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# A STUDY ON ANTIBIOTIC PROFILE OF GROUP A β-HAEMOLYTIC STREPTOCOCCUS IN ACUTE PHARYNGITIS AMONG CHILDREN BETWEEN 5-12YRS

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## ABSTRACT

**Background:** In underdeveloped nations, antibiotics are routinely administered for pharyngitis, which results in high healthcare costs and their irrational usage may cause antibiotic resistance in the population. The purpose of this study is to examine how environmental factors affect the likelihood that group incidents may occur.

**Aims**: To estimate the prevalence of Group A β-hemolytic streptococcus in cases of acute pharyngitis among children.

**Materials and methods:** The current study was a cross-sectional study conducted on 200 children between the ages of 5 to 12 years who visited the out-patient of paediatric department.

**Results**: In the current study, 4.5% of children between the ages of 5 to 12 years had GAS pharyngitis. Female children are seemed to have a greater frequency than male children. There was no appreciable incidence in any specific age group. The abrupt start of the illness and congestion of the tonsils, oropharynx, and posterior pharyngeal wall were the most prevalent clinical characteristics linked to GAS pharyngitis. Each and every one of the positive isolates had β-hemolytic colonies on sheep blood agar with Gram-positive cocci grouped in short chains on Gram stain, were susceptible to bacitracin, provided a positive PYR test result, and displayed agglutination with Group A latex reagent. Low susceptibility to ampicillin, ceftriaxone, cefipime, erythromycin, and azithromycin was indicated by the antibiotic susceptibility profile. All of the isolates shown sensitivity to vancomycin.

**Conclusion**: Thus, it is necessary to conduct periodical investigations to determine the incidence of GAS pharyngitis and to track changes in the susceptibility of GAS to antibiotics.

**Keyword:** Epidemiology, pharyngitis, Group A β-hemolyticstreptococcus, Vancomycin.

## INTRODUCTION

One of the most typical clinical manifestations is pharyngitis. [1] Sore throat is the primary symptom of pharyngitis patients who visit the outpatient clinic. [2]] The most prevalent pathogens that cause pharyngitis are viruses, followed by bacteria, candida, and viruses. Group A Beta Streptococcus being the most frequent bacterial cause of pharyngitis, accounting for 15% to 30% of cases, is streptococcus (GAS) infection. While streptococcal pharyngitis is a benign condition, it can lead to immune-mediated consequences such post-streptococcal glomerulonephritis, acute rheumatic fever (ARF), rheumatic heart disease (RHD), or suppurative tonsillo-pharyngeal problems. Rheumatic fever (RF) and streptococcal infection are closely related, and RF and its sequelae, RHD, can be avoided by promptly treating streptococcal infections with penicillin. [3]

Penicillin therapy for streptococcal sore throat is necessary for the primary prevention of Rheumatic Fever. Antibiotic use for all sore throats will remain debatable as there is upto 20% asymptomatic carrier prevalence for GAS. Despite the fact that GAS are always sensitive to penicillin, treating GAS pharyngitis and other respiratory infections with macrolides, particularly azithromycin for greater patient compliance, has grown to be a common practise. Since 1950s, macrolide resistance (MR) in GAS has been documented. The frequency of GAS that is resistant to macrolides has grown over time. Increased macrolide use is correlated with an increase in resistance rates.Macrolide resistance (MR) rates have varied from 1.1 to 98% globally, showing that surveillance data are crucial for guiding therapeutic decisions about the management of Streptococcal pharyngitis in a specific community. [4]

It's crucial to diagnose GAS pharyngitis to prevent RF and RHD. Treatment of GAS pharyngitis in children in ages of 5 to 12 years is crucial for preventing RF and RHD as RF and GAS pharyngitis are more common in this age group. Five to fifty percent of the cardiac patients in Indian hospitals had RHD. Several investigations have been conducted to determine the prevalence of GAS pharyngitis, GAS pharyngeal carriage, and GAS antibiotic susceptibility. The majority of these studies were conducted abroad. Recent research on the prevalence of GAS pharyngitis in India is not readily available.Moreover, there are no studies available that examined the susceptibility of GAS to antibiotics.[4]

As a result, there is no information available on MR of GAS in India. This suggests the need for research to be done in India to determine the disease's current prevalence and the pattern of antibiotic susceptibility, especially for macrolides. This will aid medical professionals in selecting the appropriate antibiotic and timing for treating GAS pharyngitis cases. In turn, this will achieve two objectives, namely the prevention of RF and RHD as well as the avoidance of the establishment of antibiotic-resistant GAS strains. Children who presented with pharyngitis in the out-patient department were included in the study because of the high frequency among youngsters.There is no information about MR of GAS in India as a result. In order to ascertain the disease's current prevalence and the pattern of antibiotic susceptibility, particularly for macrolides, study is recommended to be conducted in India. This will help the doctors in deciding the best antibiotic to use and when to provide it while treating GAS pharyngitis cases. In turn, this will accomplish two goals: stopping RF and RHD as well as preventing the spread of antibiotic-resistant GAS strains. Since pharyngitis affects children so often, children who came with illness in the out-patient department were included in the research.

## MATERIALS AND METHODS

The current study was a cross-sectional investigation conducted on 200 children between the ages of 5 to12 years who visited the outpatient clinic at the Niloufer Hospital in Lakdikapul, Hyderabad. The study was conducted from 16th june 2022 to17th june 2023 during a 1-year period.

**Sample size:**

The sample size was estimated using the following calculation, using 13.5% as the local prevalence of streptococcal pharyngitis:

$n=4pq\backslash L^2$

**Inclusion criteria:**

Only after receiving parental agreement children in the age group of 5 to 12 years who complained of a sore throat and/or had clear symptoms of pharyngitis and tonsillopharyngitis included in the research.

