CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Mar. 1998, p. 176–180 Vol. 5, No. 2
1071-412X/98/$04.00+0

Capsular Serotype and Antibiotic Resistance of *Streptococcus pneumoniae* Isolates in Two Chilean Cities

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Received 29 May 1997/Returned for modification 20 August 1997/Accepted 25 November 1997

We compared the incidence of nasopharyngeal colonization by *Streptococcus pneumoniae*, the serotypes causing mucosal and invasive diseases, and the antibiotic resistance of these strains in patients admitted to three large hospitals and children attending day care centers in two Chilean cities (Santiago and Temuco). The populations in both cities were similar in ethnic background, socioeconomic status, family size, and access to medical care. Significant differences in nasopharyngeal colonization rates, in serotypes causing infections, and in antibiotic resistance were found between the two cities. In children 0 to 2 years of age, 42% were colonized with *S. pneumoniae* in Santiago compared to 14% in Temuco. A total of 41 serotypes were identified in both Chilean cities studied. Six serotypes were found only in Santiago; 14 serotypes were found only in Temuco. Antibiotic-resistant serotypes 6A, 6B, 14, 19F, and 23F were detected only in Santiago. We show that important differences in the incidence of nasopharyngeal carriage, infection, and *S. pneumoniae* serotypes can exist in similar populations in different areas of the same country. Our findings are relevant for prevention strategies, antibiotic usage, and vaccine design.

Streptococcus pneumoniae has been estimated to cause 10 to 25% of all pneumonias and 90% of bacterial pneumonias (38), 20 to 40% of otitis media (4, 29), and variable percentages of sinusitis. The annual incidence of invasive infections is highest in children younger than 2 years (9, 11). The incidence of all forms of pneumococcal infections increases in high-risk populations (1).

Capsular serotypes causing nasopharyngeal colonization and infections, as well as the development of antibiotic resistance, vary according to age, geographic location, and socioeconomic status of the study population (5, 6, 37, 39). Efforts to decrease risk factors and to prepare vaccines effective against the most frequent and/or severe pneumococcal infections require the identification of serotypes causing disease in different geographic areas and populations. The formulation of one conjugate vaccine to cover serotypes in the United States and Europe and another to protect against serotypes causing infections in the developing world has been proposed on the assumption that the same serotypes account for most infections in these two areas of the world (32).

Parallel studies recently performed in Chile analyzing pneumococcal serotypes isolated in two cities with different climate and pollution indices offered an opportunity to test this assumption. By using similar methodologies, these studies were conducted in populations of similar ethnic and socioeconomic backgrounds and receiving comparable levels of health care.

Significant differences between these two cities were found in nasopharyngeal colonization rates, in serotypes causing infections, and in antibiotic resistance. Our findings suggest that further studies of the local epidemiology of pneumococcal infections need to be performed before general immunization policies can be recommended for large geographic areas.

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MATERIALS AND METHODS

Geographic areas. Our study was performed in Santiago, the Chilean capital, and in Temuco, a city 500 mi south of Santiago. Santiago, with 4,500,000 inhabitants, has a dry, temperate climate with a high index of particulate and chemical pollution, including lead from gasoline combustion, throughout the year (data from the Environmental Health Service, Chilean Ministry of Health). The city of Temuco, with 300,000 inhabitants, has a temperate, rainy climate and a low pollution index most of the time.

Study populations. The study populations in both cities consisted of middle-class and low-income patients seeking medical care from the Chilean National Health Service at public hospitals and children from the same socioeconomic groups attending day care centers. There were no differences between the study populations from each city in socioeconomic level, ethnic group, family size, or access to health care. The populations of both cities come into regular contact through readily available ground and air travel.

Sample collection. (i) Santiago. As part of a special study, nasopharyngeal cultures were obtained within 24 h of diagnosis from all healthy household contacts of 32 children admitted with invasive pneumococcal disease from April 1995 to February 1996. In addition, samples were obtained from children, 2 months to 4 years old, attending two day care centers located in the same area of Santiago where patients and household contacts were studied. These samples were collected throughout the winter months of 1995.

