JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1997, p. 785–787 Vol. 35, No. 3
0095-1137/97/$04.00+0

Serotypes of *Streptococcus pneumoniae* Causing Invasive Childhood Infections in Bangladesh, 1992 to 1995

SAMIR K. SAHA,[1]* N. RIKITOMI,[2] D. BISWAS,[1]† K. WATANABE,[2] M. RUHULAMIN,[3] K. AHMED,[2] M. HANIF,[3] K. MATSUMOTO,[2] R. B. SACK,[4]‡ AND T. NAGATAKE[2]

Departments of Microbiology[1] and Medicine,[3] Dhaka Shishu (Children's) Hospital, and Laboratory Science Division, International Centre for Diarrhoeal Disease Research,[4] Dhaka, Bangladesh, and Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan[2]

Received 7 November 1996/Accepted 13 December 1996

One hundred sixty-five invasive *Streptococcus pneumoniae* strains were isolated from children under five at Dhaka Shishu (Children's) Hospital during the period 1992 to 1995. Ninety-four strains were from cerebrospinal fluid, and 71 were from blood. More than 91% of the strains were isolated from patients aged 24 months or less. Predominant serotypes were, in descending order, 7F, 12F, 14, 15B, 4, 23F, 18, 5, and 22A. These comprised 70% of all isolates. The marked differences in serotype distribution in different countries indicate the need for a sentinel surveillance study for the countries of south Asia, particularly Bangladesh, China, India, and Pakistan.

Streptococcus pneumoniae, the leading cause of childhood pneumonia and meningitis, is responsible for 20 to 40% of the global total of 4 million child deaths from pneumonia per year (6). In Bangladesh it is a common cause of pneumonia and meningitis, with high mortality (11, 12).

The shortcomings of polysaccharide vaccines, i.e., no response in children under two (1), and lessons learned from conjugate *Haemophilus influenzae* type b vaccine have facilitated the development of pneumococcal conjugate vaccines which can accommodate only a few serotypes. Recently, protein-conjugated heptavalent and tetravalent vaccines have been developed and studied for their immunogenicity in animal models and human volunteers (14). Any plan to develop and/or introduce an appropriate narrow-spectrum pneumococcus conjugate vaccine will need serotype data from different parts of the world. Unfortunately, information from most of the developing world, which could potentially benefit the most from conjugate vaccines, is severely deficient.

We present here a serotype analysis of prospectively collected pneumococcal isolates from cerebrospinal fluid (CSF) and blood of patients with meningitis and pneumonia, respectively, in Bangladesh.

The study was carried out at Dhaka Shishu (Children) Hospital (DSH), which is the only pediatric hospital at the national level for primary and tertiary care. All children with meningitis or pneumonia, aged 0 to 5 years, were included in the study. Cases in which *S. pneumoniae* strains were isolated from CSF and blood were considered eligible for analysis. Pneumococcal strains were serotyped by the capsular swelling procedure (quellung reaction) with type-specific antipneumococcal pool, type or group, and factor sera (Statens Seruminstitute, Copenhagen, Denmark) (4). Only one isolate per disease episode was analyzed. ATCC strains 6314, 6301, and 10341 and Johns Hopkins University (JHU) strains 9, 23, and 4 (kindly provided by Mark Steinhoff) were used as known control strains. The Epi Info statistics program 6.02 (3) was used for analyzing the data.

A total of 165 invasive pneumococcus strains were isolated in the 3-year study period 1992 to 1995. Ninety-four (57%) of the strains were isolated from 412 pyogenic CSF specimens, and 71 (43%) were from the blood of 531 pneumonia patients. The numbers of male and female patients were 111 and 54, respectively, a ratio of 2.05.

Eighty-nine percent (146 of 165) of the strains were isolated from patients in the 2- to 24-month age group, and 56% (93 of 165) were from patients aged 6 to 24 months. Only 3.0% (5 of 165) of the isolates were from patients in the neonatal age group (0 to 30 days), and 8.5% (14 of 165) were from patients 2 years old or older (Table 1).

