INTRODUCTION

Streptococcus pyogenes (group A streptococcus) is one of the most common and ubiquitous human pathogens. It causes a wide variety of human diseases ranging from non-invasive diseases to severe invasive diseases. The group A streptococcus infection can be further complicated by non-suppurative sequelae, such as rheumatic fever, which usually causes throat infection and glomerulonephritis. A total number of 215 children screened for β-haemolytic streptococci from throat swabs and all culture positives are grouped by enzymatic extraction method and ASO estimation was done. All results were analysed statistically. Out of the 215 throat swabs 22.5% positive for β-haemolytic streptococci and grouped as group A 56.5% group C 31.1% group G 13.4%. All culture positives are sensitive to bacitracin 0.04 units and ASO estimated 54%. The proportion of antibiotic sensitivity was high for penicillin, vancomycin, cefotaxime and erythromycin, and the relative resistance to ampicillin and amoxicillin was seen. Our study suggests that the inappropriate use of antibiotic leads to drug resistance.

KEYWORDS: β-haemolytic, streptococcal grouping, ASO, bacitracin sensitivity.
Isolation of β-haemolytic streptococci and grouping by enzymatic extraction

MATERIALS AND METHODS

This study was done among 215 children in the age group of 5–15 years attending paediatric outpatient and inpatient wards of Government General Hospital, Rangaraya Medical College, Kakinada for a period of 24 months. Throat swabs were collected from tonsillar and posterior pharyngeal area, and the samples were processed according to the standard techniques.

Exclusion criteria

Oral antibiotic use within 3–5 days prior to the study or intramuscular antibiotic use within 28 days prior to the study.

Inclusion criteria

Sudden onset of throat pain followed by fever, headache, pain on swallowing, nausea, vomiting, thick exudates from posterior pharynx/tonsil.

Asymptomatic cases were also included in the study. Throat swabs were taken from tonsillar area and post-pharyngeal area. Specimens were processed by doing Gram staining and inoculated onto sheep blood agar and crystal violet blood agar. Isolation and identification of β-haemolytic streptococci by standard methods includes catalase test, antibiogram showing bacitracin sensitivity (Table 1). Blood samples were collected from positive culture patients, and ASO test was done.

Grouping was done to all β-haemolytic streptococci isolates by enzymatic extraction method (Streptococcal grouping latex test kit plasma tech).

RESULTS

A total number of 215 children were formed the study group. Among these, 9–10 years age group are the highest and 121 were male children. Out of 215 samples, 22.5% were culture positive for β-haemolytic streptococci. All β-haemolytic streptococci isolates were sensitive to bacitracin (0.04 units; Hi-Media). All culture positive β-haemolytic streptococci were grouped by latex test kit (plasma tech) as group A, C and G. Among these, 55.5% were group A in symptomatic cases and 69.46% in asymptomatic cases with high percentage.

All β-haemolytic streptococci screened for ASO showed 54% positivity. Antibiotic sensitivity test was performed by Kirby Bauer disc diffusion on sheep blood agar. The proportion of sensitivity was high for penicillin, vancomycin, cefotaxime and erythromycin. High proportion of resistance with cotrimoxazole was observed.

The results were analysed statistically for prevalence of β-haemolytic streptococci and antibiotic sensitivity using chi-square tests \( P > 0.05 \).

DISCUSSION

Group A β-haemolytic streptococcal pharyngitis is one of the most common bacterial diseases in human beings. Treatment and prevention of dangerous complications in this disease is of great importance. Healthy carriers of group A β-haemolytic streptococci are source for bacterial dissemination and are able to cause the disease and even lead to severe epidemics. According to different study group A, β-haemolytic streptococci is more seen in children pharynx to the adults.

Distribution of cases in the study population (215) 85 were symptomatic and 130 were asymptomatic. Out of 85 children, 18 were positive for β-haemolytic streptococci (21.15%) and out of 130 asymptomatic children 27 were positive for β-haemolytic streptococci (20.75%).

In our study, β-haemolytic streptococci were 22.5% culture positive among study group. In other studies, it has been reported as 28.5%^8.

Another prospective study from India found an incidence of group A streptococci culture positive throat in children to be 0.95% per child–year^9.

Group A streptococci was found in 20.75% of asymptomatic children as compared to 20.15% in children with acute respiratory tract infection in the present study.

In other study, it was found to be 19.5% of asymptomatic children as compared to 20% in children with acute respiratory tract infections^5. In our study, the prevalence rate is almost similar as per the above reference.

The recommended method of group A streptococcal identification is by testing β-haemolytic colonies on blood agar for group A specific carbohydrate antigen^10.