**Exclusion criteria:**

Adults, who are already taking antibiotics, those who had aphthous ulcers in the pharynx, oral candidiasis, or medication responses, as well as children whose parents didn't give consent were excluded from the research.

**Sample collection:** Two throat swabs were collected from each child. Two swabs: one for culture and the other for direct inspection under a microscope. The children were asked to sit upright and in a relaxed position for the sample collection. The child was urged to open his mouth. The child's mouth was illuminated by a strong light. He or she was instructed to take a big breath in, lean their heads back, and then open their mouths widely and phonate a "ah." The tonsillar fossae and posterior pharynx were seen by gently depressing the tongue with a tongue depressor.A sterile cotton swab (HIMEDIA Sterile Cotton Swab w/ Wooden Stick - PW005, HiMedia Laboratories Pvt. Ltd) was used to gently sweep the uvula, tonsillar pillars, and posterior throat. If present, any purulent discharge was also swabbed. The essential precautions were made to prevent the swab from coming into touch with the tongue or the lateral walls of the buccal cavity.

**Sample processing:**

A clean glass slide was immediately covered with a throat swab intended for quick microscopic inspection. Heat fixed, let to dry, and then stained using the Gram staining technique (Annexure-1). Then, using a compound microscope, this was seen.

A throat swab used for culture was promptly inoculated on 5% sheep blood agar (Annexure-2) for the main culture and crystal violet blood agar for the selective culture (Annexure-3). The streak-stab technique was used after the swab was rotated on the plates to create the well for inoculation (Annexure-4).A 0.04 units bacitracin disc (Biogram B Bacitracin 0.04 units, Microexpress®, A division of Tulip Diagnostics (P) Ltd.) was placed on the primary streaking to screen for the presence of Streptococcus pyogenes. During 18 to 24 hours, the

plates were incubated at 37°C with 5–10% CO2. To provide CO2, plates were stored in a candle jar.

After 18 to 24 hours of incubation, plates were read. Colonies exhibiting β -hemolysis were noted on the plates, and following Gram staining, a smear of these colonies was examined under a microscope. Streptococcus pyogenes was thought to be the source of smears that had chains of Gram-positive cocci. To establish the presence of Group A -βhemolytic Streptococcus, these colonies underwent bacitracin sensitivity testing, the PYR (pyrrolidonyl—naphthylamide) test, and Lancefield grouping.

**Bacitracin sensitivity test**

A sheep blood agar plate has three to four isolated colonies of the β-heamolytic Streptococcus streaking through the middle of it. The inoculum was applied on the plate as a lawn using a sterile swab. A 0.04 units bacitracin disc was applied aseptically to the infected region (performance check using Streptococcus pyogenes ATCC strain 12384). The disc was gently tapped down into the agar surface using flamed forceps. For 18 to 24 hours, the plate was incubated at 37 °C with 5- 10% CO2. The zone of inhibition was measured following incubation. Any area surrounding the disc was thought to be bacitracin sensitive. Growth that reached the disc's edge was regarded as bacitracin-resistant.

**PYR test**
Streptococcus pyogenes ATCC strain 12384, a positive control, and isolated colonies of the β-heamolytic Streptococci were inoculated on PYR agar and incubated at 37°C for 18 to 24 hours. The colonies developed on PYR agar were given a single drop of PYR reagent (HIMEDIA PYR reagent - R043, HiMedia Laboratories Pvt. Ltd).The colonies were checked for any colour changes after two minutes. Colonies that became red were seemed to be PYR positive. Using a latex agglutination kit, grouping of streptococcal bacteria ProlexTM Streptococcal Grouping Latex Kit (ProLab Diagnostics, product code: PL.030) was used to perform streptococcal grouping.

**Procedure:**
All components of the kit were brought to room temperature prior to use.

1. After gently inverting the dropper container several times, the test latex reagents were re-suspended. Before usage, the latex particles in the dropper bottles were checked to make sure they were correctly suspended.

2. The isolate to be tested was labelled on a test tube.

3. The tube received a drop of Extraction Reagent 1.

4. Using a loop, one to four beta-haemolytic colonies were chosen and suspended in Extraction Reagent 1. For testing for tiny colonies, a number of carefully isolated colonies were chosen so that the Extraction Reagent 1 solution become turbid.

5. The tube was then filled with one drop of Extraction Reagent 2.

6. For five to ten seconds, the tube was lightly tapped to mix the response.

7. Then, 5 drops of Extraction Reagent 3 were added, and the tube was gently tapped with a finger for 5 to 10 seconds to combine the ingredients.

8. In separate circles on the test card labelled for the isolate being tested, one drop of each group's latex reagent was applied.

9. One drop of extract was added next to one drop of latex reagent for each test using a Pasteur pipette.

10. Using the whole area of the circles, the latex and extract were combined using the supplied sticks.

6. The tube was softly tapped to mix the reaction for five to ten seconds.

7. To mix the contents, 5 drops of Extraction Reagent 3 were added, and the tube was gently tapped for 5 to 10 seconds with a finger.

8. One drop of the latex reagent from each group was placed in distinct circles on the test card that was labelled for the isolate being examined.

9. For each test, a Pasteur pipette was used to add one drop of extract and one drop of latex reagent.

10. With the provided sticks, the latex and extract were mixed utilising the whole area of the circles.

A 9 cm petri dish was used for the Kirby-Bauer disc diffusion method of antibiotic susceptibility testing (AST). The CLSI M02 (Annexure-7) direct colony suspension technique was used to create a 0.5 McFarland bacterial suspension, which was then implanted as a lawn culture onto MHA with 5% sheep blood. Ten different antibiotics were selected for AST. These five antibiotic discs were placed on two plates, and the plates were incubated at 37°C for the duration of the night or for 24 hours with 5% or 10% CO2. The CLSI recommendations were followed while reading and interpreting zone diameters.

