JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2002, p. 275–277 Vol. 40, No. 1
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.1.275–277.2002

Population-Based Survey of Antimicrobial Susceptibility and Serotype Distribution of *Streptococcus pneumoniae* from Meningitis Patients in Salvador, Brazil

Joice Neves Reis,[1,2] Soraia Machado Cordeiro,[1] Steven J. Coppola,[3] Kátia Salgado,[4] Maria G. S. Carvalho,[5] Lúcia M. Teixeira,[5] Terry A. Thompson,[6] Richard R. Facklam,[6] Mitermayer G. Reis,[1] and Albert I. Ko[1,3]*

Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Ministério da Saúde,[1] Faculdade de Farmácia, Universidade Federal da Bahia,[2] and Hospital Couto Maia, Secretaria da Saúde do Estado da Bahia, Salvador,[4] and Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro,[5] Brazil; Division of International Medicine and Infectious Diseases, Department of Medicine, Weill Medical College of Cornell University, New York, New York 10021[3]; and Streptococcus Laboratory, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[6]

Received 11 June 2001/Returned for modification 10 August 2001/Accepted 9 October 2001

Penicillin-nonsusceptible strains were isolated from 15% of 303 individuals with pneumococcal meningitis identified during a 4-year surveillance study in Salvador, Brazil. The estimated rate of coverage of the seven-valent conjugate vaccine was 74% among patients <5 years of age and 94% among those infected with nonsusceptible isolates, indicating that the use of conjugate vaccines may be an approach to the control of emerging penicillin resistance in Brazil.

Pneumococcal disease is responsible for over 1 million deaths each year in children under 5 years of age (22). Its public health impact has been further compounded by the global emergence of penicillin-resistant *Streptococcus pneumoniae* (1). Resistant strains have been isolated in all continents (1) and in several countries; over 50% of the clinical isolates demonstrate high-level resistance to penicillin (7, 19).

With the emergence of penicillin resistance, it is ever more urgent that surveillance be implemented in developing countries, where conditions of poverty contribute to an already large burden of pneumococcal disease (25, 26). In Latin America, surveys of reference collections of isolates (13, 24) and laboratory-based surveillance (2, 3, 5, 15) have documented penicillin resistance in up to 20% of clinical isolates. However, significant variabilities in antimicrobial susceptibility and serotype patterns have been observed between and within countries (2, 3, 15). These findings may be due, in part, to regional differences in case ascertainment and isolation procedures (12) and emphasize the importance of population-based information in guiding antibiotic control strategies and national vaccine policies.

Active surveillance for pneumococcal meningitis was established at the state infectious disease hospital in the city of Salvador, Brazil. The state health secretary requires that suspected cases of meningitis from the metropolitan region be referred to that hospital, and more than 95% of the reports of meningitis from the region are reported from that site (secretary of health for the state of Bahia, unpublished case notification records). Therefore, patients referred to that hospital represent patients with population-based cases of meningitis occurring in this city. Between December 1995 and November 1999, 317 patients were consecutively identified with cerebrospinal fluid cultures positive for *S. pneumoniae*, as determined by morphology on Gram staining of smears, susceptibility to optochin (Difco Laboratories, Detroit, Mich.), and bile solubility (BBL Microbiology Systems, Cockeysville, Md.). After enrollment of patients by protocols approved by the institutional review boards of the Brazilian Ministry of Health and Weill Medical College of Cornell University, information on the demographic characteristics and the clinical presentations of the patients was obtained for 305 patients (97% of 317 patients) during interviews or medical chart review. On the basis of the fact that 140 patients who resided within the municipal borders of Salvador (population in 1996, 2,211,467) (14) had pneumococcal meningitis, the annual incidences of pneumococcal meningitis were 1.6 and 24.7 per 100,000 person-years for all age groups and children <5 years of age, respectively. The overall mortality rates were 42% (127 of 305) for all patients and 60% (92 of 153) for those <5 years of age.

