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Evolution of Drug Resistance in *Salmonella panama* Isolates in Chile

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In a search for *Salmonella* isolates in the environment in Chile in 1975, drug-susceptible strains of *Salmonella panama* were recovered for the first time from river water and vegetables in the vicinity of Santiago. Two to 3 years later, antibiotic-resistant *S. panama* began to appear in a variety of sources (meat, animals, vegetables, etc.), giving rise to a human epidemic that involved the entire nation. Of 139 clinical isolates studied, 7 were drug susceptible, 11 were resistant only to nitrofurans, and 3 were streptomycin, spectinomycin, and nitrofuran resistant; none of these 21 isolates harbored plasmid DNA. Most isolates (n = 107) were resistant to nitrofurans (chromosomal) and to streptomycin, spectinomycin, sulfonamides, tetracycline, and mercuric and tellurite salts; this multidrug resistance was encoded on a 218-kb plasmid classified in a number of strains as being in the $IncHI_2$ group. From 1982 to 1993, 11 isolates acquired an additional self-transferable plasmid coding for resistance to any one of ampicillin (61 kb), ampicillin and trimethoprim (65 kb), ampicillin, trimethoprim, streptomycin, and sulfonamides (71 kb), ampicillin, gentamicin, kanamycin, and tetracycline (120 kb), or a nontransferable plasmid of ~6 kb encoding resistance to ampicillin or kanamycin. With the exception of ampicillin or ampicillin and trimethoprim resistance, *S. panama* isolates from foodstuffs, mainly pork meat products, and animals had resistance patterns that were the same as those found in clinical specimens. Remarkably, strains from goats and goat cheese and from shellfish isolated in particular rural regions were either drug susceptible or resistant only to streptomycin-spectinomycin encoded on a mobile genetic element and to nitrofurans. The report describes the arrival of a susceptible *S. panama* strain, its spread all over the country, and the evolution of progressively complex resistance patterns.

Salmonella panama is a human pathogen that has caused outbreaks in several European countries (2, 4, 6, 19). In Chile this serotype had not been reported prior to 1975, when its presence was detected for the first time in Santiago (36); in the following years it spread all over our territory reaching 2,200 km southward (Punta Arenas) and 1,700 km up to the northern border (Arica) (Fig. 1). During the period from 1975 to 1993, *S. panama* was isolated from many foods, animals, and water and from patients with clinical cases of infection, mainly diarrheal syndrome in infants under 15 months old, with some cases of septicemia and meningitis. The 10-year (1978 to 1987) average number of clinical strains received at the Instituto de Salud Pública de Chile per year was 29.5, while in 1982 this number rose to 63, dropping sharply afterward; in the same period, strains from food averaged 9.8 per year, while in 1982 that number rose to 38, diminishing in the following years. In 1982, several foodborne outbreaks of diverse magnitudes were caused by the consumption of goat cheese contaminated with *S. panama* (unpublished data). Although the number of clinical cases diminished subsequently, *S. panama* isolates continued to be recovered from different sources throughout the period of the present study. The number of isolates collected represents a minor proportion of the actual number of cases of infection, since only severe infections require hospital care and only the isolates causing those severe infections are submitted for bacteriological analysis; mild or subclinical *Salmonella* infections are usually not bacteriologically diagnosed (26, 34).

Resistance to antimicrobial drugs and plasmid content were studied in a number of strains isolated from different sources and locations from 1975 to 1993, and the results are described in this report. These results show the spread of this pathogen and the evolution of multiple antibiotic resistance following its introduction into Chile.

