ORIGINAL ARTICLE

Prevalence of group A streptococcal pharyngitis among schoolchildren of Barabanki district, Uttar Pradesh, India

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ABSTRACT

Background: The aim of this study was to determine the prevalence of Group A streptococcus (GAS) pharyngitis and its carriage among schoolchildren in the age group 5-15 years in Barabanki district, Uttar Pradesh, India. The study also aimed to determine the minimum inhibitory concentration (MIC) of Penicillin G, antimicrobial susceptibility pattern and inducible Clindamycin resistance in GAS. **Materials and Methods:** Three hundred schoolchildren from six different schools were included in the study. Identification of group A streptococcus was done on the basis of Bacitracin sensitivity test, pyrrolidonyl peptidase (PYRase) test and Lancefield grouping by latex agglutination test. Antibiotic susceptibility test and D-zone test were done in GAS isolates. **Results:** Out of the 300 schoolchildren, GAS was found in three (4.7%) out of 63 symptomatic children and two (0.8%) out of 237 asymptomatic children. The overall prevalence of GAS pharyngitis and of GAS carriage was 1% and 0.67%, respectively. The isolation of GAS was significantly higher in symptomatic cases when compared to asymptomatic cases (*P* value = 0.0308). All the strains were sensitive to Penicillin, Ampicillin, Quinupristin-Dalfopristin, Vancomycin and Linezolid. **Conclusion:** A low prevalence of GAS pharyngitis and GAS carriage was observed in the study, probably due to the cross-sectional nature of the study. All five GAS isolates were sensitive to Penicillin.

Key words: Carriage, children, GAS carriage, GAS pharyngitis, group A streptococcus (GAS), group A streptococcus pharyngitis, prevalence

INTRODUCTION

Group A streptococcus (GAS) is one of the most common and important pathogens causing community-associated infections.[1] GAS is a causative agent of a wide range of human infections, of which pharyngitis is very common. It accounts for approximately 15-30% of cases of pharyngitis among children aged 5-15 years in developing countries.[2] The estimated global burden of people suffering from serious GAS disease is around 18 million.[3] The prevalence of GAS pharyngitis and its carriage varies in the range of 9-34.1% in different countries.[4] The epidemiological data of disease due to GAS from developing countries are scarce. In India, prevalence of GAS pharyngitis and its carriage estimated previously varies: 4.2-13.7% and 11.2-34%, respectively.[2,5-8] GAS pharyngitis itself is not a major cause of concern as it gets resolved even without treatment within 2-5 days. The most important challenge to clinicians involves the sequelae of infection, such as acute rheumatic fever (ARF) and glomerulonephritis.

Recent studies have suggested that approximately 75% of streptococcal infections of the upper respiratory tract remain asymptomatic, which may progress to severe non-suppurative sequelae.[9] Although acute pharyngitis is one of the most frequent infections seen

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among school-aged children, GAS pharyngitis accounts for a very small percentage of such cases. The clinical presentations of GAS pharyngitis and non-streptococcal pharyngitis are similar, posing a challenge to the clinicians in differentiating them without laboratory support.[10] Thus, laboratory diagnosis of GAS pharyngitis is important for early diagnosis and proper treatment of the disease to prevent the development of complications and to prevent inappropriate administration of antibiotics used for non-streptococcal pharyngitis.

The need for active surveillance to detect the organism is borne out by the fact that the reservoir of GAS is in the pharynx of the carrier. In the present study, throat swabs were collected from schoolchildren aged 5-15 years from different schools of the district, for determining the prevalence of GAS pharyngitis and the carrier state in the community.

MATERIALS AND METHODS

Study design

The present study was an observational cross-sectional survey to determine the prevalence of GAS pharyngitis and GAS carriage among schoolchildren in the age group 5-15 years. The present study was conducted at the Department of Microbiology of the Mayo Institute of Medical Sciences, Barabanki, Uttar Pradesh, India during the period from April to October, 2014.

Study population

The study includes schoolchildren of the age group 5-15 years studying in various schools of Barabanki district, Uttar Pradesh.