Among 303 isolates tested by the broth microdilution method (21), 46 (15%) were penicillin nonsusceptible (MICs, 0.125 to 1.0 μg/ml) and all were susceptible to cefotaxime (Table 1). Patients <5 years of age had a greater chance of being infected with penicillin-nonsusceptible isolates than those ≥5 years of age (35 versus 9%, respectively; odd's ratio [OR], 4.8; 95% confidence interval [CI], 2.2 to 11.4). In addition, greater proportions of isolates from patients <5 years of age were not susceptible to co-trimoxazole (41% [60 of 145]) or tetracycline (27% [39 of 145]). The drugs involved in the most frequently identified patterns of multidrug nonsusceptibility, defined as being intermediate or resistant to two or more classes of antibiotics, were penicillin and co-trimoxazole (33 of 303 isolates [11%]) and tetracycline and co-trimoxazole (33 of 303 isolates [11%]). Children <5 years of age had a greater

* Corresponding author. Mailing address: Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz/MS, Rua Waldemar Falcão, 121, Salvador, Bahia 40295-001, Brazil. Phone: (55-71) 356-4320. Fax: (55-71) 356-2155. E-mail: aik2001@med.cornell.edu.

TABLE 1. Antimicrobial susceptibilities of 303 *S. pneumoniae* isolates from patients with meningitis identified during 4 years of active surveillance in Salvador, Brazil

Antimicrobial agent	MIC (μg/ml)[a]			No. (%) of strains that were[b]:		
	Range	MIC_{50}	$MIC_{90\%}$	S	I	R
Cefotaxime	0.008–0.5	0.016	0.125	303 (100)	—	—
Chloramphenicol	0.062–8	2.0	4.0	297 (98.0)	NA	6 (2.0)
Clindamycin	0.008–0.5	0.032	0.125	303 (100)	—	—
Erythromycin	0.008–1	0.032	0.0625	301 (99.3)	2 (0.7)	—
Ofloxacin	0.031–4	1.0	2.0	299 (99.0)	4 (1.0)	—
Penicillin	0.008–1	0.016	0.25	257 (85.0)	46 (15.0)	—
Rifampin	0.008–4	0.016	0.0625	302 (99.7)	—	1 (0.3)
Tetracycline	0.031–32	0.25	32.0	219 (72.0)	16 (5.0)	68 (23.0)
Co-trimoxazole	0.062–8	0.5	4.0	198 (65.0)	75 (25.0)	30 (10.0)
Vancomycin	0.016–1	0.5	0.5	303 (100)	NA	—

[a] MICs were determined by the broth microdilution method (21). MIC_{50} and MIC_{90}, concentrations at which the growth of 50 and 90%, respectively, of the isolates is inhibited.
[b] S, susceptible; I, intermediate; R, resistant; NA, not applicable. The breakpoints used to define susceptibility categories were those recommended by the National Committee for Clinical Laboratory Standards (21). —, no isolates were identified.

chance of being infected with penicillin- and co-trimoxazole-nonsusceptible isolates (OR, 4.3; 95% CI, 1.7 to 11.4).

The 303 isolates were distributed among 29 serogroups and 43 serotypes, as determined by the capsular swelling method with type-specific antisera (Centers for Disease Control and Prevention, Atlanta, Ga.) (Table 2). Among isolates from 145 patients <5 years of age, none were serotype 1 and 2.7% (4 of 145) were serotype 5, whereas serotype 3 was isolated from 2.1% (3 of 145) of the patients. In patients <5 years of age, 74, 77, and 82% were infected with isolates with serotypes that are represented in the 7-, 9-, and 11-valent conjugate vaccines, respectively (Table 2).