## RESULTS

**Table-1: Demographic details in present study**

| Age (Mean) | Number (Out Of 200) | Percentage |
|---|---|---|
| 5 years | 60 | 30% |
| 6 years | 26 | 13% |
| 7 years | 27 | 13.5% |
| 8 years | 24 | 12% |
| 9 years | 21 | 10.5% |
| 10 years | 19 | 9.5% |
| 11 years | 14 | 7% |
| 12 years | 9 | 4.5% |
| Total | 150 | 100% |

| **Gender** | | |
|---|---|---|
| Male child | 119 | 59% |
| Female child | 81 | 41% |

Most of the cases fell within the five-year age range on average, and most of the patients were male child.

**Table-2: Clinical presentation in the study population**

| **Feature** | **Number** | **Percentage** |
|---|---|---|
| Sore throat | 130 | 65% |
| Fever | 111 | 55.5% |
| Cold | 181 | 90.5% |
| Cough | 166 | 83% |
| Malaise | 14 | 7% |
| Others | 21 | 10.5% |
| Congestion of oropharynx | 198 | 99% |
| Congested tonsils | 195 | 97.5% |
| Enlarges tonsils | 89 | 44.5% |
| Exudate over tonsils | 9 | 4.5% |
| Membrane over tonsils | Nil | 0% |
| Congested posterior pharyngeal wall | 165 | 82.5% |
| Enlarged cervical lymph nodes | 8 | 4% |

Tonsil and oropharyngeal congestion affected nearly all of the patients (99% and 97.5%, respectively). The majority reported having a cold (90.5%), a cough (83%), and posterior pharyngeal wall congestion (82.5%).

**Table-3: Identification of GAS by direct smear, culture and bacitracin sensitivity**

| **Mean Age** | **Smear positive** | **Culture positive** | **Bacitracin sensitive** | **PYR positive** |
|---|---|---|---|---|
| 5 years | 6 | 10 | 1 | 1 |
| 6 years | 8 | 3 | 2 | 2 |
| 7 years | 6 | 5 | 1 | 1 |
| 8 years | 4 | 9 | 4 | 3 |
| 9 years | 1 | 3 | 2 | 1 |
| 10 years | 5 | 4 | 1 | 1 |
| 11 years | 3 | Nil | Nil | Nil |
| 12 years | 2 | 1 | 1 | Nil |
| Total | 35 | 35 | 12 | 9 |

**Table-4: Zone diameters with 0.04 units bacitracin disc**

| Zone diameter | Number |
|---|---|
| 10mm | 5 |

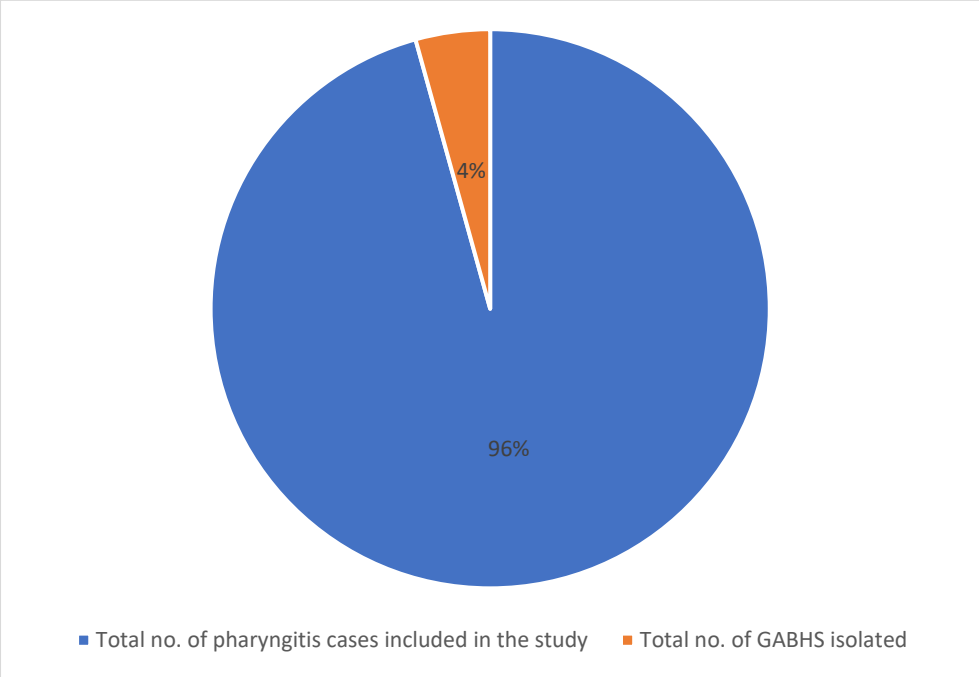
| | |
|---|---|
| 11mm | 3 |
| 12mm | 3 |
| 15mm | 1 |
| Total | 12 |

Prevalence = total no. of GABHS isolated

----------X100
total no. of pharyngitis cases included

Prevalence = 9/200 X 100 = 4.5%

**Figure-1: Prevalence of Group A beta-hemolytic streptococcus**




The following table lists the positive isolation of GABHS from pharyngitis patients across various age groups and genders:

**Table -5: Age and gender distribution in GAS positive cases**

| Age | Total cases | GAS positive |
|---|---|---|
| 5 years | 60 | 1 |
| 6 years | 26 | 2 |
| 7 years | 27 | 1 |
| 8 years | 24 | 3 |
| 9 years | 21 | 1 |
| 10 years | 19 | 1 |
| 11 years | 14 | Nil |
| 12 years | 9 | Nil |

| | | |
|---|---|---|
| Total | 150 | 9 |
| **Gender** | | |
| Male child | 119 | 3 |
| Female child | 81 | 6 |

Just one of the nine instances had a history of a gradual beginning of disease; the other eight all had abrupt onsets. It was noted that none of the symptoms had a greater prevalence when they appeared as the initial sign of GAS pharyngitis.

