

## Ten-Year Surveillance of Pneumococcal Infections in Temuco, Chile: Implications for Vaccination Strategies<sup>∇</sup>

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Received 11 October 2006/Returned for modification 8 December 2006/Accepted 20 March 2007

We monitored *Streptococcus pneumoniae* serotypes causing invasive infections in patients admitted to one hospital in southern Chile during a 10-year period (1994 to 2004). All specimens isolated from patients with invasive *S. pneumoniae* infections were serotyped at the CDC in Atlanta, GA. A total of 508 isolates belonged to 58 serotypes. There were 95 infections in patients <2 years old, 33 infections in patients 2 to 4 years old, 61 infections in patients 5 to 14 years old, 66 infections in patients 15 to 44 years old, 134 infections in patients 45 to 64 years old, and 120 infections in patients ≥65 years old. The 10 serotypes isolated with the highest frequency in all groups were, in decreasing order, 1, 3, 14, 5, 19F, 6B, 7F, 12F, 23F, and 6A. The 10 most frequent isolates in children under 2 years of age were 1, 6B, 14, 19F, 5, 23F, 6A, 9V, and 7F. In patients ≥65 years old, the most common serotypes were 3, 7F, 1, 14, 19A, 23F, 19F, 35B, 4, and 5. Penicillin resistance was detected in 14 (2.7%) clinical specimens isolated since 1998, with 13 resistant strains identified since 2001. Vaccine coverage for the 7-valent conjugate vaccine was 42% for children <2 years of age. This study is important for the design of vaccines for this region and to evaluate public health measures to decrease pneumococcal infections.

*Streptococcus pneumoniae* infections are caused by 90 serotypes grouped into 46 serogroups based on immunological similarities (12). The capsular serotypes of *S. pneumoniae* causing invasive infections vary according to the geographic location and socioeconomic status of a study population (1, 2, 13, 34, 36). Few studies have monitored serotype changes in invasive pneumococci over time, especially in the absence of increased antibiotic resistance, vaccine selective pressure, socioeconomic changes, or debilitating diseases.

In a previous communication, we reported that age was clearly a factor in the overall incidence of invasive infections, with infections being most frequent in the first years of life (14). Selective pressure has driven the emergence of worldwide antibiotic resistance of some serotypes (1, 2, 6, 16, 36). Immunization practices and antibiotic resistance due to preventive antibiotic use in special-risk populations are important factors influencing the incidence of serotypes causing infection (31). Host-related factors also contribute to susceptibility to pneumococcal infection. Underlying heart and central nervous system diseases, as well as malignancies, are frequently identified in patients developing invasive infections (16), as are underlying immune abnormalities like human immunodeficiency virus

(HIV) infection, which are major risk factors for invasive pneumococcal infections (24).

We have monitored *S. pneumoniae* serotypes associated with invasive and sterile-site infections in patients admitted to one regional general hospital in Southern Chile during a 10-year period. During this study period, the patient population remained relatively homogenous and without HIV infection. Pneumococcal immunization programs have not been implemented for any age group. Our results document that even in the absence of known selective pressures, changes in pneumococcal disease incidence and serotype distributions are associated with different age groups.

### MATERIALS AND METHODS

**Study population.** The study population consisted of patients of all ages seeking medical care and being admitted to any of the inpatient services of the Regional Hospital in Temuco (Hospital Dr. Hernán Henríquez A) in southern Chile. The lower- 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, obstetrics, and pediatric services. All samples are sent to the central laboratory of the hospital.

All patients admitted to the hospital are screened for both HIV type 1 (HIV-1) and HIV-2 serology by enzyme-linked immunosorbent assay (Abbott Laboratories, Chicago, IL). All patients with pneumococcal infections in this study were HIV seronegative.

Bacterial cultures were obtained by following the same criteria during the entire observation period. According to Emergency Service guidelines, cultures were obtained only in patients whose clinical presentation was severe enough to warrant an in-hospital observation period. Blood cultures were obtained in febrile patients with systemic symptoms. Spinal fluid was cultured in patients with clinical suspicion of meningitis or central nervous system compromise. According to protocol, no outpatients were evaluated with cultures.

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<sup>∇</sup> Published ahead of print on 28 March 2007.

