Original Article

Isolation and Characterization of *Streptococcus pyogenes* from Iraqi Children with Pharyngotonsillitis

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Abstract

Background: *Streptococcus pyogenes* known as group A streptococci (GAS) is the main causative agent of pharyngotonsillitis in children younger than 12 years old. The infection appears mostly in winter and spring. Signs and symptoms of patients with a beta-hemolytic GAS infection, include tonsillar exudate, fever, painful anterior cervical adenopathy, and the lack of a cough. Penicillin and ampicillin are the preferred clinical therapy choice for GAS pharyngitis treatment. **Objectives:** Isolate and identify GAS in throat swabs among children suffering from acute pharyngotonsillitis to estimate the prevalence rate of bacterial isolates associated with age, sex, and months variation of GAS infection. Also, it is to determine the antibiotic sensitivity patterns such as penicillin, ampicillin, erythromycin, azithromycin, ofloxacin, clindamycin, and rifampicin, which are used for the treatment of GAS associated with the acute pharyngotonsillitis in children. **Materials and Methods:** Four hundred and ten throat swabs have been obtained from patients who were clinically diagnosed as having acute pharyngotonsillitis of age ranging from (1–12) years of both sexes were admitted to various hospitals and enrolled in a sequential manner from November 2021 to April 2022 in two Governorates (Baghdad and Babylon), Iraq. These throat swabs were exposed to the bacterial culturing, and some other tests. *Streptococcus pyogenes* isolates were identified depending on the colony properties, beta-hemolysis on sheep blood agar plate, Gram's stain, catalase production test, in addition to a sensitivity to bacitracin and Pyrrolidinyl arylamidase test to confirm the identification of GAS. **Results:** The prevalence of identifying GAS isolates was 140 (34.1%) out of 410 samples. GAS strains can cause acute pharyngotonsillitis in children of all ages, but it most frequently occurred between 1 and 6 year age groups, and it was more in men 80 (57.1%) than in women 60 (42.9%). Likewise, it was found that GAS associated with acute pharyngotonsillitis with a high incidence in winter and in early spring, in which the peak of GAS infection has been seen in January (30.7%). On the other hand, some antibiotics were used to show their effect on these GAS isolates. The findings revealed that all isolates were sensitive (100%) to penicillin and ampicillin, and (83.6%) of isolates were sensitive to clindamycin, whereas some isolates shown a lesser degree of sensitivity (75% and 60%) to rifampicin and ofloxacin. However, only 49.3% of the isolates were sensitive to erythromycin, and (46.4%) were sensitive to azithromycin. **Conclusion:** GAS strains can cause acute pharyngotonsillitis in children of all ages, but it most frequently occurred in the age groups (1–6) year, and more predominant in men than in women. High percentage of infections with GAS have been noticed in winter and spring to reach its peak in January. Additionally, penicillin and ampicillin are preferred antibiotic for treatment, whereas in the most serious cases clindamycin was added to the treatment plan.

Keywords: Antibiotic resistance, beta-hemolytic streptococci, *Streptococcus pyogenes*, pharyngotonsillitis

Introduction

Acute pharyngotonsillitis is a common disease with the highest prevalence occurring in the winter months. More than half of pharyngotonsillitis cases are caused by the viruses, while 15%–30% of cases caused by beta-hemolytic group A streptococci (GAS).[1] *Streptococcus pyogenes* is able to infect humans by adhering to and colonizing the host's