Penicillin-nonsusceptible isolates were restricted to six serotypes: 14 (n = 26 [56%]), 6B (n = 10 [22%]), 23F (n = 5 [11%]), 19F (n = 2 [4%]), 19A (n = 2 [4%]), and 23B (n = 1 [2%]). Serotypes 14 (OR, 18.3; 95% CI, 8.5 to 39.3) and 6B (OR, 3.7; 95% CI, 1.6 to 8.6) were associated with decreased sensitivity to penicillin ($P < 0.05$). Among the 35 penicillin-nonsusceptible isolates from patients <5 years of age, 94% (33 of 35) had serotypes represented in the three conjugate vaccines.

The finding that a significant (15%) proportion of cases of pneumococcal infection identified during population-based surveillance were due to penicillin-nonsusceptible pneumococci has important implications for the treatment of pneumococcal meningitis in Brazil. Penicillin, often in combination with chloramphenicol, is the recommended empirical treatment regimen for meningitis (9). The current consensus is that penicillin and chloramphenicol are not adequate for the treatment of meningitis caused by resistant pneumococci, even when strains demonstrate intermediate-level resistance (4, 8, 16). In the present study, all penicillin-nonsusceptible isolates were susceptible to cefotaxime, indicating that an extended-spectrum cephalosporin alone may be an appropriate alternative treatment for penicillin-resistant pneumococcal meningitis and empirical, initial therapy for suspected bacterial meningitis.

Our findings on serotype distributions support those from laboratory-based surveys in Brazil (2, 5, 24), indicating that a limited spectrum of serotypes is responsible for invasive pneumococcal disease (10, 15, 17). However, the pattern of predominant serotypes observed in the present study differs from those reported in other regions in Brazil (2, 24) and other

TABLE 2. Serotypes of pneumococcal isolates from meningitis patients in Salvador, Brazil, by age group[a]

Capsular serotype	No. (% of total) of isolates from patients:		
	Ages <5 yr	Ages ≥5 yr	Total
Total	145	146	291
14	40 (27.4)	1 (0.7)	41 (14.0)
3	3 (2.1)	27 (18.5)	30 (10.0)
6B	17 (11.6)	9 (6.2)	26 (9.0)
19F	10 (6.8)	15 (10.0)	25 (8.6)
6A	12 (8.2)	5 (3.4)	17 (5.8)
23F	7 (4.8)	9 (6.2)	16 (5.5)
18C	9 (6.2)	5 (3.4)	14 (4.8)
4	6 (4.1)	7 (4.8)	13 (4.5)
8	6 (4.1)	7 (4.8)	13 (4.5)
10A	2 (1.4)	8 (5.5)	10 (3.4)
9N	3 (2.1)	5 (3.4)	8 (2.7)
7F	4 (2.7)	2 (1.4)	6 (2.1)
5	4 (2.7)	1 (0.7)	5 (1.7)
23B	—[b]	5 (3.4)	5 (1.7)
13	1 (0.7)	4 (2.8)	5 (1.7)
11A	—	4 (2.8)	4 (1.4)
15B	—	4 (2.8)	4 (1.4)
17F	3 (2.1)	1 (0.7)	4 (1.4)
18F	3 (2.1)	1 (0.7)	4 (1.4)
19A	2 (1.4)	2 (1.4)	4 (1.4)
Other types[c]	13 (8.9)	24 (16.4)	37 (12.7)
23-valent polysaccharide vaccine types[d]	131 (90)	115 (79)	246 (85)
7-valent conjugate vaccine types[e]	108 (74)	54 (37)	162 (56)
9-valent conjugate vaccine types[f]	112 (77)	56 (39)	168 (58)
11-valent conjugate vaccine type[g]	119 (82)	85 (58)	204 (70)