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MATERIALS AND METHODS

Strains. Three hundred five strains of *S. panama* (Table 1) were collected from different geographical locations (Fig. 1) and habitats from 1975 through 1993. Clinical strains were isolated from feces, blood, spinal fluid, urine, and wound exudate in clinical laboratories in Santiago and other cities throughout the country and were sent to the Enteric Reference Laboratory (Instituto de Salud Pública, Santiago) for bacteriological identification. Strains from food were isolated from meat products, vegetables, goat cheese, and raw shellfish by routine control in our Food Microbiology Laboratory (Instituto de Salud Pública). Strains from water were isolated in our periodical bacteriological surveys of the Mapocho River (Santiago) and in a study of the rivers around Talca, and a single strain was isolated from sewage in Antofagasta (10). Other isolates from pigs, poultry, goats, cattle, and fish meal were recovered in local laboratories in different regions of the country and were sent to our laboratory for identification. All isolates included in the present study were reidentified in our laboratory by their biochemical characteristics and antigenic formulas (17) by using specific antisera from Difco (Detroit, Mich.).

Drug resistance. Resistance to antimicrobial drugs was determined by the agar diffusion test (3) on Mueller-Hinton agar plates (all culture media used were from Difco) with Bacto Sensitivity Discs (Difco). The drugs tested were ampicillin (Ap), amikacin (Ak), chloramphenicol (Cm), carbenicillin (Cb), cephalothin (Ce), cefamandole (Cf), gentamicin (Gm), kanamycin (Km), neomycin (Nm), streptomycin (Sm), spectinomycin (Sp; Arlab, Santiago, Chile), sulfonamides (Su), tetracycline (Tc), trimethoprim (Tp), nalidixic acid (Nx), and nitrofurantoin (Fu). MICs were determined by the agar dilution method (37). Resistance to mercuric chloride (Hg) was tested as described by Smith et al. (31), and resistance to potassium tellurite (Te) was tested as described by Pohl and Thomas (25). Loss of tetracycline resistance was searched for on the agar plates described by Bochner et al. (5).

Plasmid characterization. The transferability of drug resistance was tested by mixing late-exponential-phase Penassay broth cultures of the donor strain with an appropriate recipient strain; mixtures (0.1:1 and 0.1:0.1 ml plus 1 ml of Penassay broth) were incubated in water baths for periods of 2 and 18 h, respectively, both at 28 and 37°C (33). The *Escherichia coli* strains used in the genetic experiments were described previously (9). The selection of transconjugants was made on Mueller-Hinton or MacConkey agar plates to which the appropriate drugs were added. The frequency of transfer was calculated for the donor in 2-h matings and for the recipient in overnight matings; plain MacConkey or Mueller-Hinton agar to which a single drug was added was used to

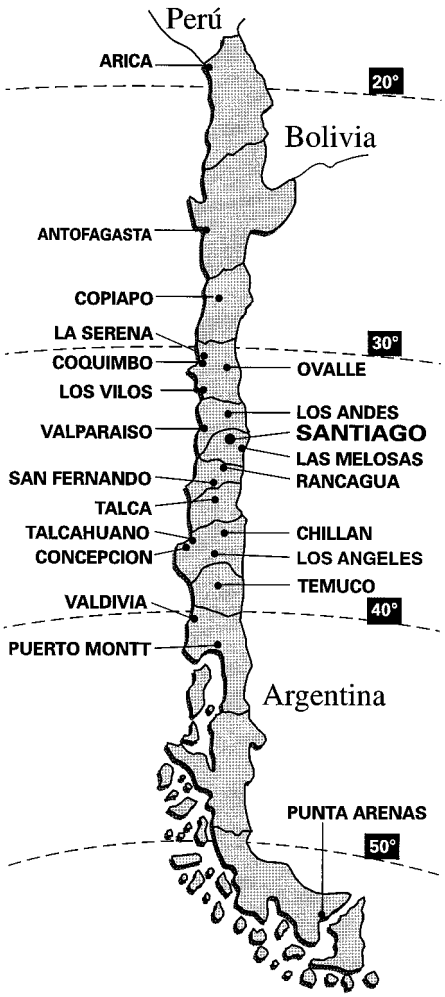

FIG. 1. Map of Chile showing the sites where *S. panama* strains were isolated.

