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Dissemination of *Streptococcus pneumoniae* Clone Colombia^5-19 in Latin America

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Streptococcus pneumoniae **serotype 5 is the third most common capsular type causing invasive diseases in children younger than 5 years in Latin America. Preliminary data on Colombian serotype 5 isolates indicated a common clonal origin associated with resistance to tetracycline (TET) and chloramphenicol (CHL). We studied 172 *S. pneumoniae* serotype 5 invasive isolates from Argentina, Brazil, Colombia, Guatemala, Mexico, and Uruguay and confirmed the presence of the Colombia^5-19 clone throughout Latin America. Fifteen subtypes of a pulsed-field gel electrophoresis pattern and 4 electrophoretic types (ET) were obtained. Most of the isolates from different geographical regions belonged to pattern A (34.3%), subtype A5 (41.9%), and ET1 (91.1%). The A pattern ($n = 59$) was resistant to TET and had variable resistance to CHL; it was present in Brazil (10.2%), Colombia (78%), Guatemala (8.5%), and Mexico (3.4%). Subtype A5 with variable susceptibility to TET and sensitive to CHL was found in Argentina (29.2%), Mexico (8.3%), and Uruguay (62.5%). Subtypes A1–A4, A7–A8, and A9–A11 (closely related to A) also shared ET1, while subtype A6 was assigned to ET1, ET2, and ET3. Eleven subtypes ($n = 21$) were found to be specific for one country each. In summary, the *S. pneumoniae* serotype 5 isolates from Latin American are genetically closely related but show different patterns of antibiotic resistance, probably as a result of horizontal transfer.**

Streptococcus pneumoniae is present in the bacterial flora of the human upper respiratory tract. *S. pneumoniae* is the most important cause of community-acquired pneumonia, meningitis, otitis media, and bacteremia, particularly in the extremes of life (1, 23). It is estimated that more than a million children younger than 5 years die each year of pneumococcal pneumonia (11, 22). The principal *S. pneumoniae* virulence factor is the capsular polysaccharide. Only 10 to 12 of the 90 capsular serotypes described are responsible for most invasive illness, but their distribution follows different geographic patterns (1, 15, 30). Additionally, resistance to different antimicrobials has been widely documented and distributed through serotypes and countries (1, 9, 25, 35).

An *S. pneumoniae* surveillance program was started in 1994 in six Latin American countries (SIREVA-Vigía project) coordinated by the Pan-American Health Organization and cosponsored by the Canadian International Development Agency (6). This regional initiative was designed to obtain information about the *S. pneumoniae* serotype distribution in order to determine the ideal composition for a conjugate vaccine that could be useful for the region. Additionally, the project was aimed at monitoring the rates of *S. pneumoniae* serotype distribution and antimicrobial resistance (3, 4, 6, 8, 16, 17, 28). After a 5-year surveillance, a high prevalence of serotype 5 (9.6%) was documented in Latin America. In Argentina and Uruguay serotype 5 ranked second (14.1 and 14.8% respectively), in Chile it ranked third (11.7%), in Colombia it ranked fourth (7.9%), and in Brazil it ranked fifth (6.7%) (7).

In Colombia the serotype 5 isolates showed a unique pulsed-field gel electrophoresis (PFGE) pattern with resistance to tetracycline and chloramphenicol, suggesting the circulation of a specific clone (33). The pneumococcal molecular epidemiology network (21) recently recognized the clone as Colombia^5-19 (K. Klugman, Minutes of the Fifth Meeting of PMEN, p. 2, 2001).

The aim of this study was to determine the genetic relatedness among *S. pneumoniae* serotype 5 invasive isolates recovered from children younger than 5 years from Argentina, Brazil, Colombia, Guatemala, Mexico, and Uruguay and to compare these results with the antibiotic resistance patterns of the isolates.

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MATERIALS AND METHODS

Bacterial isolates. *S. pneumoniae* capsular type 5 invasive isolates recovered from children younger than 5 years from Argentina, Brazil, Mexico, Guatemala, and Uruguay were submitted to the Microbiology Group at the Instituto Nacional de Salud in Colombia. The identities of all isolates were confirmed by standard methods (10). The serotyping was performed using the Quellung reaction with antisera produced by the Statens Seruminstitut (Copenhagen, Denmark) (32). Of 53 Colombian isolates analyzed, 43 had been previously studied by PFGE (33). Isolate INS-Sp Col 106 was the strain submitted to identify the clone Colombia^5-19 (Klugman, 5th Meet. PMEN, 2001). Laboratory strain R6, kindly provided by Alexander Tomasz from The Rockefeller University, was included as a molecular weight marker.

