JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1995, p. 2789–2791 Vol. 33, No. 10
0095-1137/95/$04.00+0

Distribution of Serotypes of *Streptococcus pneumoniae* Isolated from Invasive Infections over a 16-Year Period in the Greater São Paulo Area, Brazil

MARIA CRISTINA DE CUNTO BRANDILEONE,[1]* VERA SIMONSEN DIAS VIEIRA,[1] ROSEMEIRE COBO ZANELLA,[1] ILKA MARIA LANDGRAF,[1] CARMO ELIAS DE ANDRADE MELLES,[1] AUGUSTO DE ESCRAGNOLE TAUNAY,[1] JOSÉ CÁSSIO DE MORAES,[2] AND ROBERT AUSTRIAN[3]

Bacteriology Division, Adolfo Lutz Institute,[1] and Center for Epidemiological Vigilance of São Paulo State,[2] São Paulo, Brazil, and Department of Research Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania[3]

Received 29 December 1994/Returned for modification 16 February 1995/Accepted 3 July 1995

Capsular types of pneumococci from normally sterile body sites of 1,622 patients in Brazil were analyzed. Of 1,477 isolates from cerebrospinal fluid, 76.1% were of types represented in the currently available pneumococcal vaccine. The importance of age, time, and place in determining the optimal formulation of pneumococcal vaccine is considered.

Pneumococcal infections occur in all human societies and remain a major cause of morbidity and mortality, especially in infants, young children, and the elderly. In the developing countries, it is estimated that more than 1 million children die annually from pneumococcal pneumonia and that, of these, half are less than 1 year of age (31).

In 1993, in the greater São Paulo area, Brazil (capital of the state of São Paulo and 36 nearby municipalities with an estimated population of 17,000,000, in the southern region of Brazil), *Streptococcus pneumoniae* was the second most common cause of meningitis, with 1.5 notified cases per 100,000 individuals per year (Center of Epidemic Vigilance of São Paulo State). It was exceeded in frequency only by *Neisseria meningitidis*, the cause of an epidemic (4.72 cases per 100,000 individuals per annum), and nearly equaled by *Haemophilus influenzae* (0.9 case per 100,000 individuals per annum). Data on the etiology of pneumonia are not available for lack of systematic surveillance of acute respiratory infections in Brazil, but data from the Adolfo Lutz Institute in the city of São Paulo have shown that *S. pneumoniae* is the bacterium isolated most commonly in that area from patients with pneumonia.

Pneumococci are classified into 84 serotypes, each determined by a chemically distinct capsular polysaccharide (6). These macromolecules induce serum antibodies which are essential to immunity to a given serotype. In the United States, it has been found that the 23 serotypes represented in the currently licensed polyvalent vaccine, which has an aggregate efficacy of 60 to 70% in preventing bacteremic illness in adults (i.e., illness caused by types represented in the vaccine) (21), account for 85 to 90% of pneumococcal infections (25). Like other vaccines consisting solely of bacterial polysaccharides, this vaccine has shown little protectivity in infants and young children (1, 7, 12, 16, 26, 27).

At present, there is no public health program for immunization against pneumococcal infection in Brazil, although vaccine is available in private clinics for those at high risk. Because the distribution of serotypes is important in the formulation of vaccine for a given target population and may vary with age, time, and place (4, 9, 11, 13), it was thought important to augment the limited data available in Brazil (23, 28, 29). The present study is a retrospective analysis of 1,477 pneumococcal isolates from cerebrospinal fluid of patients with meningitis from 1977 through 1992 and from blood or pleural fluid of 146 patients with pneumonia from 1978 through 1992. Some of the data were included in an earlier report (29). Most of the patients admitted to the study were residents of the greater São Paulo area. Their ages ranged from birth to 81 years; for analysis, patients with isolates from cerebrospinal fluid were divided into four age groups. When isolates of the same type were obtained from several sites, they were treated as a single isolate. In one instance, pneumococci of different types were identified in the same specimen of cerebrospinal fluid.

After demonstration of their sensitivity to optochin (17), the strains were typed at the World Health Organization Collaborating Center for Reference and Research on Pneumococci at the University of Pennsylvania Medical Center, Philadelphia, by the Quellung reaction (3) with sera provided from the Statens Seruminstitut, Copenhagen, Denmark (15), and factor sera provided generously by Jørgen Henrichsen.