**Sample definition and collection.** All *S. pneumoniae* strains from invasive pneumococcal disease (IPD) were included in this study. These included strains isolated from blood, spinal fluid, pleural fluid, or ascites. Strains isolated from sputum or mucosal sites such as the conjunctiva, middle ear, or sinus cavities were not included in this study.

The same laboratory personnel performed all cultures and *S. pneumoniae* identification over the 10-year study period. Samples were collected around the clock, and the laboratory was staffed at all times. From 1994 to 2002 a culture medium made in-house was used (heart brain broth) (4).

In 2002 the automated Bac-Alert heart brain broth (BioMerieux, Lyon, France) was introduced and replaced the in-house medium. Clinical isolates were collected between February 1994 and November 2004. The analysis of serotypes was performed for the entire observation period and also for each of five 2-year periods: 1994 to 1996 (this period included some serotypes isolated in 2004), 1997 to 1998, 1999 to 2000, 2001 to 2002, and 2003 to 2004. Results for the first 5 years of surveillance have been previously published (14).

**Pneumococcal serotyping.** Pneumococcal serotyping was performed in the pneumococcal serotyping laboratory at the Centers for Disease Control and Prevention, Atlanta, GA. Before serotyping, cultures were transferred to 5% sheep blood agar plates (Difco Laboratories, Detroit, MI) overnight. All serotyping results were confirmed by quellung typing with absorbed polyclonal rabbit sera.

Antibiotic sensitivity of all isolates was determined by the Etest for penicillin. Penicillin-resistant isolates were also tested for cefotaxime, ceftriaxone, and vancomycin (12, 15). For analysis, the study population was divided into three age groups: under 5 years of age, 5 to 64 years of age, and over 64 years of age.

**RESULTS**

**Bacterial culture results.** An exact account of all blood and sterile fluid cultures performed was kept for the last 5 years of the observation period. There was no significant change in the percentages of positive cultures over this period of time. The yearly average number of blood cultures was 6,604 (range, 5,023 to 7,185), and the yearly average number of sterile fluid cultures was 1,230 (1,513 to 1,031). The average percentage of cultures positive for bacteria was 11.6% (range, 9.8 to 14.6%) for blood cultures and 2.7% (2.4 to 3.1%) for sterile fluid cultures. The percentage of cultures positive for *S. pneumoniae* was 1.14% (0.8 to 1.47%) for blood cultures and 1.1% (0.9 to 1.5%) for sterile fluid cultures.

**Epidemiology.** The total population cared for at the Temuco Regional Hospital during the 10-year study period in each of these age groups remained stable. The average yearly population in different age groups was as follows: children <2 years old, 11,311 patients (range, 10,969 to 11,671), with a yearly incidence of IPD of 80.0 cases/100,000; children 0 to 5 years old (including the previous group of children <2 years old), 28,820 patients (range, 27,454 to 29,738), with a yearly incidence of IPD of 46.7 cases/100,000; individuals 5 to 64 years old, 250,621 patients (range, 213,823 to 278,471), with a yearly incidence of 9.95 cases/100,000. For the population ≥65 years old, the average yearly number of patients was 19,050 (range, 15,780 to 21,911) with an average yearly incidence of 60.0 cases/100,000.

**Serotypes isolated over 10 years.** The total number of isolates from all age groups was 514. Only five were not typeable and were not included in this report. The 509 *S. pneumoniae* typeable isolates represented 58 serotypes isolated during the entire study period (Table 1). Of the 58 serotypes, 14 were observed only once, and five additional serotypes were observed only twice. Altogether, 27 serotypes were observed fewer than five times. The highest number of infections was observed in children under 2 years of age (*n* = 95) and in patients who were ≥45 years old (254 cases of IPD). The 10

TABLE 1. Frequency of infections due to different pneumococcal serotypes in 10-year period (1994 to 2004) in Temuco, Chile