[a] Results are shown for the 291 isolates for which information on patient's age was recorded.
[b] —, no isolates were identified.
[c] Other types include 18B (n = 3), 28A (n = 3), 7B (n = 3), 7C (n = 3), 9V (n = 3), 10F (n = 2), 16 (n = 2), 21 (n = 2), 34 (n = 2), 35B (n = 2), 1 (n = 1), 12F (n = 1), 15C (n = 1), 18A (n = 1), 22F (n = 1), 24F (n = 1), 27 (n = 1), 28B (n = 1), 35A (n = 1), 35F (n = 1), 38 (n = 1), and 48 (n = 1).
[d] Includes capsular serotypes 14, 3, 6, 19F, 23F, 4, 18C, 8, 10A, 9N, 5, 7F, 11A, 15B, 17F, 19A, 9V, 1, 12F, 20, 22F, 2, and 33F (Merck Sharp & Dohme).
[e] Includes capsular serotypes 4, 6, 9V, 14, 18C, 19F, and 23F (White Lerdele).
[f] Includes capsular serotypes 1, 4, 5, 6, 9V, 14, 18C, 19F, and 23F (Merck Sharp & Dohme).
[g] Includes capsular serotypes 1, 3, 4, 5, 6, 7F, 9V, 14, 18C, 19F, and 23F (Pasteur Merriex).

countries in Latin America (3, 13). Whereas serotypes 1 and 5 account for 8 to 12% of isolates from pediatric patients in other Brazilian cities (2), Chile (18), Colombia (3), and Uruguay (13), these serotypes were found in less than 3% of isolates from pediatric patients in Salvador. These differences may be due, in part, to true geographical differences in serotype prevalences, but they most likely reflect differences in surveillance methodologies and the patient populations sampled (11, 12).

On the basis of the results of the present surveillance study, 74% of the patients <5 years of age with pneumococcal meningitis were infected with isolates with serotypes represented in the seven-valent conjugate vaccine; this proportion is similar to that found for pediatric patient groups in the United States and other countries (23). Furthermore, conjugate vaccines may have an important additional benefit in reducing the transmission of antibiotic-resistant strains. In our study, 94% of the penicillin-nonsusceptible isolates from pediatric patients were restricted to four serotypes (serotypes 14, 6B, 23F, and 19F). These serotypes are represented in conjugate vaccines that have been shown to be effective in reducing nasopharyngeal carriage of antibiotic-resistant strains with serotypes covered by the vaccines (6, 20). In developing countries like Brazil, the significant burden associated with the treatment of penicillin-resistant pneumococcal disease in the future may justify serious consideration of the use of efficacious, albeit costly, conjugate vaccines.

This work was supported by funding from the Oswaldo Cruz Foundation, Brazilian Ministry of Health (grant 0250.250.415); the Brazilian National Research Council (grants 350.052/95–6, 300.861/96–6, 521.636/96–5, 521.132/98–3, and FINEP 4196086200); and the National Institutes of Health (grants AI-30639, TW-00919, TW-00018, and TW-00905).

We are grateful to Edilane Gouveia, Ricardo M. Pinheiro, Guilherme de Souza Ribeiro, and Tatiana Silva Lôbo (Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz/MS) for epidemiological data collection; Neide Oliveira Silva (Hospital Couto Maia) for technical assistance with pneumococcal culture isolation; the clinical staff of Hospital Couto Maia for cooperation; Brendan Flannery and Amee Manges for the review of the manuscript; and Warren D. Johnson, Jr., and Lee W. Riley for critical advice during implementation of the study.

