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Epidemic Typhoid in Chile: Analysis by Molecular and Conventional Methods of *Salmonella typhi* Strain Diversity in Epidemic (1977 and 1981) and Nonepidemic (1990) Years

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From 1977 to 1986, Chile experienced an important typhoid fever epidemic, despite statistics that indicated apparently improving levels of sanitation of drinking water and sewage disposal. The lack of antibiotic resistance among the *Salmonella typhi* strains isolated during this period, the mild clinical presentation of the disease, and the initially low level of efficacy of the *S. typhi* Ty21a vaccine in the population exposed to the epidemic suggested that this epidemic might have resulted from the dissemination of *S. typhi* strains with unique characteristics. To investigate this hypothesis, we used conventional methods (bacteriophage typing and biotyping) and molecular methods (restriction fragment length polymorphism analysis, ribotyping, IS*200* typing, and PCR amplification of the *fliC*-d gene) to study a population of 149 *S. typhi* isolates during 1977, 1981, and 1990, the years that included periods with low (when the disease was endemic) and high (when the disease was epidemic) morbidities. Our results indicate that these *S. typhi* isolates in Chile represent a number of highly diverse variants of the clone of *S. typhi* with a worldwide distribution described by Selander et al. (R. K. Selander, P. Beltran, N. H. Smith, R. Helmuth, F. A. Rubin, D. J. Kopecko, K. Ferris, B. D. Tall, A. Cravioto, and J. M. Musser, Infect. Immun. 58:2262–2275, 1990). For example, we detected 26 *Pst*I and 10 *Cla*I ribotypes among 47 and 16 *S. typhi* strains belonging to this clone, respectively. These results suggest that the Chilean epidemic was probably produced by multiple sources of infection because of deficient sanitary conditions. These findings illustrate the usefulness of molecular methods for characterizing the potential causes of the typhoid epidemics and the possible routes of transmission of *S. typhi* strains in typhoid epidemics.

A sudden, major outbreak of typhoid fever in Chile began in 1977 and lasted until 1986. The affected area was mainly the urban capital of Santiago and its neighboring regions (18, 28). The morbidity rate rose rapidly from an average of 57.6 per 100,000 population between 1949 and 1976 to 120.8 per 100,000 population in 1978 (18, 28, 29) (Fig. 1). Epidemiological studies indirectly incriminated food obtained from street vendors (5, 19) and contaminated water used to irrigate vegetables (26) as potential sources of infection with *Salmonella typhi* in Chile. However, those and other studies failed to identify definitive factors that could explain the magnitude and sudden onset of the typhoid outbreak from 1977 to 1986 (18, 26), since it was not possible to detect any potential abrupt failures in sanitation or changes in the susceptibility of the host population or in the characteristics of *S. typhi* strains (5, 18, 19, 26, 29). For example, in 1978 government statistics indicated that 99% of the urban population in Chile had access to adequately treated drinking water (18).

Multilocus enzyme electrophoresis analysis of *S. typhi* strains isolated worldwide indicate that they belong to two clones, one with a worldwide distribution and the other found only in West Africa (27). Methods such as bacteriophage typing and ribotyping are able to discriminate among different isolates belonging to the same *S. typhi* clone (3, 22, 23) and have been used as epidemiological tools to track the sources of *S. typhi* infection and to study the extent of variation in isolates belonging to the same clone (3, 23). With the goal of investigating whether the epidemic of typhoid fever in Chile from 1977 to 1986, one of the largest, most protracted, and best documented in the Western Hemisphere in the last 50 years, was produced either by *S. typhi* strains with unique characteristics or by undetected failures in sanitation, we studied *S. typhi* strains isolated in Chile by conventional microbiological assays and newly developed molecular epidemiological tools.

MATERIALS AND METHODS

Bacterial isolates. *S. typhi* isolates were obtained from stock cultures kept in soft agar at the National Reference Laboratory for Enterobacteria, Instituto de Salud Publica, Santiago, Chile, and from the pediatric Hospital Roberto del Rio, Santiago. The *S. typhi* strains selected for the study included 22 strains obtained at the beginning of the epidemic (1977; Fig. 1), 59 strains obtained during the middle of the epidemic (1981), and 68 strains obtained at the end of the epidemic (1990) (Fig. 1), for a total of 149 *S. typhi* isolates. One isolate was included twice but was counted as one isolate in every classic and molecular typing test that was performed. Inclusion of isolates from the same patient was avoided by obtaining and recording demographic and microbiological information related to the clinical sources of the *S. typhi* strains. One hundred twenty-two isolates (82%) were obtained from Santiago and its surroundings, the region of Chile that reported most of the cases of typhoid fever during the epidemic. All isolates were obtained from the following clinical samples: blood (80%), common bile duct or gallbladder bile (15%), stool (4%), and bone marrow aspirates (1%). The age distribution and the mean age of the patients who were the sources of the strains were not significantly different from those of the population known to be affected by most of the cases of typhoid in Chile (geometric mean ages, 14.1 and 15.1 years, respectively; $P > 0.2$ by the Fisher two-sided t test) (24).