The distribution of capsular serotypes in isolates from cerebrospinal fluid is shown in Table 1. Four strains were grouped but not typed because of the temporary unavailability of factor sera. As noted earlier, the distribution of types differed with age (Table 2). In the first year of life, types represented in the currently licensed vaccine accounted for 83.1% of 355 isolates and, among patients 1 to 9 years of age, for 74.9% of 290 isolates, the chief difference arising from the large number of infections caused by pneumococcus type 5 in the first year of life. If types cross-reacting with those in the vaccine are added, the fraction of strains rises to 89.6% in the youngest group and 89% in patients 1 to 9 years of age. In the group aged 10 to 49 years, the respective values are 73.4 and 81.5%. Because the pathogenesis of pneumococcal meningitis differs from that of pneumonia and bacteremia, it is not unexpected that the fraction of strains represented in the vaccine causing the former illness is somewhat less than that observed in the latter. Among the 146 patients with bacteremic infections associated with

* Corresponding author.

TABLE 1. Distribution of serotypes of *S. pneumoniae* isolated from meningitis patients in the greater São Paulo area from 1977 through 1992[a]

Serotype in Danish system	No. of isolates	% of total isolates	Serotype in Danish system	No. of isolates	% of total isolates
1[b]	170	11.5	15C[c]	8	0.5
6B[b]	108	7.3	15A[c]	8	0.5
14[b]	99	6.7	23A[c]	7	0.5
6A[c]	88	6.0	15F[c]	6	0.4
18C[b]	82	5.6	33F[b]	6	0.4
3[b]	76	5.1	35B	5	0.3
5[b]	74	5.0	31	5	0.3
23F[b]	69	4.7	7C[b]	5	0.3
19F[b]	63	4.3	21	5	0.3
7F[b]	49	3.3	40	5	0.3
12F[b]	48	3.2	39	4	0.3
4[b]	43	2.9	18B[c]	4	0.3
10A[b]	33	2.2	25	4	0.3
8[b]	31	2.1	7B	3	0.2
9V[b]	31	2.1	37	3	0.2
9N[b]	30	2.0	35A	3	0.2
19A[b]	29	2.0	45	3	0.2
16F	23	1.6	33A	3	0.2
17F[b]	23	1.6	27	2	0.1
34	22	1.5	12A	2	0.1
15B[b]	20	1.4	10F	2	0.1
20[b]	20	1.4	11B[c]	2	0.1
23B[c]	18	1.2	9[d]	2	0.1
18A[c]	18	1.2	12[d]	1	0.1
13	17	1.2	19[d]	1	0.1
38	17	1.2	35F	1	0.1
28A	15	1.0	33B	1	0.1
29	13	0.9	18F	1	0.1
11A[b]	13	0.9	19B[c]	1	0.1
2[b]	12	0.8	48	1	0.1
24F	9	0.6	19C[c]	1	0.1
22F[b]	8	0.5	18[d]	1	0.1

[a] Total number of isolates, 1,477.
[b] Serotypes (n = 23) included in the polyvalent vaccine.
[c] Serotypes cross-reactive with vaccine serotypes.
[d] Factor sera not available at the time of study.

TABLE 2. Relationship between age group and serotypes of *S. pneumoniae* isolated from patients with meningitis in the greater São Paulo area from 1977 and 1992

Serotype	No. of isolates from age group:					Total no. of isolates	%
	0–11 mo	1–9 yr	10–49 yr	50–>64 yr	Unknown		
1	23	31	98	2	16	169	11.4
2	6	0	0	1	5	12	0.8
3	3	10	32	14	17	76	5.1
4	8	7	16	1	11	43	2.9
5	38	9	13	5	9	74	5.0
6A	18	28	27	1	14	88	6.0
6B	41	30	18	2	17	108	7.3
7F	11	3	18	3	14	49	3.3
8	4	1	17	4	5	31	2.1
9N	10	4	11	2	3	30	2.0
9V	7	9	8	1	6	31	2.1
10A	7	6	12	1	7	33	2.2
11A	1	7	3	0	2	13	0.9
12F	7	2	27	3	9	48	3.3
13	0	1	8	2	6	17	1.2
14	45	26	7	2	19	99	6.7
15B	5	2	11	0	2	20	1.4
16F	2	7	8	3	3	23	1.6
17F	3	5	13	1	1	23	1.6
18A	3	5	7	0	3	18	1.2
18C	24	20	23	3	12	82	5.6
19F	22	13	15	2	11	63	4.3
19A	6	8	7	1	7	29	2.0
20	4	7	7	0	2	20	1.4
23F	20	17	18	1	13	69	4.7
23B	2	6	7	0	3	18	1.2
28A	1	2	5	3	4	15	1.0
29	8	1	4	0	0	13	0.9
34	1	2	11	3	5	22	1.5
38	2	1	7	3	4	17	1.2
Other	23	20	51	1	28	123	8.3
Total	355	290	509	65	258	1,477	100

pneumonia, the predominant types were 14, 1, 5, 6B, 9V, 4, and 6A. In this group, which is composed predominantly of children under 10 years of age and which is too small to draw firm conclusions from, the pneumococcal types represented in the vaccine accounted for 131 of the infections or 89.7%, a fraction in accordance with that found in other areas.

