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Evolution of *Streptococcus pneumoniae* serotypes and penicillin susceptibility in Latin America, Sireva-Vigía Group, 1993 to 1999

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Background. **Since 1993 the Pan American Health Organization has coordinated a surveillance network with the National Reference Laboratories of Argentina, Brazil, Chile, Colombia, Mexico and Uruguay aimed at monitoring capsular types and antimicrobial susceptibility of *Streptococcus pneumoniae* causing invasive disease in children <6 years of age.**

Methods. **The surveillance system included children 6 years of age and younger with invasive disease caused by *S. pneumoniae*. The identification, capsular typing and susceptibility to penicillin of the isolates were conducted using a common protocol, based on standard methodologies.**

Results. **By June, 1999, 4105 invasive pneumococcal isolates had been collected mainly from pneumonia (44.1%) and meningitis (41.1%) cases. Thirteen capsular types accounting for 86.1% of the isolates (14, 6A/6B, 5, 1, 23F, 19F, 18C, 19A, 9V, 7F, 3, 9N and 4) remained the most common types during the surveillance period. Diminished susceptibility to penicillin was detected in 28.6% of the isolates, 17.3% with intermediate and 11.3% with high level resistance. Resistance varied among countries and increased during this period in Argentina, Colombia and Uruguay. Serotypes 14 and 23F accounted for 66.6% of the resistance.**

Conclusion. **These surveillance data clearly demonstrate the potential impact of the introduction of a conjugate vaccine on pneumococcal disease and the need for more judicious use of antibiotics to slow or reverse the development of antimicrobial resistance.**

INTRODUCTION

Invasive pneumococcal disease is a major cause of death and disability in developing countries.[1] Since penicillin resistance in *Streptococcus pneumoniae* was first reported in the mid-1960s, resistance to this, and to other antibiotics, has increased worldwide,[2–5] emphasizing the need for preventing pneumococcal disease. The success of *Haemophilus influenzae* type b-conjugated vaccine has focused efforts on developing conjugated pneumococcal vaccines.[6–9] However, the complexity of *S. pneumoniae* as a pathogen, caused part by its 90 immunologically different capsular types, must be taken into account.[7] Additionally it is well known that there are geographic and age-related differences, as well as temporal variations, in the prevalence of disease-causing serogroups.[1, 2, 7] Consequently to establish the epidemiologic relevance of a conjugated vaccine formulation, it is mandatory to understand the local epidemiology of pneumococcal invasive disease in specific geographic settings, especially in developing countries, where the impact of invasive pneumococcal disease on children is greatest.[10]

Surveillance studies have contributed to an expanded understanding of the epidemiology of infectious diseases worldwide,[11] and specific *S. pneumoniae* surveillance studies have been conducted.[12–15] One of the most successful programs has been the one supported since 1993 by the Pan American Health Organization (PAHO), with substantial funding contributed by the

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Canadian International Development Agency. This is a multicenter, multicountry surveillance network (SIREVA-Vigía) in Latin America that monitors invasive pneumococcal disease in children <6 years of age.[16] The aim of the project is to determine the relative prevalence of capsular types and antimicrobial susceptibility of *S. pneumoniae* causing invasive disease, especially pneumonia, in children of the specified age. This information should provide reliable data for the formulation of an adequate vaccine for the region.

The objective of this study was to evaluate the course of capsular types and of susceptibility to penicillin of invasive *S. pneumoniae* isolates, collected from Latin American children <6 years of age. This study reports the surveillance results of the SIREVA-Vigía program from 1993 to June, 1999. Preliminary data on this surveillance have previously been published.[17–22]

METHODS

Participating countries and hospitals. The countries and the participating centers were selected as described previously.[16, 22] Briefly countries and hospitals were selected if they were willing to participate, served a pediatric patient population and were prepared to follow the protocol and participate in the quality control/quality assurance program. One hundred twenty-eight hospitals in 74 cities in Argentina, Brazil, Chile, Colombia, Mexico and Uruguay participated in this network.