In the current investigation, the following symptoms were most often seen in GAS pharyngitis cases:

**Table-6: Prevalence of various symptoms in GAS positive pharyngitis cases**

| **Symptom** | **Number** |
|---|---|
| Sore throat | 4/9 |
| Fever | 5/9 |
| Cold | 8/9 |
| Cough | 7/9 |
| Malaise | 2/9 |
| Others | None |

Just 2 of the 9 instances in the current investigation had a history that was similar. In the past, antibiotics were only used to treat comparable problems in 2 cases. Congestion of the oropharynx and tonsils was observed in every instance of pharyngitis that tested positive for GAS in our investigation. Eight of the nine had congestion of the posterior pharyngeal wall, and six of the nine had enlarged tonsils. None of the 9 instances exhibited swollen lymph nodes or any exudate or membrane covering the tonsils.

Gram positive cocci were seen in six out of nine instances of GAS positive pharyngitis and were organised in short chains. All nine isolates displayed agglutination with Group A latex reagent, were PYR positive, and were sensitive to 0.04 units of bacitracin. One isolate displayed agglutination with Group C latex reagent, was PYR negative, and was sensitive to 0.04 units of Bacitracin. among the 9 isolates, the incidence of various bacitracin-sensitive zones.

**Table-7: Bacitracin sensitive zone sizes in GAS isolates**

| Bacitracin sensitive zone diameter | Prevalence |
|---|---|
| 15 mm | 1/9 |
| 12 mm | 1/9 |

| | |
|---|---|
| 11 mm | 2/9 |
| 10 mm | 5/9 |

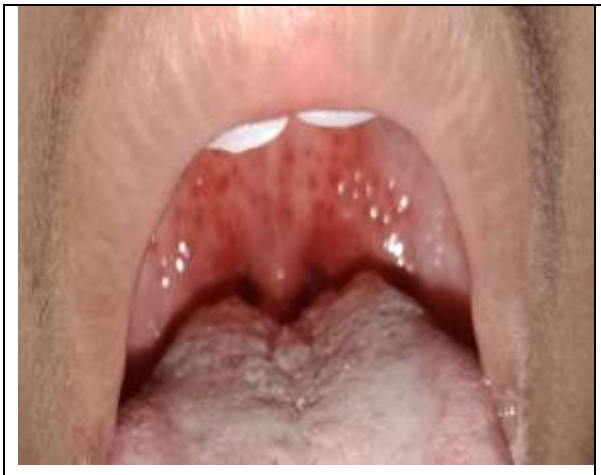
Following is the disc diffusion method's determination of the 9 isolated GAS's antibiotic susceptibility profile:

**Table-8: Antibiotic susceptibility of GAS isolates**

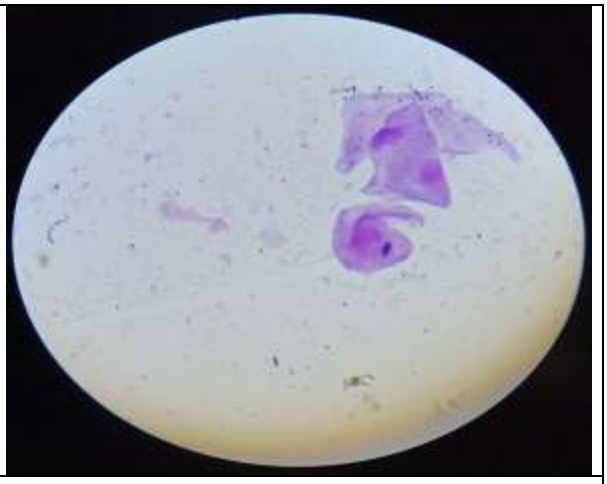
| Antibiotic | Sensitive (out of 9) | Intermediate (out of 9) | Resistant (out of 9) |
|---|---|---|---|
| Ampicillin (2µg) * | 4 | | |
| Cefotaxime (30µg) * | 7 | | |
| Ceftriaxone (30µg) * | 4 | | |
| Cefipime (30µg) * | 4 | | |
| Vancomycin (5µg) | 9 | | |
| Erythromycin (15µg) | 3 | 3 | 3 |
| Azithromycin (15µg) | 5 | 2 | 2 |
| Levofloxacin (5µg) | 8 | 0 | 1 |
| Chloramphenicol (30µg) | 7 | 2 | 0 |
| Clindamycin (2µg) | 8 | 0 | 1 |

(* Interpretation zones are not provided for intermediate susceptibility and resistance for these antibiotics in the CLSI)

**Figure-2: Images in present study**



Clinical picture of sore throat

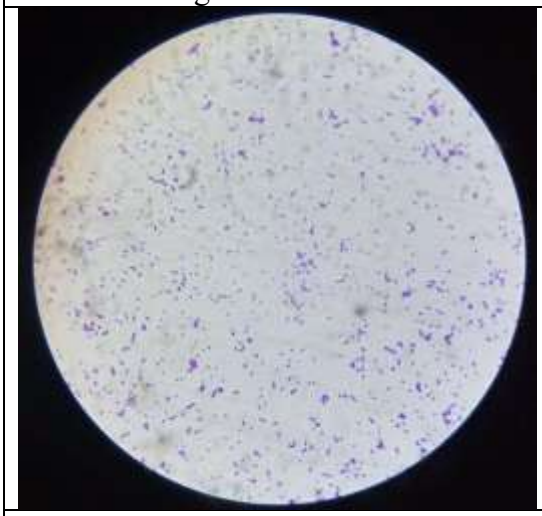


Direct smear showing epithelial cells and Gram positive cocci arranged in short chains

