

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

Marta I. C. MEDEIROS(1), Suzel N. NEME(1), Paulo da SILVA(1), Jaqueline O. SILVA(1), Ana Maria M. CARNEIRO(1), Maria Claudia CARLONI(1) & Maria Cristina de C. BRANDILEONE(2)

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 process⁵.

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 aegyptius⁴, and which was firstly described in 1985⁶, 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^{12,16}.

When studying conjunctivitis outbreaks, SHAYEGANI et al. (1982)¹⁵ 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)¹³.

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 solubility⁷, 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 reaction² using the sera produced by Statens Serum Institut, Copenhagen, Denmark.

(1) Instituto Adolfo Lutz. Laboratório I de Ribeirão Preto, SP, Brasil.

(2) Instituto Adolfo Lutz - Central, São Paulo, SP, Brasil.

Correspondence to: Marta I. C. Medeiros. Instituto Adolfo Lutz, Lab. I de Rib. Preto, Rua Minas 877, 14085-410 Ribeirão Preto, SP, Brasil. Fax (+55.16) 635-7994.

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 Standards¹⁷.

The bacteria presenting Gram-negative bacillus morphology which were suspected to be *Haemophilus* sp were biochemically identified according to KILIAN & BIBERSTEIN (1984)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 pneumococcus, 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%).

TABLE 1

Bacterial agents identified from 92 purulent conjunctivitis cases.

Bacterial agents	Positive cases	(%)
<i>Streptococcus pneumoniae</i> ^(a)	37	40.22
<i>Haemophilus influenzae</i> ^(a)	20	21.74
<i>Staphylococcus aureus</i>	01	1.09
Total of positive cases	58	58.70 ^(a)
Total of negative cases	38	41.30
Total	92	100.00

^(a) 4 cases in association of *S. pneumoniae* with *H. influenzae*.

TABLE 2

Streptococcus pneumoniae serotypes isolated from conjunctivitis.

No. of <i>S. pneumoniae</i> strains	Serotypes	(%)
02	6A	5.41
04	14	10.81
01	24F	2.70
01	19F	2.70
19	non-typable	51.35
03	dead strains	8.11
07	identification in course	18.92

TABLE 3

H. influenzae biotypes and serotypes isolated from conjunctivitis.

<i>H. influenzae</i> Biotype, serotype	Total	(%)
I, b	01	05
I, non-b*	01	05
II, non-b*	14	70
II, b	02	10
III, non-b*	02	10

*non-b – the strains were not tested with the other type antisera.

DISCUSSION

The increased resistance of *S. pneumoniae* to penicillin has been observed in Brazil¹¹, Spain⁸ as well as in several other countries¹.

In their report, LEVIN et al. (1996)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.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*⁹. In his article on the otitis-conjunctivitis syndrome, BODOR (1989)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)¹⁴, 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

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

1. APPELBAUM, P. C. – Antimicrobial resistance in *Streptococcus pneumoniae*: an overview. **Clin. infect. Dis.**, 15: 77-83, 1992.
2. AUSTRIAN, R. – The Quellung reaction, a neglected microbiology technique. **Mt. Sinai J. Med.**, 43: 699-705, 1976.
3. BODOR, F. F. – Systemic antibiotics for treatment of the conjunctivitis – otitis media syndrome. **Pediat. infect. Dis.**, 8: 287-290, 1989.
4. BRENNER, D.; MAYER, L. & CARLONE, G. – Biochemical, genetic and epidemiologic characterization of *H. influenzae* biogroup aegyptius (*H. aegyptius*) strain associated with Brazilian purpuric fever. **J. clin. Microbiol.**, 26: 1524, 1988.

5. CASTELO BRANCO, A. B.; FREITAS, D. & BELFORT, R. J. – Como diagnosticar e tratar conjuntivites. **Rev. bras. Med.**, 52: 57-64, 1995.
6. CDC – Preliminary report: epidemic fatal purpuric fever among children, Brazil. **MMWR**, 34: 217, 1985.
7. FACKLAM, R. R. & WASHINGTON II, J. A. – *Streptococcus* and related catalase negative Gram, positive cocci. In: BALOWS, A.; HAUSLER Jr., W. J.; HERRMANN, K. L. et al. – **Manual of clinical microbiology**. 5 ed. Washington, American Society for Microbiology, 1991. p. 238-240.
8. GARCIA-LEONI, M. E.; CERCENATO, P. R.; QUIRÓS, J. C. L. B. et al. – Susceptibility of *Streptococcus pneumoniae* to penicillin: a prospective microbiological and clinical study. **Clin. infect. Dis.**, 14: 427-435, 1992.
9. GIGLIOTTI, F.; WILLIAMS, W. T.; HAYDEN, F. G. et al. – Etiology of acute conjunctivitis in children. **J. Pediat.**, 98: 531-536, 1981.
10. KILIAN, M. & BIBERSTEIN, E. L. – *Haemophilus*. In: KRIEC, N. R. & HOLT, J. G. – **Bergey's manual of systematic bacteriology**. Baltimore, Williams & Wilkins, 1984. v. 1, p. 558-569.
11. LEVIN, A. S. S.; TEIXEIRA, L. M.; SESSEGOLO, J. F. et al. – Resistance of *Streptococcus pneumoniae* to antimicrobials in São Paulo, Brazil: clinical features and serotypes. **Rev. Inst. Med. trop. S. Paulo**, 38: 187-192, 1996.
12. LIMBERG, M. A. – Review of bacterial keratitis and bacterial conjunctivitis. **Amer. J. Ophthalm.**, 112: 34-36, 1991.
13. NASH, P. & KRENZ, M. M. – Culture media. In: BALOWS, A.; HAUSLER Jr., W. J.; HERRMANN, K. L. et al. – **Manual of clinical microbiology**. 5 ed. Washington, American Society for Microbiology, 1991. p. 1226-1228.
14. RASKIN, M.; ROCHA, M. M. M.; LANDGRAF, I. M. et al. – Incidência do gênero *Haemophilus* nas conjuntivites purulentas em crianças na região de Campinas, SP. **Rev. Inst. Adolfo Lutz**, 53: 59-62, 1993.
15. SHAYEGANI, M.; PARSONS, L. M.; GIBBONS, W. E. et al. – Characterization of nontypable *Streptococcus pneumoniae* – like organisms isolated from outbreaks of conjunctivitis. **J. clin. Microbiol.**, 16: 8-14, 1982.
16. TERRY, J. E. – Bacterial like organisms isolated from conjunctivitis. **J. Amer. Optom. Ass.**, 55: 587-590, 1984.
17. NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS – Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 2. ed. Villanova, National Committee for Clinical Laboratory Standards, 1995 (Approved Standard M7-A2).

Recebido para publicação em 03/09/1997

Aceito para publicação em 18/12/1997