

Penicillin-Resistant *Streptococcus pneumoniae* in Argentina: Frequent Occurrence of an Internationally Spread Serotype 14 Clone

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ABSTRACT

Six Latin-American countries participated in an epidemiological surveillance study conducted by the Pan American Health Organization in order to determine the relative prevalence of capsular types and antimicrobial resistance patterns of *Streptococcus pneumoniae* (SPN) causing invasive infections in children <5 years of age. In Argentina, the incidence of penicillin resistance (PR) was 24.4%, and it was significantly associated with serotype 14 ($p < 0.001$). The chromosomal DNA of 56 of those SPN isolates, 39 PR and 17 susceptible, was digested with *Sma*I and resolved by PFGE. Eighty-two percent (32/39) of the PR isolates shared characteristics with the widely spread International Spanish/French clone (clone B). All members of clone B except one expressed serotype 14, with the exception of one isolate that expressed serotype 19F and probably resulted from an *in vivo* capsular transformation event. Only a single isolate shared features with the 23F International Spanish/USA clone (clone A). The 17 penicillin-susceptible (PS) SPN isolates presented an enormous degree of variation in the chromosomal background, expressing 12 serotypes and 13 PFGE patterns. The data suggest that over 80% of the SPN-PR isolates in Argentina were imported, and this confirms the importance of the geographic spread of SPN clones in South America.

INTRODUCTION

THE INCREASING RATE of antimicrobial resistant *Streptococcus pneumoniae* (SPN) and the difficulties in management of some serious pneumococcal infections constitute one of the greatest public health concerns.

Previous studies have shown that geographic areas may acquire unique resistant clones. In Iceland, penicillin-resistant pneumococci seem to involve primarily a single multiresistant serotype 6B clone, probably introduced from Spain.¹⁸ In Spain, the multiresistance phenotype appears to be associated with several pneumococcal chromosome lineages and with serotypes 14, 23F, 6B, 19F, and 15F.⁴ A 23F serotype SPN, earlier identified in Spain, seems to be extensively disseminated over large geographic areas such as Portugal, France, the United States,¹⁵ Croatia,²⁰ South Africa, and Korea.¹² Some multiresistant strains of serotype 9V frequent in France may also have been imported from Spain.⁷

Between February 1993 and April 1996, the Pan American

Health Organization (PAHO), through the Sistema Regional de Vacunas (SIREVA), initiated a Latin-American surveillance program to assess the prevalence of SPN capsular types and the profile of penicillin resistance in this region.⁹ The primary purpose of this effort was to generate information for the design of an appropriate protein-polysaccharide conjugate vaccine.

As part of this project, 505 isolates causing invasive pneumococcal infections in children <5 years old were collected from 15 Argentinean hospitals located in 9 cities within the country. Pneumonia was the clinical diagnosis in 58% of the cases, meningitis in 22%, and sepsis in 10.6%. More than half of the isolates (51.2%) were recovered from blood, 22.7% were from pleural fluid, 20.7% were from CSF, and the rest of the isolates (5.4%) were from other sterile sites. The 10 most frequent serotypes, in descending order, were 14, 5, 1, 6A/6B, 7F, 9V, 19F, 19A, 16F, and 23F, representing 89.3% of the total. In this study we noted that as many as 123 (24.4%) of such isolates showed some degree of reduction in their penicillin susceptibility; 13.1% of them showed intermediate resistance (I-

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TABLE 1. PROPERTIES OF CLINICAL ISOLATES OF *S. PNEUMONIAE* FROM ARGENTINA