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upper respiratory tract mucosal surface epithelial cells.[2] It is found in a variety of infectious disorders, including minor ones like mild pharyngitis and skin infections as well as the severe ones such as streptococcal toxic shock and necrotizing fasciitis.[3] *Streptococcus pyogenes* strains are differentiated as GAS as the main causative agent of pharyngotonsillitis in pediatric patients,[4] is a gram positive coccoid-shaped bacterium, that emerges in chains. When grown on blood agar, GAS produces a distinct zone of β-hemolysis and produces a minor white to the gray colored colony. Hemolysis is not caused by uncommon GAS strains.[5] There are three mechanisms by which GAS produces serious human illness such as, suppuration, as in pyoderma and pharyngitis, immune-mediated inflammation, and toxin elaboration. Infections of the respiratory system, including acute pharyngitis, are frequent in children and adolescence, and it is most prevalent and common among youngsters aged 5–15 years old.[6] Signs and symptoms of patients with a beta-hemolytic GAS infection, including tonsillar exudate, fever, painful anterior cervical adenopathy, and the lack of a cough.[1] Recent years have seen a shift in antibiotic resistance against this bacterium, mostly as a result of the improper use of broad range antibiotics.[7] *Streptococcus pneumonia* has shown resistance to rifampicin, among other drugs.[8] Penicillin is the preferred clinical therapy choice for GAS pharyngitis treatment, whereas the first alternative antibiotic for people who are allergic to penicillin is erythromycin, followed by clindamycin, in contrast, the rate of macrolide resistance is progressively rising, which might be explained by the over usage of these antibiotics.[9]

The present study aimed to isolate and identify *S. pyogenes* (GAS) in throat swabs among children suffering from acute pharyngotonsillitis to estimate the prevalence rate of bacterial isolates associated with age, sex, and months variation of GAS infection. Also, determining the antibiotic sensitivity patterns such as penicillin, ampicillin, erythromycin, azithromycin, ofloxacin, clindamycin, and rifampicin is important, which are used for the treatment of GAS associated with the acute pharyngotonsillitis in children.

Materials and Methods

Patients and duration of the study

A cross-sectional study was carried out for 410 randomly selected patients who were clinically diagnosed as having acute pharyngotonsillitis of age ranging from (1–12) years of both sexes who were reviewers to different hospitals during the interval between November 2021 and April 2022, in two Governorates (Baghdad and Babylon), Iraq. Clinical features of pharyngotonsillitis cases and some other relevant patient data were documented.

Throat swab collection

Throat specimens were taken from the tonsils and post pharyngeal with a sterile cotton-tipped swab by a physician, and were placed in sterile disposable transport media and close it until transported to the laboratory within 2 h.

Laboratory diagnosis

Isolation and identification

The collected throat swabs were inoculated by streaked on the surface of 5% sheep blood agar plate, which enables it to easily check for colonies that are β-hemolytic, then the optimal incubated under 5% CO_2 at 37°C for 24 h. If bacterial cultures are negative should be re-examined after another 24 h of incubation. GAS colonies were identified through the formation of β-hemolysis zones surrounding colonies and depends on the morphological characteristics, Gram's stain,[10] catalase production test.[11] Cultures should be examined for bacitracin sensitivity test and pyrrolidinyl arylamidase (PYR) test to confirm the identification of GAS.

Bacitracin sensitivity test

Bacitracin sensitivity test is carried out according to the method described in Spellerberg and Brandt,[12] on a sheep blood agar plate streak two or three suspect colonies by an inoculating loop. Next, heated forceps were used to place a disk containing 0.04 U of bacitracin in the middle of each plate (area of heaviest growth). After that, incubate the plate under 5% CO_2 at 37°C for 24 h, then a zone of inhibition around the disk was measured, indicating the strain's susceptibility.

Pyrrolidinyl arylamidase test

It is a rapid colorimetric method completed within a few minutes, which was done on paper strips containing dried chromogenic substrates for the pyrrolidinyl aminopeptidase. The test is performed according to the procedure described in Abraham and Sistla.[13]

Antibiotic sensitivity testing

This test was done for all identified (140) isolates by using the following eight different antibiotics (commercial discs): penicillin (1 μg), ampicillin (10 μg), clindamycin (2 μg), rifampicin (5 μg), ofloxacin (5 μg), and erythromycin (15 μg), azithromycin (15 μg) by employing the disk diffusion method on Muller-Hinton agar enriched with 5% sheep blood, based on the standard values of the Clinical and Laboratory Standards Institute (CLSI), 2022 guideline.[14] The bacterial suspension was inoculated by streaking on the surface of the agar and the discs of antibiotic were placed by sterile forceps. Each disk was separated from the others by a distance >24 and the distance between the disk's center and the dish's border was >15 mm. The plate was incubated at 37°C for 24 h. The minimum inhibitory concentration was measured and used to categorize GAS isolates as (sensitive, intermediate, or resistant), based on the CLSI 2022 guideline.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee, according to the document number 27 (including the number and the date in November 14, 2021) to get this approval.

