Abstract

The genus Streptococcus is comprised of a wide variety of both commensal and pathogenic gram-positive bacteria which are found to exhibit a wide range of hosts, including humans, horses, pigs and cows. Streptococcal infections can cause a wide range of diseases from mild infections to severe. Repeated infection results in the non-suppurative sequelae, acute rheumatic fever, and acute glomerulonephritis. Streptococcus remains sensitive to the antibiotic penicillin which can be administered as a means to treat infection or as prophylaxis. Rheumatic fever and rheumatic heart disease continue to ravage millions of people around the world. Children and adolescents of the developing countries are especially susceptible to this disease. The aim of this study is to assess the effect of invasive Streptococcal infection with an objective to determine the prevalence of streptococcal infection in school children of 5-15 years of age in Tripura, North Eastern part of India. From September 2012 - February 2013, a study was conducted on prospective population-based laboratory surveillance of Tripura school children with isolation of Streptococci from throat swab examination of both pharyngitis and non-pharyngitis cases. Throat swabs were collected from 1165 school children, randomly selected from 3 districts of Tripura. These swabs were cultured on blood agar and Mac Conkey plates. Out of 1165 swabs, 43 were alpha haemolytic, 4 were Grp. G (Streptococcus dysgalactiae subsp. equisimilis) and 1063 were of different types than streptococcus. The study present the results of the first prospective surveillance study of Streptococcal infections in north eastern part of India.

Keywords: Acute rheumatic fever, Rheumatic heart disease, Impetigo; Pharyngitis, Post-streptococcal Glomerulonephritis

Introduction

A large genus of spherical or ovoid bacteria that are characteristically arranged in pairs or in chains resembling strings of beads. Many of the streptococci that constitute part of the normal flora of the mouth, throat, intestine, and skin are harmless commensal forms; other streptococci are highly pathogenic. The cells are gram-positive and can grow either anaerobically or aerobically, as they do not require oxygen for metabolic reactions.

The group of β-hemolytic streptococci cause a wide spectrum of clinical diseases. Pharyngitis and associated complications are the most common clinical presentations for group A streptococci (GAS). Invasive GAS infections have increased world-wide during the past decade despite the organism remaining sensitive to penicillin and other commonly used Beta lactam antibiotics (1,2) Group B streptococci (GBS) are a leading cause of infection in neonates and pregnant women and have also been recognised as a cause of invasive diseases.
in children and non-pregnant females (2, 3). Group C (GCS) and group G (GGS) streptococci are commensals of the pharynx, skin, gastrointestinal tract and female genital tract; the 'large colony'-forming strains resemble GAS in terms of their virulence (2).

Streptococcal (strep) infections are communicable diseases that develop when bacteria normally found on the skin or in the intestines, mouth, nose, reproductive tract, or urinary tract invade other parts of the body and contaminate blood or tissue. In the last decade, there has been an increase in reports of serious streptococcal infections worldwide (4). Strep infections are an occupational disease of school children between the ages of 5 and 15.

Streptococcal infections with an estimated death rate of over 500,000 individuals/year place GAS among major human pathogens, exceeded by HIV, Mycobacterium tuberculosis, Plasmodium falciparum and S. Pneumoniae and probably comparable to rotavirus, measles, Haemophilus influenzae type b (Hib), and Hepatitis B (5, 6).

Autoimmune response to infection with group A streptococcus is actually resulted into Rheumatic fever. Although the acute illness causes considerable morbidity, and some mortality, the major clinical and public health effects derive from long-term damage to the heart valves, i.e., rheumatic heart disease (RHD). Over the past century, as living conditions have become more hygienic and less crowded, and nutrition and access to medical care have improved, acute rheumatic fever (ARF) and RHD have become rare in developed countries. But, rheumatic fever/ rheumatic heart disease is the commonest cardiac disease in children and young adults and remains a major public health problem in developing countries (7).

GAS infection causes a substantial number of illnesses and deaths, especially in the developing world, with more than 500,000 deaths worldwide annually, attributable mostly to ARF and its sequelae, rheumatic heart disease, and invasive infection (8). The epidemiology of rheumatic fever (RF) is linked with that of Group A beta-haemolytic streptococcal pharyngitis; both have a maximum incidence in the age group of 5 – 15 years (9).

