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Molecular Epidemiologic Characterization of Penicillin-Resistant *Streptococcus pneumoniae* Invasive Pediatric Isolates Recovered in Six Latin-American Countries: An Overview

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ABSTRACT

The Pan American Health Organization (PAHO) has conducted a study of *Streptococcus pneumoniae* in six Latin-American countries: Argentina, Brazil, Chile, Colombia, Mexico, and Uruguay. Sterile site isolates from children aged ≤5 years showing clinical symptoms of pneumonia (as defined by the clinical criteria of WHO), meningitis, sepsis or bacteremia (without infectious foci), arthritis, and peritonitis were the source of most of the invasive pneumococcal isolates collected between the end of 1993 and 1996 in the six participating countries. Partial characterization of these isolates (antibiotic resistance and serotyping) have already been described (*Microbial Drug Resistance* 3:(2):131–163, 1997). In the next phase of the study, 326 *S. pneumoniae* isolates with reduced penicillin susceptibility were transferred to the Laboratory of Microbiology at The Rockefeller University for molecular characterization, and a summary and overview of the findings is described in this article. Some of the most interesting findings were as follows: (1) There was a surprisingly high representation of two internationally spread clones, which made up >80% of the strains with penicillin MIC of 1 μg/ml or higher; most of these isolates were recovered in large cities, supporting the likelihood that the source of these clones is through international travel. (2) The frequency of resistance to trimethoprim/sulfamethoxazole was extremely high (present in 85% of all isolates with decreased penicillin susceptibility). (3) None of these isolates was resistant to ofloxacin, and macrolide resistance was rare (present in 6.4% of the isolates). (4) There was an apparent inverse relationship between level of penicillin resistance and genetic diversity. (5) There were striking differences in the "microbiologic profiles" of the six different Latin-American countries.

INTRODUCTION

THE PRIMARY AIM of the first phase of the *Streptococcus pneumoniae* surveillance study organized by the Pan American Health Organization (PAHO) was to obtain information on the serotype distribution and level of penicillin resistance in invasive pediatric isolates from the participating Latin-American countries. The ultimate aim of this effort was to help design a pneumococcal vaccine most appropriate for these countries. As a sequel to these studies, principal investigators of the first phase of surveillance were invited to the Laboratory of Microbiology at The Rockefeller University in New York in order to

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characterize the penicillin-resistant isolates by molecular fingerprinting techniques. The purpose of this second phase of surveillance was to obtain some insights into the mechanisms of spread and possible origin of the resistant strains in Latin-America.

In this summary we shall provide an overview and comparison of the "microbiologic profiles" of penicillin-resistant *S. pneumonia* isolates from the six Latin-American countries. In addition to the 326 strains with reduced penicillin susceptibility, an arbitrarily selected group of 40 penicillin-susceptible isolates were included in the characterization.

MATERIALS AND METHODS

Organization of the molecular fingerprinting workshop at Rockefeller University

The molecular characterization of the Latin-American strains was performed during working visits of six colleagues (one from each of the participating countries) to the Laboratory of Microbiology of The Rockefeller University between January and May, 1997. After transfer to New York, the bacterial strains were replated on tryptic soy agar containing 5% sterile sheep's blood; the strains were next grown in a synthetic medium (C + Y)[6] to turbid cultures followed by preparation of stocks, which were frozen in medium containing a 10% final concentration of glycerol and were stored at −70°C as part of the collection of strains at The Rockefeller University. In order to complete microbiologic characterization of the strains, susceptibility to antimicrobial agents was confirmed by agar dilution or disk diffusion according to recommendations of the National Committee for Clinical Laboratory Standards.[11] Antimicrobial susceptibility was determined by penicillin (PEN), trimethoprim/sulfamethoxazole (SXT), ofloxacin (OFL), erythromycin (ERY), tetracycline (TET), chloramphenicol (CMP), and vancomycin (VAN). The ERY-resistant strains were also tested for susceptibility to clindamycin (CLI). Preparation of chromosomal DNA, restriction with the *Sma*I restriction endonuclease, and separation of DNA fragments by pulsed-field gel electrophoresis (PFGE) were done as described previously.[15] Interpretation of PFGE pattern similarity was done as recommended.[19]

RESULTS AND DISCUSSION

Frequency of penicillin resistance

Table 1 and Figure 1 summarize the PAHO strain collection broken down to individual countries. The tabulation shows the total number of *S. pneumoniae* isolates collected and the distribution of isolates into penicillin-susceptible strains, and isolates with low (intermediate) and high levels of penicillin resistance and the number of isolates used in the studies at The Rockefeller University. In Table 1 and Figure 1, clinical microbiologic definitions of breakpoints were used: penicillin MICs for susceptible strains ≤0.06 μg/ml; low/intermediate resistance 0.1–1 μg/ml; and highly resistant >1 μg/ml. The percentage of bacterial isolates with reduced susceptibility to penicillin varied between 15.6% in Colombia and a high value of 48.2% in Mexico. The strains studied at The Rockefeller University included 326 isolates with penicillin MIC values of ≥0.1 μg/ml and 40 penicillin-susceptible isolates selected at random from several of the national collections. Thus, the total number of isolates studied was 366 strains.

"Highly" penicillin-resistant strains: strains with penicillin MIC ≥1.0 μg/ml

More than half of the resistant isolates analyzed at The Rockefeller University (172 of 326, or 53%) showed penicillin MIC values ≥1.0 μg/ml. From the point of view of mechanism of resistance, we considered it more appropriate to compare strains on the basis of breakpoints that differ from those used in clinical microbiology. Thus, for the sake of this presentation, high-level resistant strains were defined as having penicillin MICs of ≥1 μg/ml, and low-level resistance included strains with penicillin MICs of 0.1 to 0.5 μg/ml.

Frequent presence of international clones

PFGE analysis showed that a large proportion of highly penicillin-resistant isolates (139 of 172, or 80.8%) were represented by two internationally spread clones of *S. pneumoniae*: 44 isolates (25.6%) with PFGE profiles characteristic of the international "Spanish/USA" clone (which we shall arbitrarily refer to as clone A), and 95 isolates (55.2%) with characteristic PFGE profile of a second international ("French/Spanish") clone (arbitrarily called clone B in this discussion). The overwhelming majority of isolates with clonal profile A expressed the capsular polysaccharide 23F and were also resistant to trimethoprim/sulfamethoxazole, tetracycline, and chloramphenicol and, less frequently (4 isolates, or 9%), to erythromycin as well. PFGE analysis detected 13 subtypes among the clone A isolates. Two isolates of clone A from Mexico expressed serotypes 6B and 14.