Patients. The basic inclusion and exclusion criteria were standardized by the study group to be children 6 years of age and younger with invasive disease caused by *S. pneumoniae*. This included: pneumonia, using the World Health Organization's clinical criteria, without taking into account radiographic results, meningitis, either sepsis or bacteremia without an infectious focus, arthritis or peritonitis.[22] Data collected on each case included age, gender and initial diagnosis.

***S. pneumoniae* identification and serotyping.** All pneumococci were recovered from normally sterile fluids. A common protocol for identification and capsular typing, based on standard methodologies,[23] was provided by the National Centre for *Streptococcus* (NCS). Serogrouping and serotyping of *S. pneumoniae* was performed at each of the National Public Health Laboratories using the Quellung reaction and an abbreviated typing system consisting of 12 pooled sera produced by the Statens Seruminstitut (Copenhagen, Denmark).[24] Isolates that could not be classified with this system were forwarded to the NCS for further serotyping. Isolates were classified as nontypable only if the NCS was unable to categorize them as one of the 90 known serotypes.

Susceptibility to penicillin. All isolates were screened for resistance to penicillin using a 1-μg oxacillin disc on Mueller-Hinton agar supplemented with 5% sheep blood.[25] The MIC to penicillin was determined for all strains with an oxacillin zone size of ≤19 mm. Testing was performed by broth microdilution or by the agar dilution method with *S. pneumoniae* ATCC 49619 as a reference control strain in each testing batch.[26] Interpretive standards provided by the National Committee for Clinical Laboratory Standards were used to interpret the penicillin MIC values: ≤0.06 μg/ml as susceptible; 0.12 to 1.0 μg/ml as intermediate level of resistance (ILR); and ≥2.0 μg/ml as high level resistance (HLR).[26, 27]

Quality control/quality assurance system. This international system was coordinated from Canada by the NCS. All national laboratories referred 10% of their typable isolates plus all those that they were unable to classify using the pool typing system to the NCS for confirmation. In addition an external quality assurance panel consisting of five unknown isolates was distributed twice a year to the reference centers for identification, serotyping and antimicrobial susceptibility testing.

Statistical analysis. All the data were entered and verified with the use of Epi-Info 6.0.[28] Comparisons of the proportions of each of the serotypes by diagnosis were made by chi square analysis. Also a chi square test for linear trend on proportion was used to measure the statistical significance of temporal changes in overall penicillin susceptibility and for ILR and HLR proportions for each serotype.[29, 30]

The significance of the trend of the capsular types and penicillin resistance (high or intermediate level) (PRSP) over time was measured at two points in time (1994 and 1998) calculating the expected and the observed numbers. The statistical difference between the two data was measured with Poisson approximation to the normal distribution.[29, 30] Statistical analysis were calculated with 95% confidence intervals.

A logistic regression model was constructed to evaluate the main variables that could explain the evolution of antimicrobial resistance. The dependent variable was the presence or absence of antimicrobial resistance. The independent variables were: patients' age (as a continuous variable); diagnosis (pneumonia, meningitis and others); country; serotypes; and year of isolation. Odd ratios and 95% confidence intervals (95% CI) were calculated, and linear or nonlinear analysis were done when required, specifically for variables with more than two categories. Statistical significance of every variable included in the model was assessed using the likelihood ratio test.[30] The multivariable model was constructed using STATA Version 6.0.[31]

RESULTS

Number of isolates by year and by country. Between February, 1993, and June, 1999, a total of

4105 isolates were collected. The distribution by year and country is shown in Table 1. The isolates were recovered in 27 hospitals from 19 cities in Argentina, 20 hospitals from 12 cities in Brazil, 32 hospitals from 16 cities in Chile, 20 hospitals from 12 cities in Colombia, 6 hospitals from 4 cities in Mexico and 23 hospitals from 11 cities in Uruguay. The number of participating centers within the various cities increased with time in almost all countries (in Argentina from 15 to 27, in Brazil from 16 to 20, in Colombia from 14 to 20 and in Uruguay from 11 to 23).

