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Prevalence, antimicrobial susceptibility patterns and associated factors of *Streptococcus pyogenes* among apparently healthy school children in Mekelle city primary schools, Northern Ethiopia

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ABSTRACT

Background: Streptococcus pyogenes is one of the major public health concerns causing human infections ranging from skin and throat infections to acute rheumatic fever and post streptococcal glomerulonephritis. Moreover, nowadays drug-resistant strains of *S. pyogenes* are emerging and can be transmitted through apparently healthy carriers to susceptible individuals.

Objective: To assess the prevalence, antimicrobial susceptibility pattern and associated factors *S. pyogenes* among apparently healthy school children in Mekelle city primary schools, Northern Ethiopia.

Methods: A cross-sectional study was conducted among 504 apparently healthy school children from February to May 2018. We used structured questionnaire to collect socio-demographic data. Throat specimens were collected using sterile cotton Swab and transported for culture, antimicrobial susceptibility and identification of *S. pyogenes* according to standard operating procedures. Data were analyzed using Stata 13 for descriptive statistics, bivariate and multivariate logistic regression. *P*-value <0.05 was declared statistically significance.

Results: The mean age of the study participants was 11.5 years of which 55 % of them were females. The overall prevalence of *S. pyogenes* was 8.3 %. Being female, having low monthly income, weak personal hygiene, poor hand washing habit and crowded living style were significantly associated with the occurrence of *S. pyogenes*. The isolates of *S. pyogenes* showed resistance to Penicillin (69.1 %), Amoxicillin-Clavulanic acid (62 %), Ampicillin (54.6 %),

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Ceftriaxone (47.6 %), Tetracycline (14.4 %), Cefoxitin (7.2 %). About 57.15 % isolates were multidrug-resistant.

Conclusions: This study revealed that some isolates of *S. pyogenes* among the apparently healthy school children were resistant to commonly prescribed antibiotic agents and associated with hygienic conditions and living style. Therefore, it is recommended to practice antimicrobial susceptibility test to maintain rational antibiotic use and improve hygienic and hand washing practices to decrease the likelihood of carriage rate.

1. Introduction

Group A streptococci (GAS) infections are one of the major public health problems causing 616 million pharyngeal infections annually [1,2]. Approximately 15–30 % of cases of pharyngitis among school children are caused by *S. pyogenes* particularly in developing countries where there is overcrowding living style [3]. According to the WHO report of 2005, the estimated magnitude of GAS disease was about 18.1 million, with 1.78 million new incidents and a total of 517,000 deaths annually surpassing tuberculosis [4, 5]. However, it is not universally notifiable due to weak surveillance systems in developing countries. Despite being normal flora in many parts of body, it is re-emerging human pathogen for centuries [1,6].

S. pyogenes can colonize throat of apparently healthy carriers, serving as a reservoir for pathogen transmission [7]. It is responsible to cause almost 90 % of human streptococcal infections which mostly cause acute throat infection among children rheumatic fever (RF) and post streptococcal glomerulonephritis (PSG) [6]. Moreover, 10 %–20 % of apparently healthy school children remained colonized with *S. pyogenes* to propagate it to susceptible children [7].

Antibiotic-resistant *S. pyogenes* isolated from apparently healthy school children are of great public health concerns because treatment options might be complicated [8-10]. Pharyngeal carriage rates of *S. pyogenes* among apparently healthy school children vary with geographical location, life styles and seasonal variation [2] increased among children from low-income countries [11-13].

There is limited information on the prevalence of *S. pyogenes*, drug resistance pattern and associated factors in among primary school students in the study area. Hence, this study was aimed to assess the prevalence, antimicrobial susceptibility pattern, and determinants of apparently healthy carriers for streptococcal infections among school children.

2. Methods

2.1. Study design, data and specimen collection

A cross sectional study was conducted from February to May 2018 among apparently healthy school children in Mekelle city, northern Ethiopia. Mekelle city, the capital of Tigrai Regional State, is located 787 kms north of Addis Ababa, the capital city of Ethiopia. The city has 7 sub-cities (Kedamay-Woyane, Semen, Ayder, Adi-Haki, Hawelti, Quiha and Hadnet sub-cities). There are about 22 governmental primary schools in the city. The study was conducted in 5 selected primary schools (namely Ayder primary school, Gereb-tsedo primary school, Hayelom primary school, Adi-shumduhen primary school and Ra'esi Alula Abanega) located in three of the sub-cities where the total number of school children was 6327.