Most members of clone B (87 isolates) expressed serotype 14, while 7 strains belonged to serogroup 9 (4 with serotype 9V and 3 with serotype 9A), and a single isolate expressed serotype 19F. It is possible to consider the serogroup 9 isolates and the single 19F isolate of this clone as products of *in vivo* capsular transformation events.[1,2,12] All members of this clone were also resistant to trimethoprim/sulfamethoxazole, but only 2 (2%) and 3 (3%) of the isolates presented resistance to tetracycline and erythromycin, respectively. PFGE analysis of clone B detected 32 subtype variants.

Multiresistance (i.e., resistance to more than two generically different antibiotics) was rare in clone B: two isolates (CH 188 and MEX C 58) were resistant to trimethoprim/sulfamethoxazole and erythromycin, and other two (UR 383 and UR 338) presented resistance to trimethoprim/sulfamethoxazole, tetracycline, erythromycin, and clindamycin.

The rest of the highly penicillin-resistant strains could be distributed into a large number of PFGE types as well as serotypes. Five of these strains (2.9% of total) formed a highly penicillin-resistant (MIC 4–8 μg/ml) serotype 19F cluster in Chile (clone D); the rest of 28 highly penicillin-resistant strains (16.3% of total) were represented by 20 distinct PFGE types and 6 serotypes (1, 6A, 6B, 14, 19F, and 23F) (Fig. 2).

TABLE 1. ORIGIN AND SOME RELEVANT PROPERTIES OF THE LATIN AMERICAN *STREPTOCOCCUS PNEUMONIAE* (SPN) ISOLATES

Country	*Population of country (inhab. × 10^6)*	*Period of survey*	*Total no. of strains*	*No. of strains with RPS[a] (% of the total)*	*Serotypes in order of decreasing rank*		*Total no. of strains analyzed by molecular fingerprinting (no. of strains with RPS)*
					All SPN (90% of strains)	*SPN with RPS (100% of strains)*	
Argentina	34.1	February 93–April 96	505	124 (24.4)	14, 5, 1, 6A/6B, 7F, 9V, 19F, 19A, 16F, 23F, 15, 9N, 3, 22F, 18A,	14, 23, 23F, 7, 6A/6B, 3, 9N, 19A, 19F, 15, 1, 5, NT	56 (39)
Brasil	157.1	January 93–December 96	644	136 (21.1)	14, 6A/6B, 1, 5, 18C, 23F, 19F, 9V, 19A, 3, 10A, 4, 9N, 24F	14, 6A/6B, 23F, 19A, 19F, 23B, 8	92 (91)
Chile	13.5	March 94–March 96	202	61 (30.2)	14, 1, 5, 6, 19, 23, 3, 7F, 18C, 18, 9, 15, 2, 8, 12, 22	14, 6, 19, 23, 7, 18, 5, 21	54 (36)
Colombia	33.4	January 94–December 96	409	64 (15.6)	14, 6A/6B, 5, 23F, 1, 19F, 18C, 7F, 4, 15B, 18A, 9V, 3, 12F	23F, 14, 6B, 9V, 19F, 34	59 (59)
Mexico	88.2	August 93–April 95	220	106 (48.2)	23F, 6A/6B, 14, 19F, 19A, 4, 11A, 5, 18C, 1, 10A, 2, 9V, 15A, 15B, 18A, 21, 22F, 35.	23F, 6A/6B, 14, 19F, 19A, 9V, 9A, 12F, 15B, 2, 23A, 35, 4	44 (44)
Uruguay	3.1	June 94–March 96	171	55 (32.2)	14, 5, 1, 6A/6B, 3, 7/7F, 19A, 23F, 18/18C, 9V	14, 6B, 19A, 23B	61 (57)

[a]RPS, reduced penicillin susceptibility (Pen MIC ≥0.1 mg/ml).

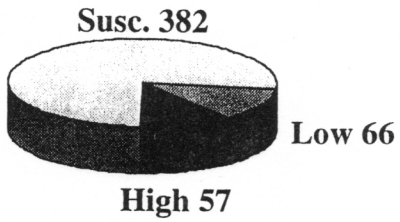
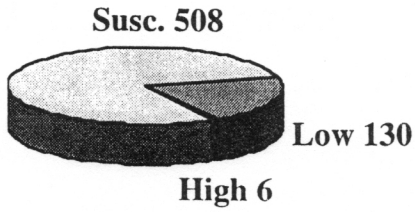
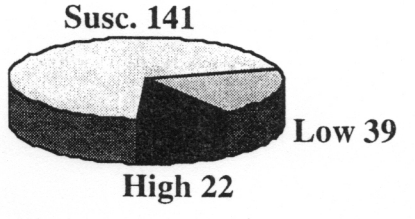
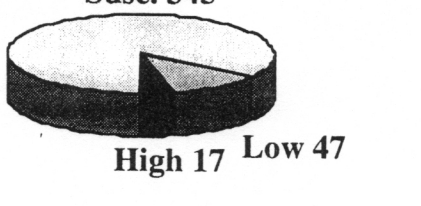
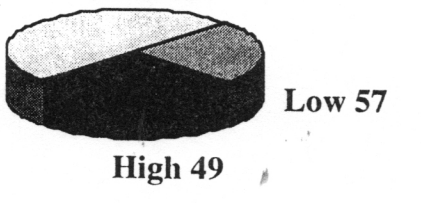
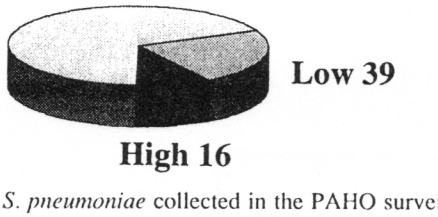
Total No. Strains		Strains with PEN MIC ≥ 0.1 ug/ml	
		Total # (%)	# studied at R.U.
ARGENTINA			
n = 505	Susc. 382 Low 66 High 57	123 (24.4)	39
BRASIL			
n = 644	Susc. 508 Low 130 High 6	136 (21.1)	91
CHILE			
n = 202	Susc. 141 Low 39 High 22	61 (30.2)	36
COLOMBIA			
n = 409	Susc. 345 High 17 Low 47	64 (15.6)	59
MEXICO			
n = 220	Susc. 114 Low 57 High 49	106 (48.2)	44
URUGUAY			
n = 171	Susc. 116 Low 39 High 16	55 (32.2)	57

FIG. 1. Invasive isolates of *S. pneumoniae* collected in the PAHO surveillance program in six Latin American countries. The numbers of penicillin susceptible (Susc) and low and high resistant pneumonococcal isolates are indicated. Also shown are the numbers of strains studied at Rockefeller University (R.U.).