enumerate the donor or the recipient strain (33). Colonies growing on the selective media were reisolated, biochemically diagnosed, and tested for their drug resistance patterns by the methods described by Anderson and Threlfall (1). When no transconjugants were obtained in direct transfer tests, a triparental mating system with the conjugative plasmids X or Δ was tried (1). From each new set of isolates a representative number of plasmids coding for resistance to Sm, Sp, Su, Tc, Hg, and Te were classified into incompatibility groups as described by Datta et al. (11). Plasmid DNA was extracted as described by Kado and Liu (16), with slight modifications. Electrophoresis of plasmid DNA was performed in 0.75% agarose (BDH Chemicals Ltd., Poole, England) gels in Tris-borate buffer (pH 8.2) in vertical slabs at 110 mV for 4 h. Plasmid sizes were estimated by comparing their relative mobilities (22) (mean of at least three independent determinations) with those of the following plasmids of known size: pTP116, 218 kb; pΔ, 92 kb; pS-a, 35 kb (12, 14, 15).

Nutritional requirements. Isolates with an apparently chromosomally inserted Sm-Sp resistance trait were tested for their nutritional requirements in search of a probable nutritional impairment (12, 24). This was done by seeding them onto a minimal medium used previously with *Salmonella typhi* (35). The medium is made by adding 2 ml of a 10× salt solution (KH_2PO_4, 3%; K_2HPO_4, 7%; NH_4Cl, 0.6%; $MgCl_2 \cdot 6H_2O$, 0.06%; [E. Merck AG, Darmstadt, Germany] for analysis of reagents) to 18 ml of molten agar (1.5% Noble agar made in double-distilled water) and pouring it into a petri dish containing 0.2 ml of a 2% freshly prepared $Na_2S_2O_3 \cdot 5H_2O$ solution and 0.2 ml of a 20% glucose solution.

RESULTS

Strains. In 1975, in a periodical search for *Salmonella* isolates in the environment, *S. panama* was detected in the Mapocho River, which runs across Santiago, and in vegetables irrigated with this water. Two years later it was again found in the Mapocho River, and in the next year it began to be isolated from clinical specimens and from diverse food products. In addition, a number of strains of different origins sent to us from several cities throughout the country (Fig. 1) were also identified as *S. panama* (Table 1).

Drug resistance. The first two isolates (isolated in 1975) were susceptible to all 16 drugs tested and to mercuric and tellurite salts; another 21 strains isolated later, mainly from goat cheese in a particular geographical region (Ovalle, 1982), were also susceptible. The remaining 282 strains proved to be resistant to Fu either as a single resistance marker (14 strains) or in more complex drug resistance patterns (Table 1).

Forty strains, mostly isolated from goats and goat cheese (Las Melosas, 1982) and from shellfish (Los Vilos, 1982), were resistant to Sm, Sp, and Fu but were susceptible to all other drugs tested (Table 1). The MIC of Sm was 64 to 125 μg/ml. These strains were prototrophic, indicating that the Sm^r Sp^r trait did not cause any change in their nutritional requirements.

Most strains ($n = 213$) were resistant to Sm, Sp, Su, Tc, Hg, Te, and Fu. The first isolate with this resistance pattern was recovered from Mapocho River water in 1977 (Table 1). Later, this resistance pattern was found throughout the country through the entire period of the study. The MICs of the different antimicrobial drugs were as follows: Sm, 125 μg/ml; Su, >1,500 μg/ml; Tc, 125 μg/ml.

Ap-Cb resistance was first found in 1982 in four clinical strains that were also resistant to Sm, Sp, Su, Tc, Hg, Te, and Fu. For two strains (VC1618 from Santiago and VC1629 from Arica), the MIC of Ap was >1,500 μg/ml; for the two other strains (VC1613 from Temuco and VC1645 from Santiago), it was 250 μg/ml.