Apparently healthy school children whose parents or guardians provided informed signed written consent/assent and willing to participate were included in our study. Simple lottery method was implemented to select sub cities and respective primary schools. systematic random sampling was employed to recruit school children from each primary schools. Data were collected using pre-tested structured questionnaire by trained data collectors and throat swab was collected using a sterile cotton swab, throat (oropharyngeal) by trained nurses from the selected apparently healthy school children.

3. Eligibility criteria

3.1. Inclusion criteria

- Apparently healthy school children whose parents or guardians provided informed signed written consent/assent.
- School children who were willing to participate

3.2. Exclusion criteria

- School children who took antibiotic treatment within 2-week interval before sample collection.
- 3.3. Sample size determination and sampling technique

The sample size was computed using the formula: Where n = sample size z = statistic for level of confidence p = previous prevalence

d = margin of error (degree of accuracy desired).

Considering 9.7 % estimated previous prevalence of beta hemolytic GAS from asymptomatic school children in Ethiopia [12], 3 % precision (d = 0.03) and 97 % level of confidence (Z = 2.17). The sample size was estimated to be:

$$\begin{split} n &= (2.17/0.03)^2 \ (0.097) \ (1\text{-}0.097). \\ n &= (72.33)^2 (0.097) \ x \ (0.0903). \\ n &= 5232.11 \ \times \ 0.087591 \end{split}$$

n = 458.

3.3.1. Adding 10 % contingency rate (458 + 46 = 504)

Allocation of sample size to the different primary schools in Mekelle city: Proportionate allocation:

 $n_j\!=\!\frac{n}{N}\!N_j$

j = 1, 2, 3 ... k.where.

k is the number of strata

n_j is sample size of the jth stratum.

N_j is population size of the jth stratum

 $n = n_1 + n_2 + \ldots + n_k$ is the total sample size.

 $N {\equiv\!\!\!=} N_1 {+} N_2 {+} \ldots {+} N_k$ is the total population size.

A. Ayder primary school (total number of students = 1671):

 $n_1 = 504 * 1671/6327, n_1 = 132$

B. Adi-Shumduhen primary school (total number of students = 1515):

n2 = 504 * 1515/6327, n2 = 121

C. Gereb-tsedo primary school (total number of students = 1452):

$$n_3 = 504 * 1452/6327, n_3 = 115$$

D.Hayelom primary school (total number of students = 1504):

 $n_4 = 504 * 1504/6327, n_4 = 116$

E. Ra'esi-Alula Abanega primary school (total number of students = 185)

 $n_5 = 504 * 185/6327, n_5 = 20$

Therefore, the number of school children to be included in the research is:

Total $n = n_1 + n_2 + n_3 + n_4 + n_5$
n = 132 + 121 + 115 + 116 + 20, n =

504

3.4. Sampling technique

Simple lottery method was implemented to select among sub cities respective primary schools. From each primary school, apparently healthy school children were included. Finally, the total number of apparently healthy school children in each school was divided to respective allocated sample size into K intervals (K = total number children in the school divided by allocated sample size to the school) using students' roster. Every K^{th} child were enrolled to participate in our study.

3.5. Cultivation and identification of isolates

Throat specimens were transported in Amies transport media to the microbiology laboratory and swabbed on Blood agar plate supplemented with 5 % sheep blood (Oxoid, UK). Plates were incubated at a temperature of 37 °C in the presence of 5 % CO₂ (using Candle Jar) for 24 h. After 24 h of incubation, each plate was inspected for any growth and hemolysis pattern and negative plates were further incubated for additional 24 h. Colonies with β -hemolysis pattern were isolated for further analysis by gram's stain and catalase test. For further identification clear zone of inhibition around the bacitracin and growth to Cotrimoxazole disk placed adjacent to bacitracin indicates the presence of *S. pyogenes* isolates [15].