Strain	City	Serotype	MIC (mg/ml)		Sml pattern	S, I or R				
			Penicillin	Cefotaxime		Chl	Ery	Sxt	Tet	Ofl
AR 740	Cap. Fed.	23F	1	0.5	A1	R	S	R	R	S
AR 320	S. Justo	14	2	1	B1	S	S	I	S	S
AR 325	Cap. Fed.	14	2	1	B1	S	S	R	S	S
AR 268	S. Justo	14	2	1	B1	S	S	R	S	S
AR 441	Cap. Fed.	14	2	1	B1	S	S	R	S	S
AR 682	S. Justo	14	2	1	B1	S	S	R	S	S
AR 754	La Plata	14	2	1	B1	S	S	R	S	S
AR 287	Misiones	14	1	0.5	B1	S	S	R	S	S
AR 705	La Plata	14	1	1	B1	S	S	R	S	S
AR 772	Cap. Fed.	14	1	1	B1	S	S	R	S	S
AR 807	Cordoba	14	1	0.5	B1	S	S	R	S	S
AR 770	Cap. Fed.	14	4	2	B4	S	S	R	S	S
AR 220	Cap. Fed.	14	2	0.25	B4	S	S	R	S	S
AR 228	Cap. Fed.	14	1	1	B4	S	S	R	S	S
AR 608	La Plata	14	4	1	B8	S	S	R	S	S
AR 419	Cap. Fed.	14	1	1	B8	S	S	I	S	S
AR 647	Mendoza	19F	4	1	B11	S	S	R	S	S
AR 601	La Plata	14	4	1	B12	S	S	R	S	S
AR 774	Cap. Fed.	14	1	1	B16	S	S	R	S	S
AR 817	Sta. Fe	14	2	1	B17	S	S	R	S	S
AR 68	Sta. Fe	14	1	0.25	B17	S	S	R	S	S
AR 786	Cap. Fed.	14	2	1	B18	S	S	R	S	S
AR 631	Sta. Fe	14	1	1	B18	S	S	I	S	S
AR 449	La Plata	14	1	0.5	B19	S	S	R	S	S
AR 432	La Plata	14	4	2	B20	S	S	R	S	S
AR 773	Cap. Fed.	14	1	0.5	B20	S	S	R	S	S
AR 461	Cap. Fed.	14	2	2	B21	S	S	I	S	S
AR 274	La Plata	14	1	0.5	B22	S	S	I	S	S
AR 831	La Plata	14	2	1	B23	S	S	R	S	S
AR 472	La Plata	14	2	1	B24	S	S	R	S	S
AR 778	Cap. Fed.	14	4	2	B25	S	S	R	S	S
AR 701	La Plata	14	4	2	B26	S	S	R	S	S
AR 758	Cap. Fed.	14	4	2	B27	S	S	R	S	S
AR 765	Cap. Fed.	19A	0.25	0.06	C	S	S	R	S	S
AR 641	Cap. Fed.	6A	0.06	0.06	D	S	S	R	S	S
AR 255	Cap. Fed.	14	2	2	E	S	S	R	S	S
AR 314	Cap. Fed.	5	0.06	0.06	F	S	S	R	S	S
AR 30	La Plata	5	0.06	0.06	F	S	S	R	S	S
AR 364	Cap. Fed.	5	0.06	0.06	F	S	S	R	S	S
AR 316	Cap. Fed.	7F	0.06	0.03	G	S	S	S	S	S
AR 805	Cordoba	14	1	0.12	H1	S	S	R	S	S
AR 428	Cap. Fed.	14	0.03	0.03	H1	S	S	R	S	S
AR 382	Cordoba	14	1	0.12	H2	S	S	R	S	S
AR 600	La Plata	14	4	1	I	S	S	R	S	S
AR 66	La Plata	6B	0.25	0.008	J	S	S	S	S	S
AR 96	La Plata	NT	0.06	0.008	K	S	S	R	S	S
AR 335	Cap. Fed.	6B	0.06	0.008	L	S	S	S	S	S
AR 683	S. Justo	1	0.01	0.06	M	S	S	R	S	S
AR 620	Sta. fe	1	0.01	0.03	M	S	S	R	S	S

TABLE 1. PROPERTIES OF CLINICAL ISOLATES OF *S. PNEUMONIAE* FROM ARGENTINA (CONT'D)