Antimicrobial susceptibility. Inhibition zones were determined by the Kirby-Bauer method, and MICs were determined by broth microdilution; the results were interpreted on the basis of the National Committee for Clinical Laboratory Standards (NCCLS) tables for the following antimicrobials: penicillin, ceftriaxone, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, erythromycin, and vancomycin (24).

MLEE. Serotype 5 isolates were typed by multilocus enzyme electrophoresis (MLEE) in Montevideo, Uruguay, as described elsewhere (29). Some modifications were made to the technique. Briefly, each isolate was grown in 5% sheep blood agar plates. The whole growth from the plates was pelleted and resuspended in 0.5 ml of lysis buffer (5 mM EDTA, 50 mM Tris [pH 7.5]) and frozen at −20°C for a minimum of 48 h. The cell debris was removed by centrifugation at 15,000 × *g* for 15 min at 4°C. Thawed lysates were absorbed into paper wicks and inserted into vertical slits cut in a 12% starch gel (Connaught Laboratories, Swiftwater, Pa.). After electrophoresis for 6 h, gel slices were stained for specific enzyme activities. The following enzymes were examined after electrophoresis in buffer system A: 6-phosphogluconate dehydrogenase, glutamate dehydrogenase, nucleoside phosphorylase, esterase, and phosphoglucose isomerase. The following enzymes were examined after electrophoresis in buffer system B: adenylate kinase, leucine aminopeptidase, L-lactate dehydrogenase, and glucose 6-phosphate dehydrogenase. The following enzymes were examined after electrophoresis in buffer system G: leucyl-alanine peptidase, leucyl-glycyl-glycine peptidase, and phenyl-leucine peptidase.

Each unique combination of migration patterns (equated with alleles at the corresponding gene loci) for the 12 enzymes studied was called an electrophoretic type (ET). All ETs were compared with each other to determine genetic relatedness (29).

PFGE. *S. pneumoniae* isolates were grown in supplemented Todd-Hewitt broth (Difco, Becton Dickinson, Sparks, Md.) (37). *S. pneumoniae* chromosomal DNA embedded in agarose disks was prepared by a previously described method (31, 37). The disks were digested with 20 U of *Sma*I (Promega, Madison, Wis.). The molecular weight marker used was the lambda ladder (New England Biolabs, Beverly, Mass.) and the R6 strain. The *Sma*I macrorestriction fragments were separated by electrophoresis (CHEF DRII apparatus; Bio-Rad Laboratories, Richmond, Calif.) at 6 V/cm with switch times ramped from 1 to 30 s over a 23-h period at 11.3°C. PFGE patterns were classified according to Tenover's criterion. Isolates that had the same number of bands with the same molecular sizes were designated genetically indistinguishable and assigned a single pattern; an isolate was considered to be closely related to the pattern when its PFGE profile differed in two or three bands, indicating a change by a single event; and an isolate was considered to be possibly related when the PFGE profile differed in four to six bands, meaning a change by two independent genetic events (34).

To analyze the PFGE results, Diversity Database (Bio-Rad Laboratories) was used to automatically identify band positions and compare two patterns by calculating the Dice coefficient of similarity, S_D. Dendrograms were generated using the unweighted pair group method of average linkage (UPGMA).

RESULTS

Bacterial isolates. A total of 172 isolates were studied. Of these, 165 (96%) were recovered between 1993 and 1999, 23 from Argentina, 12 from Brazil, 53 from Colombia, 10 from Guatemala, 10 from Mexico, and 57 from Uruguay. These isolates corresponded to 100% of the serotype 5 isolates recovered in Colombia, Mexico, Uruguay, and Guatemala and to 50 and 40% from Argentina and Brazil, respectively. Additionally, four isolates recovered in 2000 from Colombia and three isolates recovered in 1988 and 1989 from Uruguay were studied.

In Table 1 the isolates are listed by general code, country code, and year of isolation. Isolates were recovered from children suffering from meningitis ($n = 59$), pneumonia ($n = 101$), arthritis ($n = 2$), sepsis ($n = 6$), or abscess ($n = 1$) or from sources without data ($n = 3$).

Phenotypic marker and antimicrobial susceptibility. All 172 isolates were susceptible to penicillin, ceftriaxone, erythromycin, and vancomycin, except for one isolate (ARG 1206) from Argentina which was resistant to erythromycin (MIC, 32 μg/μl). Antimicrobial resistance to chloramphenicol was present in 50 (29%) isolates, resistance to tetracycline was present in 82 (47.7%), high resistance to trimethoprim-sulfamethoxazole was present in 25 (14.5%), and intermediate resistance was expressed in 114 of 172 isolates (66.3%) (Table 1).

