Drug resistance in Group A streptococcal infections of the pharynx in school children of desert part of Rajasthan

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Group A streptococcus (GAS) is major causative agent of pharyngotonsillitis. Rheumatic fever (RF) and rheumatic heart diseases (RHD) are important sequelae of this infection, which are still prevalent in developing countries. Antibiotic resistance is increasing in GAS. To screen children for pharyngeal carriage of GAS and to assess the burden of antibiotic resistance among clinical isolates of GAS. GAS was cultured from throat swabs of 443 school children with sore throat and 769 asymptomatic children in the age group of 5-14 years selected from 115 schools in rural area of Jodhpur district. Sensitivity of these bacteria to twelve antibiotics was tested. Throat swabs from 6.32% children with sore throat and 3.25% asymptomatic children yielded growth of GAS. Group G streptococci grew from throat swabs of 3.16% children with sore-throat and 2.21% asymptomatic children. Group C streptococci grew from throat swabs of 0.68% children with sore-throat. All isolates of GAS were sensitive to penicillin G, amoxicillin, cloxacillin and cephelexin. Resistance to erythromycin, chloramphenicol, gentamycin, neomycin, tetracycline, cotrimoxazole and polymyxin-B was observed in 10%, 18%, 40%, 50%, 58%, 84% and 96% isolates of GAS respectively. Varying level of antibiotic resistance was observed in GAS in desert region of Rajasthan though these were still sensitive to penicillin G, amoxicillin, cloxacillin and cephelexin.

Key words: Group A Streptococcus, Pharyngeal Carriage, Antibiotic Resistance.

INTRODUCTION

Group A streptococcus (GAS) is a major causative agent of pharyngotonsillitis. It is strictly human pathogen. Droplet transmission is major route of GAS transmission (American Academy of Pediatrics, 2006). Infection causes clinical condition ranging from a carrier state to mild or acute pharyngotonsillitis and invasive disease. The prevalence of GAS in the throat is known to be highest in school-aged children, but it varies geographically in different countries. Rheumatic fever (RF) and rheumatic heart diseases (RHD) are important sequelae of GAS infection of throat and continue to be prevalent in developing countries. Therefore epidemiological surveillance of GAS infection of throat is important in the community. Penicillin is the drug of choice for GAS infection as penicillin resistance has not been encountered till date. However, macrolides such as erythromycin have been more commonly used for the treatment of GAS infection, especially for those who are allergic to penicillin. Recently, the prevalence of erythromycin resistance has been reported from several countries (Acikgoz et al., 2003; d’Oliveira et al., 2003). Rising minimum inhibitory concentration (MIC) of penicillin to GAS have also been
found (Amabile-Cuevas et al., 2001; Cooper et al., 2006). Recently, penicillin-intermediately resistant Streptococcus agalactiae (Group B Streptococcus) that has amino acid substitutions in penicillin binding protein 2X has appeared in Japan (Kimura et al., 2008). Therefore, attention to trends of antibiotic susceptibility of GAS is also necessary. Studies on antimicrobial resistance in GAS from India are relatively scarce (Amabile-Cuevas et al., 2001; Brahmadathan et al., 2005; Jacob et al., 2006; Dhakal et al., 2010; Kumar et al., 2010). There is not much information on the screening of children for carriage of GAS in India. The purpose of this study was therefore to screen children for pharyngeal carriage of GAS and to assess the burden of antibiotic resistance among clinical isolates of GAS.

MATERIALS AND METHODS

From January 2008 to November 2009, 5431 school students were examined, from 115 schools. Written informed consent was obtained from their parents. Throat swabs were collected from all 443 children with sore throat and from 769 asymptomatic children. This work was carried out under a multicentric, Task Force Project funded by Indian Council of Medical Research. The ethical clearance was provided by Institutional Ethics Committee of Desert Medicine Research Centre, Jodhpur.