Recently worldwide population based data has been reviewed to estimate the burden of GAS diseases and highlighted deficiencies in the available data (10). According to WHO, at least 15.6 million people have RHD. Out of the 5, 00,000 individuals who acquire ARF every year, 3,00,000 go on to develop RHD; and 2,33,000 deaths annually are attributable to ARF or RHD (11). The greatest burden in streptococcal diseases is due to RHD, with a prevalence of at least 15.6 million cases, with 2, 82,000 new cases and 2, 33,000 deaths each year (8). It is estimated that approximately 600,000 children less than 15 years of age are currently suffering from chronic rheumatic heart disease, and that approximately 121,000 children are newly diagnosed with acute rheumatic fever every year.

During the past two decades, ARF/RHD has become uncommon health problems in developed countries. However, in third world countries such as India, the middle-east, sub-Saharan Africa, RF remains the leading cause of heart disease in children and young adults (12). In India, the disease burden of streptococcal infections is considerable (13). Prevalence of rheumatic heart disease and pharyngitis in India, varies from 1 to 5.4/1,000 (14) and 4.2% to 13.7%,(15, 16) school-age children respectively, which is comparable to the rates reported from developed countries (17). Streptococcus pyogenes pharyngitis has a high prevalence in North India (18), whereas pyoderma is more frequent in South India (19). In light of invasive infection, this is a completely neglected field in India and the only data available in the literature is one retrospective study of invasive β-hemolytic streptococcal infections (20). There are considerable socio-economic losses due to high cardiovascular mortality and morbidity of Indian population (21). Incidence of streptococcal pharyngitis has been reported to be 8-18
episodes per week per 1000 children in the age group of 5-15 years in India. Clinical scoring card for diagnosis of Group A Streptococcal sore throat was developed (22). For the last 20 years, in India various research programmes have been carried out at different centres mostly by ICMR and a few by WHO (23). Padmavati, in 1995 examined the status of RF & RHD in India with reference to both prevalence and incidence based on the last 10 years data and showed RHD in 1 to 5.4/1000 and RF in 0.3-0.5/1000 children. Recently the burden of group A streptococcal disease in India was addressed and treatment options standardized by the World Health Organization was also discussed (13).

Streptococcal infections are extremely common due to the overall susceptibility of these organisms to antibiotics, especially penicillin. While infections caused by the Lancefield group A streptococcus (GAS or Streptococcus pyogenes) have dominated the streptococcal medical literature, Lancefield groups C and G share many microbiologic and clinical characteristics with GAS (24). Work in the early 20th century described S. pyogenes as an exclusively human pathogen and detailed the frequency of carriers and the most characteristic infections (25). The pioneering work of Rebecca Lancefield led to the identification of a number of groups of streptococci including A, C and G, producing haemolysis on sheep blood agar, exhibiting different biochemical properties and isolated from a variety of animal species (26). Classification methods based upon the Lancefield methodology were established soon thereafter (27).

Among this diverse group, the group C and group G streptococci have assumed more important clinical roles. For a number of reasons, they can be considered together, separate from other members of the genus Streptococcus. Although less extensively studied, groups C and G streptococci are now appreciated to produce infections quite similar to GAS although they more commonly cause opportunistic and nosocomial infections than GAS. No. of recent reports have described their association with streptococcal syndromes generally caused by GAS such as streptococcal toxic shock syndrome (STSS) (28) and acute rheumatic fever (ARF) (29). This assumes significance in countries like India where ARF continues to be a major health problem.

β-hemolytic streptococcus belonging to Group G (Streptococcus dysgalactiae subsp. Equisimilis) has attracted attention as possible etiological agents of pharyngitis and post-streptococcal sequelae. Normally present on the skin, in the mouth and throat, and in the intestines and genital tract, Group G Streptococcus (GGS) is most likely to lead to infection in alcoholics and in people who have cancer, diabetes mellitus, rheumatoid arthritis and other conditions that suppress immune-system activity.