MLEE. Four different, closely related ETs were found among 157 isolates analyzed (Table 1). Variability in electrophoretic mobility was limited to three enzymes (esterase, L-lactate dehydrogenase and leucyl-glycyl-glycine peptidase), while the remaining nine were monomorphic. ET1 was the most common, represented by 143 isolates (91.1%). ET2 was shared by 11 isolates (7.0%), and ET3 and ET4 were represented by one isolate each. In Argentina, 21 isolates (91.3%) were ET1 and 2 (8.7%) were ET2. In Brazil, 10 (83.3%) were ET1 and 2 (16.7%) were ET2. In Colombia, 50 (96.2%) were ET1, 1 (1.9%) was ET2, and 1 (1.9%) was ET3. In Mexico, all 10 (100%) isolates were ET1. In Uruguay, 53 (88.3%) were ET1, 6 (10%) were ET2, and 1 (1.6%) was ET4.

Genotypic marker and PFGE. The 172 invasive isolates analyzed belonged to pattern A. Based on the number of bands and their molecular weights, 15 PFGE subtypes (A1 to A15) were classified (Table 1). Pattern A, with 59 isolates, had 13 bands ranging from 340 to 25 kb. Subtypes with two or three band differences (closely related) with respect to 109 isolates were assigned to A1 to A8, A10, A12, A14, and A15; and subtypes with four to six band differences (possibly related) with respect to 4 isolates were assigned to A9, A11 and A13. PFGE patterns A, A1, A3, A5, A6, and A12 are shown in Fig. 1.

Most of the isolates (83.7%) were concentrated into three patterns: A ($n = 59$), A5 ($n = 72$) and A6 ($n = 13$). The prevalence of PFGE subtypes was determined per country and year. Pattern A circulated in Mexico, Guatemala, Colombia, and Brazil. Subtype A5 circulated in Argentina, Uruguay, and Mexico, while subtype A6 circulated in Colombia, Brazil, Uruguay, and Argentina (Table 1; Fig. 2). Two other subtypes, A3 ($n = 3$) and A7 ($n = 4$), were present in two countries. The remaining 11 subtypes ($n = 21$), with four isolates or fewer each, were identified in only one country.

The similarity between all subtypes was more than 80% by Dice coefficient, while subtype A13 had 77% similarity to pattern A. The R6 reference strain had 60% similarity (Fig. 3).

Correlation between the phenotypic and genotypic markers. Patterns A, A1, A2, A3, and A12 grouped 68 isolates from Brazil, Colombia, Guatemala, Mexico, and Uruguay that were resistant to tetracycline and had a 340-kb characteristic PFGE band. Resistance to tetracycline was observed with lower incidences in subtypes A5 (5.5%) and A6 (61.5%) isolated from Argentina and Uruguay.

Resistance to chloramphenicol was observed exclusively in patterns A (46 of 59), A1 (3 of 3), and A3 (1 of 3). All these isolates except one were also resistant to tetracycline. In pattern A1, all three Colombian isolates were resistant, and in pattern A3, 50% of Mexican isolates were resistant. On the other hand, the isolates resistant only to tetracycline were pattern A (12 of 59) and subtypes A2 (1 of 1), A3 (2 of 3), A5 (4 of 72), A6 (8 of 13), A12 (2 of 2), A14 (3 of 4), and A 15 (1 of 1). Isolates of subtypes A7 to A11 and A13 were all susceptible to chloramphenicol and tetracycline.

TABLE 1. Serotype 5 *S. pneumoniae* isolates from Argentina, Brazil, Colombia, Guatemala, Mexico, and Uruguay