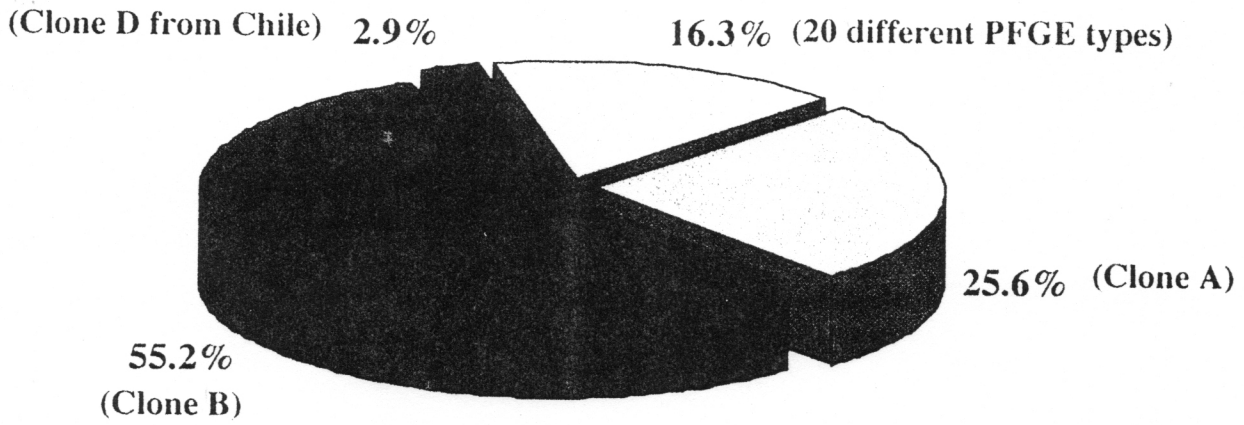


FIG. 2. Frequency of clonal types among highly penicillin-resistant pneumonococcal isolates (MIC ≥1.0 μg/ml) from Latin America. Clones A and B represent two internationally spread clones: the serotype 23F Spanish/USA clone A and the serotype 9/14 French/Spanish clone B.

Strains with low penicillin resistance: penicillin MIC 0.1–0.5 μg/ml

Close to half (154 of 326, or 47%) of the Latin-American isolates with decreased penicillin susceptibility had penicillin MICs between 0.1 and 0.5 μg/ml. A large proportion of these isolates (87 strains, or 56.5%) belonged to six local clusters of strains. We arbitrarily defined a cluster as a single PFGE type shared by at least five independent pneumococcal isolates. Clusters were arbitrarily named by capital letter assignments, all of which were restricted to national collections (i.e., cluster C of Brazil was not the same as cluster C of Columbia). These clusters usually shared not only a common PFGE but also a common serotype. The Brazilian PFGE cluster C (serotype 14) and cluster D (serotype 6B) was represented by 24 and 16 isolates, respectively. Interestingly, a single isolate in the Brazilian cluster C had only borderline to susceptible level penicillin MIC (0.06 μg/ml), in contrast to the rest of the members of this clus-

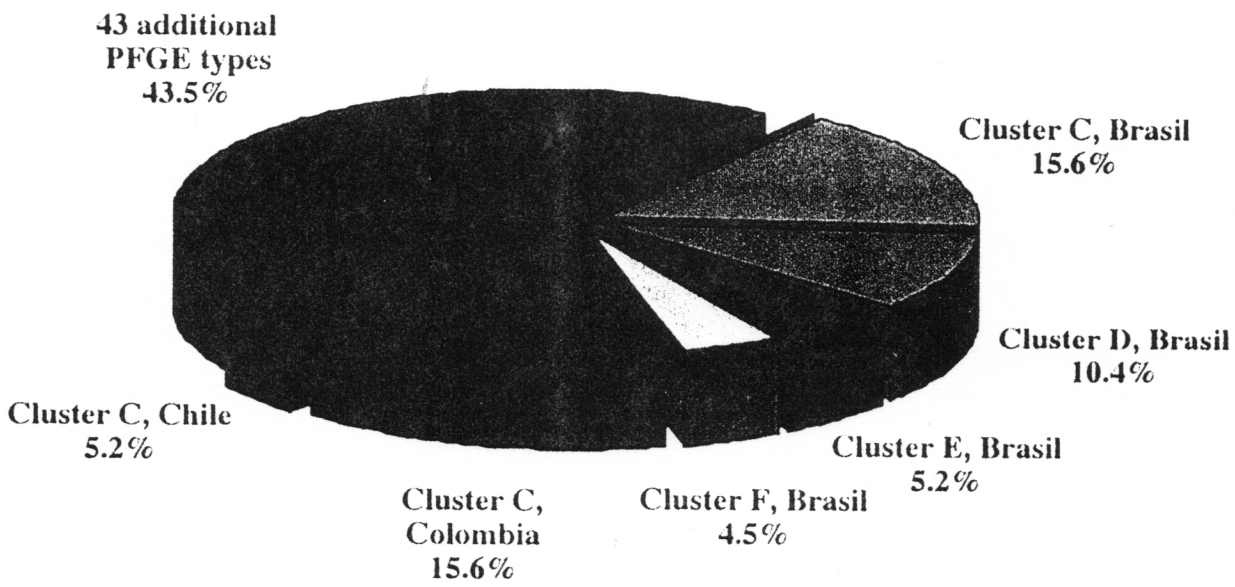


FIG. 3. Frequency of clonal types among pneumococcal isolates with low-level penicillin resistance (MIC 0.1–0.5 μg/ml) from Latin America.

ter, which had MIC values of 0.12 to 0.25 μg/ml (only one isolate with penicillin MIC 0.5 μg/ml). It is conceivable that this solitary isolate represents an ancestral cell of the Brazilian C cluster. Additional clusters included the Brazilian clusters E (8 isolates, serotype 19A) and F (7 isolates, serotype 23F) and the large cluster C of Colombia (24 isolates, serotype 23F). A single isolate from the Colombian cluster C had a capsular serotype 34 instead of the common 23F, raising the possibility that this strain might have been the product of an *in vivo* capsular transformation event.[1,2,12] The sixth cluster C, from Chile, had 8 isolates (penicillin MIC values were 0.25–0.5 μg/ml), seven with serotype 6B and one 6A.