All clinical *S. pneumoniae* isolates from patients with invasive infections and normally sterile sites were collected over the same period from patients in two pediatric hospitals serving two of the five health divisions of metropolitan Santiago.

(ii) Temuco. During a single week in the winter month of June 1996, nasopharyngeal cultures were obtained from adult health care workers and from children, 4 months to 15 years old, attending two National Health Service day care centers in Temuco. (School-age children of working parents attend day care after school.)

All clinical *S. pneumoniae* strains collected in the Hospital Regional de Temuco, a general hospital with internal medicine, surgery, obstetrics, and pediatric admissions, were collected and serotyped between April 1995 and February 1997.

TABLE 1. Nasopharyngeal carriage of *S. pneumoniae* in healthy children and adults in two Chilean cities

Age group	Santiago						Temuco		
	Contact			Day care			Day care and hospital staff[a]		
	No. tested	No. positive	%	No. tested	No. positive	%	No. tested	No. positive	%
0–2 yr	26	11	42	97	57	59	35	5	14
2–4 yr	18	8	44	103	63	61	20	5	25
5–7 yr	48	17	35				10	3	30
7–15 yr	45	11	24				6	2	33
Adults	65	6	9				38	2	5
Total	202	53	26	200	120	60	107	17	16

[a] Children, aged 0 to 15 years, were in day care; adults were healthy health care workers.

Sample definition, collection, and handling. All strains isolated from blood, spinal fluid, pleural fluid, or ascites were defined as invasive. A sputum strain isolated from one adult patient in Temuco with radiographically confirmed lobar pneumonia was also included in this group. Strains isolated from the conjunctiva, middle ear, or sinus cavities were classified as coming from normally sterile sites.

All clinical samples in both cities were obtained at the time of admission to the hospital. Samples from all sources were saved at −70°C in human group O defibrinated blood and also in 30% fat milk until typing. The same microbiologic culturing techniques were performed by similarly trained technologists in both cities.

Antibiotic usage and antibiotic sensitivity testing. With few exceptions, study patients in both cities received their medical care through the facilities of the Chilean National Health Service. Recommended antibiotic use in these clinics and hospitals was as follows: for otitis and sinusitis, amoxicillin, with cefuroxime used for patients not responding to amoxicillin; for bronchitis, amoxicillin or clarithromycin; for pharyngitis and amigdalitis, penicillin or clarithromycin; for lower respiratory infections, amoxicillin or cefuroxime, with cloxacillin for severe infections not responding to amoxicillin. Chloramphenicol or sulfa drugs are not included in treatment regimens for respiratory infections. These treatment recommendations were in place for 6 years prior to and throughout the study period.

In Santiago, MICs of penicillin, cefotaxime, vancomycin, and chloramphenicol were tested by microdilution in Mueller-Hinton medium, according to the recommendations of the National Committee for Clinical Laboratory Standards and also by E-test (AB Biodisk, Uppsala, Sweden).

In Temuco, antibiotic sensitivities of all strains were determined by the E-test for penicillin and cefotaxime.

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 erythrocyte agar plates (Difco Laboratories, Detroit, Mich.) overnight. The initial identification of strains of *S. pneumoniae* was performed by latex agglutination with antisera specific for groups and serotypes. All serotyping results were confirmed by Quellung test.

RESULTS

The percentages of *S. pneumoniae* nasopharyngeal carriers were different in Santiago and Temuco in all age groups for which comparative data was available (Table 1).

The number of cultures from pediatric inpatients positive for *S. pneumoniae* was higher in Temuco than in Santiago. In Santiago, 59 *S. pneumoniae* strains were isolated from 18,216 children admitted during the observation period (324/100,000). In Temuco, 90 cultures from 10,849 pediatric inpatients were positive for *S. pneumoniae* (829/100,000) and 89 positive cultures were obtained from 39,720 admitted adult patients (224/100,000).