Patients, demographic data, origin of isolates and clinical diagnosis. Regarding gender, information was available from 3838 (93%) patients; 59.1% were male and 40.9% were female. The distribution was similar, with a predominance of male patients in each country. Ages were available for 3965 (96.6%) patients; 3120 (78.7%) children were 2 years or younger, 771 (19.4%) were between 3 and 5 years and 74 (1.9%) were 6 years old. In the 2 years and younger age group, the distribution of patients by country was: Argentina, 83.5%; Brazil, 83.9%; Chile, 80.2%; Colombia, 79.3%; Uruguay, 74%; and Mexico, 51.9%.

Isolates were recovered from blood (1865), cerebrospinal fluid (1452), pleural fluid (600), articular and peritoneal fluid (50) and others (138). Clinical diagnoses were reported for 3873 (94.3%) patients. This information is presented for each country in Table 2. Pneumonia and meningitis were the most common diagnoses followed by sepsis or bacteremia without infectious focus and other diagnoses (peritonitis and arthritis). No major changes were observed during the study period.

Capsular types. Typing was available for 4071 (99.2%) isolates and these could be assigned to 54 capsular types; 31 (0.76%) isolates were nontypable. Distribution of the 13 most prevalent capsular types by year, for 3507 (86.1%) of the total isolates, is shown in Table 3. These same 13 types accounted for between 81.4 and 88.6% in each year of the surveillance period.

The 13 prevalent capsular types by country are shown in Table 4. Serotypes 14, 6A/6B, 5, 1 and 7F ranked as the five most common serotypes in the Southern Cone (Argentina, Chile and Uruguay), whereas in Colombia and Mexico capsular type 23F ranked as third and second, respectively. In Brazil 18C ranked fourth.

Although the prevalent capsular types remained the same during the surveillance period, in an analysis made at two times (1994 and 1998), four countries observed statistically significant variations ($P < 0.05$) for four of the eight most prevalent serotypes. Serotype 14 increased in Brazil (from 18.7% to 29.3%) and Colombia (from 20.0% to 40.3%); serotype 1 increased in Uruguay (from 11.1% to 27.9%) but decreased in Brazil (from 13.2% to 8.65%); serotype 19F increased (from 4.1% to 8.9%) in Mexico; and serotype 18C increased in Brazil (from 4.9% to 9.0%), but decreased in Colombia (from 6.4% to 1.3%).

Analysis of the association of capsular types with clinical diagnoses indicated that 11 capsular types caused 86.2% of the pneumonia cases, 78.3% of the meningitis and 76.9% of the sepsis or bacteremia cases. The data for the first two pathologies are presented in Table 5. Some serotypes were more frequently associated with pneumonia or meningitis, but the strength of the relation widely varied among countries. Correlation between capsular types and diagnosis revealed some differences by country. In pneumonia cases, even though there was a strong overall association with serotype 14 (33.1% *vs.* 20.0% of meningitis isolates), it ranged from 42.6% in Uruguay to 11.8% in Mexico. The other capsular types associated with pneumonias were, in order of frequency, 1, 5, 6A/6B and 23F (Table 5). This association was low for serotypes 1 and 5 in Mexico (3.9 and 3.3%, respectively) and for serogroup 6A/6B (3.0%) in Uruguay but was high for capsular type 23F in Colombia and Mexico (8.6 and 14.4%, respectively).

Capsular types associated with meningitis cases were, in decreasing frequency order, 14, 6A/6B, 5, 18C and 23F (Table 5). Differences were also observed according to countries; thus the association with serogroup 6A/6B was less important in Argentina (7.1%) and Chile (6.5%). Serotype 5 represented 17.0, 15.6 and 13.3% of the total cases in Chile, Uruguay and Argentina, respectively, but only 6% of the meningitis cases in Brazil and Colombia and 2% in Mexico. Conversely

TABLE 1. Number of *Streptococcus pneumoniae* isolates recovered from sites, by year and by country from 1993 to June, 1999 ($n = 4105$)

Country	1993	1994	1995	1996	1997	1998	1999	Total	
								n	%
Argentina	95	214	208	126	102	187	74	1006	24.5
Brazil	73	182	213	174	203	256	102	1203	29.3
Chile	0	79	108	58	85	71	94	495	12.1
Colombia	0	140	167	101	94	77	44	623	15.2
Mexico	0	73	120	66	54	65	45	426	10.4
Uruguay	11	64	79	69	60	43	20	352	8.6
Total	179	752	895	594	598	699	379	4105*	100.0

* No information on collection year for nine isolates.