The rest of the 67 low penicillin resistant isolates (singles or groups with ≤5) were represented by 43 distinct PFGE types and 10 different serotypes (Fig. 3).

Clinical sources of penicillin-resistant isolates

Figure 4 illustrates the recovery sites of the penicillin-resistant isolates. Most frequent were blood (146, or 45%) and cerebrospinal fluid (112 isolates, 34%), followed by pleural fluid (41 isolates, 13%) and other sites like middle ear, peritoneal, or joint fluids (27 isolates, 8%). Recovery sites of the two highly epidemic international clones A and B are also shown separately in Figure 4.

Genetic diversity of the penicillin-resistant S. pneumoniae *isolates—country by country*

Analysis by PFGE of the penicillin-resistant strains recovered in the six Latin-American countries provided microbiologic profiles characteristic for each country. Composite Figure 5 shows the distribution of penicillin-resistant isolates (separately plotting highly resistant and low-resistant isolates) into various PFGE types for Argentina, Brazil, Chile, Colombia, Mexico, and Uruguay. The Mexican isolates included only highly resistant strains. International clones A and B are marked by the arrows in Figure 5.

Comparison of these clonal profiles revealed some quite striking differences among the different countries. Most interestingly was the different representation of the international clones A and B and the very different frequencies of highly versus low resistant isolates in the six Latin-American countries. For instance, in Argentina and Uruguay, by far the highest rep-

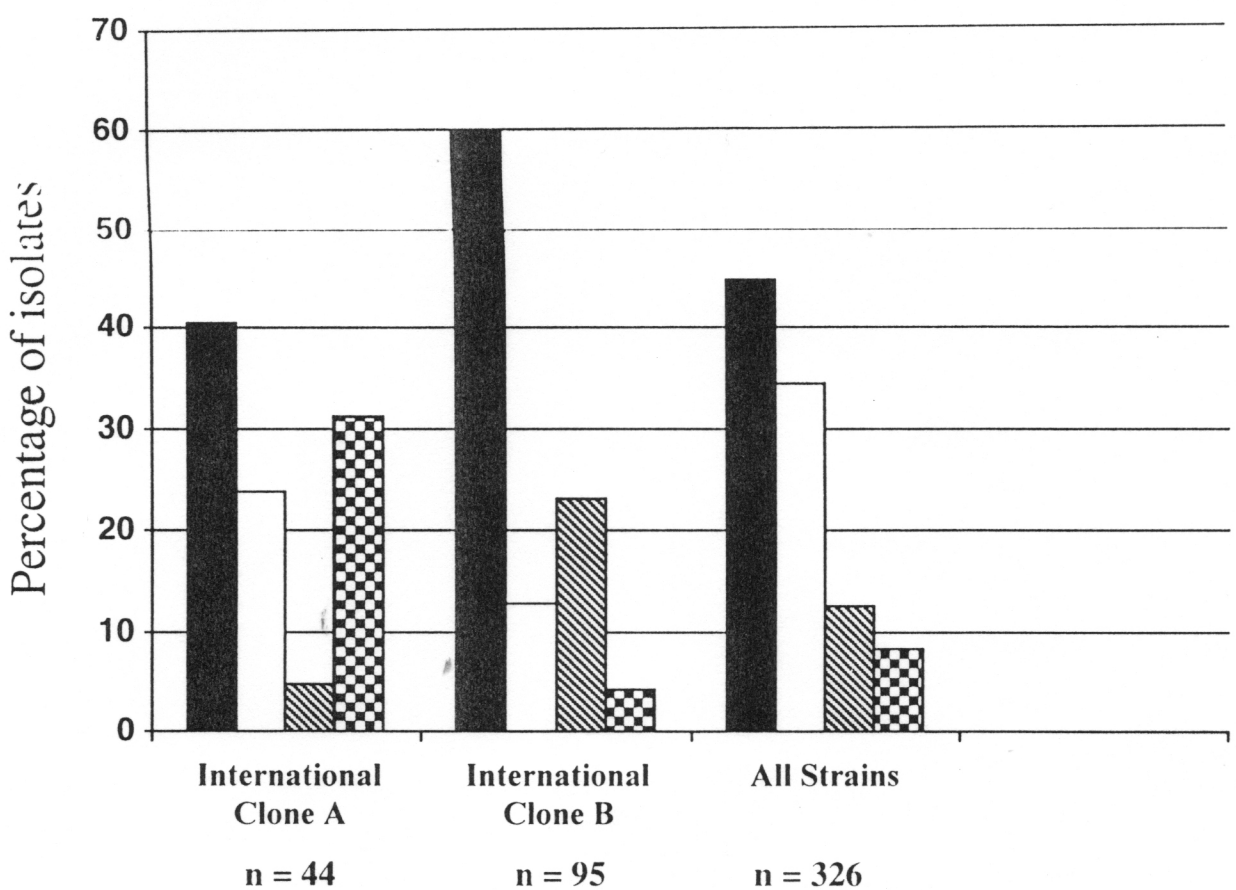


FIG. 4. Clinical sources of penicillin-resistant Latin American *S. pneumoniae* isolates. Barogram illustrates clinical sources (sterile sites) of the *S. pneumoniae* isolates. Isolates belonging to the two internationally spread clones **A** (23F) and **B** (9/14) are shown separately. The barogram shows the percentage of isolates recovered (in order from left to right) from the bloodstream (black), cerebrospinal fluid (white), pleural fluid (stripes), and other sources such as peritoneal or joint fluid (checked).