A total of 41 serotypes were identified in the two Chilean cities. The serotypes isolated in Santiago and in Temuco showed important differences (Table 2). Six serotypes were found only in Santiago; 14 serotypes were found only in Temuco. Of 27 serotypes isolated in Santiago, 10 were identified in nasopharyngeal cultures only, with serotype 15B, isolated from 11 individuals, the most frequent of these serotypes. Five serotypes were identified only from infectious sites: 1, 4, 5, 18F, and 35A. Of 35 serotypes identified in Temuco, 8 serotypes were found only in the nasopharynx of healthy individuals. Serotypes 6A and 19F were isolated mainly from healthy individuals, although they were also identified in small numbers in infectious sites. Nine serotypes were isolated only from infectious sites; none of these serotypes was isolated with a much higher frequency than the others.

In Santiago, serotypes 6A, 6B, 14, 19F, and 23F had intermediate or high levels of resistance to antibiotics (Table 3). All strains resistant to cefotaxime or chloramphenicol were also resistant to penicillin. Although those serotypes showing resistance to antibiotics in Santiago were also isolated in Temuco, no serotypes found there had developed antibiotic resistance to penicillin or cefatoxime. High levels of resistance (MIC, ≥2 mg/ml) were detected in 9% of strains isolated from patients with invasive infections and in 8% of those isolated from the nasopharynx. Of the serotypes that had developed antibiotic resistance, high levels of resistance were present in 80% of 23F strains and in none of the 19F strains (Table 3).

DISCUSSION

Nasopharyngeal colonization rates vary significantly with age, with the highest rates observed in the first 2 years of life (22). For similar age groups, colonization rates vary widely between geographic regions. For instance, nasopharyngeal colonization with *S. pneumoniae* was found in 80% of children under 5 years of age in The Gambia (22), in 56% of healthy children under 2 years of age in Nebraska (6), and in 15.2% of healthy children in Uruguay (25).

Our study shows that important differences in the prevalence of nasopharyngeal carriage can also exist in different cities of the same country. The high incidence of pneumococcal colonization and disease in developing countries has been attributed to crowding and indoor pollution (2, 16, 21). Since the ethnic and socioeconomic characteristics of the study populations in Santiago and Temuco were similar and sampling in both cities was done mostly in the winter months, the differences in carriage of pneumococci may be attributed to the known variations in climate and pollution indices between these two cities. These observations suggest that further studies specifically designed to test this hypothesis in different regions of the world are warranted.

The capsular serotypes causing nasopharyngeal colonization in healthy children in Chile were different in both cities. The serotypes isolated from healthy individuals in Temuco were also significantly more varied than those identified in another South American country, i.e., Uruguay, where serotype 6 accounted for five of eight nasopharyngeal strains isolated (25).

The studies reported here were not designed to ascertain the epidemiology of *S. pneumoniae* in lower respiratory tract in-

TABLE 2. Frequency of *S. pneumoniae* serotypes isolated in two Chilean cities

Serotype	No. of isolates in city:							
	Santiago				Temuco			
	NP[b] culture	Sterile site	Invasive	Total	NP[b] culture	Sterile site	Invasive	Total
1			3	3		3	6	9
3	1	2		3	5	6	4	15
4			1	1	1		1	2
5			4	4			3	3
6A	12	2	1	15	10	1	2	13
6B	16	1	6	23	1	2	2	5
7A	1			1				
7C					1			1
7F	1	2	2	5	1	1	3	5
8							1	1
9N	1			1			2	2
9V					2			2
10A					4	2	1	7
11A					2			2
12F					2	2	4	8
13	1			1		1	2	3
14	3	2	3	8	1	1	1	3
15B	11			11			1	1
15C	7			7		3	2	5
15F	4		1	5				
16					1	1		2
17F					1	1	1	3
18A	1			1			1	1
18C	1		1	2	1			1
18F			2	2		2	1	3
19A	7	4	1	12		1	1	2
19C	1			1				
19F	8	7	1	16	13	4	1	18
20							1	1
21	2			2		1		1
22A	1			1				
22F					1	1		2
23A	2		1	3				
23F	3		2	5	3	6	2	11
24F	2		1	3				
28A					2			2
28F					1			1
33F					2	2	1	5
34	1			1			1	1
35A		1		1	1			1
35B					1			1
Total[a]	87	21	31	138	49	33	23	144