The distribution of isolates in group A (55.5%), group C (31.1%) and group G (13.3%). Out of this, highest percentage seen in group A that is (55.5%).

Maxted^11 first reported that Bacitracin can be used to differentiate group A and other streptococci. Originally, he used filter paper squares soaked in a solution containing five units of bacitracin per ml.

Levinson and Frank used standard filter paper disks showed that a solution contain one unit of Bacitracin per ml was equally as effective in differentiating group A β-haemolytic streptococcus^12. Both these reports concluded that any zone of inhibition by bacitracin was presumptive identification of β-haemolytic streptococcus.

Table 1: Isolation and grouping of β-haemolytic streptococci among study group.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Symptomatic ( n = 80 )</th>
<th>Asymptomatic ( n = 135 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture positive for β-haemolytic streptococci</td>
<td>18 (21.15%)</td>
<td>27 (20.75%)</td>
</tr>
<tr>
<td>Grouping by enzymatic extraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>10 (55.55%)</td>
<td>14 (51.85%)</td>
</tr>
<tr>
<td>C</td>
<td>5 (27.7%)</td>
<td>5 (18.25%)</td>
</tr>
<tr>
<td>G</td>
<td>3 (16.66%)</td>
<td>8 (21.65%)</td>
</tr>
</tbody>
</table>
In our study, the diameter of zone of inhibition using bacitracin disc 0.04 units was interpreted as, more than 10 or 20 mm was taken as criterion for identification. This was compared with known group A β-haemolytic streptococcal strains for quality control.

The diameter of inhibition zones around bacitracin for streptococcal isolate was high in the range of 10–14 mm (group A 10, group C 9) and the lowest zone of inhibition was seen 5–9 mm diameter.

Using Taxo A discs and zone of inhibition 10 mm more found good correlation between bacitracin screening and serological methods11.

Coleman et al. recommended using the presence of any zone with 0.04 units disc but use of a zone of 12 mm or more with 0.1 unit disc showed similar results with both methods14.

In asymptomatic children out of 18 culture positives, 10 (55.55%) were grouped as group A followed by 5 (27.7%) group C and 3 (16.6%) group G.

In asymptomatic children, out of 27 culture positives, 14 (51.85%) were grouped as group A followed by 5 (18.29%) group C and 8 (21.63%) group G. In other studies by Helen Pollock, grouped as group A (51.7%) and group G (7.6%) from upper respiratory tract isolates15. Antibody response to streptolysin O is known to occur in about 80% of patients with acute pharyngitis cases16.

In the present study, 54% of culture positive cases showed raised ASO (>200 IU) titer. (Life screen ASO kit Latex Agglutination method). The other study reveals that in asymptomatic cases ASO titers were elevated in 34 cases out of 91 (34%) and symptomatic cases ASO titers were 27 out of 260 (10.3%) which were similar to our study17.

Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method using penicillin, macrolides and 3rd generation cephalosporins. Resistance was seen in cotrimoxazole, relative resistance with ampicillin and amoxicillin was also seen.

In a study by Navaneeth et al., all samples of pharyngeal culture of group A β-haemolytic streptococci were sensitive to penicillin, erythromycin and cephalaxin18.

In our study, 99.3% were sensitive to penicillin, 91.11% were sensitive to vancomycin, 88.88% were sensitive to cefotaxime, 86.88% were sensitive to cephalaxin, 77.77% were sensitive to levofloxacin, 73.33% were sensitive to erythromycin and 35.55% showed resistance to ampicillin, 77.11% showed resistance to amoxicillin.

The present study revealed a slight decrease in susceptibility of group A streptococcal isolates with erythromycin and resistance is at low level.

In other study, group A streptococcal strain sensitive to erythromycin directly correlate with macrolide consumption19. Our study shows relative resistance ampicillin and amoxicillin. According to our study proper antibiotic usage can prevent increase of carrier state.

CONCLUSION

Microbiological diagnosis of group A streptococcal pharyngitis can be a challenging task at each stage. Given the magnitude of problem rheumatic fever/rheumatic heart disease in India and the need for its prevention, there is no doubt that bacteriological laboratories and expertise in India and the need for its prevention, will have to upgrade the diagnosis of group A streptococcal infection in the laboratory.

In our study, the prevalence of group A streptococcal isolates were compared to other group specially among 5–15 years age groups.

Antibiotic susceptibility testing though not a mandatory, may be very useful in detecting erythromycin resistance and penicillin tolerance. Over treatment with Penicillin may lead to subsequent penicillin tolerance.

REFERENCES

14. Coleman DJ, McGhie D, Tebutt GM. Further studies on the reliability of the bacitracin inhibition test for the presumptive
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