Primary inoculation of sample on sheep blood agar showing beta-hemolytic colonies

Primary inoculation of sample on crystal violet blood agar

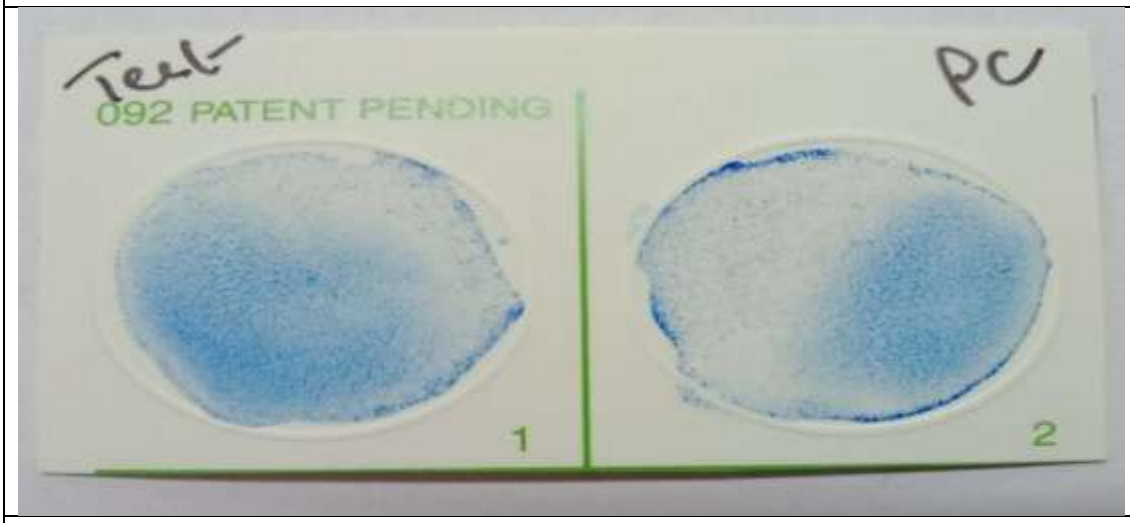
Subculture of beta-hemolytic isolate on sheep blood agar



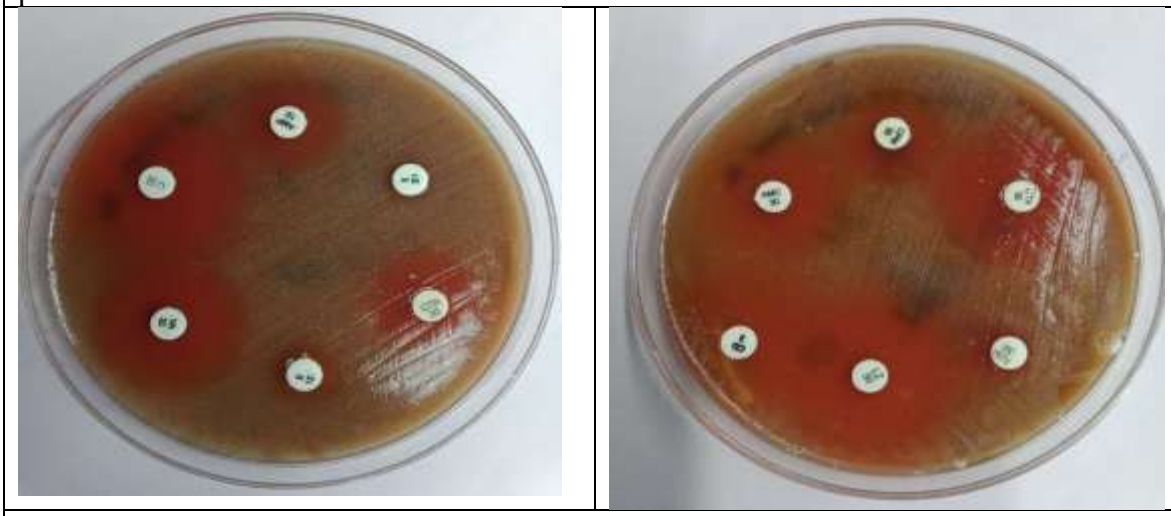
Gram stain of beta-hemolytic colony showing Gram positive cocci arranged in short chains

PYR test positive for a beta-hemolytic, bacitracin sensitive isolate, compared with a positive control

Bacitracin sensitivity performed for a beta-hemolytic isolate showing sensitivity to 0.04 units of bacitracin disc




Test isolate showing agglutination with Group A latex reagent, compared with a polyvalent positive control



Antibiotic susceptibility test on MHA with 5% sheep blood showing resistance to erythromycin and azithromycin

**DISCUSSION**

The bulk (i.e. 79% of the 200 kids) belonged to the 5 to 9 year old age range. The majority of people in all age categories were children, with a mean age of 5 years. The majority of patients were men.

The following are the rates of GAS pharyngitis found in several Indian studies:

**Table-9: Comparison of prevalence in different Indian studies**

| Study conducted by | Place of study | Age group studied | Prevalence |
|---|---|---|---|
| Sobhan Nandi et al. [5] | Periurban slum area of Chandigarh, North India | 5-15 years | 13.5% |
| Similar study done in same hospital [6] | Niloufer Hospital, Hyderabad | 5-15 years | 12% |
| Present study | Niloufer Hospital, Hyderabad | 5-12 years | 4.5% |

The frequency was found to be 23% in the Paul Little et al.[1] study done in 2012 in South and Central England. The study's sample size was substantial (606 individuals), consisting of both adults and children who were at least 5 years old.

