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Prevalence and Antibiotic Susceptibility of *Streptococcus pyogenes* Isolated from Schoolchildren in Sana'a City, Yemen

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Abstract:

Group A *Streptococcus* (GAS) is considered the most common bacteria that cause respiratory tract infections and is rapidly resistant to commonly used antibiotics. So, the current investigation was designed to find out the prevalence and antibiotics susceptibility of *Streptococcus pyogenes* among schoolchildren in Sana'a city, Yemen. Two hundred and sixty-eight (269) swab specimens were sampled from the children's throats who attending primary school in Sana'a city between February to April 2019. The *S. pyogenes* strains were isolated and identified according to standard microbiological methods. Antibacterial profile susceptibility was performed by a modified Kirby- Bauer disc diffusion procedure. The obtained results revealed that 39.03% of specimens were positive to Group A *S. pyogenes*. The frequency rate of GAS among females (40.6%) was slightly higher than males (37.4%) with a significant difference ($P < 0.05$). Also, the highest occurrence among the age group of 5-9 years old (48%) was more than the age group of 10-15 years (35.7%; $P > 0.05$). A higher rate of GAS was found among schoolchildren attending government schools (41.35%) compared to private schools (36.76%). The antimicrobial profiles revealed that *S. pyogenes* isolates were sensitive to tetracycline (74.29%) and vancomycin (67.62%). In contrast, *S. pyogenes* was highly resistant to penicillin (85.71%) and ampicillin (80%). Therefore, extensive investigation is needed to understand epidemiological factors that facilitate antibiotic-resistance dissemination and develop strengthen control and preventive strategies.

INTRODUCTION

Streptococcus pyogenes is a positive for Gram stain, spherical cocci in shape with a diameter of 0.5 to 1.0 μm that arranged in chains or pairs, non-spore-forming, and non-motile. *S. pyogenes* produces a beta-hemolytic in blood agar medium and is classified as Lancefeld Group A *Streptococcus* (GAS) (Jawetz and Adelberg's, 2014).

S. pyogenes has several factors that facilitate this bacterium for invading tissues of the body, resistant to the phagocytosis process, and destroy cells and tissues. *S. pyogenes* is the causative agent of several human diseases such as strep throat (pharyngitis), mild skin disease (impetigo), erysipelas, pneumonia, scarlet fever, rheumatic fever, necrotizing fasciitis, glomerulonephritis, myositis, and streptococcal toxic shock syndrome (Ferretti *et al.*, 2016).

The highest incidence peak of *S. pyogenes* is common between children with age-group of 5–15 years and rarely occurred in age under 3 years. Worldwide, about 15% of age schoolchildren were assessed to have GAS pharyngitis every year. The untreated cases of GAS pharyngitis are developing Rheumatic fever (RF) and life-threatening heart illness such as rheumatic heart disease (RHD). The Global Burden of Disease documented that about 33 million people have RHD and more than 319 thousand infected people die due to this disease every year (Watkins *et al.*, 2017; Oliver *et al.*, 2018).

Currently, the most concern encounters the public health is the resistance of *S. pyogenes* to commonly used antibiotics for treatment. Penicillin antibiotic remains the first-line drug used to treat strep throat and tonsillitis caused by streptococcal bacteria (Devi *et al.*, 2011).

However, the quickly growing problems of *S. pyogenes* resistance to antibiotics have occurred and augmented depending on the severity of virulence factors, individual's status immunity, geographical areas, and study design (Bourbeau, 2003; Sharma *et al.*, 2010).

A few reports documented the *S. pyogenes* prevalent among schoolchildren in Yemen. In Aden, a study by Ba-Saddik *et al.* (2014) observed that about 41.5% of examined children having (41.5%) acute pharyngotonsillitis caused by *S. pyogenes*. Also, in Ibb city, AL-Ameri and AL-Kolaibe (2015) found that 40.8% of infected children with acute pharyngitis and tonsillitis were positive for *S. pyogenes*.

Recently, a study by Othman *et al.* (2019) showed that 12.8% of asymptomatic schoolchildren in Sana'a city carried the *S. pyogenes*. Consequently, the frequency of *S. pyogenes* between schoolchildren in Sana'a city is too limited. Therefore, this work aimed to find out the prevalence and antibacterial profile of *S. pyogenes* among schoolchildren in Sana'a city, Yemen.

MATERIALS AND METHODS

Study design and area

This study is a cross-sectional study conducted on different primary schoolchildren in Sana'a city between February and April 2019. A systematic random sampling method was used to select children. Sana'a city consists of fifteen districts. It was selected eleven (11) government and private primary schools from four districts (Bni Al-Hareth, Maeen, Al-Thaorah, and Al-Sabaeen) at Sana'a city.