A clinical isolate obtained in 1982 and another one obtained in 1985 (VC1643 and VC2611, respectively; both from Santiago) showed resistance to Ap and Tp in addition to Sm, Sp, Su, Tc, Hg, Te, and Fu. The MIC of Ap was 500 μg/ml; that of Tp was >1,500 μg/ml. Prolonged storage of *Salmonella* strains yielded spontaneous segregants lacking Ap or Tp resistance.

Resistance to Km-Nm was first detected in 1983 in a stool culture (Santiago). The isolate was also resistant to Sm, Sp, Su, Tc, Hg, Te, and Fu. Another two strains with the same resistance patterns were recovered from sausages in 1984 and 1985 (Santiago). The MIC of Km was 1,500 μg/ml.

In 1987 a single strain from a blood culture showed resistance to Ap and Gm as well as to Sm, Sp, Su, Tc, Hg, Te, and Fu. A new complex resistance pattern was detected among strains collected in 1993, when three isolates from stools (Punta Arenas, $n = 2$; Santiago, $n = 1$) and two isolates from meat products (Santiago) were found to be resistant to Ap, Gm, Km-Sm, Sp, Su, Tc, Hg, Te, and Fu. The MICs were as follows: Ap, >1,500 μg/ml; Gm, 32 μg/ml; and Km, 750 μg/ml.

Plasmid content. Isolates that were susceptible to all drugs and those that were only Fu^r or Sm^r Sp^r Fu^r had no plasmid DNA (Fig. 2), resistance markers were nontransferable.

To determine if Sm^r Sp^r was encoded on a transposable genetic element, a conjugative plasmid with a selectable Tc^r marker (pΔT) (15) was introduced into *S. panama* VC694 Sm^r Sp^r Fu^r. Three clones of VC694 containing the ΔT plasmid were conjugated overnight at 37°C with an *E. coli* K-12 Nx^r strain (14R525); transconjugants resistant to Tc, Sm, Sp, and Nx were obtained at a low frequency (10^{-8} per recipient). This resistance pattern (Nx^r excluded) was retransferred to other *E. coli* strains and to several strains of *S. panama*; electrophoresis demonstrated the presence of the 101-kb ΔT plasmid in the *S.*

TABLE 1. Drug resistance markers of *S. panama* strains from different origins

Origin of strains (no. of strains)	No. of isolates with the following R pattern, yr of first isolation:								
	Susceptible, 1975	Fu, 1982	Fu						Total
			Sm, Sp, 1982[a]	Sm, Sp, Su, Tc, Hg, Te, 1977	Ap, Sm, Sp, Su, Tc, Hg, Te, 1982	Ap, Tp, Sm, Sp, Su, Tc, Hg, Te, 1982	Km, Sm, Sp, Su, Tc, Hg, Te, 1983	Ap, Gm, Km, Sm, Sp, Su, Tc, Hg, Te, 1993	
Clinical (139)									
Stool	3	5		84	2		1	3	98
Blood	2	4		7	1			1[b]	15
Spinal fluid		1		2					3
Wound exudate		1		1					2
Urine	1		1	2					4
Not stated	1		2	11	1	2			17
Foodstuffs (121)									
Meat	4	2	7	61			2	2	78
Vegetables	1		3	7					11
Shellfish			9	2					11
Goat cheese	9		12						21
Animal (28)									
Pigs				18					18
Poultry				2					2
Cattle				2					2
Goats			6						6
Fish meal (6)				6					6
Water (11)	2	1		8					11
Total	23	14	40	213	4	2	3	6	305

[a] All resistant strains were also resistant to nitrofurans.
[b] This unique strain, isolated in 1987, lacked Km resistance.