Microbiological assays. Strain identification was confirmed by standard biochemical assays (7) with group D1 antisera (Difco, Detroit, Mich.), flagellar d antisera (Difco), and antigen Vi antisera (Difco) (7). Bacteriophage typing was performed at the Instituto de Salud Publica in Chile by using well described standard techniques (8, 16). A biotyping system was designed to evaluate the

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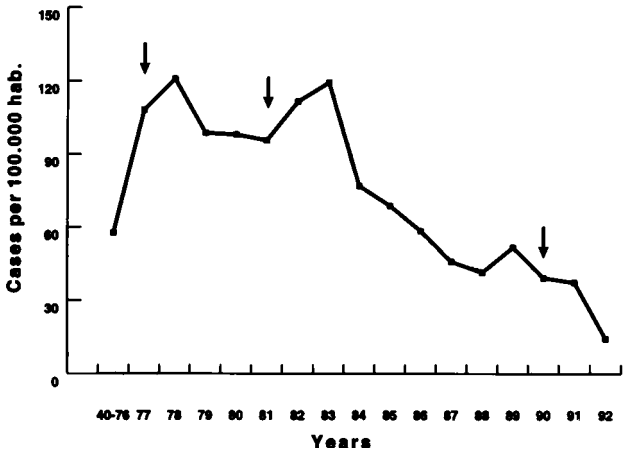

FIG. 1. Morbidity rate from typhoid fever in Chile. Arrows indicate the years that *S. typhi* isolates were obtained for the present study. Morbidity data were obtained from Anuario de Enfermedades de Notificacion Obligatoria, 1992, Ministry of Health, Santiago, Chile.

ability of the strains to ferment five different sugars (dulcitol, cellobiose, D-xylose, arabinose, and glycerol) and decarboxylate L-arginine (12, 17). Nutritional requirements were investigated to identify prototrophic and auxotrophic strains (33). The presence of type 1 fimbriae was indirectly evaluated by hemagglutination of human and animal erythrocytes (2). The susceptibilities of the strains to chloramphenicol, ampicillin, and tetracycline were determined with Luria-Bertani agar plates containing 30, 50, or 12 μg of chloramphenicol, ampicillin, or tetracycline per ml, respectively (12, 16). Enterobactin and aerobactin production was studied by cross-feeding assays with chelator-deficient mutant strains of *Escherichia coli* in iron-poor medium (9, 10). The results of these assays were analyzed by the Yates' continuity correction test or Fisher's exact test by using EPI-INFO 5.Ola software (Centers for Disease Control and Prevention, Atlanta, Ga.) or by the significance test for comparing two proportions with continuity correction. The concentration (*l*) of patterns was calculated as described previously (13, 30). This method permits one to determine the discriminatory power (*D*), which measures the probability that two strains sampled randomly from the population would fall into different types, and it is the reciprocal of the concentration; $D = 1/l$ (13, 30). The highest value ($D = 1$) indicates maximal diversity.