Code	Country code[a]	Yr	MIC (μg/ml) of[b]:			ET	PFGE type
			TET	CHL	STX		
1032	ARG708	1995	0.12	4	1/19	1	A5
1033	ARG741	1995	4	4	1/19	1	A5
1034	ARG746	1995	0.25	4	1/19	1	A5
1035	ARG793	1995	0.12	4	1/19	1	A5
1036	ARG1033	1996	0.12	4	1/19	1	A5
1037	ARG1043	1996	0.25	4	2/38	1	A5
1038	ARG1061	1996	0.12	4	1/19	1	A5
1039	ARG1096	1996	0.5	4	1/19	1	A5
1040	ARG1105	1996	0.25	4	1/19	1	A5
1041	ARG1107	1996	0.25	4	2/38	1	A5
1042	ARG1108	1996	16	2	1/19	2	A6
1043	ARG1119	1996	0.25	4	2/38	1	A5
1044	ARG1127	1996	0.25	4	2/38	1	A5
1045	ARG1128	1996	0.25	4	2/38	1	A5
1046	ARG1152	1996	0.25	4	2/38	1	A5
1047	ARG1157	1996	0.25	4	2/38	1	A5
1048	ARG1164	1996	0.25	4	1/19	1	A5
1049	ARG1179	1997	16	2	0.25/4.7	1	A5
1050	ARG1180	1997	8	2	4/76	2	A6
1051	ARG1182	1997	16	2	0.25/4.7	1	A5
1153	ARG1206	1997	32	4	1/19	1	A5
1054	ARG1224	1997	0.12	4	2/38	1	A5
1055	ARG1240	1997	0.12	4	1/19	1	A5
1010	BRA56	1996	2	4	0.25/4.7	2	A6
1011	BRA57	1996	8	4	8/152	1	A
1012	BRA71	1996	0.5	2	0.25/4.7	2	A6
1013	BRA102	1996	16	4	8/152	1	A
1015	BRA169	1997	16	4	8/152	1	A
1016	BRA171	1997	32	4	8/152	1	A2
1017	BRA210	1997	0.25	4	1/19	1	A7
1009	BRA39	1997	0.12	4	0.25/4.7	1	A7
1020	BRA410	1997	16	4	8/152	1	A
1018	BRA213	1998	16	4	16/302	1	A
1019	BRA291	1998	16	4	8/152	1	A
1007	BRA5	1998	0.25	4	1/19	1	A7
7	COL7	1994	32	32	2/38	1	A
12	COL12	1994	32	16	4/76	1	A
24	COL24	1994	32	32	2/38	1	A
30A	COL30A	1994	16	16	4/76	1	A
30B	COL30B	1994	32	32	4/76	1	A
47	COL47	1994	32	32	0.5/9.5	1	A
49A	COL49A	1994	32	16	1/19	1	A
49C	COL49C	1994	32	16	1/19	1	A
72	COL72	1994	0.12	2	0.12/2.3	3	A6
86	COL86	1994	32	32	8/152	1	A
106	COL106	1994	32	32	2/38	1	A
123	COL123	1994	32	16	0.25/4.7	1	A
133	COL133	1994	32	16	0.06/1.1	1	A
135	COL135	1994	0.25	4	2/38	1	A4
136	COL136	1994	32	16	2/38	1	A1
161	COL161	1995	16	32	2/38	1	A
175	COL175	1995	32	32	8/152	1	A
177	COL178	1995	32	32	8/152	1	A
189	COL190	1995	16	32	1/19	1	A
190	COL191	1995	32	32	1/19	1	A
201	COL202	1995	32	16	2/38	1	A
212	COL213	1995	8	16	8/152	1	A
214	COL215	1995	32	16	2/38	1	A1
221	COL222	1995	32	16	2/38	1	A
222	COL223	1995	32	4	0.5/9.5	1	A
224	COL225	1995	0.12	4	0.12/2.3	2	A6
226	COL227	1995	32	2	0.25/4.7	1	A
227	COL228	1995	32	16	2/38	1	A
252	COL253	1995	16	16	2/38	1	A
255	COL256	1995	16	32	1/19	1	A
261	COL262	1995	16	16	2/38	1	A