Strain	City	Serotype	MIC (mg/ml)		SmaI pattern	Chl	S, I or R			
			Penicillin	Cefotaxime			Ery	Sxt	Tet	Ofl
AR 459	S. Justo	22F	0.06	0.008	N	S	S	S	S	S
AR 107	S. Justo	NT	0.06	0.008	O	S	S	R	S	S
AR 35	S. Justo	NT	0.06	0.008	O	S	S	I	S	S
AR 477	Cap. Fed.	19A	0.06	0.008	P	S	S	R	S	S
AR 472	Cap. Fed.	35B	0.06	0.016	Q	S	S	S	S	S
AR 612	La Plata	19F	0.06	0.06	R	S	R ^a	R	S	S
AR 639	Cap. Fed.	I	4	2	S	R	R ^b	R	R	S

CHL, chloramphenicol; ERY, erythromycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; OFL, ofloxacin; NT, not typable; S, susceptible; I, intermediate; R, resistant.

^aClindamycin susceptible.

^bClindamycin resistant.

PR), while 11.3% presented high-level resistance (H-PR). Serotype 14 was present in almost 30% of the total of the isolates, and reduction in penicillin susceptibility was significantly associated with this serotype ($p < 0.001$).¹⁷ In fact, 75.4% of

PR-SPN were serotype 14 and trimethoprim/sulfamethoxazole was the only associated resistance.

Following the PAHO initiative, a representative of each participating Latin-American country paid a working visit to the

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

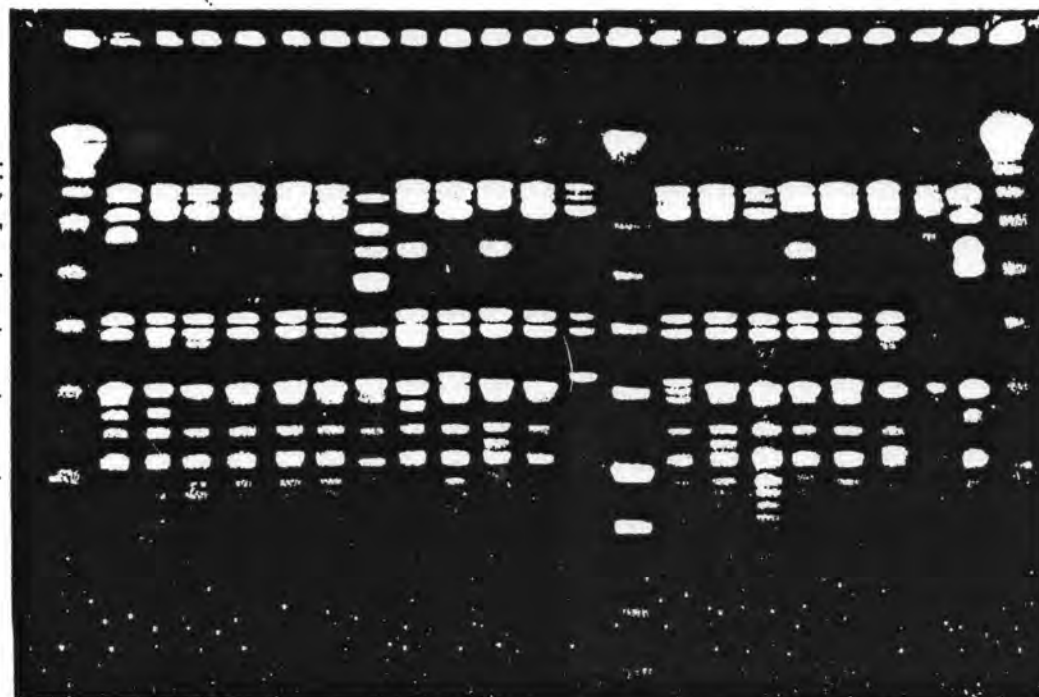


FIG. 1. Frequency representation of international clone among penicillin-resistant strains of *Streptococcus pneumoniae* from Argentina. Chromosomal DNA restricted by *Sma*I was separated by PFGE. Lanes 1, 23: lambda ladder maker. Lane 14: low molecular weight marker. Lane 2: strain AR 758. Lane 3: strain AR 701. Lane 4: strain AR 778. Lane 5: strain AR 320. Lane 6: strain AR 682. Lane 7: strain AR 754. Lane 9: strain 742. Lane 10: strain AR 786. Lane 11: strain AR 831. Lane 12: strain AR 8. Lane 13: strain AR 274. Lane 15: strain AR 287. Lane 16: strain AR 419. Lane 17: strain AR 705. Lane 18: strain AR 228. Lane 19: strain AR 772. Lane 20: strain AR 773. Lane 21: strain AR 774. Lane 22: strain AR 225.

