptococcal infection in Africa to inform GAS vaccines for rheumatic heart disease: a systematic review and meta-analysis. *Int. J. Cardiol.* **307**, 200–208 (2020).
12. Mohammed, K., Demissie, W. R. & Gare, M. B. Adherence of rheumatic heart disease patients to secondary prophylaxis and main reasons for poor adherence at Jimma Medical Center. *E J. Cardiovasc. Med.* **7** (1), 22 (2019).
13. Oliver, J. et al. Invasive group a Streptococcus disease in Australian children: 2016 to 2018 – a descriptive cohort study. *BMC Public. Health.* **19** (1), 1750 (2019).
14. Tesfaw, G., Kibru, G., Mekonnen, D. & Abdissa, A. Prevalence of group a β-haemolytic Streptococcus among children with pharyngitis in Jimma town, Southwest Ethiopia. *Egypt. J. Ear Nose Throat Allied Sci.* **16** (1), 35–40 (2015).
15. Othman, A. M., Assayaghi, R. M., Al-Shami, H. Z. & Saif-Ali, R. Asymptomatic carriage of *Streptococcus pyogenes* among schoolchildren in Sana'a city, Yemen. *BMC Res. Notes.* **12** (1), 339 (2019).
16. Anja, A., Beyene, G., Mariam, S. & Daka, Z. Asymptomatic pharyngeal carriage rate of *Streptococcus pyogenes*, its associated factors and antibiotic susceptibility pattern among schoolchildren in Hawassa town, southern Ethiopia. *BMC Res. Notes.* **12** (1), 564 (2019).
17. Orda, U. et al. Etiologic predictive value of a rapid immunoassay for the detection of group a Streptococcus antigen from throat swabs in patients presenting with a sore throat. *Int. J. Infect. Dis.* **45**, 32–35 (2016).
18. Sulis, G., Sayood, S. & Gandra, S. Antimicrobial resistance in low-and middle-income countries: current status and future directions. *Expert Rev. Anti Infect. Ther.* **20** (2), 147–160 (2022).
19. Cannon, J. W. et al. The economic and health burdens of diseases caused by group a Streptococcus in New Zealand. *Int. J. Infect. Dis.* **103**, 176–181 (2021).
20. Rani, N. Prevalence of *Streptococcus pyogenes* throat infection among schoolchildren: a cross-sectional study. *Am. J. Biomed. Sci. Pharm. Innov.* **3** (08), 06–9 (2023).
21. Anja, A., Beyene, G. & Daka, D. Asymptomatic pharyngeal carriage rate of *Streptococcus pyogenes*, its associated factors and antibiotic susceptibility pattern among schoolchildren in Hawassa town, southern Ethiopia. *BMC Res. Notes.* **12** (1), 1–6 (2019).
22. Edem, K. B., Ikpeme, E. E. & Akpan, M. U. Streptococcal Throat carriage among primary schoolchildren living in Uyo, Southern Nigeria. *J. Child. Sci.* **11** (01), e28–e34 (2021).
23. Abdissa, A. et al. Throat carriage rate and antimicrobial susceptibility pattern of group a streptococci (GAS) in healthy Ethiopian schoolchildren. *Ethiop. Med. J.* **49** (2), 125–130 (2011).
24. Population, E. M. A. Macrotrends. Gondar, Ethiopia Metro Area Population 1950–2021. (2021).
25. Rahmadhany, A., Advani, N., Djer, M. M., Handryastuti, S. & Safari, D. Prevalence and predicting factors of group A beta-hemolytic Streptococcus carrier state in primary. *Ann. Pediatr. Cardiol.* **14** (4), 471–475 (2021).
26. Spellerberg, B. & Brandt, C. Laboratory diagnosis of *Streptococcus pyogenes* (group A streptococci). *Streptococcus pyogenes: Basic Biology to Clinical Manifestations [Internet]*. (2016).
27. Food, M. a. H. C. A. a. C. A. o. E. Standard Treatment Guidelines for General Hospitals, 2014. [(2019). http://www.fmhaca.gov.et/wp-content/uploads/2019/03/STG-GeneralHospital.pdf
28. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI. supplement. M100 2021 [cited 2021 February 24]. https://www.treata.academy/wp-content/uploads/2021/03/CLSI-31-2021.pdf
29. Dumre, S. P. et al. Asymptomatic throat carriage rate and antimicrobial resistance pattern of Streptococcus pyogenes in Nepalese schoolchildren. *Kathmandu Univ. Med. J.* **7**, 392–396 (2009).
30. Carapetis, J. R. et al. Acute rheumatic fever and rheumatic heart disease. *Nat. Rev. Dis. Primers.* **2**, 15084 (2016).
31. Guilherme, L., Steer, A. C. & Cunningham, M. Chapter 2 - pathogenesis of Acute Rheumatic Fever. In: (eds Dougherty, S., Carapetis, J., Zühlke, L. & Wilson, N.) Acute Rheum Fever Rheum Heart Dis. San Diego (CA): Elsevier; 19–30. (2021).
32. Prajapati, A., Rai, S., Mukhiya, R. & Karki, A. Study on carrier rate of *Streptococcus pyogenes* among the schoolchildren and antimicrobial susceptibility pattern of isolates. *Nepal. Med. Coll. J.* **14** (3), 169–171 (2012).
33. Othman, A. M., Assayaghi, R. M., Al-Shami, H. Z. & Saif-Ali, R. Asymptomatic carriage of *Streptococcus pyogenes* among schoolchildren in Sana'a city, Yemen. *BMC Res. Notes.* **12** (1), 1–5 (2019).
34. Delpech, G. et al. Throat carriage rate and antimicrobial resistance of *Streptococcus pyogenes* in rural children in Argentina. *J. Prev. Med. Public.* **50** (2), 127 (2017).
35. Ali Al Shamisi, F. H. The Prevalence of *Streptococcus Pyogenes* and its emm Gene Types among Schoolchildren in AI Ain, UAE. (2016).
36. Akram, S., Khan, M. A., Khan, S. A. & Shah, H. B. U. Group A Beta hemolytic streptococcal carriage and antibiotic sensitivity among school going children in Mardan, Pakistan. *J. Pak Med. Inst.* **35** (1), 20 (2021).