A meta-analysis conducted by Jane Oliver et al. was published in 2018[7]. The results of this study were divided into five categories based on the clinical outcome measured, participant recruitment method (active or passive recruitment), country income level (OECD member country or non-OECD member country), age group, and country. Whenever serologically verified prevalence studies were presented, regardless of whether or not clear case confirmation had taken place.

This study found that children aged between 5 to 19 years frequently had pharyngitis with a GAS culture positive result. India is a participant in the OECD. Studies from the OECD that exclusively used passive recruiting revealed a substantially lower frequency. Patients were passively enrolled in the current trial as well. Only patients who presented to the hospital were included, then. The submerged portion of the iceberg were those who had pharyngitis but did not go to the hospital.

There might be a number of explanations for the decreased prevalence in the current research. Although it is discovered that the epidemiology of the causal agent is evolving, there isn't sufficient proof for this due to the paucity of current investigations. [8]The majority of studies used active monitoring for cases to calculate the incidence and prevalence of GAS pharyngitis, and they also included adults in their research population, which may have resulted in greater prevalence in those studies. [9] Before their presentation, patients could have taken antibiotics, but they might not have remembered to disclose it.This may also be

the cause of the lower prevalence. The current study's sample collection encountered some issues. The majority of patients' parents did not provide permission for their child's samples to be taken. Parents were still adhering to COVID taboos because the research period fell throughout the COVID and post-COVID phases. Despite explanations, they mistook the collection of throat swabs for a COVID test. Even explaining, comforting, and preparing kids for sample collection was challenging. Due to these cases' lack of involvement in the study, probable cases of GAS pharyngitis may have gone unreported, contributing to the study's low prevalence.According to the study by Thomas Z. Thompson et al.[3], sensitivities for the culture of throat swabs for GAS have been observed to range from 72 to 87% in real-world trials. Due to the limited sensitivity of culture, which was utilised in the current study's approach to identify GAS, the prevalence may have been lower. While majority of the patients in the current research were male chidren, GAS pharyngitis was more common in female children. These and other research' correlations are as follows:

**Table-10: Gender distribution in GAS positive cases in different studies**

| Study conducted by | Finding |
|---|---|
| Sobhan Nandi et al.[5] (2001) | no significant difference in the incidence of GAS sore throat was observed between males and females |
| a similar study done in this hospital (2008) | relatively higher prevalence of GAS pharyngitis among females |
| Shirin Sayyahfar et al. [4] (2015) | prevalence was relatively higher among males |
| Gregory P. DeMuri et al. [10] (2017) | prevalence was relatively higher among males |
| Present study | relatively higher prevalence among females |

Although the higher incidence or prevalence in any one gender was quite slight across all studies, its relevance is not great. In the current study, the mean age group of 5 years had the highest number of pharyngitis cases, although there was no clear-cut majority of GAS pharyngitis in any one age range.

The following are the greater prevalences in age distribution that various studies have noted:

**Table-11: Peak prevalence of GAS positive cases according to age in different studies**

| **Study conducted by** | **Year of study** | **Age group with highest prevalence** |
|---|---|---|
| Sobhan Nandi et al. [5] | Apr 1995 – Mar 1996 | 11 years |
| a similar study done in this hospital | Oct 2006 – Sep 2007 | 5-10 years |
| Study by Paul Little et al. [1] | 2012 | age <10 years |
| Shirin Sayyahfar et al. [4] | 2011 – 2013 | 3-5 years |
| Jane Oliver et al. (2018) [7] | 2017 | Children (<20 years old) |

According to the information above, each research had a different greatest frequency of GAS pharyngitis for a certain age. This discrepancy may result from differences in the economies, infrastructure for women's and children's health, housing circumstances, etc. for the nations where these studies were conducted.

Virtually all of the pharyngitis patients also had tonsillar and oropharyngeal congestion. The majority of people complained of a cold and a cough, as well as posterior pharyngeal wall congestion.The oropharynx, tonsils, and posterior pharyngeal wall congestion were the most prevalent clinical characteristics in patients with GAS positive pharyngitis. Most of them had a cold, a cough, and swollen tonsils. Except for one instance, in whom there is a history of abrupt onset of GAS-positive pharyngitis.

The following are the many clinical characteristics frequently seen in cases of GAS pharyngitis in various studies:

**Table-12: Common clinical findings of GAS pharyngitis in different studies**

| Study conducted by | Common clinical features |
|---|---|
| Paul Little et al.[1] (2012) | short duration of illness (≤3 days)<br>anterior cervical glands<br>age <10 years<br>have a moderately bad or worse sore throat fever during last 24 hours |
| Shirin Sayyahfar et al.[4](2015) | Fever<br>sore throat pharyngeal erythema |
| Gregory P. DeMuri et al. [10](2017) | sore throat<br>fever<br>pharyngeal erythema |
| Alyssa De Wyer BS et al.[9] (2020) | objective fever<br>cough<br>throat pain lasting all day<br>petechiae on the palate<br>tonsillar exudates and tonsillar swelling |
| Present study | acute onset of illness<br>congestion of oropharynx, tonsils and posterior pharyngeal wall.<br>Majority presented with cold, cough and enlarged tonsils. |

This study's clinical result that pharyngeal erythema is a frequent presentation is consistent with research by Gregory P. DeMuri et al. and Shirin Sayyahfar et al. [4,19] Clinical evidence of a disease's acute start was consistent with Paul Little's study and others' findings. [1] The inconsistent clinical results among studies may be the result of poor history-taking. The symptoms of GAS infection overlap with the symptoms of other infectious causes of pharyngitis, which may explain why the most common clinical characteristic varied between studies. Hence, to determine GAS as the pathogen, a laboratory test should be carried out unless there are clear viral clinical characteristics present. [2]