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TABLE 1—*Continued*

Code	Country code[a]	Yr	MIC (μg/ml) of[b]:			ET	PFGE type
			TET	CHL	STX		
282	COL283	1995	16	16	0.06/1.1	1	A
283	COL284	1995	16	16	1/19	1	A
299	COL300	1995	16	16	0.06/1.1	1	A
303	COL304	1995	16	16	0.25/4.7	ND[c]	A
316	COL317	1996	32	32	8/152	1	A
E1H	COLE1H	1996	32	8	2/38	1	A
E1LP	COLE1LP	1996	32	32	2/38	1	A
E16	COLE16	1996	32	32	1/19	1	A
E18	COLE18	1996	32	32	4/76	1	A
E45	COLE45	1996	32	32	2/38	1	A
E68	COLE68	1996	32	32	2/38	1	A1
E72	COLE72	1996	32	4	1/19	1	A3
E73	COLE73	1996	32	32	2/38	1	A
E86	COLE86	1997	0.25	4	2/38	1	A4
E105	COLE105	1997	32	32	2/38	1	A
E149	COLE145	1997	32	32	2/38	1	A
E161	COLE157	1997	0.12	4	2/38	1	A8
E178	COLE174	1997	32	32	2/38	1	A
E196	COLE192	1998	0.25	4	2/38	1	A8
E225	COLE220	1998	32	16	2/38	1	A
E236	COLE231	1998	32	16	8/152	1	A
E324	COLE318	1999	0.12	4	0.5/9.5	1	A8
E386	COLE386	2000	32	16	32/608	ND	A
E387	COLE387	2000	32	16	16/304	ND	A
E390	COLE390	2000	16	16	2/38	ND	A
E391B	COLE391B	2000	16	16	1/19	ND	A
1878	GUA168	1997	4	4	1/19	ND	A
1879	GUA187	1997	8	4	4/76	ND	A
1880	GUA753	1998	8	4	1/19	ND	A14
1881	GUA773	1998	8	4	1/19	ND	A14
1882	GUA783	1998	16	4	1/19	ND	A15
1883	GUA808	1998	8	4	1/19	ND	A
1884	GUA811	1998	8	4	1/19	ND	A
1885	GUA824	1998	4	4	1/19	ND	A14
1886	GUA1348	1998	8	4	1/19	ND	A14
1887	GUA1424	1998	16	4	1/19	ND	A
1025	MEX49	1994	4	16	2/38	1	A
1026	MEX110	1995	0.25	4	2/38	1	A5
1027	MEX111	1995	0.25	4	2/38	1	A5
1028	MEX112	1995	0.25	4	2/38	1	A5
1029	MEX115	1995	16	16	2/38	1	A3
1023	MEX10	1996	0.25	4	2/38	1	A5
1024	MEX16	1996	16	16	2/38	1	A
1030	MEX146	1966	8	2	2/38	1	A3
1021	MEX2NM	1998	0.25	4	0.5/9.5	1	A5
1022	MEX2PS	1998	0.25	4	1/19	1	A5
1215	URU112	1988	8	2	0.25/4.7	2	A12
1217	URU185	1988	2	2	0.25/4.7	2	A7
1216	URU332	1989	8	4	0.25/4.7	2	A12
1118	URU96	1993	0.25	4	1/19	1	A5
1119	URU N48	1993	0.12	4	0.25/4.7	1	A10
1120	URU N649	1993	8	2	0.25/4.7	1	A6
1121	URU114	1994	0.25	4	1/19	1	A5
1122	URU139	1994	0.25	4	8/152	1	A5
730	URU168	1994	0.12	4	8/152	1	A5
731	URU173	1994	0.25	4	1/19	1	A5
1123	URU176	1994	0.25	4	8/152	1	A5
1124	URU181	1994	0.12	4	1/19	1	A5
1125	URU183	1994	0.25	4	2/38	1	A5
732	URU204	1994	0.25	4	2/38	1	A5
733	URU212	1994	4	2	0.12/2.3	1	A5
1126	URU216	1994	8	4	0.25/4.7	2	A6
734	URU227	1994	8	2	0.12/2.3	1	A6
1127	URU237	1994	8	2	0.25/4.7	2	A6
735	URU243	1994	0.25	4	1/19	1	A9
1128	URU252	1994	0.25	4	2/38	1	A5