TABLE 2. Number and percent of clinical diagnoses by country: surveillance period 1993 to June, 1999 (n = 3873)*

Diagnosis	Argentina	Brazil	Chile	Colombia	Mexico	Uruguay	Total
Pneumonia	504 (58.3)†	384 (31.9)	179 (43.2)	257 (41.3)	153 (36.0)	230 (66.8)	1707 (41.6)
Meningitis	241 (27.9)	729 (60.6)	153 (37.0)	292 (46.9)	101 (23.7)	77 (22.4)	1593 (38.8)
Sepsis or bacteremia	81 (9.3)	9 (0.7)	38 (9.2)	44 (7.1)	100 (23.5)	18 (5.2)	290 (7.5)
Others	39 (4.5)	81 (6.7)	44 (10.6)	29 (4.7)	71 (16.7)	19 (5.5)	283 (6.9)
Subtotal	865	1203	414	622	425	344	3873 (94.3)

* Diagnosis was not recorded in 232 cases (5.7%), 141 from Argentina, 81 from Chile, 8 from Uruguay, 1 from Colombia and 1 in Mexico.
† Numbers in parentheses, percent.

TABLE 3. Distribution (percent) of the thirteen prevalent *Streptococcus pneumoniae* capsular types by year of diagnosis from 1993 to June, 1999

Type	1993	1994	1995	1996	1997	1998	1999	ND*	Total
14	21.2	22.8	24.0	28.8	27.5	29.2	25.4	44.4	1055
6A/6B	9.5	11.3	14.0	13.9	13.1	13.2	13.0	11.1	526
5	15.6	11.6	10.4	8.1	8.5	8.5	8.4		392
1	10.6	10.8	8.9	7.6	6.3	8.8	9.5	11.1	358
23F	3.9	5.6	6.7	6.6	6.8	5.5	8.6		258
19F	6.1	3.6	4.8	4.4	4.4	4.6	4.1		180
18C	0.6	3.7	3.3	3.7	7.6	4.6	5.1		176
19A	3.4	4.0	3.3	2.9	2.5	3.5	3.8	11.1	136
9V	3.4	3.3	2.4	3.4	2.0	3.3	5.1		126
7F	2.2	3.7	3.1	2.4	2.9	1.3	1.6	11.1	107
3	1.7	2.7	2.2	1.7	1.9	1.2	1.1		76
4	1.7	1.7	1.7	1.3	1.2	1.3	0.8	11.1	59
9N	0.6	1.5	1.0	2.4	0.7	1.4	2.4		58
Others	19.6	14.1	14.2	12.7	12.9	13.9	13.6		564
Total	179	750	892	590	589	692	370	9	4071

*ND, no information on collection year for nine isolates.

TABLE 4. The first 13 prevalent *Streptococcus pneumoniae* invasive isolates capsular types by country (n = 3507)

Serotype	% Capsular Types						n	%
	Argentina	Brazil	Chile	Colombia	Mexico	Uruguay		
14	32.6	24.9	18.2	27.1	11.0	34.7	1055	25.9
6A/6B	8.8	15.3	11.5	15.7	17.8	6.3	526	12.9
5	14.1	6.7	11.7	7.9	2.6	14.8	392	9.6
1	8.0	9.8	13.3	6.7	1.9	12.5	358	8.8
23F	3.2	5.2	4.6	10.9	17.1	2.8	258	6.3
19F	3.0	4.5	2.2	7.4	6.6	2.8	180	4.4
18C	2.2	7.4	3.0	4.5	2.8	2.3	176	4.3
19A	3.1	3.5	3.4	0.6	6.6	3.7	136	3.3
9V	3.3	4.4	1.0	1.8	3.8	2.3	126	3.1
7F	5.1	0.7	4.6	2.2	0.9	4.8	107	2.6
3	0.7	2.0	2.8	1.0	1.9	4.5	76	1.9
4	0.9	1.5	1.0	2.2	2.8	0.6	59	1.4
9N	2.6	1.7	0.6	0.8	0.2	0.6	58	1.4

there was a strong association of capsular type 23F with meningitis cases in Colombia (13%) and Mexico (13.9%).