In the current investigation, 17.5% of the direct smears included short chains of Gram-positive cocci (GPC). On Gram stain, 17.5% of the cultures exhibited β -hemolytic colonies that were generally organised in short chains. Six percent of the samples shown bacitracin sensitivity. Just 9 of the 12 bacitracin-sensitive isolates produced a positive PYR result. Streptococcus pyogenes ATCC strain 12384 served as the positive control for all PYR

assays. According to the study by Thom β as Z. Thompson et al., which cites real-world investigations, throat swab cultures for GAS have been demonstrated to have sensitivities that vary from 72 to 87%. [3]Group A streptococci are sensitive to bacitracin at relatively low doses, although other β -hemolytic streptococci can also exhibit variable bacitracin sensitivity. Even a lot of α-hemolytic streptococci are vulnerable to bacitracin at lower dosages. The PYR test is a widely used assay for the presumptive identification of both enterococci and group A β -hemolytic streptococci. [11] As a result, none of the isolates in this investigation that shown bacitracin sensitivity responded positively to the PYR test.The study's bacitracin sensitivity zones had a diameter of 10 to 15 mm. This research and one that was conducted at the same hospital in 2008 are related. In the current investigation, zones with a diameter of 10 mm were more frequently seen.

The following table shows how several antibiotic susceptibilities and other research correlate:

**Table-13: Comparison of antibiotic susceptibility of GAS isolates with different studies**

| Study | AMP/P | CTX | CTR | CPM | VA | E | AZM | LE/OF | C | CD |
|---|---|---|---|---|---|---|---|---|---|---|
| Similar study in same hospital (2008) | P-100% AMP- 100% | | | | | 95.83% | | | | |
| Study by Shirin Sayyahfar et al.[4] (2015) | P-100% | | 49% * | | | 49.1% | 44% | OF-59.3% | | 84.7% |
| Study by Gregory P. DeMuri et al.[10] (2017) | | | | | | 85% | | OF-95.6% | | 85% |
| Study by Alyssa De Wyer BS et al.[9] (2020) | P-100% | | | | 98.5% | 88.2% | | | 88.2% | |
| Present study | AMP-4/9 | 7/9 | 4/9 | 4/9 | 9/9 | 3/9 | 5/9 | LE- 8/9 | 7/9 | 8/9 |

* Antibiotic susceptibility testing done by broth dilution

The sensitivity of ceftriaxone (CTR), erythromycin (E), and azithromycin (AZM) in the current study roughly coincide with the sensitivities in the study of Shirin Sayyahfar et al., as can be seen from the above table. [4 ] Vancomycin (VA) sensitivity is highly correlated with the sensitivity found in the Alyssa De Wyer BS et al. investigation. [9] Clindamycin's sensitivity is correlated with the sensitivity levels seen in investigations by Gregory P. DeMuri et al. and Shirin Sayyahfar et al. [10]

The GAS isolates in the current investigation have limited sensitivity to ampicillin, ceftriaxone, cefipime, erythromycin, and azithromycin. Vancomycin is 100% sensitive. The conventional treatment for GAS pharyngitis is 10 days of oral penicillin V or a single intramuscular injection of benzathine penicillin G, according to the review study by Arif M. Al-Hamad[8]. Amoxicillin is frequently used to improve patient adherence. Nevertheless, when taken in the context of glandular fever, ampicillin-based antibiotics, including co-amoxiclav, may result in a rash. A first-generation cephalosporin is advised for penicillin allergies that are not life-threatening.Other treatments include macrolide or azalide antibiotics, such as erythromycin, clarithromycin, and azithromycin, or maybe clindamycin, for people with severe penicillin allergies. Except for azithromycin, for which a 3-5-day treatment course is advised because to its lengthy half-life, other oral treatment sessions that are advised last for 10 days. Azithromycin may be prescribed by doctors for individuals who don't clearly have a contraindication to penicillin or cephalosporin due to its short duration

and once-daily dosage. Regrettably, azithromycin's effectiveness in treating streptococcal pharyngitis has been severely restricted by the rise in macrolide-resistant GAS.Increased macrolide use is correlated with an increase in resistance rates. The susceptibility to macrolides is low in the current study, conducted in the city of Hyderabad, and in the study of Shirin Sayyahfar et al.[4], conducted in Iran, as a result of the increased use of macrolides for instances of pharyngitis in both locations. According to Arif M. Al-study,[8] Hamad's MR resistance is on the rise, rising from 4.5% between 2006 and 2009 to 12% between 2010 and 2014 before reaching 23.4% in 2014.

The propagation of dominant resistance clones, horizontal gene transfer, the excessive use of macrolide antibiotics, and temporal changes in the distribution of types have all been linked to variations in MR (macrolide resistance) rates. MR in GAS has been documented since the 1950s, despite the fact that beta-lactam antibiotics are uniformly responsive to all GAS. In GAS, resistance to macrolides develops through two different mechanisms:

(i) active drug efflux via a transmembrane pump encoded by mef genes

1. (ii) ribosomal modification by Erm methylase
2. The latter confers cross-resistance to streptogramins, macrolides, and lincosamides (MLSB phenotype). In the 1970s, clinically significant MR was extensively documented in a number of nations, and this was associated with a sharp rise in macrolide use.

The current study also revealed decreased sensitivity to beta-lactams and cephalosporins in addition to macrolides. This might be a result of the excessive beta-lactam and cephalosporin use in the city. Just 59.3% of participants in the Shirin Sayyahfar et al. trial were ofloxacin susceptible. Levofloxacin, an antibiotic from the same class of fluoroquinolones as ofloxacin, had a susceptibility of 8/9 in the current investigation. Just 51.5% of GAS isolates were found to be tetracycline susceptible, according to a study by Alyssa De Wyer BS et al. [9]The treatment of streptococcal pharyngitis is rendered ineffectual due to decreased sensitivity to such a broad spectrum of medicines, which simply increases the organism's antibiotic resistance. Given that the main purpose of treating GAS pharyngitis is to avoid rheumatic fever, many children will be at risk for this nonsuppurative consequence of GAS infection.