Results

In this study, four hundred and ten throat swabs have been obtained from children (male and female) suffering from acute pharyngotonsillitis of age (1–12) years. All these samples were subjected to various tests for the identification of GAS.

Among the 410 throat swabs, it was found that 140 samples were culture positive (34.1%) for GAS isolates [Table 1].

According to the results [Table 2], GAS strains can affect and causes acute pharyngotonsillitis in children of all ages, but it most frequently occurred in the age groups (1–6) year.

Furthermore, the results showed that acute pharyngotonsillitis was more in male 80 (57.1%) than in female 60 (42.9%), as shown in Figure 1.

Clinical signs and symptoms were compatible with acute pharyngotonsillitis in children patients reviewer the hospitals for a related problem of tonsils and pharyngeal. These clinical signs and symptoms are illustrated in Table 3.

Table 1: Distribution of throat swabs according to the results of diagnostic methods

Results of diagnostic methods	No. of swabs	%	Sex	
			Male	Female
Positive for GAS isolates	140	34.1	80	60
Negative for GAS isolates	270	65.9	143	127
Total	410	100	223	187

Table 2: Distribution of patients with acute pharyngotonsillitis according to the age groups and sex

Age groups in year	Patients		Sex	
	No.	%	Male	Female
1–3	58	41.4	32	26
4–6	56	40	31	25
7–9	20	14.3	12	8
10–12	6	4.3	5	1
Total	140	100	80 (57.1%)	60 (42.9%)

Data from Table 3 revealed that the fever was the most prevalent signs and symptoms presented in all GAS cases (100%), followed by pharyngeal erythema (95%), sore throat (92.9%), pharyngeal exudates (77.1%). It is also noticeable that in some cases the headache (24.3%), swollen anterior cervical lymph nodes (21.4%), abdominal pain (15.7%), and vomiting (10.7%) are concurrent findings.

Additionally, the findings revealed that acute pharyngotonsillitis caused by GAS was commenced in November (9.3%) and increased in percentage to reach its peak in January (30.7%) then beginning to decline till is becoming equal (19.3%) in February and March, then decreased percentage (7.1%) in April [Figure 2].

Furthermore, some antibiotics were used to show their effect on GAS that was isolated from children patients with acute pharyngotonsillitis [Figure 3].

Figure 3 revealed that all isolates of GAS were sensitive (100%) to penicillin and ampicillin, and (83.6%) of isolates were sensitive to clindamycin, whereas some isolates shown a lesser degree of sensitivity (75% and 60%) to rifampicin and ofloxacin, respectively. However, only 49.3% of the isolates were sensitive to erythromycin, and 46.4% were sensitive to azithromycin.

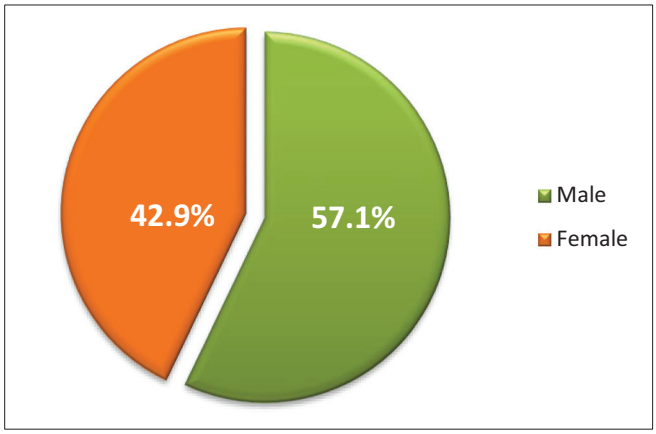

Figure 1: Distribution of patients with acute pharyngotonsillitis according to the sex