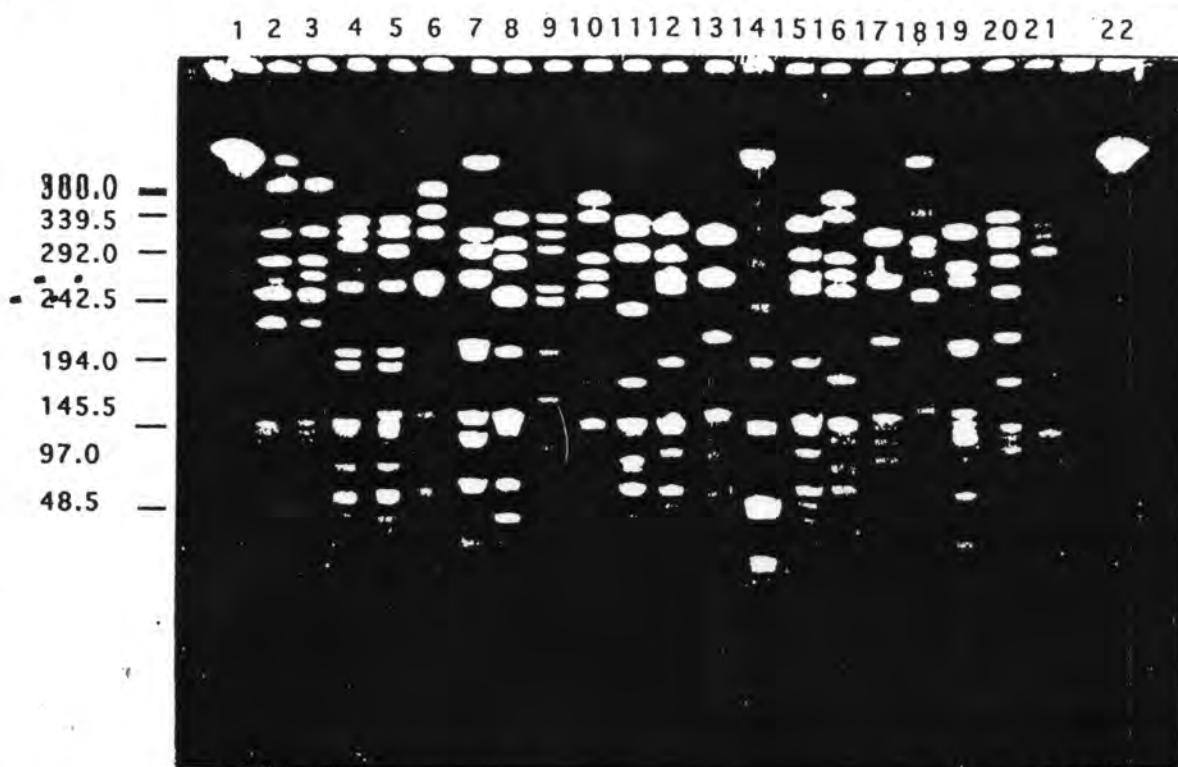


FIG. 2. Characterization of penicillin-susceptible and -resistant strains of *S. pneumoniae* from Argentina. Chromosomal DNA restricted by *Sma*I was separated by PFGE. Lanes 1, 22: lambda ladder marker. Lane 14: low molecular weight marker. Lane 2: strain Clev-2 (reference strain for international clone A). Lane 3: strain AR 740 (representative of international clone A). Lane 4, 5, 21: strains AR 770, 647, 368 respectively (representatives of international clone B). Lane 6: strain AR 612. Lane 7: strain AR 765. Lane 8: strain AR 477. Lane 9: strain AR 459. Lane 10: strain AR 35. Lane 11: strain AR 96. Lane 12: strain AR 805. Lane 13: strain AR 364. Lane 15: strain AR 428. Lane 16: strain AR 107. Lane 17: strain AR 314. Lane 18: strain AR 316. Lane 19: strain AR 641. Lane 20: strain AR 472.

