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Prevalence of Asymptomatic *Streptococcus pyogenes* Carriage and Antibiotic Susceptibility among Schoolchildren in Baghdad, Iraq

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ABSTRACT

Streptococcus pyogenes is a Gram-positive bacterium that causes infectious illnesses, such as rheumatic fever, glomerulonephritis, pharyngitis, and impetigo. This study is a cross-sectional assessment of the frequency of asymptomatic *S. pyogenes* carriage among schoolchildren in Baghdad, Iraq, and evaluates its antibiotic susceptibility patterns. From November 2022 to April 2023, throat swabs were obtained from 100 randomly selected children with acute pharyngo-tonsillitis. The samples were tested for Group A *Streptococcus pyogenes* (GAS) using standard microbiological methods, and antibiotic sensitivity assay was performed for vancomycin, tetracycline, penicillin, ampicillin, and azithromycin. Also, the levels of anti-streptolysin-O (ASO) were determined. The results revealed that 24% of the specimens tested positive for GAS, with a higher prevalence among males (26.32%) compared to females (20.93%), although no significant difference ($p > 0.001$). Age-related results indicated that younger children, particularly those under 5 years old, exhibited higher carriage rates. The prevalence was also significantly higher in urban children (50%) than in rural areas (13.89%). Antibiotic susceptibility testing demonstrated that 85% of bacterial isolates were responsive to vancomycin, while 90% were susceptible to penicillin and ampicillin. Tetracycline showed 75% sensitivity, and azithromycin had the lowest sensitivity at 40%. These findings underscore the importance of monitoring antibiotic resistance and ensuring appropriate treatment, particularly in urban settings where the prevalence of GAS infections is higher. This study highlights the need for ongoing surveillance of asymptomatic GAS carriage among schoolchildren to prevent potential outbreaks and complications.

1. INTRODUCTION

Streptococcus pyogenes (*S. pyogenes*) is responsible for various infectious disorders, including rheumatic fever, glomerulonephritis, and upper respiratory tract infections [1]. Group A *Streptococcus pyogenes* (GAS) can cause a variety of illnesses, including impetigo and respiratory tract infections such as pharyngitis [2]. GAS produces a number of cell surface components and extracellular products that are important in the pathogenesis of infection and in the immune response of the human host. Among these products is streptolysin O (SLO), an oxygen-labile toxin named for its sensitivity to oxygen. Streptolysin O is part of a family of highly conserved pore-forming cytolysins. Beyond its impact on erythrocytes, it is toxic to various cells and cell components, including polymorphonuclear leukocytes (PML), platelets, tissue culture cells, and lysosomes. The anti-streptolysin-O (ASO) test is commonly used to detect recent streptococcal infections and to assess related illnesses, such as rheumatic fever and glomerulonephritis [3].

Several factors, including host and environmental elements, influence the asymptomatic pharyngeal carriage of *S. pyogenes*, potentially affecting the severity and outcome of infection. *S. pyogenes* is most prevalent in children aged 5 to 15 years and is considered a significant health hazard among school-aged children. Each year, approximately 15% of youngsters in school globally are likely to suffer from Group A *Streptococcus pyogenes* pharyngitis. If untreated, this condition can lead to severe consequences, such as rheumatic fever (RF) and rheumatic heart disease (RHD), with the autoimmune response in RHD causing inflammation and scarring of heart valves. This can impair cardiac function, leading to heart failure or stroke. According to several studies, over 33 million people worldwide are affected by RHD, with approximately 319,400 deaths attributed to the condition annually [4]. *Streptococcus pyogenes* commonly colonizes the pharynx of asymptomatic individuals, with a carriage rate of 15–20% among schoolchildren showing no symptoms. In high-income countries, the incidence among children ranges from 8.4 to 12.9% [5]. The unauthorized use of broad-spectrum antibiotics has been a major contributor to the recent rise in antibiotic resistance against this bacterium. Resistance to rifampicin and other medications has been observed in *Streptococcus pneumonia*. On the other hand, the rate of macrolide resistance is increasing, primarily due to the overuse of these antibiotics. Erythromycin is recommended for those

allergic to penicillin, with clindamycin as an alternative, although penicillin remains the preferred treatment for GAS pharyngitis [5].

The purpose of this study was to isolate and identify *S. pyogenes* in throat swabs from children with pharyngotonsillitis to estimate the prevalence and antibiotic sensitivity patterns associated with age, sex, and seasonal variations in GAS infections. Determining the sensitivity of *S. pyogenes* to antibiotics such as vancomycin, tetracycline, penicillin, ampicillin, and azithromycin is crucial for effective treatment.

