Brief Original Article

The prevalence and antimicrobial susceptibility patterns of beta-hemolytic streptococci colonizing the throats of schoolchildren in Assam, India

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Abstract

Introduction: Studies on the carriage rate of beta-hemolytic streptococci among children form an important component of public health practice to prevent disease complications such as rheumatic fever/rheumatic heart disease, nephritis, and other local or systemic infections.

Methodology: Throat swabs collected from asymptomatic schoolchildren were inoculated into appropriate media for isolation of beta-hemolytic streptococci. They were identified by standard biochemical methods and sero-grouped. Antibiotic sensitivity was evaluated using the Kirby-Bauer disk diffusion method.

Results and Conclusion: Beta-hemolytic streptococci were isolated from 106 (7.7%) out of the 1,384 throat swabs and Group F was the predominant sero-group isolated. The highest resistance observed among all the beta-hemolytic streptococci was to trimethoprim-sulfamethoxazole.

Key Words: Beta-hemolytic streptococci; schoolchildren; Assam, India

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Introduction

The beta-hemolytic streptococci (BHS) belonging to Lancefield groups A,B,C,G and F are known to cause serious systemic infections worldwide [1-10]. BHS, particularly group A, are the cause of systemic syndromes such as streptococcal necrotizing fasciitis, toxic shock syndrome, puerperal fever, and more localized conditions such as erysipelas and tonsillitis. The BHS can colonize the throat of healthy carriers as commensals, serving as a reservoir for pathogen transmission.

There have been reports of emerging antimicrobial drug resistant strains among the β-hemolytic streptococci (BHS). Most alarming would be an increase in resistance to the older, most effective and less expensive antibiotics, such as penicillin [11-13]. Streptococci can be transmitted from person to person via aerosols after sneezing or coughing, and also via fomites. Crowded environments, which are often experienced in schools, refugee camps, and military units, are conducive to transmission. Knowledge about antibiotic resistance patterns prevalent in local geographical regions is important for clinicians to choose the most effective antibiotic for treatment. Studies that determine the carriage rate of BHS among children are important to public health practices to aid in the prevention of infections and their serious complications, such as rheumatic fever/rheumatic heart disease, nephritis, and local or systemic infections.

This study was designed to determine the carrier state of BHS, the prevalent sero-groups, and the antimicrobial susceptibility patterns to commonly used antibiotics among schoolchildren of Dibrugarh District of Assam, India.

Methodology

Study area and study subjects

Out of 1,631 schools in the Dibrugarh District, 99 schools were randomly selected for streptococcal screening after obtaining permission from the school authorities. Parents of the children were apprised about the role of BHS in colonizing the oral cavity and in their potential for causing disease. Before enrolment in the study, informed consent was obtained from the parents with the understanding that their children would undergo a simple throat swab culture to determine the presence of BHS. Children with no signs and symptoms of upper respiratory tract infection and no history of antibiotic therapy within the last two weeks were enrolled in the study. The

Table 1. Distribution by age and sex of children colonized with β-hemolytic streptococci (BHS)

Age in years	Number of subjects	Male	Female	Total BHS*
5-7	271	198	73	33 (12.17%)
7-9	260	161	99	14 (5.38%)
9-11	252	134	118	19 (7.53%)
11-13	360	168	192	26 (7.22%)
13-14	241	100	141	14 (5.8%)
Total	1384	761	623	106

*BHS = beta-hemolytic streptococci

study was approved by the Institute's Ethical Committee.

Laboratory investigations

Throat swabs from the students were inoculated on 5% sheep blood agar prepared using blood agar base (Hi Media, Mumbai, India). The inoculated plates were incubated at 37ºC in a CO_2 incubator and were observed for beta-hemolysis after 24 hours and 48 hours. BHS were processed by Gram stain, catalase test, and bacitracin sensitivity, and were sero-grouped using a latex agglutination assay (Hi Strep Latex Kit, Hi Media, Mumbai, India).