Blood was collected from the jugular vein of sheep and 25 ml of it was mixed with 5 ml of (3.8%) sodium citrate solution and stored at 2 to 8°C. One hundred ml of deionized water with 4.4 gm of Columbia blood agar base (Central Drug House Ltd. New Delhi, India) and 1 ml of Crystal Violet solution (0.01%) was autoclaved at 121°C and 15 lbs for 15 minutes and cooled to 45 to 50°C; 7 ml of citrated sheep blood was added to this. The 15 ml of medium was then poured into 90 mm Petri plates and allowed to cool. These Petri plates were incubated at 37°C for 24 hrs for sterility check and those showing any growth were discarded. Blood agar plates were then stored at 4 to 8°C in a refrigerator.

Throat swab from both asymptomatic children and those with sore-throat were taken and were inoculated on blood agar plates. The colonies of Beta hemolytic streptococci (BHS) grown were identified and isolated from the mixed culture on blood agar in bio-safety cabinet. As anaerobic condition supports the growth of BHS therefore the inoculated blood agar plates were incubated at 37°C in candle jar for 24 hours. Matt, mucoid or glossy colonies having bright circle around them appeared on blood agar, which were taken as those of beta haemolytic bacteria. To confirm GAS in culture, Streptex agglutination test was done with commercially available Streptex kit (Murex Biotec Ltd, UK). Out of 50 isolates of GAS 23 were from asymptomatic children and 27 were from symptomatic children. Antimicrobial susceptibility test of GAS was carried out using Octodisc (Himedia). The antibiotics tested were amoxycillin, cloxacillin, erythromycin, cotrimoxazole, ciphalexin, bacitracin, chloramphenicol, penicillin- G, polymyxin B, gentamycin, neomycin and tetracycline. Inoculum of isolates was prepared in 5 ml sterile peptone water containing 1% peptone and 0.5% NaCl and was then incubated for 4 to 6 hours. Turbidity of the bacterial suspension was matched with Mac Farland standard 3. Blood agar plates were inoculated by streaking the sterile swab dipped in bacterial suspension over blood agar surface. The plates were kept for 15 minutes so that excess surface moisture was absorbed. Himedia, antibiotic octodisc disc was placed on the inoculated agar plate by using sterile forceps. Plate was incubated anaerobically at 37°C. After 24 hrs the plate was examined and diameter of zone of inhibition including the diameter of disc was measured with a ruler. GAS were classified as sensitive, intermediate or resistant by referring to standard table (Table 1).

RESULTS

Twenty eight out of the 443 (6.32%) throat swabs of symptomatic children yielded growth of Group A Streptococci (GAS). Twenty-five throat swabs out of 769 non-symptomatic (healthy) children (3.25%) yielded growth of GAS. Group G streptococci (GGS) grew from throat swabs of 3.16% children with sore-throat and 2.21% non-symptomatic children. Group C streptococci (GCS) grew from throat swabs of 0.68% children with sore-throat.

Results of sensitivity of 50 clinical isolates of GAS to different antibiotics are depicted in Table 2. All 50 isolates were sensitive to penicillin G, amoxicillin, cloxacillin, bacitracin and cephelexin. Resistance to erythromycin and chloramphenicol was observed in 10% and 18% isolates respectively, while most isolates were resistant to polymyxin B and cotrimoxazole.

DISCUSSION

The proportion of GAS isolation in children with sore throat and asymptomatic cases has been reported earlier by few studies (Table 3). Two earlier studies from north India carried out in 1992 and 2001 reported higher proportion of GAS isolates from sore throat (13.5% and 13.7%). Comparison with a recent study from Haryana (2.8%) (Kumar et al., 2009), suggests a higher rate of GAS isolation (6.32%) from sore throats in the present study. Pharyngeal carriage GAS of 3.25% per cent of asymptomatic children in present study are also higher than that reported from North India in a study conducted in 2009 (Kumar et al., 2009).

Prevalence of GCS in throats of symptomatic school
children was 0.68% in present study, which was 1.6% in Orathur (Menon et al., 2004). GGS grew from 3.16% throat swabs of cases of sore-throat in present study, this rate was 0.97% in Orathur (Menon et al., 2004). Carriage rate of GGS in throats was 2.21% in present study, as compared to 8.26% in Orathur (Menon et al., 2004). GCS grew from throat swabs of 3.5% asymptomatic children in Orathur (Menon et al., 2004), whereas no GCS was observed from throat swab of these children in the present study. Very low population density (126/km$^2$ as compared to national figure of 360/km$^2$) and dry climatic conditions with very low humidity may be factors contributing to low carriage rate of streptococci in Jodhpur district in desert part of Rajasthan. Higher carriage rates of streptococci have been reported from slums and from areas with high population density and high humidity, which might favor droplet transmission.