2. MATERIALS AND METHODS

2.1 Sample collection

A cross-sectional study was conducted in Baghdad, involving 100 randomly selected patients with acute pharyngotonsillitis who visited various hospitals between November 2022 and April 2023. Throat swabs and blood samples were collected from the patients to measure ASO levels.

2.2 Exclusion criteria

The study excluded young children who had a positive ASO test result, had taken antibiotics within three weeks prior to sample collection, or had a tonsillectomy. Students beyond the age of eighteen were also not allowed.

2.3 Ethical consideration

The Faculty of Medicine's Ethics Committee granted ethical approval for the current investigation.

2.4 Microbiological analysis

Collected throat swabs were streaked onto blood agar and incubated twenty-four hours at 37°C in a 5% CO_2 atmosphere. Identification of Group A *Streptococcus* colonies was based on their morphological characteristics, Gram staining, catalase production test, and the presence of β-hemolysis zones surrounding the colonies. A bacitracin sensitivity test was conducted on the cultures to verify the GAS's identity, as shown in Figures 1 and 2.

2.5 Antibiotic susceptibility testing

The disc diffusion method was used for antimicrobial susceptibility testing (AST) in accordance with the recommendations set forth by the Clinical and Laboratory Standards Institute (CLSI) [6]. The antibiotics tested included vancomycin (30 µg), azithromycin (15 µg), penicillin (10 µg), ampicillin (10 µg), and tetracycline (30 µg), according to the 2022 guideline of the Clinical Disc Diffusion Method on Muller-Hinton Agar and Laboratory Standards Institute (CLSI). The antibiotic discs were positioned using sterile forceps, and a bacterial suspension was inoculated by streaking on the agar's surface. The distance between the center of each disc and the edge of the dish was greater than 15 mm, with each disc separated from the others by more than 24 mm. The discs were incubated at 37°C for 24 hours.

2.6 Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS; version 20) program. Comparisons were made using the Chi-square (X^2) test, and categorical data were displayed as numbers and percentages. P-value of <0.05 was considered statistically significant.

Figure 1. *Streptococcus pyogenes* on blood agar

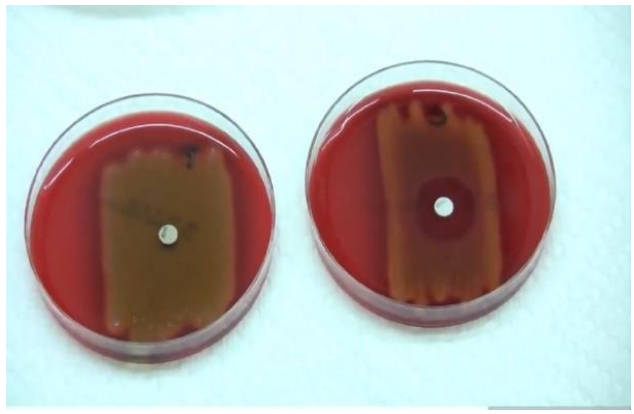

Figure 2. *Streptococcus pyogenes* on blood agar with bacitracin disk

3. RESULTS

One hundred specimens were collected from schoolchildren. The results showed that 24 specimens (24%) tested positive for GAS, while 76 specimens (76%) tested negative based on the ASO test, as shown in Table 1 and Figure 3.

Table 1. The rate of infection with Group A *Streptococcus pyogenes* among schoolchildren

Specimen	No.	Ratio%
Positive	24	24%
Negative	76	76%
Total	100	100%

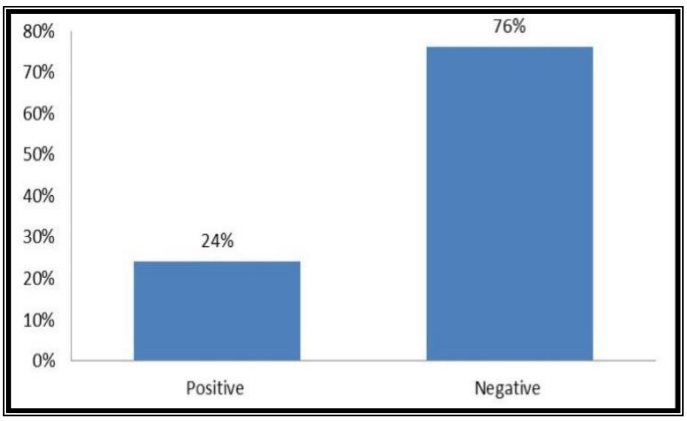

Figure 3. Distribution of Group A *Streptococcus pyogenes* among schoolchildren with the ASO test

The prevalence of GAS among schoolchildren varied by sex, age, and residency (Table 2 and Figure 4). Males exhibited a higher frequency of GAS (26.32%) compared to females (20.93%), though no significant difference ($p > 0.001$) was observed. Age-related data revealed that GAS was more prevalent in the younger age groups: 50% in children under 2 years and 46.67% in those aged 2-5 years, compared to 9.52% in the 12-18 month and 7.69% in the 6-11 month with significant difference ($p < 0.001$). Regarding residency, GAS was more common among urban children (50%) than their rural counterparts (13.89%).

