CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, May 2001, p. 556–559 Vol. 8, No. 3
1071-412X/01/$04.00+0 DOI: 10.1128/CDLI.8.3.556–559.2001

Influence of Patient Age on *Streptococcus pneumoniae* Serotypes Causing Invasive Disease

JAIME INOSTROZA,[1] ANA MARIA VINET,[1] GLORIA RETAMAL,[1] PEDRO LORCA,[1] GONZALO OSSA,[1] RICHARD R. FACKLAM,[2] AND RICARDO U. SORENSEN[3]*

Immunology Laboratory and Departments of Pediatrics and Internal Medicine, Hospital Regional de Temuco, and the Departments of Basic Sciences, Pediatrics and Internal Medicine, Universidad de la Frontera, Temuco, Chile[1]; Laboratory Section, Childhood and Respiratory Disease Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia[2]; and Department of Pediatrics, Louisiana State University Health Sciences Center, New Orleans, Louisiana[3]

Received 23 June 2000/Returned for modification 8 August 2000/Accepted 15 February 2001

All clinical *S. pneumoniae* specimens isolated from patients with invasive or sterile-site infections admitted to one regional general hospital in southern Chile were collected during a 5-year period (February 1994 to September 1999). A total of 247 strains belonging to 50 serotypes were isolated in this survey: 69 in patients under 5 years of age, 129 in patients 5 to 64 years old, and 49 from patients 65 years and older. Eight serotypes were identified in all age groups, while all other serotypes were found exclusively in one age group or in patients over 4 years of age. Serotype 3 was never found in patients under 5 years old, and serotype 14 was not found in patients >64 years of age. There was no difference in the serotypes causing infection in each one of the 5 years of the survey. Our results suggest that both bacterial virulence factors and host factors play an important role in the selection of *S. pneumoniae* serotypes causing invasive infection. Possible host factors include age-related differences in the immune response. Comparative studies with other areas of the world may help to further understanding of our observations in southern Chile.

Capsular serotypes of *Streptococcus pneumoniae* causing invasive infections vary according to geographic location and socioeconomic status of the study population (2, 3, 11, 30, 31). Information about these serotypes in different areas of the world is essential for the formulation of conjugate vaccines (24).

Bacterial factors are likely to influence the selection of serotypes causing invasive infections. When both nasopharyngeal carriage and invasive infections have been studied in the same individual, a high degree of correlation in serotypes has been found (9). On the other hand, some pneumococcal serotypes found colonizing the nasopharynx have little tendency to cause invasive disease (11, 16, 27). These observations suggest that certain pneumococcal serotypes have characteristics that represent an advantage for invasiveness. In addition, differences in infection-causing pneumococcal serotypes have been attributed to the emerging, worldwide antibiotic resistance of some serotypes (2, 3, 7, 13, 31).

The characteristics of the host may also contribute to serotype selection. Underlying central nervous system and heart diseases, as well as malignancies, are frequently identified in patients developing invasive infections (13). Recently, human immunodeficiency virus (HIV) infections have become a major risk factor for the development of invasive pneumococcal infections (19). The extent to which these factors select for infections with specific serotypes is presently unknown.

Age has a clear influence on the overall incidence of invasive infections most frequent in the first years of life (14) and also in persons older than 65 years (1). Some studies suggest that different serotypes cause infections in different age groups (20). We had an opportunity to explore this possibility further in a relatively homogenous patient population without HIV infection, where pneumococcal antibiotic resistance was not a factor during the 5-year study period. Our results document interesting differences in serotypes causing invasive disease at different ages.

MATERIALS AND METHODS

Study population. The study population consisted of patients of all ages seeking medical care and being admitted to any of the in-patient services of the Hospital Regional in Temuco, a city of 300,000 inhabitants in southern Chile. The low- and middle-income populations of this city generally seek medical care from the Chilean National Health Service at this hospital, where patients are admitted to the internal medicine, surgery, obstetric, and pediatric services. All samples were sent to the Central Laboratory of the hospital. Both HIV type 1 (HIV-1) and HIV-2 serology was performed by enzyme-linked immunosorbent assay (Abbott Laboratories, Chicago, Ill.) on all patients in this study. No HIV-seropositive patients with pneumococcal infections were identified.

Sample definition and collection. All *S. pneumoniae* strains from invasive infections or infections in normally sterile sites were included in this study. Strains isolated from blood, spinal fluid, pleural fluid, or ascitic fluid were defined as invasive, and strains isolated from the conjunctiva, middle ear, or sinus cavities were classified as coming from sterile sites. Clinical isolates were collected and serotyped between February 1994 and September 1999.