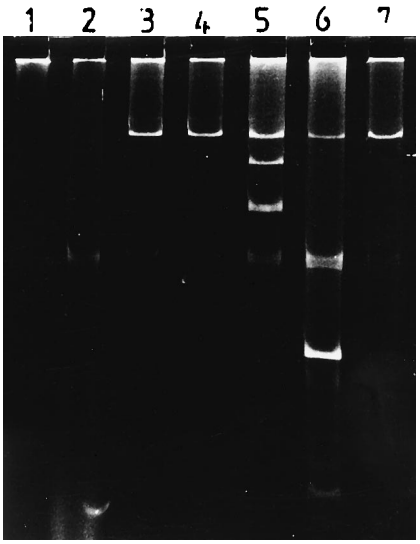

FIG. 2. Electrophoresis of plasmid DNA from *S. panama* isolates and their transconjugants. Lanes: 1, *S. panama* Fu^r; 2, *S. panama* Sm^r Sp^r Fu^r; 3, *S. panama* Sm^r Sp^r Su^r Tc^r Hg^r Te^r Fu^r (218 kb); 4, transconjugant Sm^r Sp^r Su^r Tc^r Hg^r Te^r (218 kb); 5, plasmid size standards (218 kb [pTP116], 92 kb [pΔ], 35 kb [pS-a]); 6, *S. panama* VC1618 Ap^r Sm^r Sp^r Su^r Tc^r Hg^r Te^r Fu^r (218 k and ~6 kb); 7, transconjugant Sm^r Sp^r Su^r Tc^r Hg^r Te^r (218 kb).

panama VC694 Tc^r Sm^r Sp^r and of a larger one, 109 kb, in the transconjugants with the same resistance pattern, demonstrating the construction of a transferable plasmid Tc^r Sm^r Sp^r (pVC2554) by insertion of Sm^r Sp^r in the ΔT plasmid. The frequency of transfer of this VC2554 plasmid in overnight matings among diverse *E. coli* strains was 10^{-3}, but the frequency of transfer to *S. panama* was about 10^{-7}.

The most frequent pattern, Sm^r Sp^r Su^r Tc^r Hg^r Te^r Fu^r (Table 1), with the exception of Fu^r, was transferred en bloc to *E. coli* K-12 recipient strains in overnight matings and back to susceptible strains of *S. panama* at higher frequencies at 28°C than at 37°C (Table 2); 2-h matings were regularly unsuccessful. Electrophoresis showed a single 218-kb plasmid in the *S. panama* isolates resistant to Sm, Sp, Su, Tc, Hg, and Te and in their transconjugants (Fig. 2). The MICs of Sm, Su, and Tc for the transconjugants were similar to those for the *Salmonella* isolates.

Forty-one plasmids with this resistance pattern tested for incompatibility proved to be incompatible with the $IncHI_2$ group plasmid TP116 and $IncHI_1$ plasmid TP154 and were compatible with F′ *lac* and all other incompatibility group plasmids tested. Compatibility results, thermosensitive transfer, large plasmid size, and resistance to tellurite place these plasmids in the $IncHI_2$ incompatibility group (7, 30, 31, 33).

The remaining 15 resistant strains with more complex resistance patterns (Table 1) always contained a thermosensitive, transferable, 218-kb $IncHI_2$ plasmid coding for resistance to Sm, Sp, Su, Tc, Hg, and Te plus another resistance plasmid added as follows (Table 2).