The clinical signs and symptoms in children with tonsil-related issues were consistent with acute pharyngotonsillitis. Table 3 shows that fever was the most prominent symptom, observed in 100% of cases, followed by pharyngeal exudates (75%), and sore throat (83%). Other symptoms included vomiting (20.8%), abdominal pain (20.8%), enlarged anterior cervical lymph nodes (20.8%), and headache (62.5%).

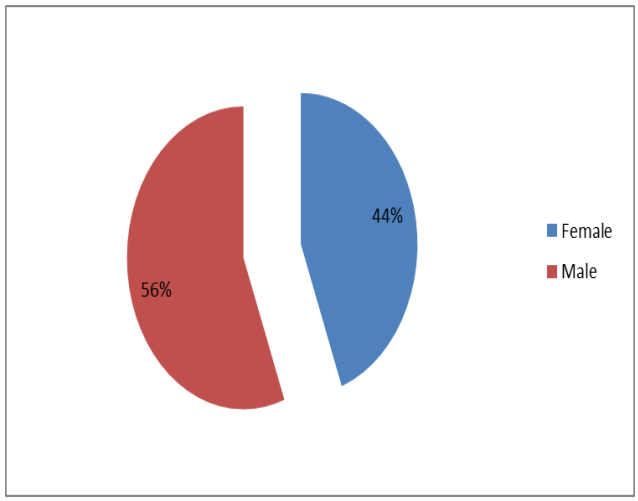

Figure 4. Distribution of patients with Group A *Streptococcus pyogenes* isolates according to the sex

Table 2. Patients' demographic attributes' frequencies

Factor		No. of Cases	Positive	Negative	P-value
Sex	Female	43(100%)	9(20.93%)	34(79.10%)	0.39
	Male	57(100%)	15(26.32%)	42(73.70%)	ns
Age	<2 years	10	5(50%)	5(50%)	<0.05*
	2-5	30	14(46.67%)	16(53.30%)	
	6-11	39	3(7.69%)	36(92.30%)	
	12-18	21	2(9.52%)	19(90.50%)	
Resid.	Urban	28(100%)	14(50%)	14(50%)	<0.05*
	Rural	72(100%)	10(13.89%)	62(86.11%)	

*: significant at $p \leq 0.05$; ns: not significant.

Table 3. Clinical signs and symptoms associated with acute pharyngeal tonsillitis

Clinical Sign	Positive Cases
Fever	24 (100.00%)
Pharyngeal erythema	20 (83.30%)
Sore throat	20 (83.30%)
Pharyngeal exudates	18 (75.00%)
Headache	15 (62.50%)
Swollen anterior cervicallymph nodes	5 (20.80%)
Abdominal pain	5 (20.80%)
Vomiting	5 (20.80%)

The results in Table 4 indicated that GAS-related acute pharyngotonsillitis began in November (12.5%), peaked in December and January (29.2%), decreased in February and March (8.3%), and finally dropped in April (4.1%).

Table 4. Distribution of Group A *Streptococcus pyogenes*-positive patients between November 2022 and April 2023

Month	Positive Cases
November	3 (12.50%)
December	7 (29.20%)
January	7 (29.20%)
February	4 (16.70%)
March	2 (8.30%)
April	1 (4.10%)

Streptococcus pyogenes isolates demonstrated susceptibility to vancomycin in 85% of cases and resistance in 15%. The isolates were also 90% susceptible to penicillin and ampicillin. Additionally, 75% of the *Streptococcus pyogenes* isolates were sensitive to tetracycline, while 40% showed sensitivity to azithromycin (Figure 5).