A regression analysis done by capsular type, pathology and country revealed that no confounding bias was operating on the above described differences (data not shown).

Susceptibility to penicillin. Susceptibility to penicillin, based on susceptibility to oxacillin, was reported for all 4105 isolates. PRSP was observed in 1173 (28.6%) of these, 709 (17.3%) with ILR and 464 (11.3%) with HLR.

Regional trends in PRSP in each country through the years (1994 to 1998) are shown in Figure 1. The highest occurrence of PRSP during the 6 years of surveillance was observed in Mexico (25.8% ILR and 21.1% HLR) and was lowest in Brazil (20.7%), where HLR is uncommon (1.6%). Argentina, Chile, Colombia and Uruguay had overall PRSP proportions of 30.6, 31.9, 23.1 and 33%, respectively.

Annual increases in PRSP and HLR were statistically significant ($P < 0.001$) in Argentina and Colombia and for the overall data (Fig. 1). In Uruguay a significant increase was observed from 1994 to 1996, followed by a significant decrease from 1996 to 1998. There were no significant changes in annual increases in Chile, Brazil or Mexico (Fig. 1).

TABLE 5. Association of the 13 prevalent *Streptococcus pneumoniae* capsular types with pneumonia and meningitis cases

Serotype*	Pneumonia			Meningitis		
	n	%	%A	*n*	%	%A
14	565	33.1	33.1	318	20.0	20.0
6A/6B	180	10.5	43.6	234	14.7	34.7
5	192	11.2	54.9	133	8.3	43.0
1	205	12.0	66.9	86	5.4	48.4
23F	75	4.4	71.3	113	7.1	55.5
19F	51	3.0	74.3	83	5.2	60.7
18C	21	1.2	75.5	125	7.8	68.6
19A	63	3.7	79.2	41	2.6	71.2
9V	55	3.2	82.4	43	2.7	73.9
7F	34	2.0	84.4	46	2.9	76.8
3	31	1.8	86.2†	24	1.5	78.3†
4	21	1.2	87.4	24	1.5	79.8
9N	15	0.9	88.3	28	1.8	81.6
Others‡	199	11.7	100.0	295	18.5	100.0
Total	1707			1593		

* The same 13 capsular types accounted for 81.0% of sepsis cases, 86.2% for other pathologies and 85.4% for the total.
† $P < 0.001$.
‡ For pneumonias, 31 different capsular types; for meningitis, 37.
%A, accumulated percentage.

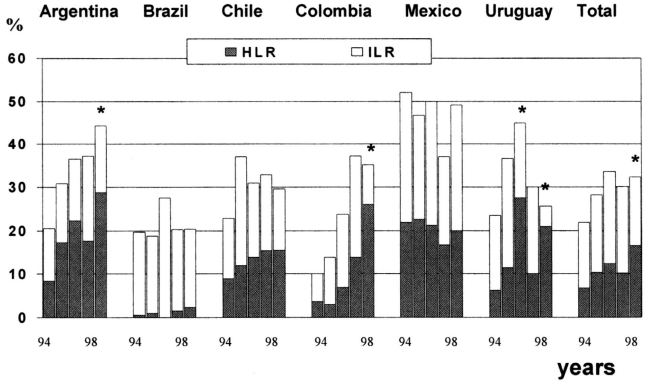

FIG. 1. Evolution of the diminished susceptibility to penicillin by country,1994 to 1998. *, $P < 0.001$.