Among 154 (93.3%) typeable strains, the most prevalent serotypes were 7F, 12F, 14, and 15B. These four serotypes were found in 54% of the cases; other serotypes were 4, 23F, 18, 5, and 22A. Serotypes of infrequent occurrence made up 23.5% of the isolates. Predominant serotypes in meningitis and pneumonia cases were similar (Table 2).

An ideal pneumococcal vaccine must be immunogenic in very young children, in whom the incidence of pneumococcal infection and mortality is highest, and must protect against the most common serotypes. Since the success of *H. influenzae* type b conjugate vaccine, a number of antigenic formulas for pneu-

TABLE 1. Major serogroups and types of invasive pneumococcal isolates in patients of different age groups

Age (mo)	No. of isolates of serotype:								Total no. of isolates	Cumulative no. of isolates (%)
	1	4	7F	12F	14	15B	23F	Other		
0–1	2	0	0	1	0	0	0	2	5	5 (3.0)
1–2	0	1	1	0	0	2	1	2	7	12 (7.3)
2–6	5	0	11	8	6	5	0	11	46	58 (35)
6–12	2	3	10	9	9	7	3	24	67	125 (75.7)
12–24	0	4	4	3	2	2	1	10	26	151 (91.5)
24–60	2	0	7	1	0	1	1	2	14	165 (100)

* Corresponding author. Mailing address: Department of Microbiology, Bangladesh Institute of Child Health, Dhaka Shishu (Children's) Hospital, Dhaka-1207, Bangladesh. Phone: 880-2-816061, -816062, or -815378. Fax: 880-2-861634.

† Present address: Department of Veterinary Public Health, Faculty of Agriculture, Tokyo University, Tokyo, Japan.

‡ Present address: Department of International Health, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Md.

TABLE 2. Groups and types of invasive *S. pneumoniae* strains isolated from Bangladeshi children with pneumonia or meningitis

Meningitis isolates (n = 94)		Pneumonia isolates (n = 71)		All isolates (n = 165)	
Serotype	% of total (cumulative %)	Serotype	% of total (cumulative %)	Serotype	% of total (cumulative %)
7F	22.3 (22.3)	7F	16.9 (16.9)	7F	20.0 (20.0)
12F	16.0 (38.0)	15B	11.3 (28.2)	12F	13.0 (33.0)
14	10.6 (48.6)	12F	9.9 (38.1)	14	10.3 (43.3)
15B	9.6 (58.2)	14	9.9 (58.0)	15B	10.3 (53.6)
1	7.4 (65.6)	23F	7.0 (65.0)	4	4.8 (58.4)
18	4.3 (69.9)	4	7.0 (72.0)	23F	3.6 (62.0)
22A	3.2 (73.1)	1	5.6 (77.6)	18	3.0 (65.0)
25	3.2 (76.3)	16F	4.2 (81.8)	5	2.4 (67.4)
4	3.2 (79.5)	6A	4.2 (86.0)	22A	2.4 (69.8)
Others	13.1 (82.6)	Others	8.4 (94.4)	Others	23.5 (93.3)
Untypeable	7.4 (100)	Untypeable	5.6 (100)	Untypeable	6.7 (100)

mococcus vaccine, some for developed and some for developing countries, have been proposed (14). However, the formulation of a vaccine based on the existing data from the developing countries may not be optimal. For example, data from India, China, and Bangladesh, countries that include the majority of the world's children, are not available.

Seventy-five percent of our patients were in the 1st year of life, and 91.5% (151 of 165) were under 2 years (Table 1). Therefore, children of less than 2 years should be the target group for conjugate vaccines, as this age group does not respond well to polysaccharide vaccines. There were only five cases (3%) in the first month of life, which is comparable to the findings of studies from other parts of the world (9, 16). Hence, the other approach, immunization of mothers during pregnancy, recently attempted by Shahid et al. (13), would be expected to provide protection for some of these children only during their first few months of life, when the incidence is very low (2, 7).

