Original Article

Prevalence of Beta-Hemolytic Streptococci Groups A, C, and G in Patients with Acute Pharyngitis

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ABSTRACT

Context: Group A beta-hemolytic streptococci (GAS) is the most frequently isolated pathogen in acute pharyngitis. However, the role of Group C (GCS) and Group G (GGS) streptococci in disease burden is under recognized. The present study is carried out to find out the prevalence of acute pharyngitis caused by the different serogroups of streptococci and antibiotic susceptibility pattern of these streptococcal isolates.

Study and Design: A cross sectional study.

Materials and Methods: A total of 218 throat swabs from patients with acute pharyngitis and 82 from healthy controls were collected and processed as per standard protocol. Samples were inoculated on blood agar and *Streptococcus* selective agar. Isolates were identifi ed by the conventional method and serogrouped by latex agglutination test using Remel Streptex kit.

Results: Beta-hemolytic streptococci (BHS) were isolated from 34 (15.59%) of pharyngitis patients and 11 (13.41%) of the healthy carrier. Among pharyngitis, GAS was isolated from 20 (9.17%), GCS 7 (3.21%), and GGS 7 (3.21%) patients. Carriage rate of GAS was 6 (7.31%) and GCS, 5 (6.09%). Vancomycin (100%), amoxyclavulanic acid (90%), levofloxacin (85%), and cephotaxime (80%) were found to be most effective antibiotics. Comparatively, higher drug resistance was observed among GCS and GGS to all the drugs used in the study except for levofloxacin.

Conclusions: Although rate of pharyngitis associated with GCS and GGS is marginally lower than GAS, their carriage rate among healthy and relative higher drug resistance emphasizes the need for periodic surveillance of infection by the different serogroups of BHS.

Key words: Beta, Groups A, C, and G, hemolytic, pharyngitis, streptococci

INTRODUCTION

Beta-hemolytic streptococci (BHS) cause a wide spectrum of clinical diseases. Pharyngitis and associated complications are the most common clinical presentation for Group A streptococci (GAS). Invasive GAS infections have increased worldwide during the past decade despite organism remaining sensitive to penicillin and other commonly used beta-lactam antibiotics.[1]

GAS have been described frequently as an emerging cause of severe invasive infections in population-based surveillance studies, whereas the descriptions of group B, C, and G streptococci (GBS, GCS, and GGS) have been less frequent. It is not possible to differentiate between invasive infections caused by GAS, GBS, GCS, or GGS solely on the clinical presentation.[2]

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BHS from groups other than Group A have been associated with outbreaks of pharyngitis, but their importance in causing endemic pharyngitis remains uncertain. Since non-Group A, BHS are inhabitants of the oropharynx during both health and disease, proof of their pathogenicity requires the isolation of strains more frequently among symptomatic patients with pharyngitis than among healthy person.[3]

While reports of the recovery of GGS/GCS from normally sterile sites are increasing, studies describing GGS/GCS throat colonization rates relative to GAS in the same population are very few.

The present study has been undertaken to estimate the prevalence of different serogroups of streptococci, among patients with the signs of pharyngotonsillitis. Furthermore, to determine antibiotic susceptibility pattern to delineate any differences between infections caused by GCS, GGS, and GAS in the same area during the same period.

MATERIALS AND METHODS

Study design

Cross-sectional study.

Inclusion criteria

All the consecutive patients suffering from acute pharyngitis, attending outpatient Department of Otorhinolaryngology and Medicine of Karnataka Institute of Medical Sciences hospital, Hubli were included in the prospective study conducted during a 1-year period, from December 2011 to November 2012. Throat swabs were collected from a total of 218 patients and also from 82 appropriate age and sex matched healthy controls.

Exclusion criteria

Patients already on antibiotic therapy were excluded. Ethical clearance was obtained.

Two swabs were collected from each patient using sterile cotton tipped swab and transported in nutrient broth. The isolation and identification of BHS were done as per standard protocol.[4]

One swab was used to make smear for Grams-stain. The Gram-stained smears were observed under the microscope for the presence of pus cells and Gram-positive cocci in pairs and chains. The second swab was inoculated on to 5% sheep blood agar plate and *Streptococcus* selective agar using "streak-stab" technique. *Streptococcus* selective media is used to inhibit the growth of throat commensals by the addition of inhibitory agents such as colistin, nalidixic acid, crystal violet, and support the growth of streptococci by enriching the media with blood. The plates were incubated for 24–48 h using a candle jar with a 5–10% CO_2. The plates were observed for the presence of any beta-hemolytic colonies. Subcultures were made from beta-hemolytic colonies using fresh blood agar plates. From the subcultured plates, identification of the beta-hemolytic colonies were done based on conventional biochemical reactions; catalase test and arginine dihydrolase test; fermentation of sugars; glucose, lactose, mannitol, ribose, sorbitol, and trehalose.[4] Presumptive identification of BHS was based on susceptibility and the resistant pattern of isolates using differential discs such as bacitracin and trimethoprim-sulfamethoxazole.