Exclusion criteria

All the students using antibiotics before seven days and eating within the two hours before specimen collecting were excluded.

Data collection

The required information such as gender, age, kind of education, school, and the district was collected by using a designed questionnaire.

Ethical approval

The procedure of this work was permitted by the Biology Department, Faculty of Science, Sana'a University Research Ethics Review Committee.

All participants were informed in the Arabic language of the purpose of this investigation before information collecting. Written informed consent was obtained from their parents and for children aged less than 10 years, while children aged 10 years and above were insisted to give a written assent.

Sample size and collection

A total of 264 swab specimens were sampled from children (aged between 5-15 years) studied in government and private schools. The specimens were collected by a sterile cotton swab that swabbed from the upper tonsil and posterior pharyngeal areas. The specimens were preserved in Amie's transport medium and immediately transported to the microbiology laboratory.

Examination and bacteria identification

The specimens were cultured on the blood agar plate and incubated anaerobically with 5% of CO₂ overnight at 37°C. After the incubation period, the colonies that showed the beta-hemolytic on blood agar were chosen and subjected for further identification by the morphological characteristics on subculture in selective and differential media, Gram's staining, sensitivity to bacitracin, and biochemical tests

(Humaid, 2018; Iqbal *et al.*, 2016; Leboffe and Pierce, 2011; Yunus *et al.*, 2016).

Antibiotic susceptibility test

All *S. pyogenes* isolates were subjected to an antibacterial susceptibility test by using the Kirby-Bauer disc diffusion procedure on the Mueller Hinton agar (MHA) according to the guideline of CLSI (CLSI, 2016; Saleem *et al.*, 2018a,b; Shahzad *et al.*, 2017). The used antibiotics in this study were tetracycline (30 µg), vancomycin (30 µg), penicillin (10 µg), and ampicillin (10 µg).

Statistical analysis

The findings were evaluated by using a SPSS program. The confidence interval (95%; CI) and probability value of $P \leq 0.05$ (significant) were used to compare the variables.

RESULTS

Two hundred and six-nine (269) specimens were collected from schoolchildren from four districts that distributed as follows: 83 (30.9%) of Bni Al-Hareth, 80 (29.7%) of Maeen, 55 (20.4%) of AL-Sabaeen, and 51 (19.0%) of AL Thaorah district from Sana'a city (Figure 1).

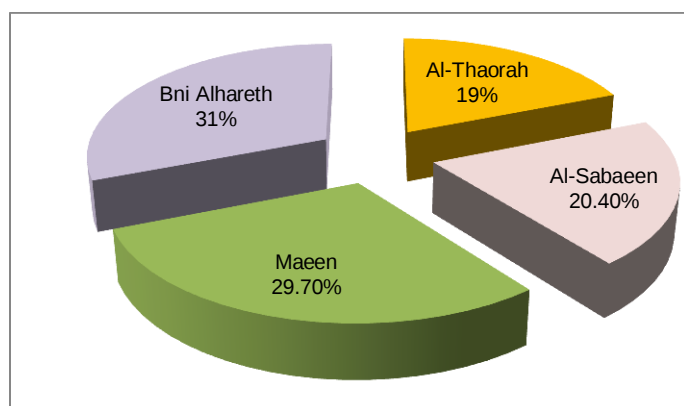


Fig. 1. Distribution of collected specimens from districts.

The present results revealed that 105 (39.03%) out of 269 specimens were positive to Group A

S. pyogenes. While 164 (60.97%) of specimens were negative (Table 1).

Table 1. The prevalence rate of GAS among school children.

GAS	No. of specimens	Ratio %
GAS positive	105	39.03
GAS negative	164	60.97
Total	269	100

Table (2) showed the distribution of GAS prevalent among schoolchildren according to gender and age. The high frequency of GAS was 56 (40.6%) recorded among females while the low rate was 49 (37.4%) among males with a statistically significant difference ($P < 0.05$).

However, the results regarding age showed that the GAS was more common among the age group of 5-9 years old (36, 48%) than group aged 10-15 years (69, 35.7%) with the statistically non-significant difference ($P > 0.05$) as summarized in Table (2).

Table 2. Prevalence of GAS among schoolchildren concerning gender and age.