Like other investigators (2, 8), we also found male predominance among the cases, with a male/female ratio of 2.05. The comparatively higher prevalence of male children in our study may reflect the social attitude whereby a male child receives more attention and is preferentially taken to the hospital.

The serotype distribution found in the present study is distinctively different from that found in children in other parts of the world. The two predominant serotypes in our study were 7F and 12F, which together made up 33% of all isolates. Neither of these types was isolated in the pneumonia cases in Pakistan (8). Serogroup 7 was the most prevalent among the isolates in Papua New Guinea (5) and ranked 7th and 11th in developed and developing countries, respectively, when all the published data were analyzed (14). Extreme diversity of pneumococcal serotype or group distribution is a common phenomenon and a great concern for workers in this field. For example, group 1 is most prevalent in Egypt (31.4%) but is rarely isolated in other developing countries, such as Pakistan (0.6%) and Mexico (0.8%), and was never isolated in Finland, a country with the world's highest average living standard. Serotype 14 is predominant in many countries but is rarely isolated in Pakistan (1.2%). Similarly, group 19 was predominant in Pakistan but rare in Egypt and was never found in Brazil (14).

This hospital-based study cannot be compared well with community-based studies. In general, serotype distribution is markedly different, and none of the proposed formulas for conjugate vaccine (Table 3) covers more than 50% of the pneumococcal patients of our population. The striking difference we found is the prevalence of serotype 12F, which is rarely reported in any other country. Another interesting observation was the similarity of the serotypes isolated in pneumonia and meningitis cases. A nonavalent conjugate vaccine containing the serogroups or types 7, 12, 14, 15, 4, 23, 18, 5, and 22 would give the optimum coverage for prevention of both meningitis (73.5%) and pneumonia (66.2%) cases.

All these studies probably indicate the importance of an alternative approach: a species-wide, protein-based vaccine for wide coverage of pneumococcal infections (10, 15). However, such species-wide vaccines are unlikely to be available in the near future. The formulation and use of a conjugate vaccine with high coverage for a particular population and/or region are expected to reduce high rates of childhood mortality in certain areas.

In conclusion, although DSH is the only national pediatric hospital for primary and tertiary care, there are general hospitals and private clinics serving the same population, and some children with meningitis are obviously admitted to those hospitals as well. Moreover, since Bangladesh is a developing country, many children are not brought to the hospital and die at home. Therefore, the actual burden of disease is possibly manyfold higher than that observed at DSH. Further, children at any particular hospital may show high degrees of concordance and may not reflect the actual situation nationwide. Finally, we would like to propose a national and subcontinental sentinel surveillance system to determine the pneumococcal serotypes causing invasive diseases in south Asia. These data

TABLE 3. Serotype coverage of proposed pneumococcal conjugate vaccine formulas,[a] on the basis of predominant types and/or groups isolated in Bangladesh

Vaccine (serotype antigen)	Serotype coverage (%)
Formula A (4, 6B, 9V, 14, 18C, 19F, 23F)	25.5
Formula B (1, 5, 6B, 14, 18C, 19F, 23F)	29.3
Formula A+B (1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F)	35.6
Developed Country Custom 7 (6B, 7F, 9V, 14, 18C, 19F, 23F)	41.7
Developed Country Custom 9 (1, 4, 6B, 7F, 9V, 14, 18C, 19F, 23F)	53.2
Developing Country Custom 7 (1, 5, 6B, 9V, 14, 19F, 23F)	26.6
Developing Country Custom 9 (1, 5, 6B, 9V, 14, 15B, 18C, 19F, 23F)	41.1
Global 9 (1, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F)	50.5
Dhaka 9 (7F, 12F, 14, 15B, 4, 23F, 18, 5, 22A)	69.8

[a] See reference 14.

would suggest the formulation of a heptavalent or nonavalent conjugate vaccine for this region, which contains the majority of the world's children.

This research was supported by the Programme Coordination Committee (PCC) of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) and DSH. The Ministry of Health and Welfare, Government of Japan, also donated generously in completing the work. We gratefully acknowledge their support and cooperation.