3.6. Antimicrobial susceptibility test

Antimicrobial susceptibility test using Muller Hinton Agar (Oxoid, UK) with 5 % sheep blood was performed by disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) [14]. Antibiotics Penicillin G (10 IU), Chloramphenicol (30 µg), Ceftriaxone (30 µg), Ampicillin (10 µg), Erythromycin (15 µg), Amoxicillin-clavulanic acid (30 µg), Ciprofloxacin (5 µg), Cefoxitin (30 µg), Azithromycin (10 µg), Clindamycin (2 µg) and Tetracycline (30 µg) (Hi-Media Ltd, India) were tested.

Colonies of *S. pyogenes* were suspended in 5 ml physiological saline and homogenized to make bacterial inoculum comparable to 0.5 McFarland turbidity standards using turbidimeter. Sterile cotton swab was dipped, and swabbed in to Muller-Hinton agar plate supplemented with 5 % sheep blood. Antimicrobial discs were placed manually approximately 24 mm apart using sterile forceps on the medium and incubated at 37 $^{\circ}$ C for 18 h [14].

3.7. Quality assurance

Questionnaire was pretested to check for understandability and simplicity among the school children and their guardians and assessed for completeness. Sample collection, handling and transport were strictly supervised. Laboratory analysis was carried out using standard operating procedures (SOPs) and reagents were checked for proper functioning and were handled according to the standard. *S. pyogenes* (ATCC 84897) was used as a control strain to check the quality of media and antibiotic discs.

3.8. Statistical analysis

Data were entered and analyzed using Stata version 13 software. Descriptive statistics was done by cross tabulating explanatory variables against the outcome and comparisons were made using Chi-square test or Fischer's exact. Bivariate and multi-variable logistic regression were employed to assess risk factors. A p-value of <0.05 was considered statistically significant. Finally, results were summarized in texts, figures and tables.

3.9. Operational definition

- Hemolysis: Refers to is the lysis of the red blood cells in the agar surrounding bacterial colonies and is a result of bacterial enzymes called hemolysins.
- School Children: Children 5–15 years old who are enrolled in school.
- GAS carriers: Children having no symptoms/signs of pharyngitis on the day of examination or in the previous 15 days, but GAS in cultures

Table 1

Isolates of *S. pyogenes* with socio-demographic characteristics and other variables among apparently healthy school children in Mekelle city primary schools, Tigray, Northern Ethiopia, 2018 (n = 504).

Variables		S. pyogenes isolates	S. pyogenes isolates		
		Positive N (%)	Negative N (%)		
Gender of children	Male	8 (3.5)	219 (96.5)		
	Female	34 (12.3)	243 (87.7)		
Age category of children	6–8	18 (24)	57 (76)		
	9–11	14 (11)	113 (89)		
	12–14	10 (3.5)	273 (96.5)		
	14–15		19 (100)		
Name of primary school	Ayder	18 (13.6)	114 (86.4)		
	Adi-shumduhen	6 (4.96)	115 (95.04)		
	Hyelom	12 (10.3)	104 (89.7)		
	Ra'esi Alula Abanega	1 (5)	19 (95)		
	Gereb-tsedo	5 (4.4)	110 (95.6)		
Monthly income of parents	<2000	32 (23.4)	105 (76.6)		
	2000-5000	10 (3.8)	255 (96.2)		
	>5000	_	102 (100)		
Living style	Crowded	10 (41.7)	14 (58.3)		
	Not crowded	32 (6.7)	448 (93.3)		
Presence of siblings <15 years	Yes	31 (8.2)	347 (91.8)		
	No	11 (8.7)	115 (91.3)		
Presence of respiratory illness	Yes	4 (13.4)	25 (86.6)		
	No	38 (8)	437 (92)		
Personal hygiene of children	Good	12 (2.5)	322 (97.5)		
	Weak	30 (25)	90 (75)		
Proper hand washing	Yes	17 (4)	417 (96)		
	No	25 (36)	45 (64)		
Average number of students in a class	≤ 30	1 (5)	19 (95)		
-		41 (8.5)	443 (91.5)		

- MDR-isolates: Isolates of S. pyogenes which are resistant to ≥ 1 antibiotic agent in at least ≥ 3 antimicrobial categories [16].
- Good personal hygiene: When the score of children from checklist result is \geq 50 %.
- Weak personal hygiene: When the mean score of children is <50 %.
- **Overcrowded living style**: Living condition of Children in households with more than 3 persons per average sized (4 m by 5 m) room (less than an area of 6.7 m² room/person) [17,18].
- Crowded classroom: A classroom consisting of a size less than 1.2 m² per student [19].
- Proper hand washing: washing hands always before and after food, before and after the toilet use and after games