Sample size

The sample size was 300 schoolchildren, calculated on the basis of the current population of approximately 5,10,000 school-going children in the age group 5-15 years in Barabanki district. Six schools were included in the study. A total of 50 school children were included from each of these six schools. Students were selected randomly from the class on the basis of the inclusion criteria of the study.

Selection criteria

The schoolchildren were included in the study according to the following criteria.

1. *Inclusion criteria*:
   a. All schoolchildren in the age group 5-15 years. Selection of the children was done randomly.
   b. Case definition:
      - Pharyngitis: Children having any symptoms/signs of pharyngitis on the day of examination or within the previous 2 weeks before examination.
      - GAS pharyngitis: Children having any of the symptoms/signs of pharyngitis and also GAS in cultures.
      - GAS carriers: Children having no symptoms/signs of pharyngitis on the day of examination or in the previous 15 days, but GAS in cultures.
2. *Exclusion criteria*:
   a. Oral antibiotic use in the past 3 days and intramuscular use in 28 days.
   b. Presence of another illness requiring antibiotics/hospitalisation.
   c. History of previous rheumatic fever or rheumatic heart disease (RHD).

Data collection

After enrolment of the children for the study on the basis of the inclusion and exclusion criteria, information was recorded on a preformed questionnaire, which included identification details, demographic variables, present medical history, physical examination and previous medical history.

Sample collection

Three throat (oropharyngeal) swabs were collected from each child. The procedure was explained, and consent and assent were obtained from the parents and the child, respectively. A sterile cotton swab in a screw-capped transport tube was used for collection of each throat swab. Using a tongue depressor, the tongue was depressed slightly to allow easier access to the pharynx. Swabbing was started with the lateral wall of pharynx and then proceeding to the posterior wall without touching the buccal mucosa or the tongue. The mucosa behind the uvula and tonsils was gently swabbed. The swab was removed carefully without touching the mucosa or tongue and re-inserted in the transport tube, labelled properly and transported to the laboratory for processing.

Sample processing

The processing of the sample was done in the following steps:

Inoculation of specimens

Out of three swabs, one was used for the preparation of Gram stain smear. The second swab was used for culture onto 5% sheep blood agar and crystal violet blood agar and incubated at 37°C for 48 h in 5-10% carbon dioxide. To enhance haemolysis, each inoculated culture plate was stabbed 3-4 times. The third swab was inoculated onto Robertson's cooked meat (RCM) broth. After 48 h of incubation, RCM broth was subcultured on 5% sheep blood agar and incubated as before.

Identification of GAS

Beta-haemolytic colonies were subjected to Gram staining and catalase test. Gram-positive cocci in chains which were catalase-negative were identified by their susceptibility to bacitracin (0.04 units) and trimethoprim-sulphamethoxazole (TMP-SMX) (1.25/23.75 μg). Pyrrolidonyl peptidase (PYRase) test using PYRA strip (Transasia, Mumbai, MH, India) was then done on the suspected isolates. The final identification of streptococcus was done by Lancefield grouping by latex agglutination test using HiStrept Latex Test kit (Himedia Labs Pvt Ltd., Mumbai, MH, India).

Antimicrobial susceptibility testing

This was performed by the Kirby–Bauer disc diffusion method using Mueller–Hinton agar (MHA) with 5% sheep blood. The antibiotic discs used were Penicillin (10 units), Ampicillin (10 μg), Clindamycin (2 μg), Erythromycin (15 μg), Vancomycin (30 μg), Linezolid (30 μg) and quinupristin-dalfopristin (15 μg). The D-zone test was done as per Clinical and Laboratory Standards Institute (CLSI) guidelines.[11]

Minimum inhibitory concentration (MIC) of Penicillin G

The MIC of Penicillin G was determined by the agar dilution method as per CLSI guidelines.[11] MHA supplemented with 5% sheep blood was used.

Statistical analysis

All data were collected and recorded in MS Office Excel Sheet. The chi-square test was applied to determine the association of isolation among different types of cases. *P* value <0.05 was considered as statistically significant.

Ethical considerations

Ethical clearance was obtained from the Institutional Ethical Committee for the study. The informed consent and assent forms were duly filled and signed by the guardian and the child, respectively.