The current investigation employed culture of throat swabs, which has a poorer sensitivity, to find instances with GAS pharyngitis. Studies have used a rapid antigen detection test (RADT) for Group A Streptococcus to identify GAS from pharyngitis patients. The use of RADT in conjunction with culture may have improved the sensitivity of GAS detection.With the Kirby-Bauer disc diffusion technique, tests for antibiotic susceptibility were conducted. The CLSI recommendations do not include intermediate susceptibility and resistance zones for several antibiotics when using this technique of AST. Such antibiotics required broth dilution, which was not carried out in our investigation. Just 9 GAS isolates were found to be positive in the current investigation. Thus, it is impossible to generalise the AST patterns of these 9 isolates. In order to isolate a high number of GAS, bigger sample sizes are required for investigations.

This study emphasises the prevalence of antibiotic resistance in GAS.

Regular sensitivity testing will enable you to comprehend the shifting patterns of susceptibility and, in turn, develop an empirical therapy strategy for GAS pharyngitis.Lower susceptibility drugs shouldn't be utilised since higher usage rates are closely related to higher resistance rates. Also, it's wise to take alternative medications to avoid the emergence of resistance to them. Hence, ongoing research on the frequency and antibiotic susceptibility profile of GAS is crucial for making an accurate diagnosis, choosing the right course of action for treating GAS pharyngitis, and avoiding the emergence of post-streptococcal sequelae. In order to boost the sensitivity of culture for GAS identification, such investigations should also include assays like RADT. Antibiotics that were not examined in this study but are typically prescribed should also have their susceptibility evaluated. To determine each antibiotic's sensitivity, intermediate susceptibility, and resistance, several AST techniques should be used.

The genes producing drug resistance in these organisms can be found utilising PCR analyses on GAS isolates. Understanding the changes in frequency, clinical presentation, and antibiotic susceptibility patterns between studies will also be aided by gene typing and gene sequencing of GAS isolates.

## CONCLUSION

In the current investigation, GAS pharyngitis is low in children due to possible reasons like: previous use of antibiotics, the absence of current research demonstrating prevalence, the challenges in sample collection, and the limited sensitivity of culture in diagnosing GAS from throat swabs. The current study revealed diminishing susceptibilities in GAS to several antibiotics often recommended by doctors; as a result, it should serve as a warning to doctors before writing an antibiotic prescription.Before beginning antibiotic therapy, the physicians must confirm the diagnosis of GAS pharyngitis either by culture or utilising a RADT and conduct a sensitivity test. The selection of an empirical antibiotic in a region will be aided by ongoing antibiotic surveillance. Only confirmed cases should be treated with suitable and effective medications because GAS is displaying resistance to medications that are often provided, preventing future development of drug resistance.

## REFERENCES

1. Little P, Hobbs FD, Mant D, McNulty CA, Mullee M; PRISM investigators. Incidence and clinical variables associated with streptococcal throat infections: a prospective diagnostic cohort study. Br J Gen Pract. 2012 Nov;62(604):e787-94.
2. Adler L, Parizade M, Koren G, Yehoshua I. Oral cavity swabbing for diagnosis of group a Streptococcus: a prospective study. BMC Fam Pract. 2020 Mar 26;21(1):57.
3. Thompson TZ, McMullen AR. Group A Streptococcus Testing in Pediatrics: the Move to Point-of-Care Molecular Testing. J ClinMicrobiol. 2020 May 26;58(6):e01494-19.
4. Sayyahfar S, Fahimzad A, Naddaf A, Tavassoli S. Antibiotic Susceptibility Evaluation of Group A Streptococcus Isolated from Children with Pharyngitis: A Study from Iran. Infect Chemother. 2015 Dec;47(4):225-30.
5. Nandi S, Kumar R, Ray P, Vohra H, Ganguly NK. Group A streptococcal sore throat in a periurban population of northern India: a one-year prospective study. Bull World Health Organ. 2001;79(6):528-33.
6. Agarwal M, Raghuwanshi SK, Asati DP. Antibiotic Use in Sore Throat: Are We Judicious? Indian J Otolaryngol Head Neck Surg. 2015 Sep;67(3):267-70.

7. Oliver J, Baker MG, Pierse N, Carapetis J. Comparison of approaches to rheumatic fever surveillance across Organisation for Economic Co-operation and Development countries. J Paediatr Child Health. 2015 Nov;51(11):1071-7.
8. Al-Hamad AM. Streptococcal throat. Therapeutic options and macrolide resistance. Saudi Med J. 2015 Sep;36(9):1128-9.
9. DeWyer A, Scheel A, Webel AR, Longenecker CT, Kamarembo J, Aliku T, Engel ME, Bowen AC, Bwanga F, Hovis I, Chang A, Sarnacki R, Sable C, Dale JB, Carapetis J, Rwebembera J, Okello E, Beaton A. Prevalence of group A β- hemolytic streptococcal throat carriage and prospective pilot surveillance of streptococcal sore throat in Ugandan school children. Int J Infect Dis. 2020 Apr;93:245-251.
10. P DeMuri G, K Sterkel A, A Kubica P, N Duster M, D Reed K, R Wald E. Macrolide and clindamycin resistance in Group A streptococci isolated from children with pharyngitis. Pediatr Infect Dis J. 2017 Mar;36(3):342-44.
11. GWProcop,DLChurch,GSHall,WMJanda,EWKoneman, P C Schreckenberger, G L Woods. Koneman'scolor atlas and textbook of diagnostic microbiology. 7$^{th}$ed. Philadelphia: Wolters Kluwer; 2017.