**Streptococcus pneumoniae AND Haemophilus influenzae AS ETIOLOGICAL AGENTS OF CONJUNCTIVITIS OUTBREAKS IN THE REGION OF RIBEIRÃO PRETO, SP, BRAZIL**

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**SUMMARY**

In the study of conjunctivitis outbreaks occurring from September 1994 to September 1996 in the region of Ribeirão Preto, conjunctival exudates of 92 patients were cultivated in Instituto Adolfo Lutz Laboratory I, Ribeirão Preto. Most cases occurred in the age range 2-7 years. The etiological agents which were most frequently isolated from the analyzed cases were: *Streptococcus pneumoniae* and *Haemophilus influenzae*, in 40.22% and 21.74%, respectively. 51.35% of the *S. pneumoniae* isolated strains were not typable. The oxacillin-resistant *S. pneumoniae* strains were submitted to the minimum inhibitory concentration test (MIC) and three of them presented intermediate resistance, whereas only one was highly resistant to penicillin.

**KEYWORDS:** *Streptococcus pneumoniae*; *Haemophilus influenzae*; Conjunctivitis; Brazilian purpuric fever.

**INTRODUCTION**

The conjunctiva is colonized by a stable bacterial microbiota which may become unstable due to the decrease of the host’s immunity or by the acquisition of a more virulent microorganism, causing the onset of bacterial conjunctivitis. Another possible cause of conjunctivitis is an inflammatory or allergic process.

Following the appearance of the Brazilian Purpuric Fever (B.P.F.), a fulminating invasive disease which is preceded by purulent conjunctivitis, caused by *Haemophilus influenzae* biogroup aegyptius, and which was firstly described in 1985, conjunctivitis surveillance was intensified in schools and day care centers aiming at controlling the disease. In outbreak cases, the culture of 10% of the conjunctivitis cases started to be carried out.

After September 1994, the occurrence of conjunctivitis outbreaks was observed in the region of Ribeirão Preto, the etiological agent of which was *Streptococcus pneumoniae*.

Although not frequently, *S. pneumoniae* has been related to conjunctivitis in adults and children.

When studying conjunctivitis outbreaks, SHAYEGANI et al. (1982) reported the isolation of non-typable *S. pneumoniae* as an etiological agent.

This study aims at characterizing the etiology of conjunctivitis in the region of Ribeirão Preto.

**PATIENTS AND METHODS**

From September 1994 to September 1996, 10% of the cases representing conjunctival exudation samples, collected from 92 patients in the age range 0-48 years, from the purulent conjunctivitis outbreaks occurring in the region of Ribeirão Preto were studied.

The samples were collected from the conjunctival sac and immediately inoculated on media chocolate blood agar (brain heart infusion blood agar base with 10% defibrinated sheep blood).

The cultures were incubated at 35/37°C for 24-48 hours in a CO₂ environment (5-10%) and humidity. The suspected colonies were microscopically investigated using Gram’s stain, modified by Hucker. Once Gram-positive diplococcus morphology was found, the strains isolated were tested as to its susceptibility to optochin and bile solubility, in order to confirm the assumed species. The strains identified as *S. pneumoniae* were sent to the Collaborative Center for Pneumococcus Reference of the World Health Organization (W.H.O.), at the School of Medicine, in Philadelphia, Pennsylvania, in order to be serotyped by the Quellung reaction using the sera produced by Statens Seruminstitut, Copenhagen, Denmark.

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All isolated S. pneumoniae strains were tested as to their assumed susceptibility to penicillin by the agar diffusion method using oxacillin discs (1 μg). Strains presenting an inhibition zone ≤ 19 mm for the oxacillin disc were submitted to the MIC test through the micro dilution method, according to the procedures of the National Committee for Clinical Laboratory Standards\(^\text{10}\).

The bacteria presenting Gram-negative bacillus morphology which were suspected to be Haemophilus sp were biochemically identified according to KILIAN & BIBERSTEIN (1984)\(^\text{10}\), as well as serotyped by the agglutination test on a slide with antisera produced by Instituto Adolfo Lutz – Central Laboratory, São Paulo.

## RESULTS

Ninety-two conjunctivitis cases were studied, 54 (58.70%) of which were positive. Among the etiological agents isolated, in 37 (40.22%) cases, S. pneumoniae was identified, 20 (21.74%) H. influenzae and 1 (1.09%) Staphylococcus aureus. In 4 cases, the association of S. pneumoniae and H. influenzae was observed (Table 1).

From the 37 S. pneumoniae strains isolated, 2 (5.41%) were of the 6A serotype, 4 (10.81%) of the 14 serotype, 1 (2.7%) of the 24F serotype, 1 (2.7%) of the 19F serotype, 19 (51.35%) were non-typable pneumococci, 7 (18.92%) are being characterized as to serotype and 3 strains (8.11%) were only characterized up to species level (Table 2).

Thirty-five S. pneumoniae strains were submitted to the susceptibility test to oxacillin and 30 presented an oxacillin-sensitive halo ≥ 20 mm, 5 presented an oxacillin-resistant halo ≤ 19 mm. From the resistant strains which were submitted to the MIC test, one presented susceptibility to penicillin (MIC = 0.06 μg/ml), 3 presented intermediate resistance (1 strain MIC = 0.5 μg/ml and 2 strains MIC = 0.25 μg/ml) and 1 was considered to be highly resistant to penicillin (MIC = 4.0 μg/ml).