TABLE 2. Plasmid content of *S. panama* isolates and their transconjugants

S. panama		Frequency of transfer at[a]:		Transconjugants	
Resistance pattern	Plasmid size (kb)	28°C	37°C	Resistance pattern	Plasmid size (kb)
Fu		NT	NT		
Sm-Sp, Fu		NT	NT	ΔT::Sm, Sp[b]	109
Sm, Sp, Su, Tc, Hg, Te, Fu	218	10^{-4}–10^{-3}	10^{-7}–10^{-6}	Sm, Sp, Su, Tc, Hg, Te	218
Sm, Sp, Su, Tc, Hg, Te, Fu, Ap	218	10^{-4}–10^{-3}	10^{-7}–10^{-6}	Sm, Sp, Su, Tc, Hg, Te	218
	~6	NT	NT		
	218	10^{-4}–10^{-3}	10^{-7}–10^{-6}	Sm, Sp, Su, Tc, Hg, Te	218
	61	10^{-4}–10^{-3}	10^{-2}	Ap	61
				Ap, Sm, Sp[c]	61/67
				Ap, Sm, Sp	74
Sm, Sp, Su, Tc, Hg, Te, Fu, Ap, Tp	218	10^{-4}–10^{-3}	10^{-7}–10^{-6}	Sm, Sp, Su, Tc, Hg, Te	218
	65	10^{-4}–10^{-3}	10^{-2}	Ap, Tp	65
	218	10^{-4}–10^{-3}	10^{-7}–10^{-6}	Sm, Sp, Su, Tc, Hg, Te	218
	71	10^{-4}	10^{-2}–10^{-1}	Ap, Tp, Sm, Su	71
Sm, Sp, Su, Tc, Hg, Te, Fu, Km	218	10^{-4}–10^{-3}	10^{-7}–10^{-6}	Sm, Sp, Su, Tc, Hg, Te	218
	~6	NT	NT	Km/X–Km/Δ[d]	~6
Sm, Sp, Su, Tc, Hg, Te, Fu, Ap, Gm, Km	218	10^{-4}–10^{-3}	10^{-7}–10^{-6}	Sm, Sp, Su, Tc, Hg, Te	218
	120	10^{-3}	10^{-2}	Ap, Gm, Km, Tc	120

[a] Overnight matings. NT, not transferable.
[b] Sm and Sp resistance gene(s) inserted in ΔT conjugative plasmid.
[c] Two plasmids, Ap^r and Sm^r and Sp^r were occasionally obtained from VC1613, while VC1645 gave a single one, Ap^r, Sm^r, and Sp^r.
[d] The Km^r plasmid transferred in tripareNTal matings with pX and pΔ.

(i) Two isolates that were resistant to Ap (VC1618, VC1629) contained an ~6-kb plasmid (Fig. 2) that was not self-transferable or mobilizable by the conjugative plasmids. In contrast, two other isolates (VC1613, VC1645) contained a 61-kb plasmid that transferred Ap^r at a frequency of 10^{-3} in 2-h matings and at a frequency of 10^{-2} in overnight matings at 37°C and at a frequency of 1 to 2 logs less than 10^{-2} at 28°C. Occasionally, in crosses at 37°C, transconjugants resistant to Ap, Sm, and Sp were obtained from both *S. panama* strains. Transconjugants from VC1613 had two plasmids; one encoded resistance to Ap (61 kb) and the other encoded resistance to Sm and Sp (67 kb); in transconjugants from VC1645, resistance to Ap, Sm, and Sp remained constantly associated on a single cointegrated transferable plasmid of 74 kb (Fig. 3). The MICs of Ap for the transconjugants were the same as those for the *Salmonella* strains.

(ii) Strain VC1643 Ap^r Tp^r had a 65-kb plasmid coding for Ap^r and Tp^r that transferred at 37°C in 2-h matings at a frequency of 10^{-3}, at a frequency of 10^{-2} in overnight matings, and at a frequency of 1 to 2 logs less than 10^{-2} at 28°C. Strain VC2611, which had the same resistance pattern as VC1643, contained a 71-kb plasmid coding for resistance to Ap, Tp, Sm, and Su transferable at a frequency of 10^{-4} at 28°C and at a frequency of 10^{-3} to 10^{-1} at 37°C in overnight matings. The MICs for the transconjugants were similar to those for the *Salmonella* isolates.

(iii) Kanamycin resistance in three *S. panama* isolates was encoded on an ~6-kb nontransferable plasmid that was mobilized by both X and Δ plasmids. The MIC of Km for the transconjugants was 500 μg/ml.