[a] Not all strains shown in Table 1 were available for serotyping.
[b] NP, nasopharyngeal.

fections in Chile. Overall, pneumococci were identified in 324 of 100,000 pediatric admissions in Santiago and in 829 of 100,000 pediatric admissions in Temuco. The reasons for this difference in incidence could not be established. Studies of the incidence of invasive disease show significantly different results in various areas of the world. In Auckland, New Zealand, the incidence was estimated at 22 per 100,000 for children younger than 15 years and 56 per 100,000 for children under 5 years (34). In Finland, the annual incidence of invasive infections in hospitalized 0- to 15-year-old children was 8.9 per 100,000 between 1985 and 1989; for children younger than 2 years, the rate increased to 45.3 per 100,000 (11). In West Africa, the incidence of invasive infection was estimated to be 554/100,000/year in children younger than 1 year and 240/100,000/year in those younger than 5 years (26).

The percentage of invasive diseases in Chile caused by pneumococci has not been evaluated recently. In a study performed in 1971 in one of the pediatric hospitals in Santiago also participating in the present study, the percentage of pneumonias caused by pneumococci was found to be very low. Bacteria were identified by lung puncture in 57% of 160 infants who had not previously received antibiotics and in 41% of pretreated patients. Only 5 of 238 bacterial strains isolated were pneumococci (24). In African countries, pneumococci were identified in much higher percentages of children with severe pneumonia: 15% in Zimbabwe (19), 51% in Nigeria (31), and in 20 to 61% in various studies in The Gambia (12, 13, 35).

Serotypes causing infections vary according to geographic area, type of infection, and age of the patients. In developing countries, such as Brazil, Uruguay, Mexico, Egypt, The Gambia, Pakistan, Papua New Guinea, and Rwanda, the predominant serotypes in rank order, 14, 6, 5, 1, 19, 9, and 23, ac-

TABLE 3. Antibiotic-resistant *S. pneumoniae* serotypes isolated in Santiago, Chile[a,b]

Serotype	No. of isolates with serotype	Penicillin				Cefotaxime[c] (intermediate resistance)		Chloramphenicol[c] (intermediate resistance)	
		Intermediate resistance		High resistance					
		No.	%	No.	%	No.	%	No.	%
6A	15	11	73	0	0	0	0	0	0
6B	23	11	48	3	13	3	13	4	17
14	8	5	62	1	12	3	38	2	25
19F	16	12	75	0	0	0	0	0	0
23F	5	1	20	4	80	3	60	2	40

[a] Only serotypes with antibiotic resistance are included in this table. All other serotypes were sensitive to all antibiotics tested. No *S. pneumoniae* isolates from Temuco were resistant.
[b] Resistance to vancomycin was also tested for all isolates. None was resistant.
[c] Only intermediate resistance was detected to cefotaxime and chloramphenicol.

counted for 75% of infections in these countries (37). In the United States and Europe, the predominant serotypes causing invasive infections differ mainly in that serotypes 1 and 5 are isolated less frequently and serotypes 4 and 9V are isolated more frequently than in developing countries (28, 30, 36).

Although the same serotypes cause the majority of infections in Chile and in other Latin American countries, important differences do exist in the percentages of the main serotypes isolated and in the total numbers of different serotypes identified. In Brazil, 42 serotypes causing infections were identified, with types 14, 6B, and 23F causing 8 to 10% of infections each (15). In Uruguay, only 12 serotypes causing invasive infections were identified, with serotype 14 causing 43% of infections, serotype 5 causing 16%, and group 9 causing 11% (25). In Mexico, 29 serotypes were identified, with serotypes 23F, 6B, 14, and 19A accounting for 20, 11, 9, and 9% of isolates, respectively (10). Our study suggests that significant differences in serotypes causing infections can exist between geographic areas in a single developing country.