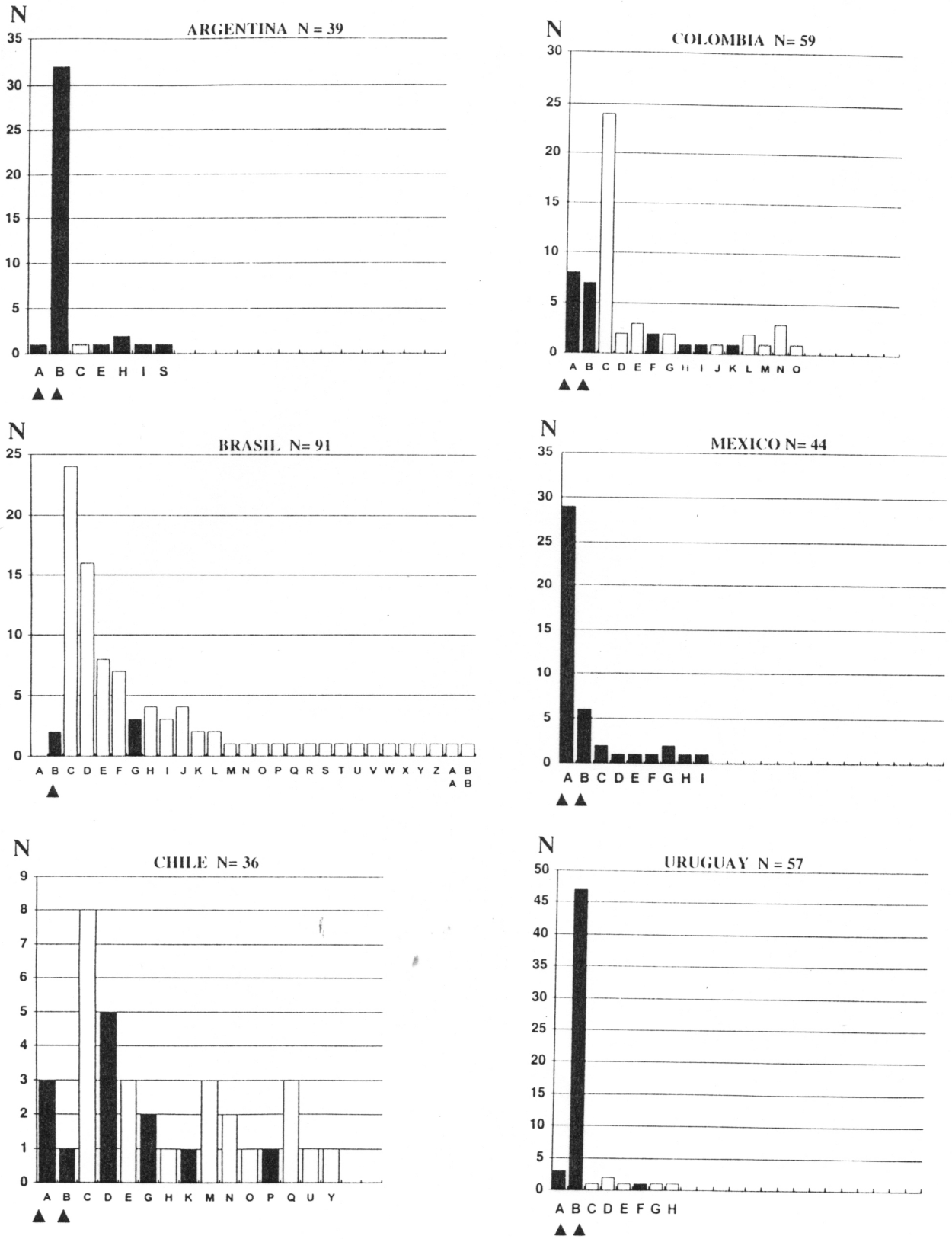


FIG. 5. Clonal "profiles" of penicillin-resistant *S. pneumoniae* isolates from six Latin American countries (Pen MIC ≥0.1 μg/ml). Pulsed field gel electrophoretic (PFGE) types are arbitrarily defined by capital letter assignments. Common letter assignments are made for the internationally spread clones with PFGE types **A** (Spanish/USA clone, serotype 23F) and **B** (French/Spanish clone, serotype 9 or 14); they are also marked with arrowheads. Black bars represent strains with penicillin MIC of ≥1 μg/ml; white bars represent strains with penicillin MIC 0.1–0.5 μg/ml. N: number of isolates.

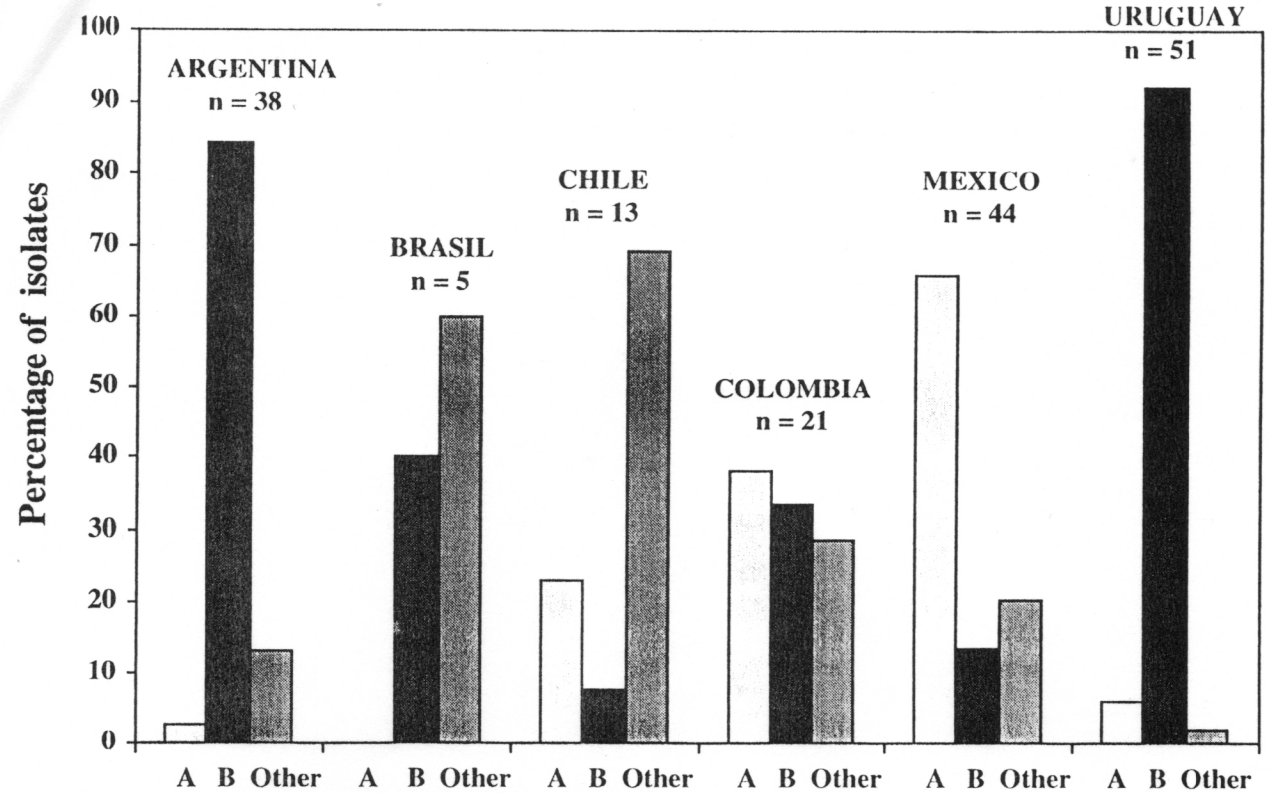


FIG. 6. Representation of two international clones (A and B) of *S. pneumoniae* among highly penicillin resistant (MIC $\geq$1.0 μg/ml) isolates from six Latin American countries.