As to the Haemophilus species, there was a predominance of H. influenzae biotype II, followed by biotype III and I (Table 3).

The age range of greater conjunctivitis prevalence was between 2 and 7 years with 36.96%, followed by 0 to 2 years (29.35%).

## DISCUSSION

The increased resistance of S. pneumoniae to penicillin has been observed in Brazil\(^\text{11}\), Spain\(^\text{12}\) as well as in several other countries\(^\text{11}\).

In their report, LEVIN et al. (1996)\(^\text{11}\) detected the presence of intermediate-resistance strains. In this study, in addition to intermediate-resistance strains, a strain with MIC = 4.0 μg/ml, which is considered to be highly resistant, was also found. This confirms the gradual resistance increase of S. pneumoniae to penicillin observed by LEVIN et al.\(^\text{11}\).

In this study, S. pneumoniae is associated with conjunctivitis cases, although its role as the cause of this disease is not so well established as that of H. influenzae\(^\text{11}\). In his article on the otitis-conjunctivitis syndrome, BODOR (1989)\(^\text{3}\) did not observe the appearance of mixed infection by H. influenzae and S. pneumoniae. In this report, this association was observed in 4.35% of the diagnosed cases.

As to seasonality, the greatest frequency of S. pneumoniae conjunctivitis was observed in spring and summer, which differed from what was found by RASKIN et al. (1993)\(^\text{3}\), who observed the occurrence of this agent in the cold months of the year.

## CONCLUSION

This study reinforces the necessity of a continuous conjunctivitis surveillance, especially during outbreaks, not only as a prophylactic measure aiming at preventing the appearance

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### TABLE 1

<table>
<thead>
<tr>
<th>Bacterial agents</th>
<th>Positive cases</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae(^\text{a})</td>
<td>37</td>
<td>40.22</td>
</tr>
<tr>
<td>Haemophilus influenzae(^\text{a})</td>
<td>20</td>
<td>21.74</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>01</td>
<td>1.09</td>
</tr>
<tr>
<td>Total of positive cases</td>
<td>58</td>
<td>58.70(^\text{a})</td>
</tr>
<tr>
<td>Total of negative cases</td>
<td>38</td>
<td>41.30</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>100.00</td>
</tr>
</tbody>
</table>

\(^\text{a}\) 4 cases in association of S. pneumoniae with H. influenzae.

### TABLE 2

<table>
<thead>
<tr>
<th>S. pneumoniae strains</th>
<th>Serotypes</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>02</td>
<td>6A</td>
<td>5.41</td>
</tr>
<tr>
<td>04</td>
<td>14</td>
<td>10.81</td>
</tr>
<tr>
<td>01</td>
<td>24F</td>
<td>2.70</td>
</tr>
<tr>
<td>01</td>
<td>19F</td>
<td>2.70</td>
</tr>
<tr>
<td>19</td>
<td>non-typable</td>
<td>51.35</td>
</tr>
<tr>
<td>03</td>
<td>dead strains</td>
<td>8.11</td>
</tr>
<tr>
<td>07</td>
<td>identification in course</td>
<td>18.92</td>
</tr>
</tbody>
</table>

### TABLE 3

<table>
<thead>
<tr>
<th>H. influenzae biotypes and serotypes isolated from conjunctivitis.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H. influenzae</strong></td>
</tr>
<tr>
<td>Biotype, serotype</td>
</tr>
<tr>
<td>I, b</td>
</tr>
<tr>
<td>I, non-b*</td>
</tr>
<tr>
<td>II, non-b*</td>
</tr>
<tr>
<td>II, b</td>
</tr>
<tr>
<td>III, non-b*</td>
</tr>
</tbody>
</table>

*non-b – the strains were not tested with the other type antisera.
of the B.P.F., but also in order to characterize other bacteria which are disseminated by this means, since conjunctivitis is highly contagious.

The appearance of non-typable *S. pneumoniae* as a cause of conjunctivitis has been repeated, which indicates that additional studies to reach the complete characterization of these strains are necessary. The isolation of one highly penicillin-resistant strain and of 3 with intermediate resistance as conjunctivitis etiological agents reinforces the importance of monitoring the susceptibility level of this species which is present in the community.

RESUMO

*Streptococcus pneumoniae* e *Haemophilus influenzae* como agentes etiológicos de surtos de conjuntivite na região de Ribeirão Preto, SP, Brasil

No estudo de surtos de conjuntivite ocorridos no período de setembro de 1994 a setembro de 1996, na região de Ribeirão Preto, foram semeadas no Instituto Adolfo Lutz Laboratório I, Ribeirão Preto, exsudatos conjuntivais de 92 pacientes, sendo que a maioria dos casos estava na faixa etária de 2-7 anos. Os agentes etiológicos mais frequentemente isolados dos casos analisados foram: *Streptococcus pneumoniae* e *Haemophilus influenzae* em 40,22% e 21,74% respectivamente. 51,35% das cepas de *S. pneumoniae* isoladas foram não tipáveis. As cepas de *S. pneumoniae* oxacilina resistente foram submetidas ao teste de concentração inibitória mínima (CIM), sendo que três apresentaram resistência intermediária e apenas uma foi altamente resistente à penicilina.

REFERENCES