Serotype <sup>a</sup>	No. of isolates						Total
	<2	2-4	5-14	15-44	45-64	≥65	
1	14	6	13	14	13	7	67
3			3	2	16	15	36
4	1	1		1	1	4	8
5	8	4	5	5	7	3	32
6A	5	1	1	1	3	3	14
6B	14	2	2	1	4	3	26
7A					1		1
7F	2		3	6	5	10	26
8			2	1	3	3	9
9A	1				1		2
9L					1		1
9N				1	3	3	7
9V	3	2		2	5	1	13
10A	1			1	4	1	7
10B						1	1
10F				1			1
11A		1	2	2			5
11F				1			1
12F	2	1	3	5	8	3	22
12B					2	1	3
13			1	3	5	2	11
14	9	1	7	5	5	7	34
15A	1				1	1	3
15B	1			1	4	2	8
15C	1	1	2	1	1	2	8
16			3	2	5	1	11
17F			1	1	1	2	5
18A	1				2	1	4
18C	2	2	1		2	3	10
18F				1	1	1	3
19A	2	1	2		2	6	13
19F	9	3	2	2	6	5	27
20	1		1		3		5
21	1						1
22A						1	1
22F	1		1		2	2	6
23A						2	2
23B				1		1	2
23C					1		1
23F	7	5	3	1	1	5	22
24A					1		1
24F			1				1
25					1		1
27	1					2	3
28F	1						1
29				1		1	2
31	1				1	1	3
33							
33F	3			2	1		6
34	1		1			1	3
35A					3	1	4
35B					2	4	6
35F	1	1			3	3	8
36					2	1	3
37		1					1
38					1	3	4
47F						1	1
48			1	1			2
Total	95	33	61	66	134	120	509

<sup>a</sup> Serotype 2 is included in the 23-valent vaccine but was never isolated.

serotypes identified with the highest frequency were, in decreasing order, 1, 3, 14, 5, 19F, 6B, 7F, 12F, 23F, and 6A.

**Serotypes and age.** The overall distribution of serotypes causing infection in the six different age groups is shown in

Table 1. Some serotypes were isolated exclusively in children who were <2 or in patients who were ≥65 years old. The most commonly isolated serotypes in children <2 years old were serotypes 1, 6B, 14, 19F and 5; serotypes 3, 7F, 4, and 18C were never isolated from this age group. In children 2 to 4 years of age the most frequent serotype was, again, serotype 1, followed by 23F. Again, no serotypes 3 and 7F were isolated. Serotypes 3 and 7F appear in patients 5 years and older. In patients ≥65 years old, serotypes 3 and 7F become the most frequently isolated serotypes, followed by serotypes 1 and 14. Serotypes 5 and 6B were isolated in only three patients each in this age group.

The serotypes identified most frequently in each age group tend to be isolated in each of the five 2-year periods. For children under 2 years old, the most frequent serotypes isolated throughout the study were 1, 6B, 14, and 19F. In contrast, for patients ≥65 years old, the most frequent were serotypes 3, 7F, and 1. All other serotypes were isolated in only some of the 2-year periods. Notably, serotype 5 was isolated in children <2 years of age six times in 1997 to 1998 but only two additional times in the remaining observation period. In patients ≥65 years old, serotype 14 was not identified in the first 4 years of the study but was isolated regularly starting in 1999. Sporadic isolation of some serotypes was also frequent in each age group.

**Antibiotic resistance.** Antibiotic resistance was identified in only one clinical isolate in 1988. This serotype, 23F strain, was isolated from a child younger than 5 years of age. It was highly resistant to penicillin and cefotaxime but not to vancomycin (7). Thirteen additional resistant serotypes were isolated starting in 2001. The resistant strains included serotype 14 (four isolates), serotype 11A (two isolates), and serotypes 5, 6B, 9V, 18F, 19A, 19F, and 23F. All strains were sensitive to cefotaxime, ceftriaxone, and vancomycin. Notably, two penicillin-resistant 11A isolates were isolated in two consecutive years. Since detection of antibiotic resistance in 2001, the incidence of resistance has remained constant.

**Vaccine coverage.** The estimated vaccine coverage offered by different vaccines to patients of different age groups in the five 2-year periods of this study is summarized in Table 2. Overall, the coverage offered by the different vaccines for each age group remained stable over the entire observation period. The 7-valent conjugate vaccine coverage for children <2 years of age ranged from 40 to 60%, with much lower coverage in all other age groups. Of 23 serotypes in the polysaccharide vaccine, one (serotype 2) was never isolated. All other vaccine serotypes were isolated on at least five occasions with 67, 36, and 34 isolates recovered for serotypes 1, 3, and 14, respectively. However, coverage by the 23-valent vaccine was only 61 to 68% for patients ≥65 years of age.