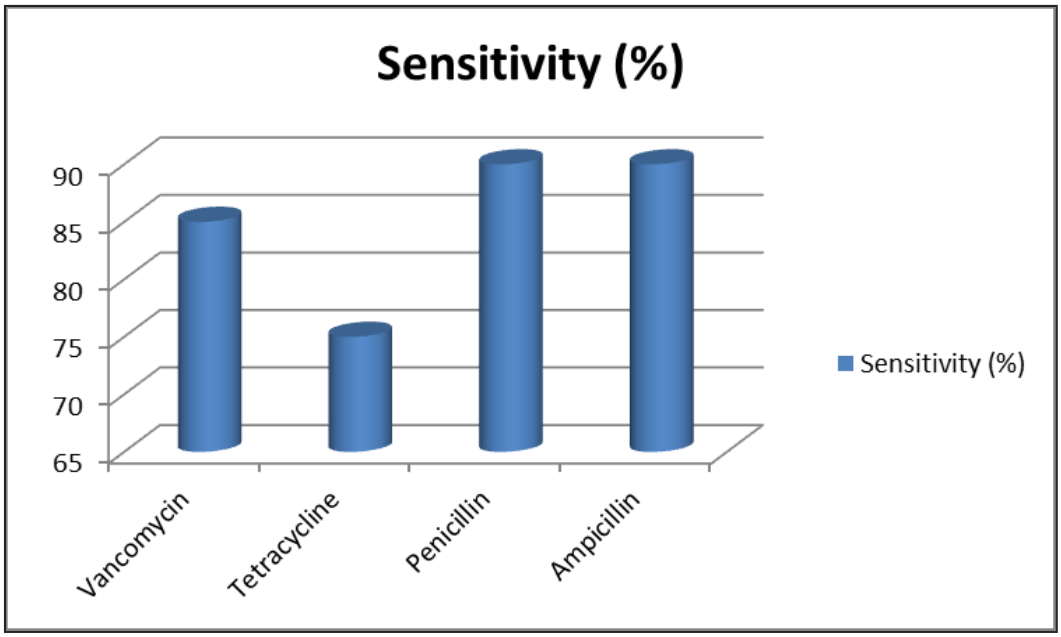

Figure 5. The pattern of antibacterial susceptibility of Group A *Streptococcus pyogenes* isolates

4. DISCUSSION

In this study, the prevalence of GAS carriage and antibiotic susceptibiity among schoolchildren were investigated. Demographic differences, clinical symptoms, and patterns of antibiotic susceptibility were examined among asymptomatic kids in Baghdad, Iraq. The results provide some important new insights into the prevalence of GAS and the factors that contribute to it.

This study found that isolates of bacteria from blood cultures in patients with tonsillitis and these results was in agreement with the other reports which indicated that positive blood culture.

4.1 Prevalence and demographic variations

An infection rate of 24% for GAS was detected among school-aged children. Previous research indicated that the infection rate of GAS among asymptomatic school students ranged from 10 to 50% [7]. This demonstrates the importance of GAS as a prevalent infection in this group. In Iraq, a significant GAS infection rate of 34.1% was documented among children with acute pharyngotonsillitis, with a higher prevalence in boys (26.32%) compared to girls (20.93%) [8]. A comparable result was reported in Iran [9, 10]. Similar high *S. pyogenes* carriage rates were reported from Argentina (14.2%), Egypt (16%), Ethiopia (9.7%), India (16.3%), Nepal (10.8%), Turkey (14.3%) and Uganda (16%). In contrast, these findings differ from a prior study conducted in Sana'a city Yemen [11] and in Spain [12, 13], which found that 90% of patients under the age of 5 with acute pharyngotonsillitis tested positive for GAS from pharyngeal specimens, with no difference in prevalence between sexes. The findings of the current study align with the report by Abd Al-Kareem et al. [14], which found that *Streptococcus pyogenes* infections were more common in male children [14]. Similarly, *Streptococcus pyogenes* infections were more common in male children than in female children, according to Kalaf [15]. The gender distribution brings up a number of possible causes and aspects that could be involved in this discrepancy. Possible explanations include biological factors, such as differences in hormonal levels and immune responses between the sexes, which may influence susceptibility to *Streptococcus pyogenes* infections. Al-Hasnawi et al. [16] conducted local studies in Baghdad, Iraq, which revealed a prevalence rate of 53% for GAS. This study was in disagreement with the study by Avire et al. [17], they reported the females were more susceptible and response for infection compared with males. The discrepancies in results may be attributed to geographical influences, variations in weather, time of sample collection (seasonal variations), and differences in inquiry methodology.

Abd Al-Kareem et al. [14] found most children infected with *Streptococcus pyogenes* resided in densely populated urban areas, aligning with the results of the present study. Urban population density, increased human interaction, and restricted access to medical facilities are some of the conditions that may contribute to higher transmission rates. The prevalence of *Streptococcus pyogenes* infections is influenced by socioeconomic disparities that affect living conditions, healthcare access, and hygiene habits. Urban areas tend to have larger population densities [12]. Additionally, urban environments, including crowded housing complexes, schools, and daycare centers, can facilitate close contact among individuals, potentially accelerating the spread of the bacteria [13].