Laboratory of Microbiology at The Rockefeller University in New York in order to test the genetic relatedness of the SPN isolates, using primarily pulsed-field gel electrophoresis (PFGE) of *Sma*I digested chromosomal DNA prepared from the isolates. We briefly describe here the results obtained with the Argentinean isolates.

MATERIALS AND METHODS

Isolates, serotyping, and antibiotic susceptibilities

Fifty-six SPN isolates, including 39 PR (MIC ≥ 0.12 $\mu\text{g/ml}$) and 17 PS (MIC ≤ 0.06 $\mu\text{g/ml}$), were analyzed for chromosomal relatedness. Genotypic features such as serotype, geographic sites of isolation, and resistance profile are shown in Table 1. Pneumococcal isolates were typed by the quellung reaction, using sera provided by the Statens Serum Institute, Copenhagen, Denmark.¹⁹ Antibiotic susceptibilities to penicillin, cefotaxime, chloramphenicol, erythromycin, trimethoprim/sulfamethoxazole, tetracycline, ofloxacin, and vancomycin were determined by the agar dilution method according to NCCLS recommendations, using Mueller-Hinton agar supplemented with 5% sheep's blood and

incubated without increased CO₂ concentration (unless indispensable for its development) for 20–24 h to 35°C. The erythromycin-resistant strains were also tested for clindamycin by disk diffusion.

PFGE analysis

Genetic analysis was performed by *Sma*I digestion of chromosomal DNA followed by PFGE, as described previously.²⁰ The gels were run in a CHEF-DRII apparatus (Bio-Rad). PFGE patterns (>6 bands of differences) were assigned with capital letters and subtypes or variants (1–6 bands of differences) were named with a capital letter followed by a number.²¹

Reference strains: S. pneumoniae

ATCC 49619 was used as a reference strain for MIC and disk diffusion. Unencapsulated SPN R6 was used as a molecular weight marker. Representatives of PR-SPN belonging to the international clones were used for comparing the genetic similarity between Argentinean strains: Clev-2 (representative type 23F of Spanish/USA clone), M13P (representative type 14 of Spanish/French clone), and ICE186 (representative type 6B of Spanish/Icelandic clone). These strains are part of The Rockefeller University collection.

RESULTS

Among the 39 PR strains studied, only seven different patterns, arbitrarily named A, B, C, E, H, I, and S, were identified (Table 1). Of these isolates, 32/39, or 82%, belonged to the widely spread International Spanish/French clone (Clone B). Figure 1 shows the relative homogeneity observed in this group of bacteria. In contrast to the situation in France or Spain, most of the members of Clone B from Argentina were serotype 14 but not serotype 9V. Only one isolate (AR 647) from Mendoza expressing the 19F capsular type showed a closely related genetic background with the dominant B profile. Seventeen different subtypes were detected in the B PFGE pattern, and the most representative of these was profile B1, which was present in 10 (26%) strains. They were all susceptible to chloramphenicol, tetracycline, erythromycin, and ofloxacin but resistant to trimethoprim-sulfamethoxazole and had similar minimal penicillin inhibitory concentrations (MICs between 1 and 4 $\mu\text{g/ml}$). Clone B was recovered from all of the cities studied.

AR 740 from Capital Federal was the unique isolate in this study that shared characteristics with the 23F serotype international Spanish/USA clone (Clone A). It was indistinguishable from reference strain Clev-2. This isolate presented a penicillin MIC of 1 $\mu\text{g/ml}$ and was also resistant to chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole but susceptible to erythromycin and ofloxacin. The other 6 PR isolates were represented by 5 different PFGE types and 3 serotypes (1, 14, and 19A).