REFERENCES

1. **Appelbaum, P. C.** 1996. Epidemiology and in vitro susceptibility of drug-resistant *Streptococcus pneumoniae*. Pediatr. Infect. Dis. J. **15:**932–934.
2. **Brandileone, M. C., V. S. Vieira, S. T. Casagrande, R. C. Zanella, M. L. Guerra, S. Bokermann, J. C. De Moraes, E. R. Baldacci, C. B. Chamone, M. A. Oliveira, D. G. De Matos, T. M. Arruda, M. F. Coelho, S. M. D'Avila, A. R. Dos Santos, and J. L. Di Fabio.** 1997. Prevalence of serotypes and antimicrobial resistance of *Streptococcus pneumoniae* strains isolated from Brazilian children with invasive infections. Pneumococcal Study Group in Brazil for the SIREVA Project. Regional System for Vaccines in Latin America. Microb. Drug Resist. **3:**141–146.
3. **Castaneda, E., A. L. Leal, O. Castillo, F. De La Hoz, M. C. Vela, M. Arango, H. Trujillo, A. Levy, M. E. Gama, M. Calle, M. L. Valencia, W. Parra, N. Agudelo, G. I. Mejia, S. Jaramillo, F. Montoya, H. Porras, A. Sanchez, D. Saa, J. L. Di Fabio, and A. Homma.** 1997. Distribution of capsular types and antimicrobial susceptibility of invasive isolates of *Streptococcus pneumoniae* in Colombian children. Pneumococcal Study Group in Colombia. Microb. Drug Resist. **3:**147–152.
4. **Committee on Infectious Diseases of the American Academy of Pediatrics.** 1997. Therapy for children with invasive pneumococcal infections. Pediatrics **99:**289–299.
5. **Critchley, I. A., C. Thornsberry, G. Piazza, M. Jones, M. L. Hickey, A. L. Barth, C. Mendes, F. F. Rossi, H. S. Sader, L. M. Teixeira, and D. F. Sahm.** 2000. Antimicrobial susceptibility of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* collected from five centers in Brazil, 1997–98. Clin. Microbiol. Infect. **6:**178–184.
6. **Dagan, R., R. Melamed, M. Muallem, L. Piglansky, D. Greenberg, O. Abramson, P. M. Mendelman, N. Bohidar, and P. Yagupsky.** 1996. Reduction of nasopharyngeal carriage of pneumococci during the second year of life by a heptavalent conjugate pneumococcal vaccine. J. Infect. Dis. **174:** 1271–1278.
7. **Fenoll, A., I. Jado, D. Vicioso, A. Perez, and J. Casal.** 1998. Evolution of *Streptococcus pneumoniae* serotypes and antibiotic resistance in Spain: update (1990 to 1996). J. Clin. Microbiol. **36:**3447–3454.
8. **Friedland, I. R., and G. H. McCracken, Jr.** 1994. Management of infections caused by antibiotic-resistant *Streptococcus pneumoniae*. N. Engl. J. Med. **331:**377–382.
9. **Guia de Vigilância Epidemiológica.** 1998. Meningites em geral. Cap 5.20. Ministério da Saúde, Fundação Nacional de Saúde, Brasilia, D.F., Brazil.
10. **Hausdorff, W. P., J. Bryant, P. R. Paradiso, and G. R. Siber.** 2000. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. Clin. Infect. Dis. **30:**100–121.
11. **Hausdorff, W. P., J. Bryant, C. Kloek, P. R. Paradiso, and G. R. Siber.** 2000. The contribution of specific pneumococcal serogroups to different disease manifestations: implications for conjugate vaccine formultation and use, part II. Clin. Infect Dis. **30:**122–140.
12. **Hausdorff, W. P., G. Siber, and P. R. Paradiso.** 2001. Geographical differences in invasive pneumococcal disease rates and serotype frequency in young children. Lancet **357:**950–952.