When both nasopharyngeal carriage and infections have been studied for the same individual, a high degree of correlation has been found (17). However, one study found serotypes 1, 14, and 12 more frequently in patients than in healthy controls, suggesting that these serotypes have a higher propensity to invade (22). Our observations in two Chilean cities show that some serotypes are isolated more frequently from the nasopharynx than from infectious sites, while other serotypes cause infections more frequently. Serotype 15B was the predominant example found only in the nasopharynx of healthy individuals in Santiago. In Temuco, serotypes 6A and 19F were found predominantly in the nasopharynx, while serotype 3 was frequently isolated from normally sterile sites and invasive infections. This is an interesting observation since other studies have found this serotype to be a frequent isolate from the respiratory tract (18, 20) but an infrequent cause of invasive *S. pneumoniae* infections (30). The variability in the serotypes causing nasopharyngeal colonization or infection in different geographic areas should be explored further before conclusions regarding invasiveness can be reached with certainty.

Serotypes found to have developed antibiotic resistance in Santiago are the same serotypes that have developed resistance to antibiotics in the United States (14, 39), where serotypes 6B, 23F, 14, 9V, 19A, and 19F account for nearly 85% of strains resistant to at least one drug class. The same serotypes have also developed antibiotic resistance in other areas of the world (3, 7, 23, 27), suggesting a relationship between capsular serotypes and changes in antibiotic-binding proteins leading to antibiotic resistance in *S. pneumoniae.*

The emergence of pneumococcal strains with resistance to penicillin and other antibiotics poses a challenge to developing new approaches for the prevention of pneumococcal infections (8). There is significant variation in antibiotic resistance in different geographic areas (5, 6, 14, 39), which is also reflected in the difference between Santiago and Temuco. The reason for this difference is not immediately apparent. The same capsular serotypes found to have developed antibiotic resistance in Santiago were also isolated in Temuco but were not found to have developed resistance. Since the development of resistance is likely to occur under the selective pressure exerted by antibiotic use (14), differences in antibiotic use may explain the observed differences in resistance (33). Although the same treatment schedules are used in both Chilean cities, we cannot rule out greater antibiotic use in Santiago. Antibiotic consumption records are not available for the study populations.

Other mechanisms for the development of antibiotic resistance may also be operative, since antibiotic exposure is not always linked to the development of antibiotic resistance (6). Once produced, the mutations coding for resistance are stable and resistant strains can spread in the absence of exposure to antibiotics. The absence of antibiotic-resistant strains in Temuco suggests that transmission of pneumococcal serotypes from one area to another is not important between these two cities.

Current recommendations for vaccine formulation are based on serotypes and serogroup distribution for invasive and sterile-site pneumococcal infections. The existing conjugate vaccine formulations for developing countries would cover over 70% of strains isolated in Santiago and 62% of strains isolated from children in Temuco (32). As antibiotic resistance increases, the design of vaccines may need to include strains frequently isolated from patients with mucosal infections. While not life threatening, mucosal infections are much more frequent than invasive infections (2,333 cases of otitis for each case of meningitis and 35 cases of otitis for each case of pneumonia) (8). Continued monitoring of *S. pneumoniae* strains causing mucosal and invasive infections in children and adults in Chile and of the development of antibiotic resistance will be needed to define future immunization strategies for the country as a whole and for its different regions.

ACKNOWLEDGMENTS

This research was supported in part by an unrestricted grant from Lederle-Praxis Biologicals and by project 1950635, FONDECYT-Chile.

We thank Terry Thompson for his advice and support in the serotyping of streptococcal strains at the Centers for Disease Control and

Prevention and Patricia Giangrosso for assistance in the preparation of the manuscript.

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