Susceptibility of isolates to penicillin-G (10U), erythromycin (15 µg), cefotaxime (30 µg), chloramphenicol (30 µg), trimethoprim-sulphamethoxazole (TMP-SMX) (1.25/23.75µg), and ofloxacin (5 µg) were evaluated by the Kirby-Bauer disk diffusion method [14]. The antibiotic disks were obtained from Hi Media, and *Streptococcus pneumoniae* ATCC 49619 was utilized as the quality control strain. An overnight broth suspension of each test isolate was adjusted to a 0.5 McFarland's turbidity standard and was used to inoculate Mueller Hinton agar plates supplemented with 5% sheep blood. Antibiotic disks were applied to the plates, incubated at 37ºC for 18 hours in a CO_2 incubator after which the diameters of the zones of inhibition were measured. Zone diameters were interpreted as susceptible, intermediate, or resistant according to the manufacturers' instructions and Clinical Laboratory Standards Institute (CLSI) guidelines [15].

Results

In total, 1,384 throats swabs were obtained from 761 boys and 623 girls ranging from five to 14 years old and 106 BHS (7.65%) were isolated (Table 1). Students five to seven years of age were most frequently colonized with BHS (12.17%) (Table 1) and the distribution of Lancefield groups within these age categories is shown in Table 2. Results indicate that group F (53.77%) was the predominant colonizer across all age groups. BHS groups G and A had similar colonization rates of 19.81% and 16.98%, respectively. The least common colonizers were group C (5.66%) and group B (3.77%) (Table 2).

The susceptibility patterns of the different BHS subgroups isolated from the schoolchildren are depicted in Table 3. The group A isolates were 100% susceptible to penicillin, chloramphenicol and ofloxacin, and very susceptible to erythromycin (94.4%). Susceptibility to cefotaxime and TMP-SMX were 72.2% and 22.2%, respectively. The four group B isolates tested were 100% susceptible to penicillin, chloramphenicol, cefotaxime, TMP-SMX and ofloxacin and one isolate was intermediate to erythromycin. All six group C BHS were 100% susceptible to erythromycin, chloramphenicol and ofloxacin; however, penicillin, cefotaxime and TMP-SMX had low susceptibility rates of 50%, 66.7% and 0%, respectively.

Susceptibility to the antimicrobial agents tested was lowest among the 57 group F BHS isolated (Table 3). The following pattern was observed: penicillin 54.4%, erythromycin 87.7%, cefotaxime 43.85%, chloramphenicol 86% TMP-SMX 31.6%, and ofloxacin 89.47%. Among the 21 group G isolates, 80.95% were susceptible to penicillin, erythromycin, chloramphenicol and ofloxacin. Poor susceptibility was observed with cefotaxime (42.85%) and TMP-SMX (14.3%).

Discussion

This study determined that the prevalence of BHS is 7.7% among asymptomatic schoolchildren within the Dibrugarh District of Assam, India. A similar carrier rate of 8.3% was reported among 1,796 asymptomatic patients in an urban hospital in Croatia [7] and a prevalence of 6-21.6% was reported from India [16,17]. In this study, we observed that the

Table 2. Sero-group distribution of the 106 β-hemolytic streptococci (BHS) isolated

Age in years	Group A BHS* *n* (%)	Group B BHS *n* (%)	Group C BHS *n* (%)	Group F BHS *n* (%)	Group G BHS *n* (%)
5-7	4 (3.77)	3 (2.83)	4 (3.77)	16 (15.09)	6 (5.66)
7-9	2 (1.89)	1 (0.94)	1 (0.94)	8 (7.55)	2 (1.89)
9-11	2 (1.89)	0	1 (0.94)	12 (11.32)	4 (3.77)
11-13	7 (6.60)	0	0	12 (11.32)	7 (6.60)
13-14	3 (2.83)	0	0	9 (8.49)	2 (1.89)
Total	18/106 (16.98)	4/106 (3.77)	6 /106(5.66)	57/106 (53.77)	21/106 (19.81)

*BHS = beta-hemolytic streptococci

Table 3. Susceptibility patterns of the isolated beta-hemolytic streptococci (*n* = 106)