Till date, no report is available in Tripura about the streptococcal infection in school children; hence the present study was designed to study the prevalence of β-hemolytic Streptococcal infection among School children in Tripura, North Eastern part of India. This study also provides an opportunity to establish the direction of further investigations and to focus interventions in Tripura.

Materials and Methods

Present study was conducted after the clearance of institutional ethical committee (IET) of Agartala Govt. Medical College and G. B. P Hospital, Agartala. Cross sectional prospective study was conducted to find out the GAS isolates in Tripura, north eastern part of India at Agartala Govt. Medical College and G. B. P Hospital, Agartala. The school children of 5-15 years age group in different schools from 3 districts of Tripura were considered for the study. Besides, patients were not included those who have taken antibiotics prior to this programme. In aseptic condition, throat swabs were collected in duplicate using torch light under direct vision and with the aid of a tongue depressor. Swabs were rubbed quickly but thoroughly over both tonsils and tonsillar fossae. Since the sites of collection
were not very far from the microbiology laboratory so within two hours collected samples were transported to the lab at room temperature.

**Sample processing** - Two types of plates were used for culturing the sample; Blood agar and Mac Conkey. Blood agar plates were kept in candle jar to have microaerophilic condition whereas the Mac Conkey plates were allowed to incubate for 24-48 hours at 37°C.

After 24-48 hours incubation, bacterial colonies grown on blood agar plates showing beta haemolytic colonies were further processed from the mixed colony and incubate at the same atmosphere for 24 hours. Following day Gram staining was prepared from the suspected beta haemolytic colonies and catalase test was also performed for further confirmation. Based on the gram staining reaction and catalase test result, grouping was performed with the help of Streptex kit.

**Streptococcal Grouping** : Streptex agglutination was carried out on colonies from purity plates. The extraction enzyme provided in the Streptex kit was reconstituted by the addition of 11 ml sterile distilled water and was stored at 4°C. With cultures from solid media, colonies were suspended in 4 ml extraction enzyme and incubated at 37°C for 1 hr. The enzyme extract was used for agglutination reactions without any centrifugation. One drop of extract was added to one drop of each of the latex particles coated with group-specific immunoglobulin to groups A, B, C, D, F and G on the clean glass grouping tiles provided. After thorough mixing, the tile was rocked gently for a maximum of one minute and then the agglutination pattern was read. Where agglutination was weak or occurred with more than one latex suspension the test was repeated using the extraction enzyme alone in parallel with the extract (30).

The number of positive throat swabs were then analyzed and represented in tabular form. As a part of external quality control, the methods and materials were supervised by expert of PGI, Chandigarh within our study period.

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Fig. 4. Grouping of β-haemolytic colonies through Streptex Kit

Fig. 5. Distribution of Cases from September, 2012 to February 2013

Prevalence of β-haemolytic Streptococcal infection
Results and Discussion

In the present study, 1165 throat swabs were randomly collected from school children of 3 districts of Tripura within a 6 months’ time period. Socio demographic profile of the study subjects evaluated on the basis of sex, age and religion which is shown in the figure 5. Out of 1165 throat swabs, female children were more (608) in comparison to male (508). Whereas, 10-15 yrs children were much higher in number (1033) as compared to 5-10 yrs. children (146). So far religion is concerned, number of Hindu children were maximum as compared to Muslim and Christian. In the following figure 5, school children were more from urban area (34) than rural (2). Within same time period, 30 Govt. Schools were visited.

Laboratory investigation reports

Out of 1165 throat swabs cultured, 52 cases were with no growth observed in blood agar. Mixed bacterial colonies were present in Mac Conkey. Out of total cultured samples (1165) 91.24% cases exhibited different types of bacteria other than Streptococcus. Whereas, 3.94% cases exhibited Alpha haemolytic and 0.34% exhibited with Beta haemolytic (GGS). The results of throat swab cultures were shown in table 1.

Streptex kit analysis of 4 haemolytic colonies showed only the presence of group G.