Characteristic	No. examined(%)	GAS positive (%)	GAS negative (%)	95% CI	P value
Gender	Male	131 (48.7)	49 (37.4)	82 (62.6)	0.700-1.867
	Female	138 (51.3)	56 (40.6)	82 (59.4)	0.870-1.275
Total	269 (100)	105 (39.03)	164(60.97)		<0.05
Age in years	5-9	75 (27.9)	36 (48)	39 (52)	0.348-1.026
	10-15	194 (72.1)	69 (35.7)	125 (64.43)	0.634-1.027
Total	269 (100)	105 (39.03)	164 (60.97)		>0.05

CI= Confidence Interval, $P \leq 0.05$ (significant)

Regarding the prevalence of GAS according to the district, it was observed that the highest frequency of GAS was 50 (18.59%) recorded in Al-Sabaeen district, followed by 37(13.75%) in

Bni Al-Hareth, 13(4.83%) in Maeen, and 5 (1.86%) in Al-Thaorah summarized in the Table (3).

Table 3. Prevalence of GAS among schoolchildren in relation to district

District name	Frequency of specimens (%)	GAS positive (%)	GAS negative (%)
Bni Al-Hareth	83 (30.9)	37 (13.75)	46(17.1)
Maeen	80 (29.7)	13 (4.83)	67(24.91)
Al-Thaorah	51 (19)	5 (1.86)	46(17.1)
Al-Sabaeen	55(20.4)	50 (18.59)	5(1.86)
Total	269 (100)	105 (39.03)	164 (60.97)

This finding showed that the high prevalence of GAS positive was 55(41.35%) found among schoolchildren of government schools. Whereas

the lower prevalence was 50(36.76%) reported among students studying in private schools ($P < 0.05$) as listed in Table (4).

Table 4. Prevalence of GAS in relation to the type of school

Type of school	No. examined (%)	GAS positive (%)	GAS negative (%)	P value
Government	133 (49.4)	55 (41.35)	78 (58.65)	<0.05
Private	136 (50.6)	50(36.76)	86 (63.24)	
Total	269 (100)	105 (39.03)	164 (60.97)	

The isolated *S. pyogenes* was sensitive to vancomycin at 67.62% and resistant at 32.38% with no significant differences ($P > 0.05$). Also, 74.29% of isolated *S. pyogenes* were sensitive

to tetracycline and resistant at 25.71% ($P < 0.05$). The high resistant of *S. pyogenes* was observed to penicillin with 85.71% followed by ampicillin at 80% as shown in Table (5).

Table 5. Antibacterial susceptibility pattern of GAS isolates

Antibiotics	Susceptibility pattern	No.	Percentage %	P value
Vancomycin	Sensitive	71	67.62	>0.05
	Resistant	34	32.38	
Tetracycline	Sensitive	78	74.29	<0.05
	Resistant	27	25.71	
Penicillin	Sensitive	15	14.29	<0.05
	Resistant	90	85.71	
Ampicillin	Sensitive	21	20.0	<0.05
	Resistant	84	80.0	

DISCUSSION

The current work found that the prevalence rate of Group A *S. pyogenes* was 39.03% recorded among participants' schoolchildren. This result is in agreement with other studies that showed that the prevalence rate of GAS was ranged between 10 to 50% among asymptomatic schoolchildren (WHO, 2004).

A similar investigation by Ba-Saddik *et al.* (2014) found that the high prevalence of GAS was 41.5% recorded among children with acute pharyngotonsillitis in Aden city. Also, AL-Ameri and AL-Kolaibe (2015) showed that 40.8% of infected children with acute pharyngitis and tonsillitis were positive for *S. pyogenes* in Ibb city. The lower rate of GAS infection was 12.8% recorded among the asymptomatic schoolchildren in Sana'a city by Othman *et al.* (2019).

Therefore, the high prevalence rate of GAS found in this work might be resulting from different factors such as student overcrowding in class, malnutrition, and absence the awareness of personal hygienic status. The use of broad-spectrum antibiotics for infection treatment maybe increase the antibiotic resistance rate and other resistant pathogens (Ashraf and Iqbal, 2020).

Moreover, the continued war and aggression by the Saudi coalition against Yemen since 2015 and so on have greatly contributed to the destruction of the health system infrastructure that leads to a high increase in the prevalence of infectious disease (Abdullah *et al.*, 2020; Edrees and Anbar, 2020).

It was observed in this work that the slightly higher prevalence of GAS among females (40.6%) than males (37.4%) with a significant difference ($P < 0.05$). This finding is consistent with a result by Othman *et al.* (2019) found the statistically significant higher frequency of GAS among females (66.15%) than males (38.10%). Similar results documented that the females had more exposure to *S. pyogenes* infection than males (Vijaya *et al.*, 2013; Nayiga *et al.*, 2017; Anja *et al.*, 2019).