Several additional observations emerge. One is the decline in the proportion of infections caused by pneumococcus type 1, from approximately 14% in the first 12 years of study to 5% in the last 4 years; a similar observation was made earlier in the United States, where in 1993, pneumococcus type 1 accounted for only 0.7% of 1,197 typed isolates from blood cultures (5a). Similarly, pneumococcus type 5, isolated rarely in North America (5, 20, 25) and northern Europe (10) in recent years, has been a common cause of infection in developing countries (8, 18, 19, 22, 24), especially in the infant population, making its polysaccharide a possible candidate for inclusion in a conjugate vaccine for these areas.

The continuing, albeit slow, shifts in the frequency of pneumococcal types causing serious infection together with the increasing frequency of illness caused by drug-resistant strains (2, 14, 30) make regular monitoring of the distribution of such serotypes essential. Only through the vigilance of laboratories throughout the world can an optimal formulation of vaccines to prevent such illness at different times and in different places be achieved.

We thank the research teams from the Instituto da Criança do Hospital das Clínicas, Santa Casa de Misericórdia, Hospital Universitário, and Instituto de Infectologia Emílio Ribas of São Paulo.

REFERENCES

1. **Advisory Committee on Immunization Practices.** 1989. Pneumococcal polysaccharide vaccine. Morbid. Mortal. Weekly Rep. **38:**64–68, 73–76.
2. **Applebaum, P. C.** 1992. Antimicrobial resistance in *Streptococcus pneumoniae*: an overview. Clin. Infect. Dis. **15:**77–83.
3. **Austrian, R.** 1976. The Quellung reaction, a neglected microbiologic technique. Mt. Sinai J. Med. **43:**699–705.
4. **Austrian, R.** 1981. Some observations on the pneumococcus and on the current status of pneumococcal disease and its prevention. Rev. Infect. Dis. **3**(Suppl.)**:**S1–S17.
5. **Austrian, R.** 1993. Preventing pneumococcal infection in Philadelphia. Philadelphia Med. **89:**10–16.
5a. **Austrian, R.** Unpublished data.
6. **Austrian, R., C. Boettger, M. Dole, L. Fairly, and M. Freid.** 1985. *Streptococcus pneumoniae* type 16A, a hitherto undescribed pneumococcal type. J. Clin. Microbiol. **22:**127–128.
7. **Baltimore, R. S.** 1992. New challenges in the development of a conjugate pneumococcal vaccine. JAMA **268:**3366–3367.
8. **Berman, S.** 1991. Epidemiology of acute respiratory infections in children of developing countries. Rev. Infect. Dis. **13**(Suppl. 6)**:**S454–S462.
9. **Broome, C. V., R. R. Facklam, J. R. Allen, D. W. Fraser, and R. Austrian.** 1980. Epidemiology of pneumococcal serotypes in the United States. J. Infect. Dis. **141:**119–123.
10. **Burman, L. A., B. Trollfors, R. Norrby, E. Falsen, S. Haidl, and J. Henrichsen.** 1986. Serotype distribution of *Streptococcus pneumoniae* strains isolated