<table>
<thead>
<tr>
<th>Month</th>
<th>Beta Hemolytic</th>
<th>Alpha Hemolytic</th>
<th>Bacteria other than Streptococcus</th>
<th>No Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept, 2012</td>
<td>0</td>
<td>4</td>
<td>148</td>
<td>10</td>
</tr>
<tr>
<td>Oct, 2012</td>
<td>0</td>
<td>6</td>
<td>766</td>
<td>34</td>
</tr>
<tr>
<td>Nov, 2012</td>
<td>0</td>
<td>5</td>
<td>213</td>
<td>8</td>
</tr>
<tr>
<td>Dec, 2012</td>
<td>0</td>
<td>11</td>
<td>294</td>
<td>0</td>
</tr>
<tr>
<td>Jan, 2013</td>
<td>0</td>
<td>19</td>
<td>228</td>
<td>0</td>
</tr>
<tr>
<td>Feb, 2013</td>
<td>4 (Group G)</td>
<td>1</td>
<td>104</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>46</td>
<td>1063</td>
<td>52</td>
</tr>
</tbody>
</table>

Out of these 4 cases, 3 were males and one was female.

In our study, carriage rate of Group G Streptococcus (GGS) was higher in males than females. In case of age group, GGS infection was high in the age group of 11 years. Similar studies were conducted in various part of the world with similar findings. In the present study, though GAS was not found but the carriage rate in GGS (Group G Streptococcus) was higher in boys (75%) than girls (25%) which were similar to other reports (31,32). Age has been found to be an important factor in the microbiological etiology of pharyngitis, the peak incidence of GAS pharyngitis occurring in children aged 5–10 years (33). In the present study, the Group G Streptococcal infection was higher in 10–15 yrs. age group. All the 4 cases of Group G found in this study were of 11 yrs. and of more than 11 yrs. which was similar to other reports (33,34). In Varanasi (India), the prevalence of beta-haemolytic streptococcal sore throat was 13.6% in a rural area (35). In our study, though GAS could not be isolated but the carriage rate in GGS was predominant in urban area (80%).

The variations in the carriage rates among different schools in the same village may be explained on the basis of the location which was studied (36). Location wise present study revealed higher rate in govt. school (92%) than private school (8%).

Throat cultures have always been considered as the “gold standard” for diagnosing the presence of GAS (37). The healthy carriers of GAS are the sources of a bacterial dissemination and they are able to communicate the disease and even lead to severe epidemics (31). Different studies reported that, GAS is seen more in the pharynges of children as compared to that in adults (31). In the present study, there is a complete absence of Group A Streptococci among the school going children (5-15 yrs.) within the period of 6 months. Early use of antibiotics may be one of the factors for complete disappearance of group A Streptococcus in this

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The prevalence of beta-haemolytic Streptococcus in healthy individuals in different parts of India has been reported ranging between 11.2-34% (33). Various studies were conducted in different parts of South India like Vellore, South India – 2.3% (1977) (15), Chennai, South India - 5.2 % (2005) and 8.4 % (2006) (38,39), Coimbatore, S.India – 5.09 % (2012) (40) and 4. B.G. Nagara, Karnataka – 1.9% (2013) (41). In Northern India, the incidences of sore throat and GAS sore throat were -7.05 and 0.95 episodes per child/year (2001) respectively (17).

In Japan, a case of neonatal toxic shock syndrome due to S. dysgalactiae subsp. Equisimilis was reported (28). Invasive infections caused by this species are also increasingly seen in Japan (42) while one case of necrotizing fascitis and another case of toxic shock syndrome were reported from Norway (43) Due to these reasons, it is believed that GGS strains may be involved in the pathogenesis of post-streptococcal sequelae. Meanwhile, our study was based on a relatively small sample size within only 6 months time period. Hence it is imperative to carry forward to further study in a broader spectrum in different seasons to look for the presence of seasonal variability. We should explore the application of newer technologies to isolate GAS from the study subject. The results of the present study highlighted the importance of regular surveillance programmes to keep the Streptococcus infections and their carriage in check. The children who were found to be carriers could be adequately treated with antibiotics. This would further facilitate the control and the development of non-suppurative sequelae such as acute rheumatic fever and post streptococcal glomerulonephritis, which are debilitating and difficult to treat.

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References