4. Results

4.1. Socio-demographic characteristics of study participants

Out of the total 504 school children, 55 % of them were females. The mean age of study participants was 11.5 years (SD: ± 2.295). About 25.2 % of the participants were living in a room encompassing more than three family members. Additionally, 87.7 % of the children participated in our study were washing their hands every day before and after eating food, before and after using the toilet and after playing games (Table 1).

4.2. Prevalence of S. pyogenes among apparently healthy school children

The overall prevalence of *S. pyogenes* was 8.3 % which varies depending on the categories of variables in our study. Prevalence among female children was 12.3 %. An increased prevalence was observed among the school children aged 6–8 years, 24 % (Table 1).

4.3. Antimicrobial susceptibility test of S. pyogenes isolates

In our study, the isolates of *S. pyogenes* were resistant to Penicillin (29, 69.1 %), Amoxicillin-clavulanic (26, 62 %) and Ampicillin (23, 45.8 %). Most of the *S. pyogenes* isolates were sensitive to Ciprofloxacin (40, 95.2 %) and Clindamycin (39, 92.8 %) (Table 2).

4.4. Multi drug resistance profile of S. pyogenes isolates

Overall, 9 (21.43 %) isolates of *S. pyogenes* were resistant to one class of antimicrobial agent; 9 (21.43 %) were resistant to two classes of antimicrobials and 24 (57.15 %) isolates were resistant to > 3 classes of antimicrobials (Fig. 1). Hence, 24 (57.15 %) isolates of *S. pyogenes* were identified to be MDR.

4.5. Associated factors for S. pyogenes asymptomatic carriage

In our study, about 10 independent variables were considered during cross tabulation (bivariate analysis). Variables with a P < 0.25 in the bivariate analysis were entered in to multivariate logistic regression model and then variables with P less than 0.05 were considered statistically significant. Factors like; sex of child (being female, AOR = 12, 95 % CI: 0.125, -0.043, P < 0.001), low monthly income of parents (income <2000, AOR = 5, 95 % CI: 0.164, -0.041, P \leq 0.002) crowded living style of children (AOR = 8, 95 % CI: 0.318, -0.115, P < 0.001), weak personal hygiene (AOR = 4, 95 % CI: 0.169, -0.052, P < 0.001) and poor hand washing (AOR = 2.3, 95 % CI: 0.191, -0.053, P \leq 0.002) were found to be statistically associated with *S. pyogenes* (Table 3).

Table 2

Antimicrobial susceptibility patterns of S. pyogenes isolates among apparently healthy school children in Mekelle city primary schools, Northern Ethiopia, 2018 (n = 504).

Antibiotic discs	Susceptibility test of S. pyogenes isolates					
	Susceptible N (%)	Intermediate N (%)	Resistant N (%)			
Penicillin	2 (4.8)	11(26.1)	29 (69.1)			
Ampicillin	4 (9.5)	15 (35.7)	23 (54.8)			
Amoxicillin-Clavulanic acid	16 (38)	0 (0)	26 (62)			
Chloramphenicol	31 (73.9)	11 (29.1)				
Ceftriaxone	17 (40.5)	5 (11.9)	20 (47.6)			
Ciprofloxacin	40 (95.2)	0 (0)	2 (4.8)			
Erythromycin	36 (85.6)	4 (9.5)	2 (4.8)			
Tetracycline	30 (71.2)	6 (14.4)	6 (14.4)			
Azithromycin	38 (90.4)	3 (7.2)	1 (2.4)			
Clindamycin	39 (92.8)	3 (7.2)				
Cefoxitin	38 (90.4)	1 (2.4)	3 (7.2)			

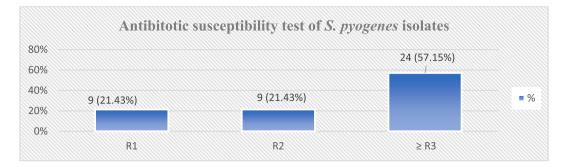


Fig. 1. Multidrug-resistance pattern of S. pyogenes isolates among apparently healthy school children.