Analysis of the PRSP and HLR in two years (1994 and 1998) for each country showed a significant increase of PRSP ($P < 0.05$) in Argentina (from 20.6% to 44.4%), Chile (from 22.8% to 29.6%) and Colombia (from 10.0% to 35.1%). Such increase was not observed in Brazil (from 19.8% to 20.3%) or Uruguay (from 23.4% to 25.6%). However, the analysis of HLR in the same years revealed a significant increase in five of the six countries ($P < 0.05$): Argentina, from 8.4% to 28.8%; Brazil, from 0.5% to 2.3%; Chile, 8.9 to 15.5%; Colombia, from 3.6% to 26.0%; and Uruguay, from 6.3% to 20.9%. In Mexico there was a slight decrease in HLR that was not significant (from 21.9 to 20.0%, $P = 0.1$).

Capsular types and PRSP. A regional analysis showed PRSP among 29 of the 54 capsular types described; 1072 of 1173 (91.4%) nonsusceptible isolates belonged to just 7 serotypes: 14 (51.7%); 23F (14.9%); 6B (11.7%); 19A (4.4%); 19F (3.8%); 6A (2.9%); and 9V (2.0%). These 7 PRSP types were present in all 6 countries, with the following exceptions: 19A was not reported from Colombia; 6A was not reported from Uruguay; and 9V was not reported from Brazil.

The proportion of serotypes carrying resistance varied widely among countries; in Uruguay 28% (7 of 24) of capsular types had PRSP, in Colombia 26% (9 of 34), in Argentina 22% (10 of 45), in Brazil 31% (13 of 42), in Mexico 47% (19 of 40) and in Chile 60% (21 of 35).

Serotype 14 was the predominant capsular type associated with PRSP overall, but there were significant variations among the six countries. In Mexico only 17.1% of the isolates with PRSP belonged to this serotype, whereas in Uruguay type 14 accounted for 90.5% of the PRSP isolates. Percentages for the other countries were 77.6% for Argentina, 49% for Brazil, 45.8% for Colombia and 27.9% for Chile. Variations by country were also observed for PRSP type 23F. In Colombia 31.2% of the PRSP isolates belonged to type 23F, 29.6% in Mexico, 15.5% in Brazil, 11.7% in Chile, 4.5% in Argentina and 0.8% in Uruguay.

The level of penicillin resistance varied with the serotype. Of 607 serotype 14 PRSP isolates, 297 (48.9%) had HLR and 310 (51.1%) had ILR; of 175 serotype 23F, 83 (47.4%) had HLR and 92 (52.6%) had ILR; of 136 serotype 6B, 17 (12.5%) had HLR and 119 (87.5%) had ILR and of 52 serotype 19A, 4 (7.7%) had HLR and 48 (92.3%) had ILR. An analysis of the association of HLR with each of the 4 main PRSP serotypes using serotype 19A as a baseline revealed that serotypes 14 and 23F were 6 times more likely to have HLR than serotype 19A (serotype 14, relative risk, 6.4, 95% CI 2.5 to 16.4; and serotype 23F, relative risk , 6.2, 95% CI 2.4 to 16.1). Proportion of HLR for serotype 6B did not differ significantly from resistance of serotype 19A.

PRSP and serotype evolution. The evolution of serotypes 14 and 23F, the two most important PRSP serotypes, is shown in Figure 2 using data from 1994 and 1998. PRSP type 14 increased in 5 of the 6

countries ($P < 0.05$), Argentina (from 58% to 84.6%), Brazil (from 31.2% to 42,7%), Chile (from 30.8% to 42%), Colombia (from 7.1% to 67.7%) and Uruguay (from 70% to 100%). In Mexico there was no change (57.1%). In contrast the prevalence of PRSP type 23F decreased in Brazil (from 87.5% to 43.8%), Chile (from 75% to 60%) and Colombia (from 61.5% to 50%).

Relation of PRSP to diagnosis and capsular type. Capsular types of the PRSP isolates recovered from pneumonia cases in order of frequency were: 14 (64%, with 34% HLR); 6B (11%, with 1.3% HLR); 23F (8.8%, with 5% HLR); 19A (4.3%, with 0.2% HLR); and 9V (2.1%, with 1.8% HLR).

The PRSP isolates recovered from meningitis were serotype 14 (40.5%, with 13% HLR), 6B (18%, with 1.5% HLR), 23F (19.7%, with 6.5% HLR), 19F (5.9%, with 1.2% HLR) and 19A (4.4%, all of them with ILR).