37. Chaudhary, K. K., Khanal, H., Singh, G. K., Chaudhary, S. R. & Sah, O. P. Assessment of Group a Streptococcus and Antimicrobial Resistance Pattern in School going children in Morang District, Nepal. *Birat J. Health Sci.* **5** (3), 1148–1154 (2020).
38. Khas, L. A., Noorbakhsh, S., Movahedi, Z. & Ashouri, S. *Streptococcus pyogenes* and its immunological disorders in an endemic era: a review article in Iran. *Curr. Pediatr. Res.* (2017).
39. Vijaya, D., Sathish, J. & Janakiram, K. The prevalence of group a streptococci carriers among asymptomatic schoolchildren. *J. Clin. Diagn. Res.* **7** (3), 446 (2013).
40. Monemo, P. et al. Pharyngeal carriage of Beta-haemolytic *Streptococcus* species and seroprevalence of anti-streptococcal antibodies in children in Bouaké, Côte d'Ivoire. *Trop. Med. Infect. Dis.* **5** (4), 177 (2020).
41. Edrees, W. H. & Anbar, A. Prevalence and antibiotic susceptibility of *Streptococcus pyogenes* isolated from in Sana'a City, Yemen. *PSM Vet. Res.* **6** (2), 22–30 (2021).
42. Mukundan, A. & Vijayakumar, S. Pharyngeal carriage of group A Streptococci among schoolchildren. *J. Int. Med. Dent.* **4** (1), 18–26 (2017).
43. Nayiga, I., Okello, E., Lwabi, P. & Ndeezi, G. Prevalence of the group a streptococcus pharyngeal carriage and clinical manifestations in schoolchildren aged 5–15 yrs in Wakiso District, Uganda. *BMC Infect. Dis.* **17** (1), 1–8 (2017).
44. Abd El-Ghany, S. M., Abdelmaksoud, A. A. & Saber, S. M. Abd El Hamid D. H. Group A beta-hemolytic streptococcal pharyngitis and carriage rate among Egyptian children: a case-control study. *Ann. Saudi Med.* **35** (5), 377–382 (2015).
45. Dumre, S. et al. Asymptomatic throat carriage rate and antimicrobial resistance pattern of *Streptococcus pyogenes* in Nepalese schoolchildren. *Kathmandu Univ. Med. J.* **7** (4), 392–396 (2009).
46. Avire, N. J. & Whiley, H. A review of *Streptococcus pyogenes. Public. Health Risk Factors Prev. Control* ;**10**(2). (2021).
47. Rahmadhany, A., Advani, N., Djer, M. M., Handryastuti, S. & Safari, D. Prevalence and predicting factors of group A beta-hemolytic streptococcus carrier state in primary. *Ann. Pediatr. Cardiol.* **14** (4), 471 (2021).
48. Kebede, D., Admas, A. & Mekonnen, D. Prevalence and antibiotics susceptibility profiles of *Streptococcus pyogenes* among pediatric patients with acute pharyngitis at Felege Hiwot Comprehensive Specialized Hospital, Northwest Ethiopia. *BMC Microbiol.* **21** (1), 1–10 (2021).
49. Wondimu, M. T. et al. Oropharyngeal pathogenic Bacteria: carriage, Antimicrobial susceptibility pattern and associated risk factors among febrile patients. *Int. J. Pharm. Sci. Dev. Res.* **8** (1), 006–12 (2022).
50. Qiu, F. et al. Impacts of cigarette smoking on immune responsiveness: up and down or upside down? *Oncotarget* **8** (1), 268–284 (2017).

Acknowledgements
We would like to thank all participants of this research, all the study participants, and the directors of each elementary school for their active and voluntary participation and cooperation. We also thank all individuals who have in one way or another contributed to the completion of this research.

Author contributions
Yalewayker Gashaw contributed to the conception of the research idea, data collection, laboratory work, analysis, and interpretation, thesis preparation, and preparation of the manuscript. Baye Gelaw contributed to the conception of the research idea, supervision, thesis preparation, and review of the manuscript. Alem Getaneh contributed to the analysis and interpretation, supervision, thesis preparation, and review of the manuscript. Desie Kasew contributed to the analysis and interpretation, thesis preparation and supervision and Mitkie Tigabie contributed to the laboratory work, and review of the manuscript. All authors read and approved the final manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-82009-2.

Correspondence and requests for materials should be addressed to Y.G.