Pneumococcal serotyping. Serotyping of *S. pneumoniae* strains was performed by one of us (J.I.) in the pneumococcal serotyping laboratory at the Centers for Disease Control and Prevention (Atlanta, Ga.). Before serotyping, cultures were transferred to 5% sheep red cell agar plates (Difco Laboratories, Detroit, Mich.) overnight. All serotyping results were confirmed by Quellung test.

Antibiotic sensitivities of all strains were determined by the E-test for penicillin, cefotaxime, and vancomycin.

* Corresponding author. Mailing address: Department of Pediatrics-LSUHSC, 1542 Tulane Ave., Box T8-1, New Orleans, LA 70112-2822. Phone: (504) 568-2578. Fax: (504) 568-7598. E-mail: rsoren@lsuhsc.edu.

RESULTS

Epidemiology. For analysis, the study population was divided into three age groups: under 5 years of age, 5 to 64 years old, and over 64 years old. The total population cared for at the Temuco Regional Hospital during the 5-year study period in each of these age groups was as follows: <5 years, 34,631; 5 to 64 years, 248,305; >64 years, 19,180. The yearly incidence of pneumococcal infections per 100,000 individuals during the same period was as follows: <5 years, 193/100,000; 5 to 64 years, 54/100,000; >64 years, 234/100,000. A separate analysis of children under 2 years of age revealed 53 infections with a yearly incidence of 348/100,000 and 16 infections in children 2 to 4 years old with an incidence of 76/100,000. A breakdown of the data for each age group in each of the 5 study years revealed that the yearly incidence of invasive pneumococcal infections in each age group remained constant (data not shown).

Serotypes and age. Two hundred and forty-seven *S. pneumoniae* strains with a total of 50 serotypes were isolated during the entire study period (Table 1). Sixty-nine, 129, and 49 strains causing infections in children under 5 years of age, patients 5 to 64 years old, and patients over 64 years old, respectively, were found.

There was no difference in the serotypes causing infection in each of the 5 years of the survey. A separate analysis also revealed that there were no differences in serotypes causing infection between the 5- to 15- and the 16- to 64-year-old patients (data not shown).

In the study population as a whole, the five most frequent serotypes were 1 with 35 isolates, 5 with 22, 3 with 19, 19F with 15, and 23F with 13. However, the distribution was markedly different in young children and older patients. In children under 5 years of age, there were nine strains of serotype 1, eight of 5, seven each of 6B and 23F, six of 19F, and five of 6A, while in patients 65 years and older serotypes 3 with seven strains, 7F with five, and 1, 19F, and 38 with three strains each were found most frequently.

Only 11 serotypes were isolated in all age groups, namely, 1, 5, 6A, 6B, 7F, 12F, 15B, 15C, 19A, 19F, and 23F. All other serotypes were found exclusively in one or two age groups, e.g., patients over 4 or under 64 years only.

Children under 5 years of age differed from patients 5 years and older. Notably, 20 serotypes present in the other age groups were not isolated from the youngest group, i.e., 3, 7A, 8, 9N, 10A, 10B, 11A, 11F, 13, 16, 17F, 18F, 20, 22F, 24F, 29, 35A, 35B, 36, and 38. Of these, serotype 3 caused of 19 infections in patients 5 years and older (15%). Five serotypes were isolated exclusively in the youngest age group, i.e., 9A, 21, 28F, 31, and 34, each causing one infection.

Twenty-two serotypes present in the other age groups were not isolated in patients over 64 years of age: 4, 7A, 8, 9A, 9V, 10A, 11A, 11F, 13, 14, 16, 17F, 18A, 20, 21, 23B, 23C, 24F, 28F, 31, 33F, and 34. Particularly notable was the absence of infections with serotype 14 in patients 64 years and older, since this serotype was isolated from nine infections in younger patients.

Three serotypes were isolated exclusively from patients older than 64 years, namely, 10B, 22F, and 27, each causing one infection.