Resistance to penicillin in GAS has not been reported from anywhere in world, and was also not found in our study. All GAS isolates were sensitive to bacitracin; one way to differentiate beta-hemolytic GAS from other beta-hemolytic streptococci is by determination of their sensitivity to bacitracin. Streptococcus pyogenes (group A beta-hemolytic) is sensitive to bacitracin and do not grow around the antibiotic-containing disc. The other beta-hemolytic streptococci are not sensitive to bacitracin. We also did not find resistance to cephelexin and in a meta-analysis of clinical trials, cephalosporin including cephelexin have shown superior bacteriological cure rates than penicillin (Casey and Pichichero, 2004). We observed erythromycin resistance in 10% GAS, it has also been reported from Tamilnadu, where it rose from...
Table 3. The proportion of GAS isolation in children with sore throat and asymptomatic cases

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year of study</th>
<th>Place</th>
<th>Proportion in sore throat</th>
<th>Proportion in asymptomatic throat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koshi G (Koshi et al., 1970)</td>
<td>1970</td>
<td>South India</td>
<td>10%</td>
<td>-</td>
</tr>
<tr>
<td>Koshi G (Koshi and Benjamin, 1977)</td>
<td>1977</td>
<td>Vellore</td>
<td>-</td>
<td>2.3%</td>
</tr>
<tr>
<td>Gupta R (Gupta et al., 1992)</td>
<td>1992</td>
<td>Delhi</td>
<td>13.7%</td>
<td>-</td>
</tr>
<tr>
<td>Nandi S (Nandi et al., 2001)</td>
<td>2001</td>
<td>Chandigarh</td>
<td>13.5%</td>
<td>-</td>
</tr>
<tr>
<td>Menon T (Menon et al., 2004)</td>
<td>2004</td>
<td>Orathur, Tamil Nadu</td>
<td>10%</td>
<td>7.8%</td>
</tr>
<tr>
<td>Kumar R (Kumar et al., 2009)</td>
<td>2009</td>
<td>Villages of Haryana</td>
<td>2.8%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Bramhachari PV (Bramhachari et al., 2010)</td>
<td>2010</td>
<td>Mumbai</td>
<td>-</td>
<td>1.5%</td>
</tr>
<tr>
<td>Dhakal R (Dhakal et al., 2010)</td>
<td>2010</td>
<td>Pondicherry</td>
<td>-</td>
<td>4.5%</td>
</tr>
<tr>
<td>Lloyd CA (Lloyd et al., 2006)</td>
<td>2006</td>
<td>Chennai</td>
<td>-</td>
<td>8.4%</td>
</tr>
</tbody>
</table>

5.8% in 1998 to 13.2% in 2002 (Brahmadathan et al., 2005). It was reported to be 9.04% from Chennai in 2006 (Jacob et al., 2006), 29.4% from Delhi in 2006 (Cooper et al., 2006) and 5.2% from Nepal in 2009 (Dhumre et al., 2009). The same was 10% in Sweden, 17% in Finland and 22% in UK (De Azavedo et al., 1999). Resistance to tetracycline was 58% in our study and was 27.4% in Chennai (Lloyd et al., 2007). Resistance to chloramphenicol and cotrimoxazole has also been reported in Chennai (Lloyd et al., 2007) and Nepal (Dhumre et al., 2009). We also observed very high prevalence of resistance to neomycin, polymyxin-B, gentamycin and cotrimoxazole which was expected and is also found in many studies. The resistance of GAS to erythromycin, cephalosporins and tetracycline might be due to their increasing clinical use and resentment for injectable penicillin which may occasionally induce hypersensitive reaction. The antimicrobial prescribing practices directly affect the pattern of antibiotic resistance; the physicians therefore, need to be motivated to use penicillin for GAS infections. At the same time over the counter sale of antibiotics in India needs to be seriously viewed and checked to prevent the problem of antibiotic resistance (Lloyd et al., 2007).

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