Data were analyzed to determine whether trends in serotype and resistance to penicillin could be influenced by other variables such as changes in the proportion of pneumonia and meningitis cases by year (data not shown). The results suggested that the increase of capsular type 14 isolates, as well as the decrease of 23F, was a real change and that there was no confounding factor caused by annual differences in the pneumonia:meningitis ratio.

Factors related to antimicrobial resistance. Some factors such as gender and disease were related with resistance: isolates from male patients had a 40% higher likelihood to be resistant than isolates from female patients; whereas isolates recovered from pneumonia cases showed a 75% increase on the risks of being resistant compared with other invasive diseases. Serotypes and countries were as well independently related with resistance (data not shown).

DISCUSSION

Before the development of the SIREVA-Vigía project, there were few published data concerning capsular types and penicillin susceptibility of *S. pneumoniae* causing invasive disease in Latin American children.[32, 33] The findings presented here, as well as several previously published reports, testify to the success of this surveillance project.[34–37] The predominant age groups in all the participating countries, were children 2 years old or younger; this finding is consistent with other reports in the literature from developing and developed countries.[1, 2, 10, 13, 14]

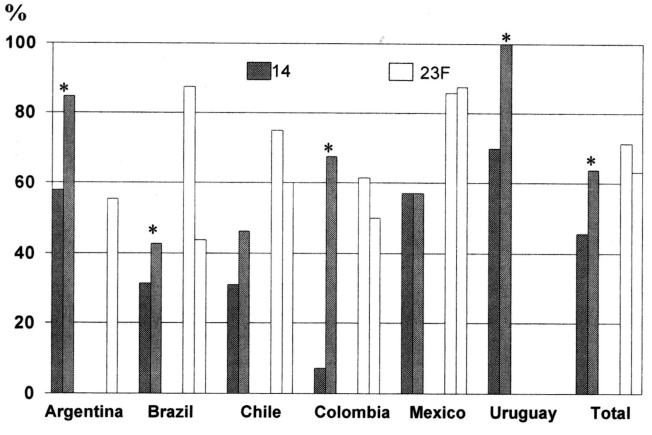

FIG. 2. Evolution of serotypes 14 and 23F with PRSP at two points in time, 1994 and 1998. *, serotype 14, $P < 0.05$.

In this study 13 types represented 86% of the total collection, and these types were consistently the most prevalent throughout the surveillance period. Six of the 13 prevalent types, 14, 5, 6A, 6B, 1 and 7F, showed a similar distribution in the Southern Cone countries (Chile, Uruguay and Argentina). Moving toward northern latitudes there was a gradual decrease in the prevalence of types 1 and 5, paralleled by a gradual increase of serotype 23F. Serotype 1 was present in a relatively high proportion, ranking fourth overall; this is similar to observations in some European countries[8] as well as Israel,[38] India[13] and some regions in Africa,[8, 39] but this serotype is uncommon in the United States and Canada.[12, 40] In North America serotype 5 is also uncommon.[12, 40]

The lack of change in the 13 prevalent capsular types during the 6.5 years of the study suggests that our data could be used confidently as a baseline to assess changes in serotype distribution and penicillin resistance that could be associated with new interventions. Similar stability of serotype distribution has been described previously[12, 41]

It has been suggested that some capsular types have strong association with certain pathologies.[1, 6, 8] In the overall regional analysis serotype 14 predominated as a cause of pneumonia (33.1%) and meningitis (20%), serotype 1 was most frequently isolated from pneumonia cases and serotype 18C was most often recovered from patients with meningitis. Capsular type differences observed by country may be a reflection of the different pathologies in those regions; whether these variations correlate to differences in the clinical spectrum of the disease remains to be determined.