TABLE 1. Distribution of *S. pneumoniae* serotypes isolated from invasive and sterile-site infections according to age[a]

Serotype[b]	No. (%) of strains from subjects of age (yr):			Total
	<5	5–64	>64	
1*+	9 (13)	23 (17.8)	3 (6.1)	35
2*				0
3*+		12 (9.3)	7 (14.3)	19
4*+	1 (1.4)	1 (0.7)		2
5*+	8 (11.6)	12 (9.3)	2 (4.1)	22
6A	5 (7.2)	2 (1.5)	2 (4.1)	9
6B*+	7 (10.1)	3 (2.2)	2 (4.1)	12
7A		1 (0.7)		1
7C				0
7F*+	1 (1.4)	5 (3.8)	5 (10.2)	11
8*		6 (4.6)		6
9A	1 (1.4)			1
9N*		3 (2.0)	2 (4.1)	5
9F				0
9V*	1 (1.4)	1 (0.7)		2
10A*	1 (1.4)	3 (2.2)		4
10B			1 (2.0)	1
11A*		3 (2.2)		3
11F		1 (0.7)		1
12F*	2 (2.9)	4 (3.0)	1 (2.0)	7
13		5 (3.8)		5
14*+	4 (5.7)	5 (3.8)		9
15B*	1 (1.4)	1 (0.7)	1 (2.0)	3
15C	2 (2.9)	3 (2.2)	2 (4.1)	7
16		4 (3.1)		4
17F*		2 (1.4)		2
18A	1 (1.4)	1 (0.7)		2
18C*	2 (2.9)		1 (2.0)	3
18F		2 (1.4)	1 (2.0)	3
19A*	1 (1.4)	3 (2.2)	2 (4.1)	6
19F*+	6 (8.7)	6 (4.5)	3 (6.1)	15
20*		2 (1.4)		2
21	1 (1.4)			1
22F			1 (2.0)	1
23B		1 (0.7)		1
23C		1 (0.7)		1
23F*+	7 (10.1)	4 (3.0)	2 (4.1)	13
24F		1 (0.7)		1
27			1 (2.0)	1
28A				0
28F	1 (1.4)			1
29		1 (0.7)	1 (2.0)	2
31	1 (1.4)			1
33				0
33F*	3 (4.3)			3
34	1 (1.4)			1
35A		3 (2.2)	1 (2.0)	4
35B		2 (1.5)	2 (4.1)	4
35F	2 (2.9)		2 (4.1)	4
36		1 (0.7)	1 (2.0)	2
38		1 (0.7)	3 (6.1)	4
Total	69	129	49	247

[a] All pneumococcal serotypes included in the 23-valent polysaccharide vaccine are included. Of the vaccine serotypes, only serotype 2 was never identified in our patient population. Isolation of serotypes took place from February 1994 to September 1999.

[b] *, serotype in the 23-valent pneumoccal polysaccharide vaccine; +, serotype in the II-valent pneumococcal conjugate vaccine.

Serotype 4 was isolated only twice, once in a child under 5 years and once in the 5- to 64-year-old group.

Antibiotic resistance was identified in only one clinical isolate, from a child younger than 5 years. This was a serotype 23F strain highly resistant to penicillin and cefotaxime but not to vancomycin (8).

TABLE 2. Vaccine coverage of invasive pneumococcal infections in different age groups

Vaccine	No. (%) of covered clinical isolates from patients of age (yr):		
	<5 (n^a = 69)	5–64 (n = 129)	>64 (n = 49)
7-valent conjugate (formula B)			
No cross-reactivity	43 (62)	53 (41)	8 (16)
Serogroup cross-reactivity	50 (72)	68 (53)	15 (31)
9-valent conjugate			
No cross-reactivity	45 (65)	55 (43)	18 (37)
Serogroup cross-reactivity	53 (76)	73 (57)	25 (51)
11-valent conjugate			
No cross-reactivity	46 (67)	72 (56)	31 (63)
Serogroup cross-reactivity	54 (78)	91 (71)	38 (78)
23-valent polysaccharide			
No cross-reactivity		99 (77)	31 (63)
Serogroup cross-reactivity		108 (84)	38 (78)

[a] n, total number of clinical isolates.

The estimated coverage offered by various vaccines to patients of different age groups in this study is shown in Table 2. Table 1 shows the serotypes included in the 23-valent polysaccharide vaccine and also those included in the proposed 11-valent conjugate vaccine. Including protection offered by serogroup cross-reactivity, the estimated coverage of the three conjugate vaccines for infections in children younger than 5 years ranged from 72 to 78%, denoting only a small increase in coverage going from the heptavalent to the 11-valent vaccine. In patients over 64 years, the coverage from conjugate vaccine ranged from 31 to 78%. This estimated coverage for the 11-valent conjugate vaccine is the same for this age group as that estimated for the 23-valent polysaccharide vaccine.