(iv) Strains resistant to Ap, Gm, and Km had a 120-kb transferable plasmid (Table 2) encoding resistance to Ap, Gm, Km, and Tc. The MICs for these strains were the same as those for the *Salmonella* donor strains except for that of Tc, which was 32 μg/ml.

DISCUSSION

It was in the environment, in the Mapocho River in Santiago, where *S. panama* was first detected in Chile in 1975 as a susceptible strain and again in 1977, this time as a multiresistant isolate, later evolving toward progressively more complex resistance patterns. *S. panama* was not isolated from humans in Chile until 1978, when a small number ($n = 5$) of strains were isolated first in Santiago and months later in Concepción; these strains had the same multiresistance pattern that was found in the 1977 river isolate. The number of clinical isolates rose to 63 in 1982, declining afterward. Since it is not required that cases of enterocolitis be reported to public health authorities, no epidemiological data are available; the data reported here were obtained from testing of the *S. panama* strains received at the Instituto de Salud Pública.

A large proportion of the isolates were resistant to nitrofurans; in *E. coli* this is the result of a mutation, and the genes involved have been located on its chromosome (21, 28). In an extensive review of the molecular biology of *Salmonella typhimurium* (24), the best-studied *Salmonella* serotype at the genetic level, no reference is made to Fu^r, and no reference is made to Fu^r in the VIIth edition of its linkage map (27), although Fu^r has been described in *S. typhimurium* as being a nontransferable resistance (15). In the literature on *S. panama* that we have reviewed (2, 4, 6), there is no description of Fu^r, but in the isolates tested in the present study, it behaved as a mutation; that is, it was not transferable or mobilizable and no

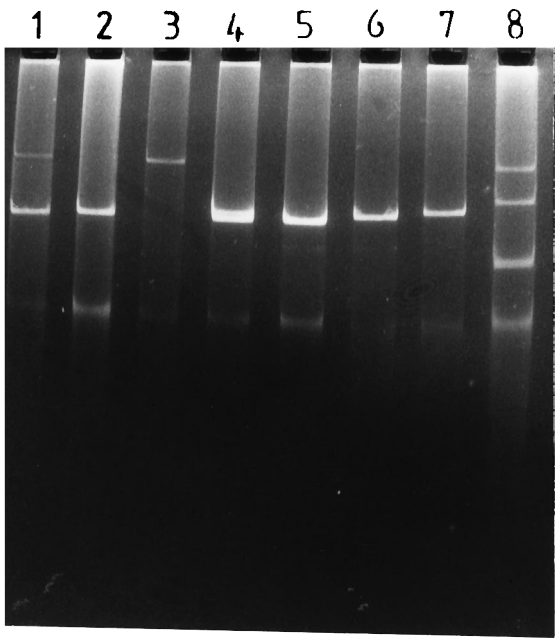

FIG. 3. Electrophoresis of plasmid DNA from *S. panama* isolates and their transconjugants. Lane 1, *S. panama* VC1613 or VC1645 Ap^r Sm^r Sp^r Su^r Tc^r Hg^r Te^r Fu^r (218 and 61 kb); lanes 2 to 4, transconjugants from VC1613 (lanes 2, Ap^r [61 kb]; lane 3, Sm^r Sp^r Su^r Tc^r Hg^r Te^r [218 kb]; lane 4, Ap^r Sm^r Sp^r [61 kb and 67 kb]); lanes 5 and 6 retransfers from Ap^r Sm^r Sp^r transconjugant (lanes 5, Ap^r [61 kb]; lane 6, Sm^r Sp^r (67 kb); lane 7, transconjugant from VC1645 Ap^r Sm^r Sp^r (74 kb); lane 8, plasmid size standards as described in the legend to Fig. 2.

plasmid DNA was present. Prior to the arrival of *S. panama*, Fu was used in Santiago as a therapeutic agent against infantile enterocolitis, and this practice probably aided in the selection of this trait. Fourteen of the 305 strains studied were resistant only to nitrofurans; multiresistant strains were always Fu^r. In fact, a multidrug-resistant strain susceptible to Fu was never recovered, suggesting that it was a Fu^r mutant which later acquired resistance to other drugs by other mechanisms.