Table 3: Clinical signs and symptoms in relation to the identified acute pharyngotonsillitis

Clinical symptoms	No. of cases (n = 140)	%
Fever	140	100
Pharyngeal erythema	133	95
Sore throat	130	92.9
Pharyngeal exudates	108	77.1
Headache	34	24.3
Swollen anterior cervical lymph nodes	30	21.4
Abdominal pain	22	15.7
Vomiting	15	10.7

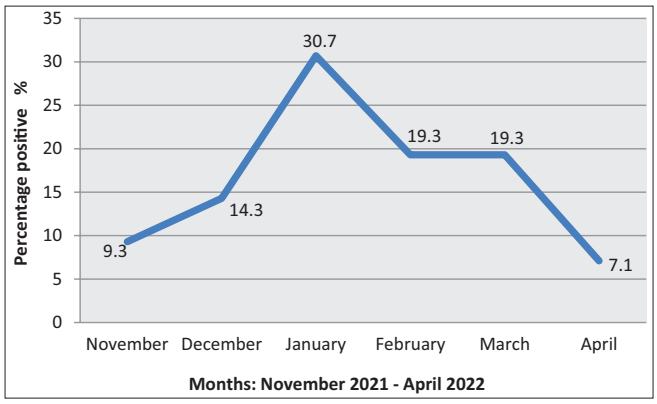

Figure 2: Distribution of GAS positive patients for the period from November 2021 to April 2022

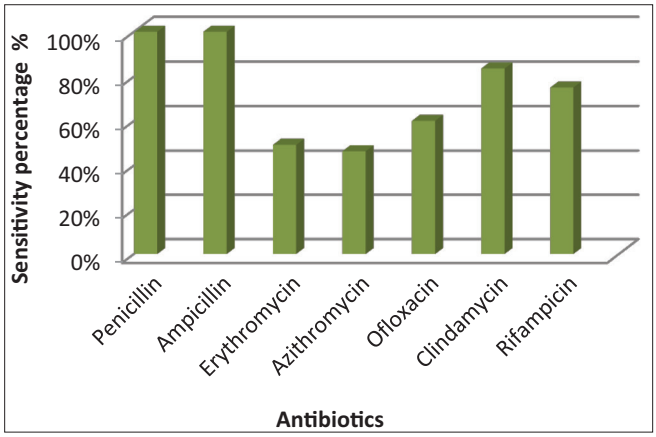

Figure 3: Sensitivity percentage of GAS isolates to some antibiotics

Discussion

Infection caused by *S. pyogenes* (GAS) remains a serious public health issue, particularly in developing countries, despite early antibiotic usage and advancements in health care that have decreased the frequency of complications.[15] GAS the common bacteria are found on the skin and in the throat, but without exhibiting any symptoms of sickness, people may have GAS in their throats or on their skin.[16]

In the present study, GAS was isolated from children with acute pharyngotonsillitis at a rate of (34.1%), and it is most often seen in the age group between 1 and -6 year, and more in men (57.1%) than in women (42.9%). A similar result was recorded in Iran by Jasir *et al.*[17] who indicated that the rate of GAS isolated from children with acute pharyngotonsillitis was 34.1%. In contrast, these results disagree with the previous study recorded in Spain by Espadas-Maciá *et al.*[18] demonstrated that 90% of patients with acute pharyngotonsillitis less than 5 years of age were GAS positive that isolated from pharyngeal specimens, with no differences in incidence between men and women. The local previous study in Baghdad city, Iraq carried out by Al-Hasnawi *et al.*[6] showed that the prevalence rate of GAS 53% when cultured on blood agar and the prevalence rate (42%) when detected by rapid antigen-detection test among children mostly in the age group 6–8 years. These variations in the findings are probably due to geographical region effects, differences in weather, the timing of sample collection (months), and the investigation method.