DISCUSSION

Epidemiology. Our results confirm the high incidence of pneumococcal infections in young children observed in other studies (6, 29). A recent study of invasive infections in children under 5 years of age in west Africa estimated that the incidence of invasive infection was 240/100,000/year in children younger than 5 years and 554/100,000/year in children younger than 1 year (18). Our data confirm a very sharp drop in the incidence of invasive pneumococcal infections after 2 years of age, with an incidence in children 2 to 4 years old only slightly higher than that in individuals 5 to 64 years old. This may reflect the absence of high-risk groups, i.e., children with sickle cell disease and/or HIV infections, in our study population.

An analysis of serotypes causing infections in these different age groups suggests that there are risk factors in young children and in elderly populations that may be serotype specific. Two serotypes stand out as representative of age differences in susceptibility because of their relatively high frequencies in the general population: serotype 3, absent in young children, and serotype 14, absent in the elderly.

Several studies have shown that serotype 3 is a frequent isolate from the respiratory tract (10, 12) but an infrequent cause of invasive *S. pneumoniae* infections in children (17, 20, 23). In a recent study of invasive infections in children under 5 years of age in Santiago, Chile, serotype 3 caused only 3.3% of infections (three-pneumonias) (15).

The absence of serotype 14 in isolates from elderly patients in our study is surprising, since this was a common serotype isolated from patients over 60 years old in the United States and New Zealand (4, 17).

A study in Israel also found differences in the serotypes isolated in children under 13 years and in adults (20) but a distribution of serotypes different from that found in Chile. For example, while serotype 6B was isolated almost exclusively in adults in Israel, it caused 11.3% of infections in children under 2 years of age in Temuco.

The relatively high frequency of infections with high-numbered serotypes (28F to 38) in children under 5 years and in adults over 64 years in Temuco is interesting. Serotypes 28F, 31, 33F, 34, 35F caused 11% of invasive and sterile-site infections in children under 5 years in Temuco. Yet none of these serotypes is included in the conjugate vaccines, and only serotype 33F is included in the polysaccharide vaccine. Nor were any of these serotypes identified in young children with invasive infections in Santiago, Chile, further documenting the importance of local variations in serotype predominance (15).

Taken together, our data and those reported by others support the notion that age plays a role in the serotypes causing infection. Socioeconomic and geographical differences do not explain our results for a homogeneous patient population living in the same geographical area (11). Year-to-year variation in serotype distribution was not a factor in our study or in other studies of infections over time (20, 30). Furthermore, antibiotic resistance due to preventive-antibiotic use in special-risk populations (26) and underlying immune system abnormalities such as those due to HIV infection (19) were also ruled out as an explanation for our results for different age groups. Lastly, our population was not immunized with any form of pneumococcal vaccine that could have altered the immune response and therefore the incidence of infections with some serotypes.

Whether or not differences in the immune response may account for some of these observations is questionable. Transplacental transmission of immunoglobulin G (IgG) antibodies is very efficient for serotypes 3 and 14 (5), so that this is an unlikely explanation for the absence of serotype 3 infections and the high incidence of serotype 14 infections in young children. Furthermore, transplacentally transmitted antibodies decrease rapidly during the first 6 months of life (22). In patients with recurrent infections, serotype 3 is clearly more immunogenic in children than serotype 14, while in adults serotype 14 induces a very high concentration of IgG antibodies (25). For serotype 14 antibodies detected by enzyme-linked immunosorbent assay also correlate well with antibody avidity and opsonophagocytic activity in the elderly (21). Since antibodies to other serotypes have low opsonophagocytic activity in the elderly, our observations may be explained by the functional properties of antibodies against pneumococcal serotypes in this age group. However, this would not explain the high incidence of serotype 14 infection in adults in New Zealand (17). We conclude that age differences are probably due to multiple factors, defying a clear interpretation at this point.

Our findings are relevant for prevention strategies, antibiotic usage, and vaccine design. Current recommendations for vac-

cine formulation are based on serotypes and serogroup distribution for invasive and sterile-site pneumococcal infections (24). Conjugated vaccines are recommended for children under 5 years of age (28). If our results are confirmed in other studies, the age of the patient population to be immunized in different regions of the world will have to be considered. One important observation is that serotype 3, included in all conjugate vaccines, and serotype 7F, included in the 11-valent conjugate vaccine, are very infrequent causes of infections in children under 5 years of age in Temuco, Chile. Continued surveillance of pneumococcal infections at different ages is necessary to design the most-effective vaccines to be used at the most-appropriate ages.

ACKNOWLEDGMENTS

We thank Terry Thompson for his advice and support in the serotyping of streptococcal strains at the CDC and Patricia A. Giangrosso for assistance in the preparation of the manuscript.

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