R1- Resistant to one class of antibiotics only, R2-Resistance to two classes of antibiotics, \geq R3-Resistance to at least three classes of antibiotics, MDR = multidrug-resistance (an isolate of *S. pyogenes* resistant to three or more classes of antibiotics tested).

5. Discussion

S. pyogenes throat carriage is an important public health concern worldwide, as the infection leads to post streptococcal sequelae and complicated severe infections of different parts of our body [6]. Notably antibiotic resistant *S. pyogenes* isolates are emerging and becoming major public health problems which can't be easily treated with available treatments [2]. Individuals colonized with *S. pyogenes* serve as a source of spread of infections to other susceptible individuals in a community. Hence, we have conducted this research to determine the prevalence, antibiotic susceptibility and associated factors with *S. pyogenes*.

The overall prevalence *S. pyogenes* is 8.3 % (95 % CI: 6 %–11 %). Which is in line with previous studies conducted in Nepal, (10.9 %) [13] and, (10.8 %) [20], India, (7.8 %) [21] and, (9.8 %) [22], Tanzania, (8.6 %) [23] and, (6.9 %) [24] as well as Ethiopia, (9.7 %) [12]. On the contrary our finding revealed higher prevalence rate than previous reports from India, (2.3 %) [25] and, (0.67 %) [26], Turkey, (1 %) [27]. These variations might be due to time gap, differences of sample size employed, the difference in trend of continuous screening strategies among school children and inclusion of the age category of the school children in the previous studies.

However, our study reported a lower prevalence than previous studies conducted in Nepal, (14.5 %) [28], Argentina, (14.2 %) [29], India, (27.2 %) [30], Turkey, (14.3 %) [31], Egypt, (16 %) [32] and Uganda, (16 %) [33]. The difference might be due to sample size, some of the studies included orphanage and children from rural communities for these study participants are expected to be crowded living style, some studies have conducted the research exclusively in rural primary schools and the difference in the detection techniques.

More than half of *S. pyogenes* isolates were observed to be resistant to the frequently prescribed antibiotics. For the tested antibiotic agents, 26 (62 %) to Amoxicillin-Clavulanic acid, 29 (69.1 %) to Penicillin, 23 (54.6 %) to Ampicillin, 20 (47.6 %) to Ceftriaxone, 6 (14.4 %) to Tetracycline, 3 (7.2 %) to Cefoxitin resistant isolates of *S. pyogenes* were observed. This indicates high occurrence of emergence of antibiotic resistant *S. pyogenes* isolates in the community. Moreover, out of the total 42 *S. pyogenes* isolates, 40 (95 %), 39 (92.8 %), 38 (90.4 %), 36 (85 %), 31 (73.9 %), and 30 (71.2 %) were reported to be resistant to Ciprofloxacin, Clindamycin, Azi-thromycin and Cefoxitin, Erythromycin, Chloramphenicol and Tetracycline respectively. Previous studies from Nepal showed 6.7 % and 13.2 % resistance to Clindamycin and Erythromycin respectively [28], (7.8 %) to Ampicillin and (5.8 %) to Erythromycin [20], 7.8 % to Chloramphenicol, 5.2 % to Ciprofloxacin and Erythromycin [13].

Resistance of *S. pyogenes* isolates to Erythromycin (11.1 %) and Tetracycline (55 %) was also reported previously from Argentina [29]. In another study conducted in Egypt, resistant isolates to Erythromycin (12.5 %) and Tetracycline (37.5 %) were reported [31]. Additionally, a study in India has reported *S. pyogenes* isolates resistant to Erythromycin (4.17 %) [26] were reported. There was also a study conducted in, Ethiopia (Addis Ababa), antibiotic resistant *S. pyogenes* to Tetracycline (47.8 %) was reported [12]. The increased resistance pattern in our study might be due to overuse of antibiotics for longer period of time to treat Streptococcal pharyngitis. Unlike the studies previously conducted in Argentina [29], Egypt [31], and India [26], our study reported 57.15 % MDR isolates of *S. pyogenes*. This higher rate of drug resistance might be due to over-prescription of broad-spectrum antibiotics and empirical treatment without performing drug susceptibility.