## DISCUSSION

**Epidemiology.** The incidence of pneumococcal infection varies widely in the world and even varies within countries (20). The incidence of invasive pneumococcal disease is influenced by age, immunization status, and ethnic background (11). Our results further confirm the high incidence of pneumococcal infections in young children that has been observed in other studies (5, 33). This observation suggests that there should be

TABLE 2. Coverage of vaccine serotypes in 10-year period (1994 to 2004) in Temuco, Chile

Vaccine	Age range of patient group (yr)	No. of patients positive for any serotype(s) covered by vaccine/total no. infected (% coverage) <sup>a</sup>		Coverage range (%) <sup>a,c</sup>
		No cross-reactivity	Cross-reactivity <sup>b</sup>	
7 valent <sup>d</sup>	<2	45/95 (42)	51/85 (53)	40–60
	2–4	16/33 (49)	17/33 (52)	40–63
	5–14	15/61 (25)	16/61 (26)	11–50
	15–44	12/66 (18)	13/66 (20)	8–30
	45–64	24/134 (18)	27/134 (20)	5–28
	>65	28/120 (23)	31/120 (26)	13–41
10 valent <sup>e</sup>	<2	69/95 (73)	74/95 (78)	60–82
	2–4	26/33 (79)	27/33 (81)	50–89
	5–14	36/61 (58)	37/61 (61)	50–86
	15–44	37/66 (56)	38/66 (58)	46–64
	45–64	50/134 (37)	53/134 (37)	16–66
	>65	48/120 (40)	51/120 (43)	26–50
13 valent <sup>f</sup>	<2	78/95 (80)		70–82
	2–4	26/33 (85)		50–81
	5–14	42/61 (69)		50–83
	15–44	40/66 (61)		46–71
	45–64	71/134 (53)		32–81
	>65	72/120 (60)		43–65
23 valent <sup>g</sup>	<2	80/95 (84)	85/95 (90)	84–100
	2–4	29/33 (88)	30/33 (81)	71–93
	5–14	51/61 (84)	52/61 (85)	71–93
	15–44	51/66 (77)	52/66 (79)	62–81
	45–64	96/134 (72)	99/134 (74)	53–88
	>65	85/120 (71)	88/120 (73)	61–86

<sup>a</sup> Values indicate amount of coverage that would have been afforded had the vaccines been in use during the surveillance period.

<sup>b</sup> Coverage of serotype 6A included. The 13-valent vaccine already provides protection against serotype 6A; hence, no values were calculated for this vaccine.

<sup>c</sup> For these values, coverage of serotype 6A was not taken into account. For each age group, the coverage was evaluated for five 2-year periods, and the range of the five resulting values is given here.

<sup>d</sup> The 7-valent vaccine provides protection against serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F.

<sup>e</sup> The 10-valent vaccine provides protection against serotypes 1, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

<sup>f</sup> The 13-valent vaccine provides protection against serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

<sup>g</sup> The 23-valent vaccine provides protection against serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F.

a continuous program of monitoring invasive pneumococcal infections.

Recently, HIV infection has been identified as an important risk factor for invasive pneumococcal disease (24). This factor was ruled out for our population because there were no HIV-positive individuals in the survey.

**Pneumococcal serotypes causing infection over a 10-year period.** Of the 58 serotypes found among individuals over the 10-year period, 27 serotypes were found fewer than five times, suggesting that some serotypes are rarely associated with invasive infection even when present in a community. Bacterial factors are likely to influence the serotype spectrum that is associated with invasive infections. When both nasopharyngeal carriage and invasive infection isolates have been studied from the same individual, a high degree of correlation in serotypes has been found (8). On the other hand, some pneumococcal serotypes found colonizing the nasopharynx have little tendency to cause invasive disease (13, 19, 32). These observations

suggest that certain pneumococcal serotypes have characteristics that are advantageous for invasiveness.

Serotype distribution within age groups changed over the 10-year period of this survey. However, some differences among age groups remained throughout the entire study period, suggesting the importance of continuing to search for specific susceptibilities for some serotypes, especially in young children and the elderly.