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TABLE 1—*Continued*

Code	Country code[a]	Yr	MIC (μg/ml) of[b]:			ET	PFGE type
			TET	CHL	STX		
1129	URU284	1995	0.12	4	0.5/9.5	1	A5
1130	URU297	1995	0.12	4	1/19	1	A5
1131	URU304	1995	0.25	4	2/38	1	A5
736	URU305	1995	8	4	0.12/2.3	1	A5
1132	URU316	1995	0.25	4	2/38	1	A5
737	URU325	1995	0.25	4	2/38	1	A5
738	URU350	1995	0.25	4	2/38	1	A5
1133	URU391	1995	8	4	0.25/4.7	2	A6
1134	URU392	1995	0.25	4	1/19	1	A5
1135	URU393	1995	0.12	4	2/38	1	A5
739	URU420	1996	8	4	0.25/4.7	1	A6
740	URU427	1996	0.12	4	4/76	1	A5
742	URU480	1996	0.12	4	1/19	1	A5
743	URU499	1996	0.12	4	1/19	1	A5
1136	URU504	1996	0.25	4	0.5/9.5	1	A5
1137	URU519	1996	0.25	4	1/19	1	A5
1138	URU524	1996	0.25	4	2/38	1	A9
1139	URU708	1997	0.5	4	2/38	1	A13
744	URU812	1997	0.25	4	1/19	1	A5
745	URU887	1997	0.25	4	1/19	1	A5
746	URU888	1997	0.25	4	2/38	1	A5
1140	URU910	1997	0.25	4	2/38	1	A5
747	URU954	1997	0.25	4	2/38	1	A5
1141	URU973	1998	0.25	4	1/19	1	A5
1142	URU988	1998	0.25	4	1/19	1	A5
1143	URU1016	1998	0.25	2	0.5/9.5	4	A11
1144	URU1033	1998	0.25	4	2/38	1	A5
1145	URU1040	1998	0.25	4	1/19	1	A5
1146	URU1042	1998	0.25	4	1/19	1	A5
1147	URU1060	1998	0.25	4	1/19	1	A5
1148	URU1093	1999	0.25	4	2/38	1	A5
1149	URU1113	1999	0.25	4	1/19	1	A5
1150	URU1123	1999	0.25	4	1/19	2	A6
1151	URU1127	1999	0.25	4	1/19	1	A5
1152	URU1161	1999	0.25	4	1/19	1	A5
1153	URU1193	1999	0.25	4	0.5/9.5	1	A5
1154	URU1205	1999	0.25	4	1/19	1	A5
1155	URU1208	1999	0.25	4	1/19	1	A5
1156	URU1213	1999	0.25	4	1/19	1	A5
1157	URU1215	1999	0.25	4	1/19	1	A5

[a] ARG: Argentina; BRA: Brazil; COL: Colombia; GUA: Guatemala; MEX: Mexico; URU: Uruguay.
[b] All 172 isolates were susceptible to penicillin, ceftriaxone, erythromycin, and vancomycin. TET, tetracycline resistant (≥8 μg/ml); CHL, chloramphenicol resistant (≥4 μg/ml); STX, trimethoprim-sulfamethoxazole resistant (≥4 μg/ml) and intermediate (1 and 2 μg/ml) (25).
[c] ND, not determined.

DISCUSSION

As previously reported, *S. pneumoniae* serotype 5 is one of the most frequent causes of invasive pneumococcal disease in Latin American children younger than 5 years (3, 4, 7, 8, 16, 28). In a recent review by Hausdorff et al. (14), in more than 70 data sets originating worldwide, between five and eight serogroups comprised at least 75% of pneumococcal isolates from young children. Moreover, they found that throughout Asia serotype 5 ranked fourth, ranking second in China (12.8%) and third in Israel (13.4%). In Africa, serotype 5 ranked sixth, comprising 14.6% of isolates in Rwanda, 9.5% in Gambia, and 14.3% in Kenya. In Europe, serotype 5 ranked 10th, with the most representative country being Spain (5.7%) (14). However, in the United States and Canada, serotype 5 does not appear among the 12 most important circulating serotypes. In a subsequent analysis, Hausdorff et al. reported that serotype 5 was isolated in third place from middle ear fluid and blood and second from cerebrospinal fluid in Asia (13).

In Colombia, where serotypes 5, 14, and 23F have the highest incidence in invasive disease among children (5), the circulation of the major clones Spain^{23F}-1 and Spain^{9v}-3, as well as a unique clone 23F associated with resistance to penicillin, has been described (37). In 1999, we reported the presence of a Colombian serotype 5 that was later confirmed as a specific clone (Colombia^{5}-19) (33; Klugman, 5th Meet. PMEN, 2001). A study done in Israel showed the presence of serotype 5 isolates, which have the same PFGE pattern as Latin American isolates (R. Dagan, personal communication).

The isolates analyzed in the present study had the same PFGE A pattern and 91% showed the same ET, suggesting a common origin, previously described as the Colombian^{5}-19 clone. Moreover, 86% of isolates belonged to only 3 of the 15