Molecular characterization. Because preliminary data indicated that molecular biology-based methods detected a wide diversity of strains among *S. typhi* isolates, representative *S. typhi* strains from the three groups previously analyzed by the bacteriophage typing and biotyping procedures described above were chosen for use in molecular studies. They included 16 strains from 1977, 13 strains from 1981, and 18 strains from 1990 for *Pst*I ribotyping. The 13 *S. typhi* strains obtained in 1981 and the 18 strains obtained in 1990 were also used in *Eco*RI and IS*200* ribotyping assays. From this group, 3 strains obtained in 1981 and 13 strains obtained in 1990 were also analyzed by *Cla*I ribotyping. Seventeen of the 47 *S. typhi* strains were used in the hybridization experiments with porin and iron uptake DNA probes and for the amplification of the *fliC*-d gene. Thus, this group of *S. typhi* included strains from epidemic and nonepidemic years and isolates with similar or different bacteriophage types, biotypes, and nutritional requirements. Genomic DNA was isolated by using guanidine chloride (Sigma) (4). DNA samples containing 1 to 2 μg were digested with either *Pst*I, *Cla*I, or *Eco*RI (New England Biolabs, Beverly, Mass.), separated by agarose gel electrophoresis (0.7%) at 20 V for 16 to 18 h, transferred to a nylon membrane (Zeta Probe; Bio-Rad, Richmond, Calif.), and hybridized with either a 2.15-kb *Eco*RI DNA fragment containing part of the rDNA operon genes (6), a 692-bp PCR-amplified DNA fragment spanning most of the insertion sequence IS*200* (11, 25), or a 1.9-kb *Hpa*I DNA fragment used as an enterobactin synthesis gene probe (9, 16). Other DNA probes included a 1.9-kb *Ava*I DNA fragment as an aerobactin receptor gene probe, a 2.2-kb *Pvu*II DNA fragment as an aerobactin synthesis gene probe, and a 800-bp *Pst*I-*Hpa*I DNA fragment as a 36-kDa porin gene probe (1, 9). DNA probes were labeled and detected by using the Genius I DNA labeling and detection kit (Boehringer Mannheim). Prehybridization and hybridization procedures used formamide-sodium dodecyl sulfate solutions as described in the Zeta Probe instruction manual. PCR amplification of the flagellin *fliC*-d gene was performed as reported previously (20). In the analysis of ribotypes and IS*200* types, we decided that one band difference represented a different *S. typhi* variant because we found that strains in this collection diverging by one band also differed in the expression of other properties. Cluster analysis of *Pst*I ribotypes was performed by the unweighed pair group method with arithmetic averages (31).

RESULTS

Microbiological assays. Bacteriophage types E_1 ($n = 50$; 36%) and 46 ($n = 43$; 31%) were the most prevalent types identified in the sample of strains analyzed and comprised 67% of the 138 strains analyzed by bacteriophage typing. The frequencies of the two main bacteriophage types remained unchanged among the three groups of *S. typhi* strains assayed, regardless of the year that the strains were isolated. Thirteen biotypes (biotypes A to M) were identified among the 149 *S. typhi* strains, with biotype A ($n = 102$; 68.5%) and biotype B ($n = 19$; 12.8%) predominating and accounting for more than 80% of the observed biotypes. Both of these biotypes were characterized by the ability to decarboxylate L-arginine and to utilize D-xylose and by the ability to utilize glycerol for biotype A, and by the ability to utilize cellobiose for biotype B. Eight strains (5.4%) were biotype C and resembled biotype A strains but were unable to utilize glycerol. Biotype D ($n = 5$) and biotype E ($n = 4$) strains were the next most frequently observed strains 3.4 and 2.7%, respectively). Biotype D strains were similar to biotype A strains except that they did not utilize glycerol and were able to ferment arabinose. Biotype E strains resembled biotype A strains but did not decarboxylate L-arginine. Biotype G strains ($n = 3$), biotype F strains ($n = 2$), and biotype H through M strains ($n = 1$ each) comprised the rest of the observed profiles and represented 7.2% of the total.

One hundred thirty-three of 149 strains (90%) were able to grow in a sulfur-containing minimal medium supplemented with tryptophan. Fourteen (9%) *S. typhi* strains were able to grow in this minimal media in the absence of tryptophan, and one strain did not grow in any of these media ($n = 1$; 1%). Prototrophic *S. typhi* strains could not be identified among the strains belonging to phage type E_1 ($P < 0.005$ by Fisher's two-tailed test). All of the isolates were able to synthesize enterobactin but not aerobactin in cross-feeding assays. Only one strain displayed resistance to chloramphenicol after 48 h, but the presence of plasmid-mediated resistance could not be demonstrated by conjugation or by physical methods (data not shown).

The presence of type 1 fimbriae was indirectly demonstrated in 125 of 128 *S. typhi* strains (97.6%) analyzed by mannose-sensitive hemagglutination. This property was not studied for the 22 *S. typhi* isolates obtained at the beginning of the epidemic. None of the 128 *S. typhi* strains expressed mannose-resistant hemagglutinins.

The calculated values for the discriminatory powers of the different typing systems applied in the study are represented in Table 1. Bacteriophage typing and biotyping were associated with concentrations of 0.23 and 0.50, respectively, and discriminatory powers of 0.77 and 0.50, respectively.