The absence of serotype 3 pneumococcal infections among children under 5 years of age was observed throughout the survey, although this serotype was a frequent cause of infection in all patients above 5 years of age. This observation is consistent with several studies that have shown that serotype 3 pneumococci are frequently recovered from the respiratory tract (9, 15) but are infrequent causes of invasive *S. pneumoniae* infections in children (21, 27, 29). In a study of invasive infections in children under 5 years of age in Santiago, Chile, serotype 3 caused only 3.3% of infections (17). The literature suggests that in young children serotypes 1, 5, and 7F play a dominant role in IPD. In the outpatient study population only serotypes 1 and 5 were isolated in patients under 5 years of age, while serotype 7F was isolated among older patients (10).

The absence of serotype 14 in isolates from elderly patients in the first part of our study was surprising (14) because this was a serotype commonly isolated from patients over 60 years of age in the United States and New Zealand (3, 21). However, serotype 14 was isolated from the elderly in the second part of our surveillance, suggesting that the initial observation was not due to an intrinsic resistance of older individuals to this serotype.

All the data presented in this study were based on quellung typing with adsorbed polyclonal rabbit sera. Serotype identity may vary if different typing methods are used, like monoclonal antibody typing (18, 35). For example, using this methodology a new serotype (6C) was identified; this serotype was not detected using the traditional typing method (23).

In our population there is no herd immunity since no immunization against pneumococcal infection has been introduced. Any change in serotype distribution is likely due to natural changes in the serotypes in circulation and/or the development of natural immunity in the population.

**Antibiotic resistance.** During the first 5 years of the study, only one clinical isolate (serotype 23F) was found to be highly resistant to penicillin and cefotaxime but not to vancomycin (7). This high-level resistance was not identified in any strain during the remainder of the study. Most antibiotic-resistant strains isolated since 2001 in Temuco have been described to have developed antibiotic resistance worldwide (11). Serotypes commonly associated with antibiotic resistance worldwide are 6A, 6B, 9V, 14, 19A, 19F, and 23F. A higher rate of nasopharyngeal colonization and exposure to antibiotics may have contributed to the development of antibiotic resistance in these serotypes (11). The identification of two 11A isolates resistant to penicillin confirms the potential for this serotype to develop penicillin resistance, as observed earlier (25). All 11A isolates that developed penicillin resistance belonged to a single clone (ST156). We plan to determine if the resistant isolates identified in our study are similar to those described earlier by multilocus sequence typing.

Interestingly, after 2001, we did not observe an increase in

antibiotic resistance. The development of resistance is likely to occur under the selective pressure exerted by antibiotic use (6). The observed differences in resistance may be attributed to differences in the antibiotic (31). The guidelines for use of antibiotics for common respiratory infections recommended by the Chilean National Health Service have not changed in Temuco, where most patient care is delivered at clinics following these guidelines (13). Sale of antibiotics without prescription is not allowed, and antibiotics are generally restricted by prescribing physicians. This may explain why there has not been a steady increase in antibiotic resistance in this area.

Vaccine coverage is 61% for the available 7-valent conjugate vaccine in our survey area, which is relatively low. Overall, this vaccine covers 70% of infections. However, this coverage decreases with age, as seen in our study (11), reaching only 29% coverage in elderly persons  $\geq 65$  years of age. Our results confirm that information concerning the seroepidemiology of pneumococcal disease in different areas of the world is essential for the formulation of widely applicable conjugate vaccines (30).

**Summary and conclusions.** The observations made in this 10-year survey are relevant for prevention strategies, antibiotic usage, and vaccine design. Current recommendations for conjugate multivalent vaccine formulations are based on the serotypes and serogroup distribution for invasive and sterile-site pneumococcal infections in young children and infants (30). Conjugated vaccines are recommended for children under 5 years of age (26).

One important observation is that serotypes 4, 9V, and 18C, representing three of the 7-valent conjugate vaccine serotypes, caused no more than five infections in children under 5 years of age in our study population in Temuco, Chile. Continued surveillance of pneumococcal infections among different age groups is necessary to design the most effective vaccines to be used at the most appropriate age. The spontaneous variability in serotype isolation observed in this surveillance is of interest. Sensitive techniques like multilocus sequence typing (for follow-up of clones) will be used in the future to track these changes (22, 28).

#### ACKNOWLEDGMENTS

We thank Judith Rodriguez and Elizabeth Gordon for assistance in the preparation of the manuscript.

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