In contrast, Nayiga *et al.* (2017) found the males having more frequent GAS than females. The high frequency of GAS among females in the present study maybe refers to the social attitudes towards children female in Yemen country that required from them performance some works on home for helping and supporting their mother in everyday tasks.

However, this result revealed that the group aged 5-9 years old having more frequent GAS than the age group of 10-15 years with a statistically non-significant difference ($P > 0.05$).

A similar finding was reported by Anja *et al.* (2019) who observed that the high GAS prevalent was between the age-group of 5–8 years.

This is might be due to the uncompleted immune system, behavior of children during playing together with a close contact for a long time as well as the absence of hygiene awareness. Also, the children always prefer the ice cream after they leave the class which affects the throat.

In current work, the highest rate of GAS occurrence was found schoolchildren living in Al-Sabaeen district followed by Bni Al-Hareth, Maeen, and Al-Thaorah. The high variation in results is might be due to the sample size, method of sample collection, and other factors possible explanation of the difference.

This finding showed that the GSA prevalence was more among children presenting to government schools compared to children presenting in private schools with a statistically significant difference. This result is an agreement to obtained result by Nayiga *et al.* (2017). It is well-known that the students coming from poor families always attending government schools due to a lack of enough money to overcome the private expenses. So, the government schools become crowded with students that facilitate the *S. pyogenes* transmission among students.

The antibiotics susceptibility results found that the 67.62% and 74.29% of isolates of *S. pyogenes* were sensitive to vancomycin and tetracycline, respectively. The higher rate was reported by Mahdi *et al.* (2017) found that the *S. pyogenes* was 96.3% recorded sensitive to vancomycin antibiotics. Also, Agrawal *et al.* (2014) observed that completely (100%) of isolated *S. pyogenes* was sensitive to vancomycin. Anja *et al.* (2019) showed that 42.9% of *S. pyogenes* isolates were susceptible to tetracycline. The present result is in disagreement with Camara *et al.* (2013) who observed the complete (100%) resistance of isolated *S. pyogenes* to tetracycline.

However, the high resistance of *S. pyogenes* isolates was 85.71% recorded against penicillin.

The lower rate was reported by Telmesani *et al.* (2002) recorded that only 48% of isolates of *S. pyogenes* were sensitive to penicillin in Saudi Arabia. Similarly, a study by Mahdi *et al.* (2017) reported that the *S. pyogenes* isolates were resistant to penicillin at 40.74%. In contrast, the higher rate of *S. pyogenes* resistance to penicillin was 100% recorded by Al-Saimary (2011) and Anja *et al.* (2019).

Therefore, the high resistance of isolated *S. pyogenes* to penicillin that is using as the first-line antibiotic of sore throat treatment poses several serious problems to healthcare and the community. The increasing resistance of pathogenic bacteria to commonly and effective antibiotics leads to an increase the patient morbidity and mortality, prolonged duration of illness, and cost for disease controlling measurement (Al-Haik *et al.*, 2017; Alhlale *et al.*, 2020; Edrees and Banafa, 2021).

Several reports were conducted in Yemen that reported the high prevalence of resistant-pathogenic bacteria to commonly used antibiotics resulting from the availability of antibiotics as over-the-counter-drugs and misuse of these drugs (Alhlale *et al.*, 2019; Edrees and Al-Awar, 2020). Also, the prevalence of antimicrobial-resistant microorganisms has been noticed in different regions in Yemen that will be become hospital-acquired infection as well as community-infection acquired (Al-Khawlanly *et al.*, 2021; Edrees *et al.*, 2021). Various antibiotics, plant parts, or complex compounds could be an important source of new therapeutic agents to deal microbial infections that may help to deal the development of resistance (Iqbal and Ashraf, 2019; 2020; Mouffouk *et al.*, 2019).

CONCLUSION

The high occurrence of *S. pyogenes* found among schoolchildren in this work reflects the bad situation in the healthcare system in Yemen during the last years. In addition, the high resistance of *S. pyogenes* to penicillin antibiotic may be resulting from some factors that contribute to increasing resistance to the first-line antibiotic. This increases significant

concerns about the dissemination of antibiotic-resistant strains and the effect upon treatment options in penicillin-sensitive children. Further study is required to understand the epidemiological factors and to introduce the guidance for public health for antibiotics therapeutic strategies and implementing preventive vaccines.

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CONFLICT OF INTEREST

The authors declare that this article's content has no conflict of interest.

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