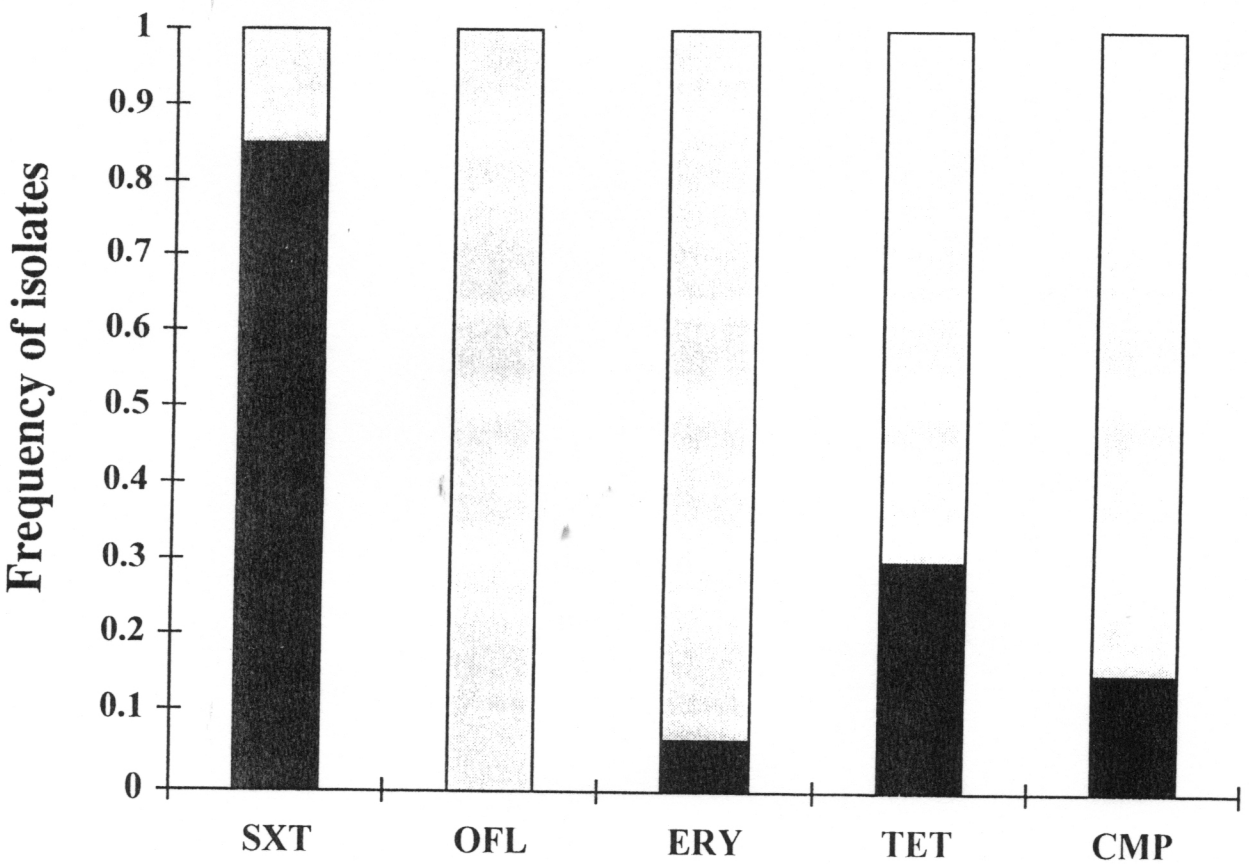


FIG. 7. Resistance to antibiotics other than penicillin among 326 Latin American *S. pneumoniae* isolates with reduced susceptibility to penicillin. The fraction of all penicillin-insensitive isolates carrying additional resistance factors is indicated by the black bars.

resentation was that of international clone B. In contrast, international clone A was by far the most frequent among the Mexican highly penicillin-resistant isolates. Among 91 Brazilian isolates examined, the dominant clone was one of the low resistant clusters (cluster C); no representatives of international clone A were detectable, and only two isolates showed the PFGE profile of international clone B. The overwhelming majority of the Brazilian isolates showed low-level penicillin resistance and a very large genetic variation of background (as many as 27 different PFGE types). The microbiologic profile of isolates from Chile and Colombia were intermediate by comparison.

Figure 6 illustrates in a graphic manner the relative contributions of the international clones A and B as well as strains with other PFGE patterns to the highly penicillin-resistant isolates from the six Latin-American countries.

Variation in genetic background and serotype: comparison of penicillin-susceptible, low and "highly" resistant strains

As mentioned before, the total number of Latin-American isolates with reduced penicillin susceptibility was 326, out of which 154 strains were of low penicillin resistance level (MIC 0.1 to 0.5 μg/ml), and 172 were highly penicillin resistant (MIC $\geq$1 μg/ml). In the case of isolates from Uruguay alone, isolates with penicillin MIC $\geq$0.5 μg/ml were considered highly penicillin resistant. In addition to these isolates, the complete collection examined at The Rockefeller University also included 40 penicillin-susceptible isolates, which were obtained from four countries: 18 from Chile, 17 from Argentina, 4 from Uruguay, and 1 from Brazil.

The variabilty of genetic background (number of PFGE types) and serotype and, to a lesser extent, the frequency of resistance to antibiotics other than penicillin showed striking variations with the penicillin MIC value of the isolates (Table 2).

The highest variability was observed among the 40 penicillin-susceptible strains: PFGE analysis showed that >80% of the 40 isolates had distinct PFGE patterns (33 different PFGE types in 40 isolates). These penicillin-susceptible strains expressed as many as 18 different serotypes (1, 5, 6A, 6B, 7F, 12F, 13, 14, 15, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 34, 35B, and nontypable).