The data generated allowed calculation of the expected coverage of three conjugated vaccines by country and region for prevention of pneumonia and meningitis (Table 6). The 7-valent vaccine[42] would cover only 58% of these pathologies, because serotypes 1 and 5 are not included in the formulation. These two serotypes are relevant in the region but not so in North America, the pediatric population for whom that vaccine was designed.[42, 43] The overall percentage would increase to 76.2 and 80.7% with the 9- and 11-valent vaccines respectively. This increase would be observed in all countries but Mexico. The expected coverage is better in some countries (Colombia) than others

(Chile). In four of six countries the 9-and 11-valent vaccines would provide better coverage for pneumonias than for meningitis. This may be explained by the greater number of capsular types causing meningitis.

Our analysis clearly establishes the substantial increase of PRSP by year and by country with the exception of Mexico and Brazil where no changes were observed. Mexico consistently showed the highest proportion of PRSP throughout the 6.5 years of surveillance. It could be assumed that the level of HLR was reached before the beginning of this project, as was the case described in Spain, where HLR reached a high but stable level.[41] In contrast Brazil, which collected isolates from several regions, has shown the lowest proportion of PRSP during the same surveillance period. Similar data have been provided by some European countries,[44, 45] Canada[40] and India.[13] Increase of PRSP has been described in several recent surveillance reports worldwide, highlighting the concern for adequate and successful management of pneumococcal infections.[12, 14, 15, 46]

Different countries in the Region presented varying numbers of serotypes associated with PRSP, ranging from 7 capsular types (in Uruguay) to 21 (in Chile). Nonetheless in the overall analysis, PRSP was closely related to the same 7 capsular types that have been associated with resistance worldwide.[2–5, 8, 14] Of most importance were capsular types 14, 23F and 6B which were responsible for 78% of the resistant isolates. However, this association has marked geographic variation within the region. The 3 vaccine formulations under trial have good coverage of PRSP capsular types; however, type 19A, missing in all formulations, is relevant in all countries with the exception of Colombia.

The results of the logistic regression model confirmed that there was a significant increase in the risk of acquiring an infection caused by a resistant strain throughout the surveillance years independently of changes in serotypes, age, gender or pathology.

Complementary molecular analysis of PRSP isolates was done as part of a collaborative project with Rockefeller University in New York.[47–52] The results showed that some of the resistant international clones were circulating in the region, mainly the Spain^{23F}-1 clone and the Spain^{9V-14}-3. In general these results are in agreement with the findings that a few clones are responsible for the majority of the resistant population.[41, 44]

Despite the success in improving the knowledge of *S. pneumoniae* ecology in the region, the surveillance did not generate complete information regarding the universe of invasive pneumococcal disease, a well recognized limitation of laboratory-based surveillance. Regional efforts are taking place to address this issue; this information is needed for the design of future intervention measures.

We have implemented a Latin American laboratory surveillance system that has successfully improved our knowledge of the epidemiology of *S. pneumoniae* invasive disease in children. The success of this surveillance system depends on the continued strength of the national reference centers in each country.[16–22]

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TABLE 6. Conjugated vaccines for *Streptococcus pneumoniae*: expected coverage of the three vaccine formulations for pneumonia and meningitis by country

Vaccine*	Pathology	% of Expected Coverage						
		Argentina	Brazil	Chile	Colombia	Mexico	Uruguay	Total
7-valent	Pneumonia	58.3	60.2	35.7	67.7	55.6	52.2	56.7
	Meningitis	45.7	64.4	35.3	71.5	64.4	42.9	59.0
	Total	52.8	63.1	42.0	69.6	61.9	51.7	58.0
9-valent	Pneumonia	82.6	84.9	59.2	90.3	62.8	82.2	80.0
	Meningitis	67.3	76.3	58.8	80.4	66.4	63.6	72.8
	Total	74.8	79.6	67.1	84.2	66.4	79.0	76.2
11-valent	Pneumonia	86.9	87.8	62.6	91.8	64.7	90.4	83.8
	Meningitis	75.1	78.8	65.4	83.9	69.3	69.8	77.2
	Total	80.4	82.3	74.2	87.5	69.2	88.4	80.7

* Conjugated vaccines[9]: 7-valent, serotypes 4, 6B, 9V, 14, 18C, 19F, 23F; 9-valent, 7-valent + serotypes 1 and 5; 11-valent, 9-valent + serotypes 3 and 7F.

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APPENDIX

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