Molecular characterization. Chromosomal DNA samples of previously bacteriophage typed and biotyped *S. typhi* isolates digested with *Pst*I, separated in agarose gels, and hybridized with a probe made up of the gene coding for rRNA (rDNA) showed a total of 20 different bands (range, 5 to 8 bands per strain; average, 5.85 bands), with molecular sizes ranging from 7 to >30 kb. The 20 different DNA bands identified were ordered numerically, and a matrix of present or absent bands was constructed for every ribotype identified to facilitate comparisons (10a). In this manner 26 *Pst*I ribotypes, designated ribotypes A to Z, could be identified among 47 independent *S. typhi* isolates analyzed in the three groups of *S. typhi* strains tested (Fig. 2; Table 2). The reproducibility of the assay was 100% among isolates tested twice ($n = 9$) or three times ($n = 8$), and all of the tested strains were typeable. Ribotype F was the most frequently identified pattern ($n = 11$) and included

TABLE 1. Discriminatory powers of different typing systems to differentiate among Chilean *S. typhi* isolates

No. of *S. typhi* strains	Typing system	*D*[a]
138	Phage typing	0.77
149	Biotyping	0.50
47	*Pst*I ribotyping	0.93
16	*Pst*I ribotyping of *S. typhi* strains from 1977	0.96
13	*Pst*I ribotyping of *S. typhi* strains from 1981	0.91
18	*Pst*I ribotyping of *S. typhi* strains from 1990	0.92
16	*Cla*I ribotyping	0.93
31	IS*200* typing	0.67

[a] *D*, discriminatory power.

the two strains isolated from the same patient (lanes p and q in Fig. 2C; Table 2). Ribotype K ($n = 6$) was the next most frequently observed pattern; this was followed by ribotype N ($n = 3$). Ribotypes H, J, L, and M were represented by two strains each, and 19 other *Pst*I ribotypes were represented by only one strain each (Table 2).

The most frequently observed ribotypes were distributed homogeneously among the three samples of strains obtained in different years (1977, 1981, and 1990). *Pst*I ribotype F was observed in three strains in 1977 (18.7%), in three strains in 1981 (23.0%; including two isolates from the same patient), and in five strains in 1990 (27.7%); *Pst*I ribotype K was observed in two strains in 1977 (12.5%), in three strains in 1981 (21.4%), and in one strain in 1990 (5.5%). Finally, *Pst*I ribotype N was observed only once in each group of strains (6.2, 7.6, and 5.5% for strains obtained in 1977, 1981, and 1990, respectively). The differences in the proportions for *Pst*I ribotypes F and K in different years were not significant. The distribution of *Pst*I ribotypes at the beginning of the typhoid fever epidemic (1977), during the middle of the epidemic (1981), and after the incidence of disease declined to values before the epidemic (1990) showed a high level of diversity (high *D*; 0.96, 0.91, and 0.92, respectively; Table 1 and Fig. 2).

The predominant bacteriophage types among *Pst*I ribotype F or K strains were similar to those observed for the whole group (E_1 and 46; Table 2). *Pst*I ribotype F strains were associated with biotype A ($n = 8$; 73%), biotype C ($n = 2$; 18%), and biotype D ($n = 1$; 9%) strains (Table 2), and their distributions did not differ from the proportions of these biotypes observed among strains in the whole sample. *Pst*I ribotype K was associated with biotype A ($n = 3$; 50%), B ($n = 1$; 17%), and D ($n = 2$; 33%) strains (Table 2), and their frequencies did not differ significantly from those observed among strains in the whole group except for the high proportion of biotype D strains observed in ribotype K (33%), which differed from the low frequency of biotype D described for among strains in the whole group (3.4%; $P < 0.005$). According to these results, *Pst*I ribotype F strains could be further divided by biotyping or bacteriophage typing and could be grouped into several subtypes: bacteriophage type E_1, biotype A strains ($n = 3$), bacteriophage type 46, biotype A strains ($n = 3$), and bacteriophage type 46, biotype C strains ($n = 2$) (Table 2). These three predominant groups accounted for 72% of the 11 strains belonging to this rDNA type. The two strains isolated from the same patient were identified as belonging to the *Pst*I ribotype F, biotype C, phage type 46 subgroup. In contrast, all *Pst*I ribotype K strains could be subtyped into different groups by combining their biotype and phage type profiles (Table 2). Prototrophic *S. typhi* strains were found in 1977 and 1990 and in a group of strains in 1981 that were not analyzed by molecular biology-based methods (data not shown).