In our study being female, low monthly income of parents/guardians, crowded living style of children, weak personal hygiene and poor hand washing habit of the children were statistically associated with the asymptomatic carriage *S. pyogenes*. Being female was one of the determinants to acquire *S. pyogenes*. Female school children were observed to be 12 times more likely to have carriage of *S. pyogenes* compared to males (AOR = 12, 95 % CI: 0.125, -0.043, P < 0.001). Supporting findings have been reported from Nepal [13,28,34], Japan [35] and India [36]. The difference might be due to comparably weak personal hygiene observed among female school children than male.

School children in crowded living style were eight times more likely to have *S. pyogenes* carriage (AOR = 8, 95 % CI: 0.318, -0.115, P < 0.001). Previous studies from Nepal [20,28], Turkey [31] and Yemen [37] supported our finding. This might be due to the fact that group living for longer time and crowded living style will let the *S. pyogenes* transmit easily to other susceptible individuals.

The other predictors identified to be associated with the carriage of *S. pyogenes* were, weak personal hygiene (AOR = 4, 95 % CI: 0.169, -0.052, P < 0.001) and poor hand washing habit (AOR = 2.3, 95 % CI: 0.191, -0.053, P ≤ 0.002. Some studies with similar determinant factors were reported from Nepal [20] and Turkey [27]. In addition to the above factors, school children from parents

Table 3

Bivariate and multivariable logistic regression analysis of factors associated with *S. pyogenes* isolates among apparently healthy school children in Mekelle city primary schools, Northern Ethiopia, 2018 (n = 504).

Variables	Categories	S. pyogenes isolates		Bivariate analysis		Multi variable analysis		
		Pos (%)	Neg (%)	Р	97 % CL	AOR	97 % CI	Р
Sex of children	Male	8 (3.5)	219	< 0.001	(0.000-0.002)	1	(-0.125, -0.043)	<
			(96.5)					0.00
	Female	34	243			12		
		(12.3)	(87.7)					
Age category of	6–8	18 (24)	57 (76)	< 0.001	(0.000 - 0.007)	1.8	(-0.188, 0.071), (-0.246, 0.025),	0.073
children	9–11	14 (11)	113 (89)			2.1	(-0.270, 0.049)	0.05
	12–14	10	273			1.3		0.11
		(3.5)	(96.5)					
	14–15	-	19 (100)			1		
Name of primary	Ayder	18	114	0.041	(0.024–0.063)	1.4	(-0.262, -0.011), (-0.089, 0.084),	0.05
school		(13.6)	(86.4)			1.1	(-0.082, 0.014), (-0.155, 0.09)	0.786
	Adi-shumduhen	6	115			1.6		0.234
		(4.96)	(95.04)			1.1		0.895
	Hayelom	12	104			1		
		(10.3)	(89.7)					
	Ra'esi Alula	1 (5)	19 (95)					
	Abanega							
	Gereb-tsedo	5 (4.4)	110					
			(95.6)					
Educational level of parents	Illiterate	6 (8.8)	60 (91.2)	0.021	(0.004-0.280)	1.5	(-0.048, 0.128), (-0.082, 0.069),	0.32
	Read and write	22	145			1.4	(-0.050, 0.100), (-0.112, 0.058)	0.85
		(13.2)	(86.8)					0.46
	1-8	5 (3.9)	122			0.9		0.49
			(96.1)					
	9–12	6 (11)	49 (89)			1.3		
	College & above	3 (3.5)	84 (96.5)			1		
Monthly income of	<2000	32	105	< 0.001	(0.000 - 0.007)	5	(-0.164, -0.042), (-0.078, 0.002)	0.00
parents		(23.4)	(76.6)					0.440
	2000-5000	10	255			1.8		
		(3.8)	(96.2)					
	>5000	0 (0)	102			1		
			(100)					
Living style	Crowded	10	14 (58.3)	< 0.001	(0.000 - 0.013)	8	(-0.318, -0.115)	<
		(41.7)						0.00
	Not crowded	32	448			1		
		(6.7)	(93.3)					
Presence of siblings	Yes	12	322	< 0.001	(0.000-0.013)	1.2	(-0.263, 0.416)	0.625
<15 years		(2.5)	(97.5)					
	No	30 (25)	90 (75)			1		
Personal hygiene of children	Good	31	347	< 0.001	(0.000-0.007)	1	(-0.169, -0.059)	<
		(8.2)	(91.8)					0.00
	Weak	11	115			4		
		(8.7)	(91.3)					
Proper hand washing	Yes	17 (4)	417 (96)	< 0.001	(0.000-0.007)	1	(-0.191, -0.053)	0.00
	No	25 (36)	45 (64)			2.3		
Number of students in	≤ 30	41	19 (95)	0.583	(-0.172-			
a class	1 (5)	(8.5)	443		0.102)			
			(91.5)					