4.2 Clinical features of GAS infection

The present study revealed that fever is the primary symptom of GAS infection, followed by sore throat, pharyngeal exudates, throat erythema, and occasionally headache, as well as enlarged anterior cervical lymph nodes, nausea, and abdominal pain. Similar clinical signs were observed in an old study conducted in Tehran, Iran, regarding acute pharyngotonsillitis. According to earlier study by Rhumaid et al. [7], the two most common symptoms of streptococcal pharyngitis are a sore throat and fever exceeding 38°C, accompanied by typical signs such as exudates, cervical lymphadenopathy, and irritation of the tonsils and pharynx.

4.3 Seasonal trend in Group A *Streptococcus pyogenes* infection

The findings also indicated variations in the monthly incidence of GAS infection. A six-month observation period revealed a high rate of GAS infections resulting in acute pharyngotonsillitis, with most cases occurring in the winter and spring. December and January recorded the highest rates of GAS infection at 29.2%. This pattern is similar to the seasonal increase in respiratory diseases, which is probably caused by warmer weather, less ventilation, and more people crammed into a smaller space [1]. GAS transmission is influenced by environmental conditions, as seen by the reduced occurrence in warmer months like March and April. Several factors, including genetic variations and environmental conditions, may have contributed to this peak period, or it could be attributed to the fact that most samples were collected during the colder months.

4.4 Antibiotic susceptibility and resistance

The isolated *Streptococcus pyogenes* exhibited 85% sensitivity to vancomycin and 90% resistance to penicillin and ampicillin. Additionally, 75% of the isolated *Streptococcus pyogenes* strains were sensitive to tetracycline, while sensitivity to azithromycin was observed in 40% of the isolates. Ibrahim and Raad [18] and Mahdi et al. [19] reported an increased sensitivity rate to vancomycin, with *Streptococcus pyogenes* demonstrating 96.3% susceptibility, as noted by Kebede et al. [20]. Furthermore, Agrawal et al. [21] found that vancomycin was effective against 100% of the isolated *Streptococcus pyogenes*. The current findings contrast with those of Camara et al. [22], who reported that isolated *Streptococcus pyogenes* exhibited complete (100%) resistance to tetracycline. Additionally, 85.71% of their *Streptococcus pyogenes* isolates showed significant resistance to penicillin, while Mahdi et al. [19] reported that 40.74% of *Streptococcus pyogenes* isolates were resistant to penicillin. This high level of resistance poses significant challenges for healthcare providers and the community, as penicillin is commonly considered the first-line antibiotic for treating sore throats. According to Alhlale et al. [23] and Edrees et al. [24], higher patient morbidity and death as well as longer illness duration are caused by pathogenic bacteria's growing resistance to widely used and efficient antibiotics [25, 26]. Several studies from Iraq show that a considerable prevalence of bacteria that are resistant to commonly administered antibiotics has been caused by the availability of antibiotics as over-the-counter drugs and their abuse [7, 27, 28].

4.5 Implications and recommendations

The findings of this study highlight the need for preventative measures to lessen the effects of pediatric GAS infections. Regular screening programs are essential, particularly for high-risk groups such as younger children and those residing in urban areas, to identify asymptomatic carriers who may contribute to the transmission of the pathogen. To lessen the emergence and spread of antibiotic resistance, public health education initiatives should stress the need of good cleanliness and the prudent use of antibiotics. To ensure that narrow-spectrum antibiotics, such as penicillin, continue to be the recommended first-line treatment for GAS infections, antibiotic stewardship programs must be reinforced to control the distribution of antibiotics. Such regulations can aid in preserving these drugs' effectiveness and halting the development of resistance. The genetic and environmental factors influencing demographic and seasonal variations in GAS prevalence also require more investigation. To successfully stop the spread of GAS infections, this research can help guide focused efforts and improve public health tactics.

5. CONCLUSION

Streptococcus pyogenes causes acute pharyngitis and tonsillitis in children. Males have a significantly greater infection rate than females, albeit this difference is not statistically significant. According to the current study's found significant difference ($p > 0.001$) in the prevalence between the age groups of less than two years and two to five years. Additionally, GAS infections are more common in urban populations than in rural ones. Cases of acute pharyngitis and tonsillitis caused by GAS typically increase in November, peaking in January, with this pattern continuing throughout the winter and spring. Furthermore, ampicillin, tetracycline, vancomycin, and penicillin are highly recommended for treatment.Multiple drug resistance was observed in isolated GAS isolates (two or more medications). Antibiotics are readily available, and their abuse may be the cause of this.

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