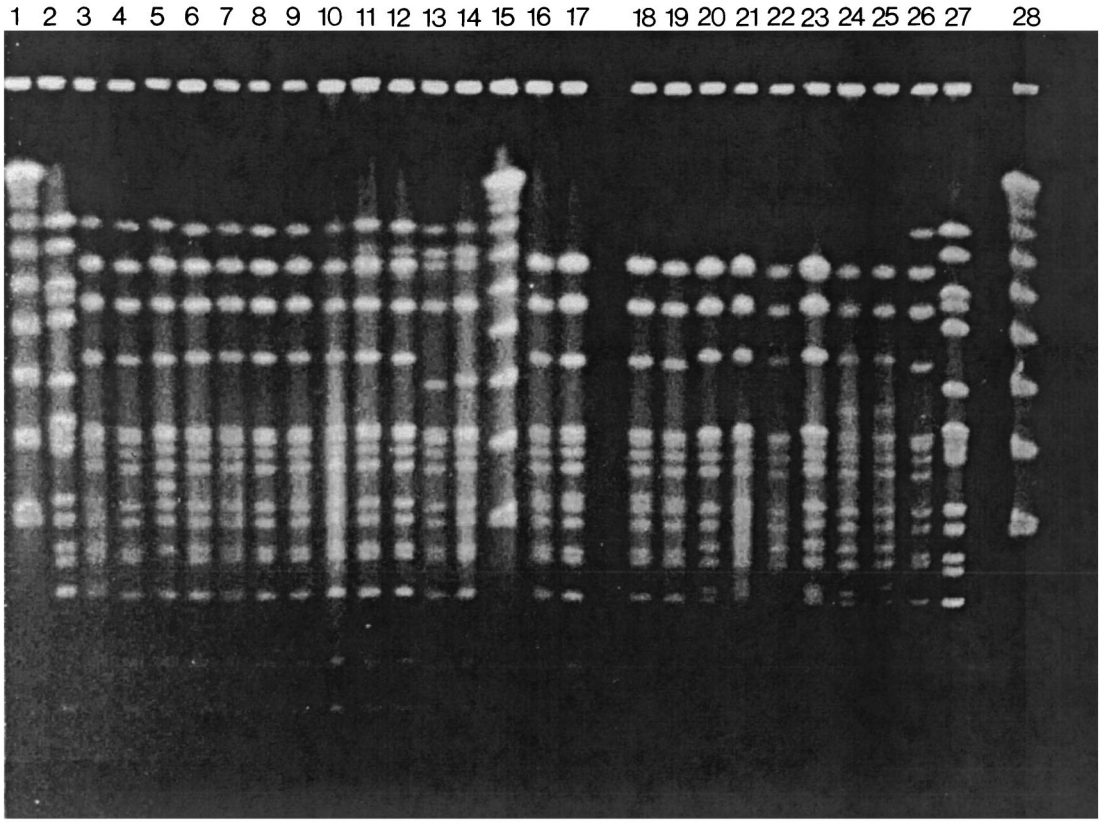

FIG. 1. PFGE subtype patterns of *S. pneumoniae* serotype 5 invasive isolates. Chromosomal DNA fragments were digested with *Sma*I. Lanes 1, 15, and 28 (counting from the left) correspond to Lambda ladder; lanes 2 and 27 contain strain R6. Lanes 3 to 10 and 26 show PFGE pattern A: Col 7, Col E330, Col E390, Bra 102, Bra 291, Gua 168, Gua811, Mex 49, and Col 133. Lanes 11 and 12 show PFGE pattern A1: Col E68 and Col 136. Lanes 13 and 14 show PFGE pattern A3: Col E72 and Mex 146. lanes 16 to 19 show PFGE pattern A5: Arg 1061, Arg 1105, Uru 1215, and Mex 110. Lanes 20 to 23 show PFGE pattern A6: Uru 216, Arg 1108, Col 72, and Bra 71. Lanes 24 and 25 show PFGE pattern A12: Uru 332 and Uru 112.