from blood and cerebrospinal fluid in Sweden. Scand. J. Infect. Dis. **18:**45–48.
11. **Finland, M., and M. W. Barnes.** 1977. Changes in the occurrence of capsular serotypes of *Streptococcus pneumoniae* at Boston City Hospital during selected years between 1935 and 1974. J. Clin. Microbiol. **5:**154–166.
12. **Giebink, G. S.** 1985. Preventing pneumococcal disease in children: recommendations for using pneumococcal vaccine. Pediatr. Infect. Dis. **4:**343–348.
13. **Gray, B. M., G. M. Converse III, and H. C. Dillon, Jr.** 1980. Epidemiologic studies of *Streptococcus pneumoniae* in infants: acquisition, carriage, and infection during the first 24 months of life. J. Infect. Dis. **142:**923–933.
14. **Haglung, L. A., G. R. Istre, D. A. Pickett, D. F. Welch, D. P. Fine, and the Pneumococcus Study Group.** 1993. Invasive pneumococcal disease in Central Oklahoma: emergence of high-level penicillin resistance and multiple antibiotic resistance. J. Infect. Dis. **168:**1532–1536.
15. **Henrichsen, J.** 1979. The pneumococcal typing system and pneumococcal surveillance. J. Infect. Dis. **1**(Suppl. 2)**:**31–37.
16. **Keyserling, H. L., E. L. Anderson, and J. L. Martin.** 1993. Immunogenicity of a tetravalent (types 6B, 14, 19F, 23F) pneumococcal conjugate vaccine in infants. Pediatr. Res. **33:**172A. (Abstr. 1016.)
17. **Lund, E., and J. Henrichsen.** 1978. Laboratory diagnosis, serology and epidemiology of *Streptococcus pneumoniae*, p. 242–262. *In* T. Bergan and J. R. Norris (ed.), Methods in microbiology. Academic Press, London.
18. **Mogdasy, M. C., T. Camou, C. Fajardo, and M. Horta.** 1992. Colonizing and invasive strains of *Streptococcus pneumoniae* in Uruguayan children: type distribution and patterns of antibiotic resistance. Pediatr. Infect. Dis. J. **11:** 648–652.
19. **Monto, A. S.** 1989. Acute respiratory infection in children of developing countries: challenge for the 1990s. Rev. Infect. Dis. **11:**498–505.
20. **Orange, M., and B. M. Gray.** 1993. Pneumococcal serotypes causing disease in children in Alabama. Pediatr. Infect. Dis. J. **12:**244–246.
21. **Robbins, J. B., R. Austrian, C. J. Lee, S. C. Rastogi, G. Schiffman, J. Henrichsen, P. H. Makela, C. V. Bromme, R. R. Facklam, R. M. Tiesjema, and J. C. Parke, Jr.** 1983. Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. J. Infect. Dis. **148:**1136–1159.
22. **Selwyn, B. J.** 1990. The epidemiology of acute respiratory tract infection in young children: comparison of findings from several developing countries. Rev. Infect. Dis. **12**(Suppl. 8)**:**S870–S888.
23. **Sessegolo, J. F., A. S. S. Levin, C. E. Levy, M. Asensi, R. R. Facklam, and L. M. Teixeira.** 1994. Distribution of serotypes and antimicrobial resistance of *Streptococcus pneumoniae* strains isolated in Brazil from 1988 to 1992. J. Clin. Microbiol. **32:**906–911.
24. **Shann, F.** 1986. Etiology of severe pneumonia in children in developing countries. Pediatr. Infect. Dis. J. **5:**247–252.
25. **Shapiro, E. D., and R. Austrian.** 1994. Serotypes responsible for invasive *Streptococcus pneumoniae* infections among children in Connecticut. J. Infect. Dis. **169:**212–214.
26. **Shapiro, E. D., A. T. Berg, R. Austrian, D. Schroeder, V. Parcells, and A. Margolis.** 1991. The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. N. Engl. J. Med. **325:**1453–1460.
27. **Sniadack, D. H., R. F. Breiman, and W. W. Willians.** 1992. Pneumococcal vaccine in the 1990s, p. 58–61. *In* M. L. Cohen (ed.), Infectious diseases in clinical practice, vol. 1. CDC/NCID report. Centers for Disease Control and Prevention, Atlanta.
28. **Taunay, A. E., R. Austrian, I. M. Landgraf, M. F. P. Vieira, and C. E. A. Melles.** 1990. Sorotipos de *Streptococcus pneumoniae* isolados de líquido cefalorraquidiano no periodo de 1977–1988 na cidade de São Paulo, Brasil. Rev. Inst. Med. Trop. São Paulo **32:**11–15.
29. **Teixeira, L. M., J. R. Andrade, and N. J. Lourenço.** 1988. Serotypes and antimicrobial susceptibility of *Streptococcus pneumoniae* isolated in Rio de Janeiro, Brazil. Rev. Microbiol. **19:**93–99.
30. **Ward, J.** 1981. Antibiotic-resistant *Streptococcus pneumoniae*: clinical and epidemiologic aspects. J. Infect. Dis. **3:**254–266.
31. **World Health Organization.** 1993. Programme for the control of acute respiratory infections: pneumococcal conjugate vaccines. Report of a meeting. WHO/ARI/94.34. World Health Organization, Geneva.