13. **Hortal, M., G. Algorta, I. Bianchi, G. Borthagaray, I. Cestau, T. Camou, M. Castro, M. de los Santos, R. Diez, L. Dell'Acqua, A. Galiana, A. Giordano, P. Giordano, G. Lopez-Ghemi, N. Milanese, C. Mogdasy, R. Palacio, W. Pedreira, A. Pisano, and L. Pivel.** 1997. Capsular type distribution and susceptibility to antibiotics of *Streptococcus pneumoniae* clinical strains isolated from Uruguayan children with systemic infections. Pneumococcus Study Group. Microb. Drug Resist. **3:**159–163.
14. **Instituto Brasileiro de Geografia e Estatística.** 1996. Anuário Estatístico do Brasil, vol 56. Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro, R.J., Brazil.
15. **Kertesz, D. A., J. L. Di Fabio, M. C. de Cunto Brandileone, E. Castaneda, G. Echaniz-Aviles, I. Heitmann, A. Homma, M. Hortal, M. Lovgren, R. O. Ruvinsky, J. A. Talbot, J. Weekes, and J. S. Spika.** 1998. Invasive *Streptococcus pneumoniae* infection in Latin American children: results of the Pan American Health Organization Surveillance Study. Clin. Infect Dis. **26:**1355–1361.
16. **Klugman, K. P.** 1997. Impact of antimicrobial resistance and antibiotic choice on the outcome of pneumococcal meningitis. S. Afr. Med. J. **87:**912.
17. **Ko, A. I., J. N. Reis, S. J. Coppola, E. L. Gouveia, S. M. Cordeiro, T. S. Lobo, R. M. Pinheiro, K. Salgado, C. M. Ribeiro Dourado, J. Tavares-Neto, H. Rocha, M. Galvao Reis, W. D. Johnson, Jr., and L. W. Riley.** 2000. Clonally related penicillin-nonsusceptible *Streptococcus pneumoniae* serotype 14 from cases of meningitis in Salvador, Brazil. Clin. Infect. Dis. **30:**78–86.
18. **Levine, M. M., R. Lagos, O. S. Levine, I. Heitmann, N. Enriquez, M. E. Pinto, A. M. Alvarez, E. Wu, C. Mayorga, and A. Reyes.** 1998. Epidemiology of invasive pneumococcal infections in infants and young children in metropolitan Santiago, Chile, a newly industrializing country. Pediatr. Infect. Dis. J. **17:**287–293.
19. **Marton, A.** 1992. Pneumococcal antimicrobial resistance: the problem in Hungary. Clin. Infect. Dis. **15:**106–111.
20. **Mbelle, N., R. E. Huebner, A. D. Wasas, A. Kimura, I. Chang, and K. P. Klugman.** 1999. Immunogenicity and impact on nasopharyngeal carriage of a nonavalent pneumococcal conjugate vaccine. J. Infect. Dis. **180:**1171–1176.
21. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard M7–A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
22. **Obaro, S. K., M. A. Monteil, and D. C. Henderson.** 1996. The pneumococcal problem. BMJ **312:**1521–1525.
23. **Sleeman, K., K. Knox, R. George, E. Mille, P. Waight, D. Griffiths, A. Efstratiou, K. Broughton, R. T. Mayon-White, E. R. Moxon, and D. W. Crook.** 2001. Invasive pneumococcal disease in England and Wales: vaccination implications. J. Infect. Dis. **183:**239–246.
24. **Teixeira, L. M., M. G. Carvalho, T. M. Castineiras, S. A. Fracalanzza, A. S. Levin, and R. R. Facklam.** 1997. Serotyping distribution and antimicrobial resistance of *Streptococcus pneumoniae* isolated in Brazil (1992–1996). Adv. Exp. Med. Biol. **418:**269–271.
25. **Thomas, K., M. K. Lalitha, M. C. Steinhoff, N. K. Arora, A. Ratan, S. K. Awasthi, P. K. Misra, J. Amita, R. Daivanayagam, P. Manjula, D. Jain, V. Agarwal, P. Ravindran, T. Cherian, P. Raghupathy, P. Moses, M. V. Jesudason, K. N. Bramhadathan, L. Jayseelan, and T. J. John.** 1999. Prospective multicentre hospital surveillance of *Streptococcus pneumoniae* disease in India. Lancet **353:**1216–1221.
26. **Wright, P. F.** 1989. Approaches to prevent acute bacterial meningitis in developing countries. Bull W. H. O. **67:**479–486.