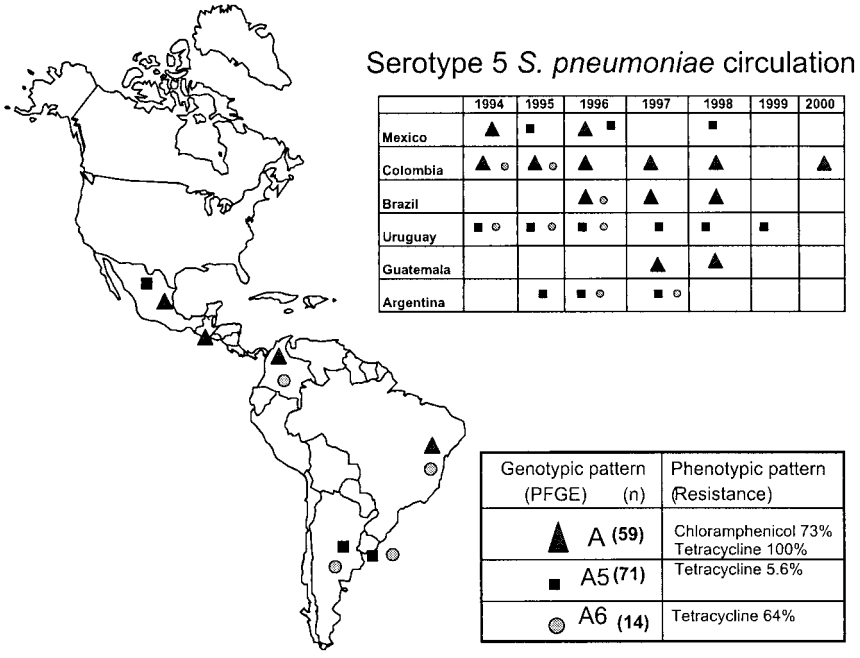

FIG. 2. Geographic and temporal distribution of the *S. pneumoniae* serotype 5 clone.

FIG. 3. Phylogenetic tree of Latin American serotype 5 *S. pneumoniae* isolates on the basis of PFGE patterns (UPGMA).

subtypes identified (A, A5, and A6), each showing a different geographic distribution. Pattern A was isolated in Colombia, Guatemala, Mexico, and Brazil, while subtype A5 circulated in Argentina, Uruguay and Mexico. These subtypes have been disseminating in the region at least since 1994. Although, in Colombia the pattern A frequency had its highest peak in 1995, it is still present until today. Interestingly, none of the three Uruguayan isolates from 1988 and 1989 belonged to the predominant subtypes or had the predominant ET1. The similarity between pattern A and the two other most common subtypes, A5 and A6, which are closely related, was higher than 92%. Subtypes A11 and A13, possibly related to pattern A, showed less than 85% similarity (Fig. 3).

Correlation between PFGE and MLEE results was 100% with respect to the two most common PFGE subtypes (A and A5), but a lower correlation was observed among other PFGE types and ETs. One possible explanation is that the two methods detect different genetic events. PFGE identifies changes in restriction sites, which, according to Hall and Duke (12), would be due to DNA insertion or deletion arrangements of mobile elements, instead of point mutations. On the other hand, MLEE analyzes the allelic variation of enzymatic loci, which have a low mutation rate (18). Spratt et al. analyzed three serotype 5 Colombian isolates by multilocus sequence typing (MLST) and reported identical profiles for two isolates while the third isolate showed a single locus variant of the clone (personal communication). Additionally, one Uruguayan serotype 5 isolate had a single locus variant with the clone's profile but at a different locus from the Colombian isolate (http://www.MLST.net). MLST provides a highly discriminating typing method to analyze closely related genetic population and could be useful to identify differences between the Latin-American serotype 5 PFGE subtypes and ETs.

The close genetic relatedness between *S. pneumoniae* serotype 5 isolates suggests two possible explanations. The first is that this serotype is infrequently isolated from healthy carriers (36) and thus lacks the opportunities for genetic exchange with its own or other related species (20). In Latin America and Israel, serotypes 5 and 1 are some of the most common causes of invasive disease, but they are rarely isolated from the nasopharynges of healthy children (26, 27). A second possibility is that the clone may have been recently established and there has not been enough time for differentiation or dissemination.

The presence of a 340-kb DNA band from Colombian serotype 5 isolates has been associated with high resistance to tetracycline and chloramphenicol (19, 33). Our findings showed that all the isolates with this *Sma*I DNA fragment were resistant to tetracycline and 71.4% were resistant to chloramphenicol, showing an indirect correlation between the presence of the 340-kb band and resistance to chloramphenicol and tetracycline among Latin American serotype 5 isolates. These results suggest that there could be a different insertion site of the mobile elements along the chromosomal DNA. Most tetracycline- and chloramphenicol-resistant isolates shared the A pattern, suggesting clonal dissemination, while the presence of *tetM* and *cat* genes among other PFGE subtypes suggests horizontal transfer or differentiation events that occurred after the common lineage became established in the region. It is also important to take into account the observation that antibiotic use differs among Latin American countries (16).

It is important to point out that serotype 5 has not been included in the heptavalent conjugate vaccine. The formulation was made based on studies in North America, where serotype 5 is not as frequent as in Latin America or Asia (2, 13, 14, 30). Thus, it would be beneficial if, when formulating a new vaccine, the geographic distribution and the serotypes prevalent worldwide are considered.

The results of this study provide strong support for a unique genetic origin of the Latin American serotype 5 invasive *S. pneumoniae* isolates. It is important to perform molecular studies with serotype 5 isolates from other countries to analyze relatedness. Additionally, continuing surveillance to explore predominant clones would be helpful to monitor the influence of selective pressure when conjugate vaccines are introduced into the Latin American population (2).

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