Cluster analysis of the observed *Pst*I ribotypes and their bands performed by an average linkage method (Fig. 3) indicated that the ribotypes identified in the years when the disease was epidemic (1977 and 1988) ($n = 29$) and in the year when the disease was endemic (1990) ($n = 18$) were philogenetically distant. For example, the two most frequently observed *Pst*I ribotypes, F and K, differed in 6.8 of 20 possible matching bands, with an average relative band mismatch of 0.34. Cluster BUFCDL (at the top of Fig. 3) was associated with five of the seven (71%) prototrophic strains included in the molecular characterization (Table 2) and did not contain any strain belonging to biotype B, although these findings did not reach statistical significance. Similar analysis with bacteriophage types, other biotypes, and IS*200* types (see below) did not reveal any specific grouping along the dendrogram.

*Cla*I-digested *S. typhi* chromosomal DNA samples hybridized with the same 2.15-kb *Eco*RI fragment probe used for *Pst*I

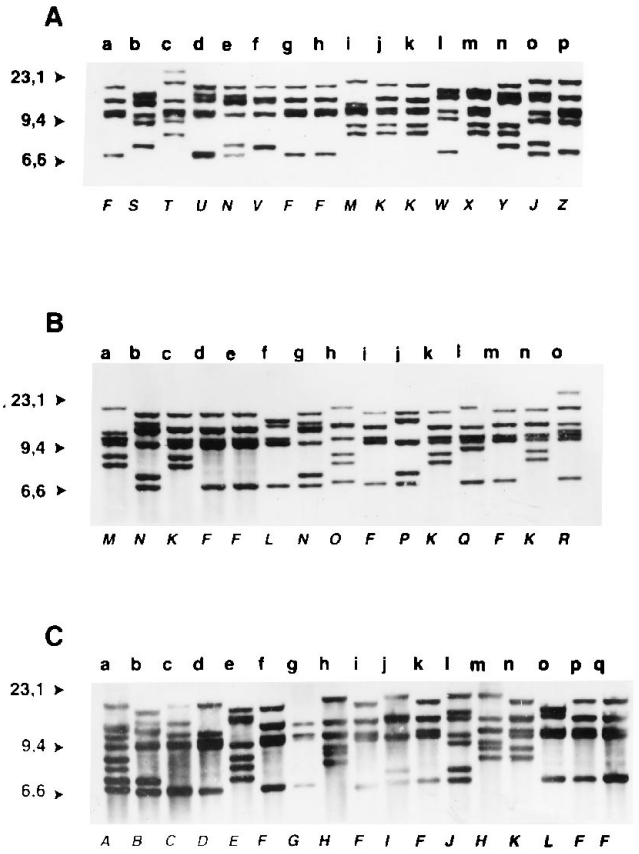

FIG. 2. *Pst*I ribotyping of Chilean *S. typhi* isolates. (A) *S. typhi* isolates obtained in 1977. Lanes: a, strain 3741; b, 3744; c, 3746; d, 3748; e, 3753; f, 3859; g, 3861; h, 3902; i, 3865; j, 3866; k, 3867; l, 3868; m, 3883; n, 3896; o, 3901; and p, 3952. (B) Lanes a to e, *S. typhi* isolates obtained in 1990; lanes f to o, *S. typhi* isolates obtained in 1981. Lanes: strain a, 36; b, 38; c, 42; d, 65; e, 66; f, 73; g, 80; h, 81; i, 87; j, 88; k, 90; l, 93; m, 97; n, 109; o, 112. (C) Lanes a to m, *S. typhi* isolates obtained in 1990; lanes n to q, *S. typhi* isolates obtained in 1981. Lanes: a, strain 5; b, 9; c, 10; d, 41; e, 13; f, 16; g, 22; h, 32; i, 47; j, 53; k, 57; l, 63; m, 72; n, 74; o, 78; p, 118; q, 119. Letters at the top of each lane correspond to different *S. typhi* strains. The ribotype denomination is indicated by capital, italic letters at the bottom of each lane. Numbers at the left are molecular size standards (in kilobases).

TABLE 2. Distribution of *Pst*I ribotypes, phage types, nutritional requirements, biotypes, and IS*200* types among *S. typhi* strains isolated in 1977, 1981, and 1990