In a large majority of isolates ($n = 213$), these other drugs to which isolates were resistant were Sm, Sp, Su, Tc, Hg, and Te, and the entire pattern of resistance to Sm, Sp, Su, Tc, Hg, Te, and Fu was present in isolates recovered from 1977 through 1993. These isolates had a plasmid of the same size, the same resistance pattern, including Hg and Te, thermosensitive transferability, and, when tested, the same HI_2 incompatibility group. Its presence in this rare *Salmonella* serovar, which was never before found in Chile, in a strain with the unusual Fu^r marker, with only a remote probability of being repeatedly introduced into the country, all strongly suggest that it is a single strain containing the same plasmid. Later, 15 isolates acquired a second plasmid encoding for transferable resistance to any one of Ap; Ap and Tp; Ap, Tp, Sm, and Su; Ap, Gm, Km, and Tc; or nontransferable resistance to Ap or Km.

Given the wide ecological distribution of *S. panama*, these diverse resistance plasmids could have been acquired in different habitats, for instance, within the human gastrointestinal tract. In Chile, antibiotics can be bought over the counter, and certainly, our human population carries a stock of drug-resistant enterobacteria, mostly *E. coli*, whose proportion and complexity increases notoriously in individuals subjected to antimicrobial therapy, with a high percentage of Ap^r and a lower percentage of Tp^r (9). An increasing frequency of Tp^r has been reported in clinical isolates of *E. coli* in children in Chile and in *S. typhi* in Peru (13, 23), but the presence of Tp^r in *S. panama* was, until now, very rare. Acquisition of drug resistance could also occur in animals, and interestingly, the antimicrobial drugs authorized for use in feeds include almost all of the antimicrobial drugs to which *S. panama* became resistant. Of 300 asymptomatic pigs slaughtered for consumption in 1980 in Rancagua (about 90 km south of Santiago), 14.4% were contaminated with *Salmonella* isolates, and four-fifths of these were drug-resistant *S. panama* isolates (unpublished data); 2 years later *S. panama* was still present in pigs from Rancagua, but it was not found in other seven cities near Santiago (36). There is also the possibility that different drug resistance markers are incorporated through contact with environmental bacteria carrying resistance and transfer plasmids (20, 32). This has recently been demonstrated in laboratory experiments (18).

These multiple possible origins of resistance plasmids could explain the diversity of resistance patterns found in *S. panama* strains isolated in Chile and other countries. *S. panama* isolates in France were Ap^r, Km^r, and Tc^r, each of which was encoded on a different plasmid (29), and in Romania they were Ap^r Cm^r Km^r Sm^r Su^r Tc^r (IncM) or Ap^r (IncI2), whereas those from Belgium were Ap^r Cm^r Km^r Sm^r Su^r (IncFII) or Ap^r Tc^r (IncN) and those from The Netherlands were Tc^r (IncN) or Ap^r or Sm^r (2, 4, 6), all of which are different from the patterns of our isolates.

Interestingly, susceptible strains or non-plasmid-encoded Sm and Sp-resistant strains were present mainly in animals or in their products from three limited small rural zones (Ovalle, Las Melosas, Los Vilos) with few inhabitants, where animals are fed without drugs.

The arrival and spread of *S. panama* in Chile emphasizes the need for strict bacteriological controls on the national and international trade of foods and feeds. As in other countries (34), we have detected several *Salmonella* serotypes in imported food (poultry and other meat products) (8); although we did not find *S. panama* among them, this could be the way that it originally came into the country. The World Health Organization has pointed out the danger of microbiological pathogens spreading across countries (38) and the need for preventive control measures.

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