We also thankfully acknowledge the technical assistance rendered by Maksuda Islam, research associate, Microbiology Laboratory of DSH.

REFERENCES

1. **Baltimore, R. S.** 1992. New challenge in the development of a conjugate pneumococcal vaccine. JAMA **268:**3366–3367. (Editorial.)
2. **Dagan, R., D. Englehard, and E. Piccard.** 1992. Epidemiology of invasive childhood pneumococcal infections in Israel. JAMA **268:**3328–3332.
3. **Dean, J. A., D. Coulombier, D. C. Smith, et al.** 1994. Epi Info version 6.02: a word processing, database, and statistics system for epidemiology on microcomputers. Centers for Disease Control and Prevention, Atlanta, Ga.
4. **Facklam, R. R., and J. A. Washington II.** 1991. Streptococcus and related catalase-negative gram-positive cocci, p. 238–257. *In* A. Balows, W. J. Hausler, Jr., K. L. Hermann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
5. **Gratten, M., and J. Montgomery.** 1991. The bacteriology of acute pneumonia and meningitis in children in Papua New Guinea: assumptions, facts and technical strategies. P. N. G. Med. J. **34:**185–198.
6. **Institute of Medicine.** 1986. New vaccine development: establishing priorities, vol. 2. Diseases of importance in developing countries. National Academy Press, Washington, D.C.
7. **Klein, J. O.** 1981. The epidemiology of pneumococcal diseases in infants and children. Rev. Infect. Dis. **3:**246–253.
8. **Mastro, T. D., A. Ghafoor, N. K. Nomani, et al.** 1991. Antimicrobial resistance of pneumococci in children with acute lower respiratory tract infection in Pakistan. Lancet **i:**156–159.
9. **Okolo, A. A., and J. A. Omene.** 1985. Trends in neonatal mortality in Benin City, Nigeria. Int. J. Gynecol. Obstet. **23:**191–195.
10. **Paton, J. C., R. A. Lock, C.-J. Lee, J. P. Li, A. M. Berry, T. J. Mitchell, P. W. Andrew, D. Hansman, and G. J. Boulnois.** 1991. Purification and immunogenicity of genetically obtained pneumolysin toxoids and their conjugation to *Streptococcus pneumoniae* type 19F polysaccharide. Infect. Immun. **59:**2297–2304.
11. **Rahaman, M., F. Huq, and D. A. Sack.** 1990. Acute lower respiratory tract infections in hospitalized patients with diarrhea in Dhaka, Bangladesh. Rev. Infect. Dis. **12:**S899–S906.
12. **Setarunnahar, A., A. Chowdhury, and F. Hoq.** 1985. Aetiological agents of meningitis in Bangladeshi children. Indian J. Med. Microbiol. **6:**81–85.
13. **Shahid, N. S., M. C. Steinhoff, S. S. Hoque, T. Begum, C. Thompson, and G. R. Siber.** 1995. Serum, breast milk, and infant antibody after maternal immunization with pneumococcal vaccine. Lancet **346:**1252–1257.
14. **Sniadack, D. H., B. Schwartz, H. Lipman, et al.** 1995. Potential interventions for the prevention of childhood pneumonia: geographic and temporal differences in serotype and serogroup distribution of sterile site pneumococcal isolates from children—implications for vaccine strategies. Pediatr. Infect. Dis. J. **14:**503–510.
15. **Talkington, D. F., A. Koenig, and H. Russell.** 1992. The 37-kDa protein of *Streptococcus pneumoniae* protects mice against fatal challenge, abstr. E-29, p. 149. *In* Abstracts of the 92nd General Meeting of the American Society for Microbiology 1992. American Society for Microbiology, Washington, D.C.
16. **Wenger, J. D., A. W. Hightower, R. R. Facklam, S. Gaventa, and C. V. Broome.** 1990. Bacterial meningitis in the United States. Report of a multistate surveillance study. J. Infect. Dis. **162:**1316–1323.