RESULTS

Of the 300 school-aged children, only 63 had signs and symptoms of pharyngitis. Out of these 63 symptomatic children, beta-haemolytic streptococcus (BHS) was found in 14 (22.2%); of them, GAS was found in three (4.7%) children. Of 237 asymptomatic children, BHS and GAS were found in 11 (4.6%) and two (0.8%) children, respectively. The overall prevalence of GAS pharyngitis and of GAS carriage was 1% and 0.67%, respectively. The distribution of symptomatic and asymptomatic cases on the basis of sex is seen in Table 1. Table 2 shows that the isolation of GAS isolates was significantly higher from symptomatic cases as compared to asymptomatic cases (*P* value = 0.0308).

Table 3 shows the distribution of various BHS isolates among cases of GAS pharyngitis and GAS carriage. Group F streptococcus was most commonly isolated from both types of cases of GAS pharyngitis and GAS carriage. Among these 25 isolates, eight isolates were Bacitracin-sensitive and TMP-SMX resistant. Of these eight isolates, only five were GAS by Lancefield grouping. All five were PYRase test-positive.

The antibiotic susceptibility patterns of all the five GAS isolates are documented in Table 4. All the strains were sensitive to Penicillin, Ampicillin, quinupristin-dalfopristin, Vancomycin and Linezolid. The MIC of Penicillin for all five GAS isolates was <0.064 μg/mL. None of the isolates showed inducible Clindamycin resistance.

DISCUSSION

GAS is an important cause of pharyngitis among children aged 5-15 years. Although pharyngitis is self-limited and resolves within 1 week after its onset, the main cause of

Table 1: Sex wise distribution of the cases

Sex	Number (%)	Symptomatic cases			Asymptomatic cases		
		Number (%)	BHS (%)	GAS (%)	Number (%)	BHS (%)	GAS (%)
Boy	157 (52.3)	34 (21.6)	8 (23.5)	2 (5.8)	123 (78.3)	7 (5.7)	2 (1.6)
Girl	143 (47.7)	29 (20.1)	6 (20.7)	1 (3.4)	114 (79.7)	4 (3.5)	0 (0)
Total	300	63 (21)	14 (22.2)	3 (4.7)	237 (79)	11 (4.6)	2 (0.8)

Table 2: Association of GAS isolation from symptomatic cases

Cases	GAS isolated		Chi-square	*P* value
	Yes	No		
Symptomatic cases	3	60	4.6618	0.0308*
Asymptomatic cases	2	235		

*Significant; *P* value <0.05 is considered as significant

Table 3: Pattern of distribution of various beta-haemolytic Gram-positive cocci isolates

BHS	Isolates from cases of GAS pharyngitis (%)	Isolates from cases of GAS carriage (%)	Total (%)
Group A streptococcus	3 (21.4)	2 (18.2)	5 (20)
Group B streptococcus	1 (07.1)	1 (09.1)	2 (08)
Group C streptococcus	2 (14.3)	3 (27.3)	5 (20)
Group F streptococcus	6 (42.9)	4 (36.3)	10 (40)
Group G streptococcus	2 (14.3)	1 (09.1)	3 (12)
Total	14	11	25

Table 4. Antibiotic susceptibility pattern of GAS isolates

Antibiotics	Number of isolates sensitive to antibiotics	Percentage of sensitive isolates
Penicillin	5	100
Ampicillin	5	100
Erythromycin	4	80
Clindamycin	4	80
Quinupristin-dalfopristin	5	100
Vancomycin	5	100
Linezolid	5	100

concern are the post-infectious complications, mainly ARF.[12] Long-standing cases of ARF may develop into RHD, which is an important cause of cardiovascular morbidity and mortality in India.[13] Studies suggest that the majority of streptococcal infections of the upper respiratory tract remain asymptomatic until they progress to ARF and RHD.[9]