Year	Strain	Ribotype	Phage type	Prototrophic or auxotrophic[a]	Biotype	IS*200* type
1977	3741	F	E_1	AUX	A	ND[b]
1977	3861	F	46	AUX	A	ND
1977	3902	F	46	AUX	A	ND
1977	3901	J	E_1	AUX	B	ND
1977	3866	K	46	AUX	D	ND
1977	3867	K	46	AUX	A	ND
1977	3865	M	E_1	AUX	A	ND
1977	3753	N	46	AUX	B	ND
1977	3744	S	A	AUX	A	ND
1977	3746	T	NT[c]	AUX	L	ND
1977	3748	U	NT	PRO	A	ND
1977	3859	V	46	AUX	B	ND
1977	3868	W	46	AUX	A	ND
1977	3883	X	M1	AUX	A	ND
1977	3896	Y	46	AUX	A	ND
1977	3952	Z	E_1	AUX	B	ND
1981	87	F	E_1	AUX	A	D
1981	97	F	Vi deg.[e]	AUX	A	C
1981	118/119[d]	F	46	AUX	C	C
1981	74	K	E_1	AUX	B	D
1981	90	K	E_1	AUX	A	D
1981	109	K	Forma	AUX	A	D
1981	73	L	46	AUX	A	C
1981	78	L	W	AUX	A	D
1981	80	N	F_1	AUX	A	D
1981	81	O	46	AUX	C	C
1981	88	P	46	AUX	A	C
1981	93	Q	46	AUX	A	C
1981	112	R	51	AUX	B	G
1990	5	A	I+IV	PRO	A	A
1990	9	B	E_1	AUX	A	B
1990	10	C	46	PRO	A	C
1990	41	D	46	PRO	A	C
1990	13	E	ND	AUX	C	D
1990	16	F	ND[e]	AUX	D	D
1990	47	F	I+IV	PRO	A	C
1990	57	F	E_1	AUX	A	D
1990	65	F	46	PRO	C	C
1990	66	F	46	AUX	A	C
1990	22	G	A	AUX	A	E
1990	32	H	E_1	AUX	A	D
1990	72	H	E_1	AUX	C	D
1990	53	I	46	AUX	A	F
1990	63	J	F_1	PRO	K	D
1990	42	K	E_1	AUX	D	C
1990	36	M	E_1	AUX	E	D
1990	38	N	E_1	AUX	A	D

[a] PRO, prototrophic; AUX, auxotrophic.
[b] ND, not done.
[c] NT, nontypeable.
[d] 118/119, isolates from the same patient and counted as one.
[e] Vi deg., degraded (12).

ribotyping and identified 10 ribotypes among a subset of 16 isolates obtained in 1981 and 1990 (data not shown). Strains belonging to ribotype F and H by *Pst*I ribotyping clustered in similar groups, and two strains assigned to different ribotypes by *Pst*I ribotyping (lanes c and d in Fig. 2B) were clustered in the same group by *Cla*I ribotyping. *D* calculated for *Cla*I ribotype analysis was similar to that obtained by *Pst*I ribotyping (0.93; Table 1). *Eco*RI ribotype analysis revealed only two patterns among 31 strains obtained in 1981 and 1990 (data not shown), and in none of the strains was the pattern associated with the West African *S. typhi* clone (Tp2 clone) described by Selander et al. (27).

IS*200* hybridization analysis of *S. typhi* chromosomal DNA digested with *Pst*I resulted in seven different patterns (patterns A through G) among 31 *S. typhi* isolates obtained in 1981 and 1990, with hybridization patterns C ($n = 12$) and D ($n = 14$) being predominant (Fig. 4; Table 2). Other patterns were represented by one strain each. *D* obtained for IS*200* typing was 0.67. Ten *S. typhi* bacteriophage type 46 strains hybridized with the IS*200* probe, and nine of them were associated with IS*200*