Three serotype 14 SPN shared variants of a PFGE profile named H; two of these were PR (MIC = 1 $\mu\text{g/ml}$), and the third was susceptible to this drug (MIC = 0.03 $\mu\text{g/ml}$).

Among the 17 PS-SPN analyzed, we found a high degree of variability in the chromosomal background: as many as 13 different PFGE types and 12 serotypes (1, 5, 6A, 6B, 7F, 10F, 14, 19A, 19F, 22F, 34, 35B, and NT). In Fig. 2 is possible to observe the degree of heterogeneity in the chromosomal DNA digested with *Sma*I of susceptible strains.

We did not find vancomycin or ofloxacin resistance in any of the 56 isolates here studied. We detected only two strains with erythromycin resistance, one of which (AR 639) was also resistant to clindamycin.

DISCUSSION

Most of the PR-SPN (75.4%) collected in Argentina during the Latin-American surveillance project organized by PAHO belonged to serotype 14,¹⁷ and the only associated resistant found was to trimethoprim-sulfamethoxazole. A similar situation was encountered in Uruguay,⁸ where 89% of the penicillin resistant SPN were of serotype 14. This serotype was less frequent among resistant isolates from Brazil (48%)² and Colombia (25.6%).³ Serotype 14 SPN was often isolated in France and Israel,¹⁰ and lately as the most frequent one in Spain,⁴ but these were almost always multiresistant strains.

A sample of 35 Argentinean PR serotype 14 SPN isolates revealed that all but 4 (88%) were variants of the international clone with PFGE pattern B. Clone B was represented by 32 isolates (82%) in 39 PR-SPN studied, and most of them (31 strains) expressed capsular type 14. A single isolate with chro-

mosomal background closely related to the serotype 14 predominant clone was shown to have capsular determinants of serotype 19F. This phenomenon suggests horizontal transfer of genetic information for the synthesis of capsular type 19F to an SPN originally of serotype 14. Such events of *in vivo* capsular transformation have already been documented.^{1,4,7} Clone B was detected in 6 cities in Argentina, some located more than 1,200 km from one another (see Fig. 3) and the same clone was already detected in European countries^{4,7,13} as well as in certain Latin-American countries, namely, Uruguay, Chile, Brazil, Colombia, and Mexico (see other articles in this issue).

Serotype 23F SPN was poorly represented (2.2%) among the strains collected in the Argentinean surveillance.¹⁷ Two of them, isolated in Capital Federal, were multiply antibiotic re-



FIG. 3. Collection sites participating in the multicenter study of *Streptococcus pneumoniae*.

sistant (resistant to penicillin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole) and one of those strains analyzed here showed identical PFGE profile to international clone A described in several European countries and also in the United States.¹⁵

In contrast to the common serotype, resistance profile, and genetic similarity of penicillin resistant isolates, the smaller groups of 17 penicillin susceptible pneumococci examined were distributed to 13 unrelated PFGE patterns and 12 serotypes. Such diversity among susceptible SPN has already been described in other reports.^{6,18,20}

Basically, there could be two ways of increasing the prevalence of PR-SPN isolates in a certain region: (1) *in vivo* selection of strains with modified PBPs,²² either by replacement of part of *pbp* genes by interspecies or homologous recombinational events,^{5,11} or by the acquisition of point mutations in *pbp* genes as demonstrated *in vivo*.^{14,16} (2) Alternatively, a new resistant clone may be introduced^{12,15,18,20} with advantages over local strains to spread in an environment in which antibiotics are often misused. In the first case, isolates with a common chromosomal background but with different degrees of resistance may coexist. Actually, three isolates with different penicillin susceptibility levels appeared to be closely related genetically (PFGE pattern H). This may be an example of resistant strains emerging from a susceptible precursor, as previously suggested.^{11,14} However, no such potential precursor of the most prevalent resistant clone was evident among the limited number of susceptible isolates examined in this collection. Furthermore, the overwhelming majority of PR-SPN analyzed (85%) depicted a similar genetic background to the international clone already described. Therefore, it is reasonable to assume that the second hypothesis was more likely to have occurred in Argentina, raising questions about the mode of transmission and the nature of the bacterial carriers.

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