The present study has demonstrated an overall prevalence of GAS pharyngitis of 1% among 300 schoolchildren aged 5-15 years, with 4.7% prevalence of GAS pharyngitis among symptomatic cases. The isolation of GAS was significantly higher among symptomatic cases (*P* value = 0.0308). This finding is consistent with those from studies conducted by Menon *et al.*, Kumar *et al.*, Rajkumar *et al.* and Lloyd *et al.*, which showed a prevalence of 2.5%, 2.8%, 4.0% and 5.6% respectively.[6,7,9,14] Nandi *et al.*, Jain *et al.* and Kushwaha *et al.* demonstrated higher prevalence: 18.8%, 12.6% and 39.1%, respectively.[6,15,16] The prevalence of GAS isolates among asymptomatic cases was 0.8%, which is very low compared to the studies conducted by Muthusamy *et al.* (5.09%), Anbu *et al.* (5.2%), Menon *et al.* (5.8%), Lloyd *et al.* (8.4%), Gupta *et al.* (13.7%), Devi *et al.* (16.98%) and Kushwaha *et al.* (27.2%).[9,14,16-20] In a study conducted by Kumar *et al.* it was observed that during summer, the GAS carriage was only 0.47%, while during winter it increases to 2.12%.[6] The present study was conducted during the summer months, which may be a reason for the lower prevalence of GAS carriage in the study group.

In the present study, among 25 BHS isolates, two (8%), three (12%), five (20%) and 10 (40%) isolates were group B, G, C and F streptococcus, respectively. Similarly, a higher isolation of group F (53.7%) was observed in a study conducted by Devi *et al.*, with 3.77%, 5.66% and 19.81% group B, group C and group G, respectively.[20] Muthusamy *et al.* isolated six cases (27.7%) and one case (3.7%) of non-A non-B BHS and group B streptococcus, respectively.[17] Anbu *et al.* found one (4.5%), three (13.6%) and five (22.7%) cases of group B, C and G streptococcus among 22 BHS isolates, respectively.[18] The finding of the present study suggests that the majority of the children had normal commensal flora in their throat. However, a few members of the normal commensal flora have been found to be associated with primary atypical pneumonia and endocarditis.[21]

The antibiotic susceptibility pattern of the GAS isolates demonstrated 100% sensitivity to most of the antibiotics, such as Penicillin, Ampicillin, quinupristin-dalfopristin, Vancomycin and Linezolid, as seen in studies conducted by Devi *et al.* and Muthusamy *et al.*[17,20] In addition, in the present study the MIC of Penicillin, determined by the agar dilution method, had showed that the GAS isolates were sensitive to the lowest concentration of antibiotic used. This indicates that the first-line and reserved drugs for treatment of GAS pharyngitis are effective against GAS isolates and thus only early diagnosis of cases of GAS pharyngitis would be helpful for treatment of such cases to prevent serious complications of the disease. Although Erythromycin has been used as an alternative for patients allergic to Penicillin, a proportion of the isolates were found to be resistant in the present study as well in the studies conducted by Devi *et al.* and Muthusamy *et al.*[17,20] Brahmadathan *et al.* had demonstrated an increase in Erythromycin resistance among GAS isolates causing tonsillitis and pharyngitis.[22] This may be attributed to an increase in the use of macrolide antibiotics for treatment of upper respiratory tract infections. In the present study, one (20%) isolate was resistant to Clindamycin, but none of isolates showed inducible Clindamycin resistance as determined by the D-zone test. This indicates that the mechanism of Clindamycin resistance is only constitutive. Similar results were obtained in a study conducted by Megged *et al.*[23]

CONCLUSIONS

A low prevalence of GAS pharyngitis and GAS carriage was observed in the study, probably due to the cross-sectional nature of the study conducted during one season only. Many studies have found that the peak of GAS pharyngitis is mainly seen during winter and in early spring. GAS isolation from symptomatic cases was significantly higher as compared to asymptomatic cases. Thus, it may be advisable that surveillance of GAS pharyngitis are conducted for a period of 1 year to determine the exact incidence of the disease and to take preventive measures. This study has demonstrated resistance to Erythromycin among GAS isolates, thus suggesting that antibiotic susceptibility testing should be done to provide guidance for empirical therapy in cases of acute infections.

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Conflicts of interest

There are no conflicts of interest.

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