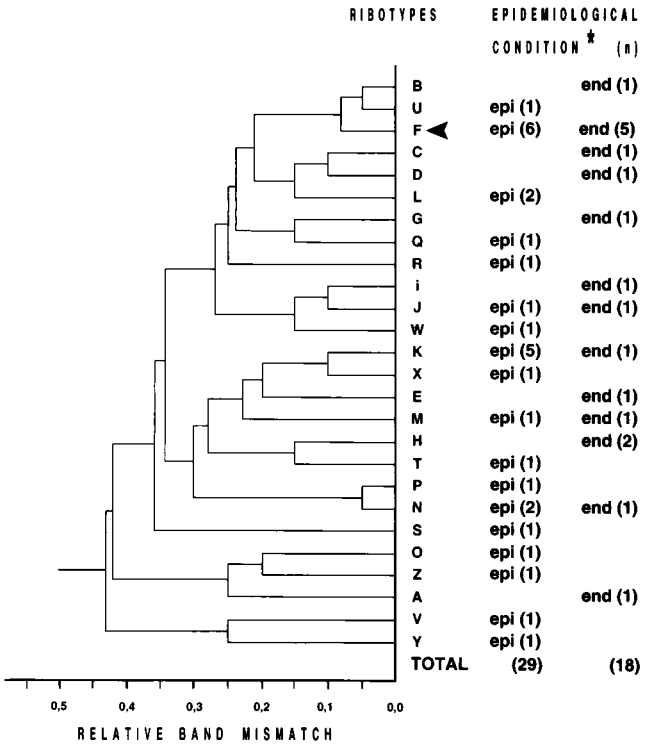

FIG. 3. Cluster analysis of *Pst*I ribotypes identified in 47 independent *S. typhi* isolates isolated in an epidemic or endemic situation in Chile. One band mismatch is approximately equivalent to 0.05 units. The arrowhead indicates the most commonly identified ribotype. *, epi and end, epidemic and endemic, respectively.

type C ($P < 0.0005$ by Fisher's two-tailed test). Complementary studies revealed that all phage type 46 *S. typhi* strains belonging to biotype C showed IS*200* pattern C ($n = 6$; data for 4 strains are included in Table 2).

Chromosomal DNA samples digested with the *Pst*I, *Cla*I, or *Eco*RI restriction enzyme produced similar patterns of digestion by agarose gel electrophoresis (data not shown). A subset of chromosomal DNA samples from 16 *S. typhi* strains obtained in 1981 and 1990 and digested with *Pst*I demonstrated a conserved band hybridizing to a 1.9-kb *Hpa*I fragment probe encoding part of the enterobactin synthesis gene operon and to an 800-bp *Pst*I-*Hpa*I DNA fragment encoding part of the 36-kDa porin gene. *Pst*I-digested chromosomal DNAs of the same *S. typhi* strains did not contain DNA sequences homologous to a 1.9-kb *Ava*I fragment probe encoding part of the aerobactin synthesis gene or a 2.2-kb *Pvu*II DNA fragment probe encoding for the aerobactin receptor. Amplification of the *fliC*-d gene in these strains by PCR also identified an expected 1.6-kb fragment (data not shown).

DISCUSSION

Our results demonstrate two predominant bacteriophage types (types E_1 and 46) among the *S. typhi* isolates studied. This could suggest the dissemination in Chile of restricted and unique *S. typhi* strains, but an important diversity of the *S. typhi* strains in this sample may be indicated by the observed low *D* of bacteriophage typing (0.77) and, conversely, by the low concentration of bacteriophage types (0.23) (13, 30). This result, however, does not rule out the emergence and dissemination of *S. typhi* strains with epidemic potential because of the potential acquisition of plasmids and lysogenic conversion among strains of different bacteriophage types. Molecular characterization of these *S. typhi* isolates showed the high *D* (or diversity) of the different ribotype patterns present in Chilean *S. typhi* strains in 1977 (0.96), 1981 (0.91), and 1990 (0.92) and indicated that different ribotypes of *S. typhi* were circulating in Santiago during the years when the disease was epidemic (1977 and 1981) and in the year when the disease was endemic (1990). Cluster analysis of different ribotypes identified among epidemic and endemic *S. typhi* strains demonstrated that the *Pst*I ribotypes associated with both the epidemic and the endemic are phylogenetically distant (Fig. 3) and that the differences observed were not due to isolated band mismatching. Moreover, diversity is also demonstrated in these strains by the different patterns observed by *Cla*I ribotyping and IS*200* DNA hybridization studies. These results reasonably exclude the dissemination of a unique *S. typhi* variant with specific virulence properties as being responsible for the epidemic and also rule out the possibility that the initial low level of effectiveness of the *S. typhi* Ty21a vaccine in Chile could be explained by infections produced by *S. typhi* strains markedly different from the strain in live attenuated vaccine (14, 28, 29). Dissemination of transferable genetic elements encoding for virulence factors in *S. typhi* strains could also explain an outbreak produced by bacteria with widely diverse chromosomally encoded properties. However, such a possibility is highly improbable because of the scarcity of plasmids reported among Chilean *S. typhi* strains (8 to 17%) (15, 21; the present work) and the absence from these strains of phenotypic properties usually encoded by extrachromosomal elements, such as multiple antibiotic resistance and colicin production (data not shown).