Serogrouping of BHS was done using Remel Streptex kit manufactured by Remel Europe Limited. Clipper Boulevard West, Crossways, Dartford, Kent, DA2 6PT, United Kingdom. Streptex is a rapid latex agglutination test system for use in the qualitative detection and identification of the Lancefield group of streptococci. Reagents are provided for Groups A, B, C, D, F, and G covering the majority of clinical isolates. Interpretations of results were done on the strength of agglutination.[5]

Antimicrobial susceptibility testing was done on MHA with 5% sheep blood agar by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standards Institute guidelines.[6] Microbial Type Culture Collection (MTCC) number 1926 *Streptococcus pyogenes* obtained from MTCC and Gene Bank, Institute of Microbial Technology, Chandigarh was used as a control. Isolates were tested for ampicillin 10 μg, amoxicillin/clavulanic acid 20/10 μg, erythromycin 15 μg, clindamycin 2 μg, levofloxacin 5 μg, tetracycline 30 μg, cephotaxime 30 μg, and vancomycin 30 μg.

Statistical analysis was done by using Fisher's exact test.

RESULTS

Age group of patients was ranging from 1 to 80 years. Mean age was 24.03. A maximum number of cases 58 (26.6%) were in the age group of 1–10 years. Male to female ratio was 1:1.29.

Majority 110 (50.45%) of patients presented with fever and cough, 47 (21.55%) with sore throat, 83 (38.07%) with throat pain, 36 (16.51%) with difficulty in swallowing, and

2 (0.91%) with change of voice. The severity of infections among different serogroups is presented in Table 1.

BHS were isolated from 34 (15.59%) patients with pharyngitis and 11 (13.41%) healthy carriers. Among pharyngitis cases, the incidence of GAS was 20 (9.17%), GCS 7 (3.21%), and GGS 7 (3.21%). Carriage rate of GAS was 6 (7.31%) and GCS 5 (6.09%).

Antibiotic resistant pattern of Group A, Group C, and Group G Streptococci isolated from throat swab presented in Table 2.

DISCUSSION

S. pyogenes (GAS), a human pathogen and *Streptococcus dysgalactiae* subsp. *equisimilis* (GGS/GCS) are evolutionarily related, share the same tissue niche in humans, exchange genetic material, share up to half of their virulence associated genes, and cause a similar spectrum of diseases. Yet, GGS/GCS are often considered as a commensal bacterium and its role in streptococcal disease burden is under recognized.

The etiological agents of acute pharyngitis include viruses, isolated in approximately 40–60% of cases and primary bacterial pathogens, which account for approximately 5–30% of cases. In around 30% of cases, no pathogen is isolated.[7] The most important source of concern is infection with Group A beta-hemolytic *S. pyogenes,* that is, associated with acute glomerulonephritis and acute rheumatic fever.[8]

In the present study, among adults overall the prevalence of pharyngitis was found to be 7.04% and among children 12.91%. A similar observation was made by Kumar *et al.* among children,[9] comparatively higher incidence rate was reported by Bramhachari *et al.*[3]

We have isolated 83 (38.07%) bacterial pathogens from pharyngitis cases. Among these BHS were predominant organisms 34 (40.96%). The prevalence of BHS was 15.59%. Slightly, the lower prevalence rate of GAS was observed by Sindhulina *et al.* from CMC Vellore.[10] No significant gender difference was observed. Our findings correlate with Nandi *et al.*[11] Majority of the cases 152 (69.72%) belonged to an urban area.

Maximum number 157 (72.01%) of pharyngitis cases occurred in the month of October (winter season). Some studies have noted two peak incidences in May and September with sporadic cases of pharyngitis throughout the year.[9,12]

By latex agglutination test, 20 (58.82%) isolates were identified as GAS, 7 (20.58%) as GCS and, 7 (20.58%) as GGS. The prevalence of GAS was 20 (9.17%). Different studies have quoted rates ranging from 2.8% to 40%.[3,9]

The roles of GCS and GGS in causing endemic pharyngitis are still controversial, although GCS are implicated in the outbreak of pharyngitis and associated disorders. GCS and GGS have also been described as frequent invasive pathogens in elderly patients, often in association with alcohol abuse, diabetes mellitus, malignant diseases, cardiac, and peripheral vascular diseases.[2]

The prevalence of GCS was 7 (3.21%) and GGS 7 (3.21%). The frequency of GCS and GGS isolates from acute pharyngitis from different regions ranges from 0.7% to 6.24% and 3.74% to 5.1%, respectively.[12,13]

BHS were isolated from 11 (13.41%) of asymptomatic healthy controls. The percentage of asymptomatic GAS carriers was 6 (7.31%) and GCS was 5 (6.09%). GGS was not isolated from the healthy control group. Our study is comparable with Lloyd *et al.*[14] carriage rate of GAS ranging from 1.3% to14.9%,[3,8,14] GCS ranging from 0.97% to 11%,[3,14] and GGS ranging from 2.4% to 11%[3,14] from asymptomatic individuals have been reported by various studies.