Antimicrobial Agent S, I, R*	**BHS** Groups**				
	Group A *n* = 18 (%)	**Group B *n* = 4 (%)**	**Group C *n* = 6 (%)**	**Group F *n* = 57 (%)**	**Group G *n* = 21 (%)**
Penicillin G					
S	18 (100)	4 (100)	3 (50)	31 (54.4)	17 (80.95)
I	0	0	3 (50)	19 (33.3)	2 (9.52)
R	0	0	0	7 (12.3)	2 (9.52)
Erythromycin					
S	17 (94.4)	3 (75)	6 (100)	50 (87.7)	17 (80.95)
I	1 (5.6)	1 (25)	0	5 (8.8)	4 (19.05)
R	0	0	0	2 (3.5)	0
Cefotaxime					
S	13 (72.2)	4 (100)	4 (66.7)	25 (43.85)	9 (42.85)
I	2 (11.1)	0	1 (16.7)	14 (24.56)	3 (14.3)
R	3 (16.7)	0	1 (16.7)	18 (31.6)	9 (42.85)
Chloramphenicol					
S	18 (100)	4 (100)	6 (100)	49 (86)	17 (80.95)
I	0	0	0	6 (10.5)	4 (19.05)
R	0	0	0	2 (3.5)	0
Trimethoprim-Sulfamethoxazole					
S	4 (22.2)	4 (100)	0	18 (31.6)	3 (14.3)
I	2 (11.1)	0	1 (16.7)	2 (3.5)	2 (9.5)
R	12 (66.7)	0	5 (83.3)	37 (64.9)	16 (76.2)
Ofloxacin					
S	18 (100)	4 (100)	6 (100)	51(89.47)	17(80.95)
I	0	0	0	3 (5.26)	4 (19.05)
R	0	0	0	3 (5.26)	0

*S-Susceptible, I-Intermediate, R- Resistant
**BHS = beta-hemolytic streptococci

highest prevalence rate was for group F BHS. These findings confirm those of other studies from various geographic locations that have demonstrated a higher prevalence of non-group A streptococci among healthy carriers [17]. In contrast, a report from India indicated a predominance of group G among various populations [17]. Therefore, the carriage rate of specific Lancefield groups of BHS can vary by geographic location.

In our study, BHS most frequently colonized the five to seven years age group, in agreement with reports from Nepal [18] and United Arab Emirates [19]. It can be speculated that a contributing factor to a lower colonization rate among older children may be related to host immunity that increases over time.

Both group A and non group A BHS are associated with acute pharyngitis [1,3,7]. However, group A pharyngitis is the most serious infection because of the severe sequelae, namely acute rheumatic fever and post streptococcal glomerulonephritis. Of importance, group G streptococci has been isolated from rheumatic fever patients and also from skin lesions of patients with acute glomerulonephritis. Group F, the predominant BHS observed in this study, has been associated with the following infections: dental abscess, brain abscess, meningitis, bacteremia, endocarditis, and pericarditis [2,10,20].

Penicillin is used most often as the drug of choice for streptococcal pharyngitis. The emergence of resistance to this antibiotic would therefore be a major public health concern. The group A BHS in our study were 100% susceptible to penicillin and the lack of resistance among group A strains was also reported by others [16,17]. In contrast, only 54.4% of group F strains were penicillin-susceptible and these were also less susceptible to the other antimicrobial agents tested.

TMP-SMX had the highest antimicrobial resistance rate among the BHS tested in this study. Group A BHS exhibited 66.7% resistance to TMP-SMX, which was higher than the 12.2% rate demonstrated in a study from North India [11]. The elevated resistance to this drug may be due to the fact that it is commonly prescribed in rural settings of the Assam state of India. Since all groups of BHS can cause serious disease, their identification and rates of resistance among asymptomatic carriers as well as those who are infected should be monitored. Knowledge of the drug susceptibility patterns of BHS and the prevalence of the Lancefield groups in specific geographic regions would assist clinicians in appropriate patient management and therapeutic regimen.

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