This analysis performed by conventional and molecular biology-based methods appears to indicate that *S. typhi* strains in Chile belong to the clone with a worldwide distribution described by Selander et al. (27). Their analysis of 334 isolates of *S. typhi* included 73 isolates from Chile (27). For example, *Eco*RI-digested *S. typhi* genomic DNA samples that hybridized with an rDNA probe (31 strains) did not reveal the specific ribotype associated with the alternative West African *S. typhi*

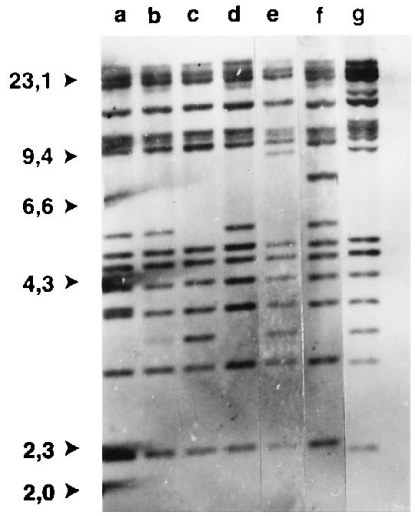

FIG. 4. Representative patterns among 31 Chilean *S. typhi* isolates by *Pst*I-IS*200* typing. Lanes: a, IS*200* type A ($n = 1$); b, IS*200* type B ($n = 1$); c, IS*200* type C ($n = 12$); d, IS*200* type D ($n = 14$); e, IS*200* type E ($n = 1$); f, IS*200* type F ($n = 1$); g, IS*200* type G ($n = 1$). Numbers at the left are molecular size standards (in kilobases). Pattern B has a band of approximately 3.6 kb that is not well reproduced in the figure and that is not present in pattern D.

clone also described by Selander et al. (27) and confirm their previous observation that Chilean *S. typhi* strains belong to the clone that is predominant worldwide (27). Also, PCR amplification of the *fliC*-d gene revealed identical flagellin gene amplicons among these strains and did not show evidence of a smaller amplified fragment found in strains containing the *fliC*-j gene that corresponds to an internal deletion of the *fliC*-d gene and that has been found only among strains isolated in Indonesia (20). The present study also extended previous observations suggesting that in *S. typhi* strains, which has a restricted host range and which is able to infect humans only, there appears to be little variation in the properties potentially linked to virulence, such as iron uptake and flagella, in contrast to extensive variations in ribotypes, suggesting a link between observed virulence properties and host range (9, 20, 27).

Previous studies performed among *S. typhi* isolates in Malaysia (23) have also found several *Pst*I ribotypes in endemic and epidemic *S. typhi* strains, although with a lower *D* (0.83 versus 0.93 in the present study). Some of those ribotypes are similar to a few reported here (ribotypes F, K, and L), although they are not predominant ribotypes. Another study performed by *Cla*I ribotyping of *S. typhi* strains from an Italian epidemic (22) demonstrated that ribotype diversity was low among strains belonging to the same bacteriophage type associated with a well-identified source of infection (22). Thus, ribotyping of *S. typhi* isolates from different continents and performed with different enzymes suggests that different *S. typhi* variants are being disseminated in the environment under conditions of disease endemics and that typhoid fever outbreaks can be associated with a few predominant ribotypes of *S. typhi* (22) or with many ribotypes of *S. typhi* (23; the present study). The paucity of *S. typhi* ribotypes could derive from one or a few sources of infection in epidemiological situations with a low background of infections caused by *S. typhi*, as happened in southern Italy, while the variety of ribotypes found in Malaysia and Chile could result from multiple sources of infections in situations of high endemicity of typhoid fever (18, 23, 29). Although bias could have been introduced in the present study because of a lack of a random selection of samples, the age distribution of patients, the geometric mean of their age, and the bacteriophage type distributions of the strains are in agreement with those of previous reports on typhoid fever in Chile (18, 24) and validate the present sample as being representative.

In the epidemic described here, the information generated by the analysis of *S. typhi* strains with molecular epidemiology tools could have been used to abort its protracted course. The wide genetic variation in *S. typhi* strains etiologically involved in the epidemic indicated that it was probably the result of failures in sanitation, notwithstanding official government statistics displaying presumably satisfactory sanitation indexes (18, 19). This hypothesis is supported by the fact that in 1991, after the emergence of cholera in Chile, the incidence of typhoid fever declined drastically after the government implemented sanitary and education policies to forestall the dissemination of cholera in the country (Fig. 1). These and recently reported (25, 32) findings suggest that molecular biology-based tools may play an increasingly important role in the characterization of *S. typhi* isolates and in the identification of potential routes of infection in areas where typhoid is endemic and epidemic. The present study also illustrates the complementarity between molecular biology-based methods such as ribotyping and classical microbiological typing systems such as biotyping and bacteriophage typing that permit the subtyping of predominant rDNA types.

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