Besides, the results revealed that the fever was the most prevalent signs and symptoms presented in all GAS cases, followed by pharyngeal erythema, sore throat, pharyngeal exudates. It is also noticeable in some cases with the headache, swollen anterior cervical lymph nodes, abdominal pain, and vomiting. Acute pharyngotonsillitis infection has been reported in the current study with an almost similar signs and symptoms reported in another previous study in Tehran, Iran.[19] In contrast, other previous studies reported[20,21] indicated that a fever higher than 38°C and sore throat are the two most typical signs of streptococcal pharyngitis, whereas, the other symptoms such as exudates, cervical lymphadenopathy, and inflammation of the pharyngeal and/or tonsillar are common signs.

Moreover, the results revealed that the variation in the monthly incidence of GAS infection. The epidemiological behavior of the GAS that causes acute pharyngotonsillitis throughout a 6-month period was observed. The infection appears mostly in winter and spring, in which the peak of GAS infection has been seen in January (30.7%). It may be due to several factors contributed to the epidemic period, including environmental factors, and genetic variation, or most samples perhaps were collected throughout the cold months. These results agreed on with the result found out by Ebell *et al.*[22] who also discovered that winter and early spring are the two seasons when Streptococcal pharyngitis most frequently occurs. However, the findings of the present study are in contrast with previous studies[18] that discovered no evidence of seasonal variation.

As stated in our results section, all isolates showed penicillin and ampicillin sensitivity. Thus, penicillin and ampicillin continue to be the preferred treatment for GAS associated with acute pharyngotonsillitis, and no reports of resistance to these antibiotics have been made globally to yet.

Also, 83.6% of isolates were sensitive to clindamycin. This result is in agreement with results study in Romania done by Gavriliu *et al.*[23] who have indicated that only 85% of the GAS were sensitive to clindamycin. Another study recorded in Korea by Kim and Uh[24] observed that the 67.5% of GAS were sensitive to clindamycin. Once GAS has been recognized, empirical therapy can be replaced with penicillin, whereas in the most serious cases clindamycin was added to the treatment plan.[25]

Nearly half of the cases, sensitive to erythromycin and 46.4% sensitive to azithromycin, whereas 60% sensitive to

ofloxacin which was the only fluoroquinolone estimated in this study. In 2015, an Iranian study found that 49.1% sensitive of GAS isolates to erythromycin, 44% sensitive to azithromycin, and 59.3% sensitive to ofloxacin; this is probably due to improper usage of these antibiotics in recent years.[19] Other studies confirm that the resistance to erythromycin, which has become more common in later years, particularly in Spain, where the rate of resistance to 14- and 15-membered macrolides ranges from 10% to 41%.[26] In contrast, 75% of GAS strains were sensitive to rifampicin. A similar result was recorded by Choby[19] who observed that 74.6% of GAS strains were sensitive to rifampicin. Other previous studies showed that all of the GAS strains were rifampicin-sensitive.[27] The interpretation of these findings probably related to the fact that the rifampin is one of the primary agents for the treatment of tuberculosis and utilized in the treatment plan for various illnesses caused by multi-resistant microorganisms, like MRSA and *Acinetobacter* spp. Another reason for rising resistance to rifampicin may be the rate of development of these multidrug-resistant bacteria in recent years. The only way to control and eliminate GAS infection may be by vaccination, but there are currently no commercially available vaccinations.[28]

Conclusion

This study concluded that GAS strains can cause acute pharyngotonsillitis in children of all ages, but it most frequently occurred in the age groups (1–6) year, and more predominant in men than in women. High percentage of infections with GAS has been noticed in winter and spring to reach its peak in January. Additionally, penicillin and ampicillin are preferred antibiotic for treatment, whereas in the most serious cases clindamycin was added to the treatment plan. Early identification and treatment are essential because of the disease's potential severity and the probable need for severe supportive care in some cases.

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Conflicts of interest

There are no conflicts of interest.

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