Substantially less variation was detected among the isolates with low level penicillin resistance: only about 30% of the isolates showed distinct PFGE patterns (49 different PFGE types

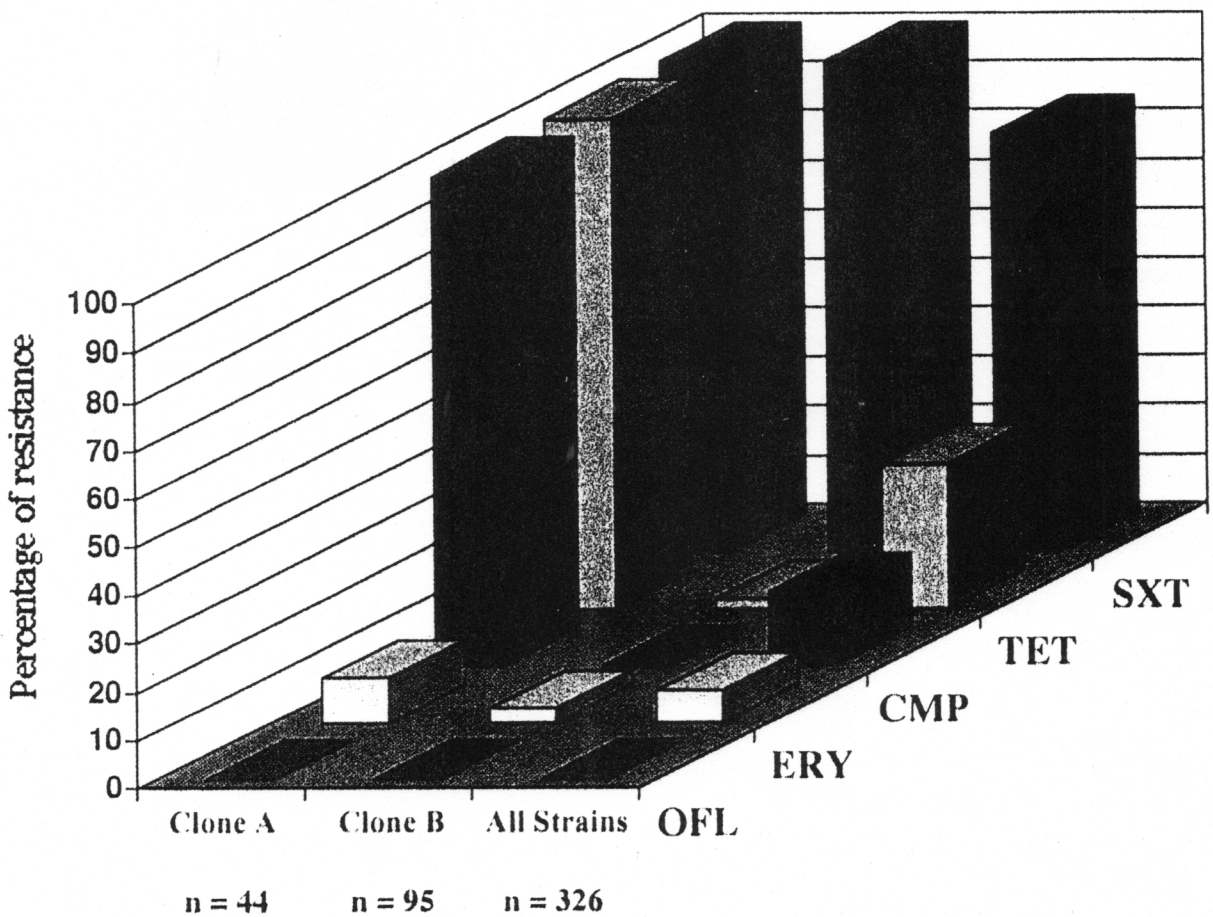


FIG. 8. Distribution of antibiotic resistance traits among Latin American isolates of *S. pneumoniae*.

in 154 isolates), and these strains expressed only 10 different capsular polysaccharides (6A, 6B, 11A, 14, 19A, 19F, 21, 23B, 23F, and 34).

The least variability was shown by the highly penicillin-resistant isolates: only ~10% of the isolates showed different PFGE patterns (23 PFGE types in 172 isolates), and these strains belonged to 8 capsular types (1, 6A, 6B, 9A, 9V, 14, 19F, and 23F). A closer examination of these strains actually showed an even more marked relative homogeneity: the overwhelming majority (139 of 172, or 81%) of the highly penicillin-resistant bacteria belonged to the two international clones A and B, the great majority of which expressed two major serotypes (23F and 14).

Resistance to antibiotics other than penicillin

Among the 326 isolates with penicillin MIC≥0.1 μg/ml, the frequency of strains with resistance to antibiotics other than penicillin was the following: 0.85 for trimethoprim/sulfamethoxazole, 0.30 for tetracycline, 0.15 for chloramphenicol, and 0.06 for erythromycin (Fig. 7). Of the 21 erythromycin-resistant pneumococci, 15 appeared to carry a constitutive MLS mechanism (resistance to erythromycin plus clindamycin), while 6 of the strains appeared to carry the efflux mechanism (resistance to erythromycin, susceptibility to clindamycin, without induction).[16] None of the isolates showed resistance to ofloxacin or vancomycin (data not shown). Figure 8 shows the percentage of resistance to trimethoprim/sulfamethoxazole, tetracycline, chloramphenicol, erythromycin, and ofloxacin in all of the 326 isolates and in the 44 and 95 strains belonging to the international clones A and B, respectively. As mentioned before, all isolates that shared the PFGE pattern A of the USA/Spanish international clone were resistant to trimethoprim/sulfamethoxazole, tetracycline, and chloramphenicol, but only 9% (4 strains) presented erythromycin resistance. In contrast, the French/Spanish international clone with PFGE pattern B displayed 100% trimethoprim/sulfamethoxazole resistance, but only two isolates (2%) presented tetracycline resistance and 3 (3%) erythromycin resistance. None of the isolates belonging to this clone showed chloramphenicol resistance.