Table 1: Severity of infections among serogroups

Pharyngotonsillitis	Group A *n* (%)	Group C *n* (%)	Group G *n* (%)
Pharyngitis	15 (75)	3 (42.85)	5 (71.42)
Pharyngotonsillitis-unilateral infection grade I		1 (14.28)	1 (14.28)
Pharyngotonsillitis-bilateral infections grade II	4 (20)	1 (14.28)	1 (14.28)
Pharyngotonsillitis-bilateral infections grade III	1 (5)	2 (28.57)	
Total	20	7	7

Table 2: Antibiotic resistant pattern of Group A, Group C and Group G streptococci isolated from patients throat swab

Serogroups	Group A *n* (%)		Group C *n* (%)		Group G *n* (%)
Antibiotics	Pharyngitis	Healthy	Pharyngitis	Healthy	Pharyngitis
Amp	5 (25)	3 (50)	3 (42.85)	3 (0.6)	2 (28.57)
Amc	2 (10)	1 (16.66)	1 (14.28)	1 (0.2)	2 (28.57)
E	8 (40)	3 (50)	4 (57.14)	4 (0.8)	7 (100)
Cd	8 (40)	3 (50)	4 (57.14)	4 (0.8)	7 (100)
Te	6 (30)	4 (66.66)	2 (28.57)	2 (0.4)	3 (42.85)
Le	3 (15)	2 (33.33)	1 (14.28)	2 (0.4)	0 (0)
Ctx	4 (20)	2 (33.33)	1 (14.28)	2 (0.4)	3 (42.85)
B	0 (0)	0 (0)	2 (28.57)	3 (0.6)	3 (42.85)
SXT	20 (100)	6 (100)	0 (0)	0 (0)	0 (0)

A: Ampicillin, Amc: Amoxyclavulanic acid, E: Erythromycin, Cd: Clindamycin, Te: Tetracycline, Le: Levofloxacin, Ctx: Cephotaxime, Ba: Bacitracin, SXT: Sulfamethoxazole-trimethoprim

All 20 (100%) GAS, 5 (71.42%) of GCS, and 4 (57.14%) of GGS isolates from acute pharyngitis were sensitive to bacitracin. All 6 (100%) GAS and 2 (40%) GCS of isolates from healthy control throat swabs were also sensitive to bacitracin. All the GAS isolates from acute pharyngitis and healthy controls were resistant to sulfamethoxazole-trimethoprim. All the GCS and GGS isolates were sensitive to trimethoprim–sulfamethoxazole. Menon *et al*. reported that 9.8% strains of GAS were resistant to bacitracin, whereas 85.7% strains of GGS and 90% strains of GCS were sensitive to bacitracin.[15]

Antibiotic susceptibility test revealed the majority of GAS and GCS isolates from acute pharyngitis patients and healthy control group were sensitive for amoxyclavulanic acid, levofloxacin, and cephotaxime. Maximum resistance was observed to erythromycin and clindamycin. All the GGS isolates were sensitive to levofloxacin, resistant to clindamycin and erythromycin. Most of the GGS isolates were sensitive to amoxyclavulanic acid, tetracycline, and cephotaxime. The majority of GAS 4 (66.66%) and 2 (40%) of GCS isolates from healthy controls were resistant to tetracycline. A similar observation was made by Ho *et al*. with isolates resistant to erythromycin and tetracycline.[16] All the isolates were sensitive to vancomycin.

Most of the studies have quoted all the GAS isolates being sensitive to penicillin, cefotaxime, and vancomycin.[17] Al-Charrakh *et al*. quoted high bacterial resistance to beta-lactam antibiotics-ampicillin, third generation cephalosporins-cefotaxime, ceftriaxone, and fourth generation cephalosporin-cefepime.[18]

Macrolides have been effective in the treatment of individuals with *S. pyogenes* infection and a good alternative in patients who are allergic to penicillin. Unfortunately, because of the general use of these agents, macrolide-resistant *S. pyogenes* have been isolated by many different studies from India and other countries. We have observed 26 (55.31%) of BHS isolates resistant to both erythromycin and clindamycin. Resistance to erythromycin ranging from 3.4%[19] to 91.8%[20] has been quoted by various studies. Comparatively, the low rate of resistance was observed with clindamycin ranging from 0.6%[19] to 80%[20] and tetracycline ranging from 24%[19] to 50%.[17] None of the isolate in our study revealed inducible clindamycin resistance.

In comparison with GAS, GCS exhibited a higher range of drug resistance to all the antibiotics tested in our study, except for levofloxacin and tetracycline. However, statistically significant difference was not observed with any of the drugs. GGS also exhibited a higher range of drug resistance to all drugs tested except for levofloxacin. The statistical significant difference was observed toward erythromycin and clindamycin.

CONCLUSION

Healthy carriers of BHS are sources for bacterial dissemination and even lead to severe epidemics. BHS pharyngitis is one of the most common bacterial diseases in a human being. Invasive infections caused by GAS, GBS, GCS, and GGS are still a major challenge for clinicians. Antimicrobial resistance among these is an emerging concern. Continued epidemiological and microbiological surveillance is necessary to assess the development of infections by different serogroups of BHS for better management of patients and to improve preventative strategies.

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Conflicts of interest

There are no conflicts of interest.

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