with low monthly income were five times more likely to develop *S. pyogenes* carriage than school children from parents with high income (AOR = 5, 95 % CI: 0.164, -0.041, P \leq 0.002). Previous report from Turkey [34] was in tandem with our finding. This might be due to the economic and social support parents provided to their children which attributes better hygienic conditions and increase in health seeking behavior to the safety of each child.

6. Conclusion

The prevalence of *S. pyogenes* among school children was 8.3 %. Presence of *S. pyogenes* among the school children were statistically associated with being female, low monthly income of their parents, overcrowded living style of children, poor personal hygiene and poor hand washing habit of children. Improved hygienic practices (like being safe and clean, appropriate toilet use and improve personal behavior), washing hands properly and high income seem to be important factors to decrease the likelihood of *S. pyogenes* carriage. The isolates of *S. pyogenes* were resistant to most commonly prescribed antibiotics like Penicillin, Amoxicillin-Clavulanic acid, Ampicillin and Ceftriaxone. Whereas majority of the isolates were sensitive to Ciprofloxacin, Clindamycin and Azithromycin. All in all, our study showed increased prevalence of MDR of *S. pyogenes*.

6.1. Strength and limitation of the study

Screening school children for the presence or absence of *S. pyogenes* which contributes to decrease asymptomatic disease transmission is one of the best prevention strategies. To meet our main objective, we have followed the best recommended standard procedures of isolation and identification. Moreover, using transport medium (amies transport medium) was the best method employed. The most important limitation of the study was school children who identified being carrier for the isolates of *S. pyogenes* failed to receive treatment. Their parents wouldn't be convinced to treat the children because they were not having symptoms of infection. **Recommendations**.

From our finding, the following recommendations are forwarded. National health system policy and federal ministry of health should consider to introduce regular screening strategy for carriage rate of *S. pyogenes* and integrate link system to health facilities among the asymptomatic school children to be treated with appropriate antibiotics. Children living in overcrowded conditions should be prioritized for screening and diagnostic centers should practice antimicrobial susceptibility testing during the management of *S. pyogenes* to reduce the further emergence of multidrug-resistance. In addition to this health education should be given to the school children on the basis of personal hygiene and hand washing as well as transmission of *S. pyogenes*.

6.2. Data sharing statement

All datasets generated and/or analyzed during the current study are summarized in the manuscript. Additionally, raw data will be made available on request.

Ethical approval

Ethical approval was obtained from College of Health Science Research Ethics Review committee (RERC) of Mekelle University prior to data collection. After having the letter of co-operation from the division of Biomedical Science; permission was obtained from Mekelle city Ministry of Education and Mekelle city primary school administrators. Informed written consent and assent was obtained from each parent or guardians and children. This study was conducted in accordance with the declaration of Helsinki.

Consent to publication

Not available.

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CRediT authorship contribution statement

Hadush Negash Meles: Writing – review & editing, Writing – original draft, Supervision, Methodology, Data curation, Conceptualization. Brhane Berhe Aregawi: Writing – review & editing, Writing – original draft, Investigation, Data curation. Miglas Welay Gebregergis: Writing – review & editing, Writing – original draft, Conceptualization. Haftamu Hailekiros: Writing – original draft, Supervision, Methodology, Funding acquisition. Yemane Weldu: Writing – original draft, Supervision. Puganzhenthan Thangaraju: Writing – review & editing, Writing – original draft, Methodology, Data curation. Muthu Thiruvengadam: Writing – review & editing, Writing – original draft, Data curation. Naiyf S. Alharbi: Writing – original draft, Validation, Data curation. Muthupandian Saravanan: Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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