Resistance to antibiotics other than penicillin showed a tendency to be more frequent in the highly penicillin-resistant isolates (Table 2). Thus, virtually all (164 out of 172, or 95.3%) of highly penicillin-resistant isolates were also resistant to trimethoprim/sulfamethoxazole. Trimethoprim/sulfamethoxazole resistance was present in 73.4% of strains with low penicillin resistance, and 47.5% of penicillin-susceptible bacteria also carried the trimethoprim/sulfamethoxazole trait. Chloramphenicol resistance was more frequent among highly penicillin resistant strains (close to 28%) than among strains with low penicillin resistance (0.6%),

TABLE 2. ANTIBIOTYPES, SEROTYPES, AND PFGE PATTERNS OF *S. PNEUMONIAE* ISOLATES

Total no. of strains: 366	*Pen MIC*[a] *<0.1*	*Pen MIC 0.1–0.5*	*Pen MIC ≥1.0*
No. of strains (%)	40 (10.9)	154 (42.1)	172 (47.0)
No. of serotypes	18 (1, 5, 6A, 6B, 7F, 12F, 13, 14, 15, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 34, 35B, NT)	10 (6A, 6B, 11A, 14, 19A, 19F, 21, 23B, 23F, 34	8 (1, 6A, 6B, 9A, 9V, 14, 19F, 23F)
No. of PFGE types	33	49	23
No. of strains with unique PFGE type (%):	26 (65.0)	29 (18.8)	13 (7.5)
No. (%) of strains belonging to			
PFGE A (International)			44 (25.6)
PFGE B (International)			95 (55.2)
BRASILIAN CLUSTERS	1 BR C (2.5)	BR C 24 (15.6) BR D 16 (10.4) BR E 8 (5.2) BR F 7 (4.5)	
CHILEAN CLUSTERS		CH C 8 (5.2)	CH D 5 (2.9)
COLOMBIAN CLUSTERS		COLA C 24 (15.6)	
No. of strains resistant to drugs other than penicillin (%)	23 (57.5)	125 (81.2)	170 (98.8)
No. of strains with SXT resistance (%)	19 (47.5)	113 (73.4)	164 (95.3)
No. of strains with OFL resistance (%)	0 (0)	0 (0)	0 (0)
No. of strains with ERY resistance (%)	1 (2.5)	6 (3.9)	15 (8.7)
No. of strains with TET resistance (%)	9 (22.5)	37 (24.0)	60 (34.9)
No. of strains with CMP resistance (%)	0 (0)	1 (0.6)	48 (27.9)

[a]MIC in μg/ml.

FIG. 9 International clones among penicillin-resistant *S. pneumoniae* isolates from six Latin American countries. The presence of the serotype 23F Spanish/USA clone (red squares), the serotype 9 French/Spanish clone (yellow squares) and the serotype 14 French/Spanish clone (blue squares) is shown on the map of the countries participating in the PAHO surveillance project.

and chloramphenicol resistance was not detectable among the 40 penicillin-susceptible strains examined. Erythromycin resistance was present in 8.7% of the highly penicillin-resistant, 3.9% of low penicillin-resistant strains, and 2.5% of penicillin-susceptible bacteria. This rough correlation with penicillin resistance level was less clear in the case of resistance to tetracycline: close to 35% of highly penicillin-resistant and 24% of low penicillin-resistant strains, but also 22.5% of penicillin-susceptible pneumococci carried tetracycline resistance trait.

Resistance to β-lactam antibiotics, trimethoprim/sulfamethoxazole, tetracycline, chloramphenicol, and sometimes erythromycin was part of a multidrug resistance package associated with the international clone A.

Summary and highlights of findings

Table 2 provides a tabulation of the most important findings of the study with the 366 Latin-American *S. pneumoniae* isolates performed at The Rockefeller University. Some comments about the highlights of these results may be appropriate.

1. The surprisingly high incidence in the 23F Spanish/USA clone A and the usually serotype 14 French/Spanish clone B among the Latin American isolates further documents the extensive geographic spread of these two genetic lineages (Fig. 9), which have already been identified as major components of the penicillin-resistant pneumococcal flora in collections originating in Spain,[10] France,[3] Germany,[13] Italy,[7] Portugal,[21] Croatia,[18] and Bulgaria[14] as well as the United States,[8] South Africa,[5] and South Korea.[9,17] Interestingly, the most frequent recovery sites for those international clones were large population centers and capitals of the participating Latin American countries. This observation suggests the possibility that these resistant clones are introduced into various countries through international travel. The microbial factors likely to contribute to the epidemicity of these clones are presently unknown. It is also important to note that *S. pneumoniae* isolates belonging to the two international clones were recovered with high frequencies from sterile sites like blood and cerebrospinal fluid, (Fig. 4), indicating that these strains carry and express genetic determinants of pneumococcal virulence.

2. The high frequency of resistance to trimethoprim/sulfamethoxazole and the rarity of macrolide resistance and apparently complete absence of resistance to fluoroquinolones (ofloxacin) are probably a reflection of the type of antibiotic usage in Latin-American countries.

3. The apparently inverse relationship between penicillin MIC value and genetic variability has already been noted in the analysis of other collections of penicillin-resistant *S. pneumonia*: strains from the Czech and Slovak Republics,[4] from Croatia,[14] and from Portugal.[21] Increasing the penicillin MIC values of isolates tended to bring along decreasing genetic variability (as assessed by the number of PFGE types), fewer serotypes, and a higher probability for multidrug resistance. While the exact mechanism of this relationship is not clear, one contributing factor appears to be the extensive geographic spread of a relatively few multiresistant lineages. The high frequency of clone A in Mexico and clone B in Argentina and Uruguay suggest that most of these highly resistant strains are imported to these countries. In contrast, the relatively low level of penicillin resistance and enormous genetic variability observed among the Brazilian isolates suggests that these may be resistant strains originating from a large variety of genetic backgrounds *in situ* in Brazil.

4. The origin of the unique microbiologic profiles of highly penicillin-resistant isolates in several of the Latin-American countries (for instance, in Argentina, Uruguay, and Mexico) strongly suggests unique avenues of importation of these strains. The extremely high prevalence of the serotype 23F Spanish/USA clone A in California[8,20] provides an example for the exchange of this clone through the geographic vicinity of Mexico and the southwestern United States.

5. The occasional appearance, in collections of penicillin-resistant pneumococci, of isolates that express serotypes not typical for the particular clone has already been described in the literature.[1,2,12] The Latin-American collection provides some additional examples, for instance, the appearance of serotypes 9A, 9V, and 19F isolates of *S. pneumoniae* belonging to international clone B, which usually expresses serotype 14; serotype 6B and 14 isolates with PFGE pattern A, which is usually associated with serotype 23F; and the single Colombian isolate expressing capsular serotype 34 instead of the expected serotype 23F common to this particular cluster. These pneumococcal isolates are most likely the products of spontaneous *in vivo* capsular transformation events.[1,2,12]

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