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Propagation of TEM- and PSE-Type β-Lactamases among Amoxicillin-Resistant *Salmonella* spp. Isolated in France

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A survey conducted between 1987 and 1994 at the University Hospital of Besançon, France, demonstrated a dramatic increase (from 0 to 42.5%) in the prevalence of amoxicillin resistance among *Salmonella* spp. Of the 96 resistant isolates collected during this period (including 77 Typhimurium), 54 were found to produce TEM-1 β-lactamase, 40 produced PSE-1 (equivalent to CARB-2), one produced PSE-1 plus TEM-2, and one produced OXA-1 in isoelectric focusing and DNA hybridization experiments. Plasmids coding for these β-lactamases were further characterized by (i) profile analysis, (ii) restriction fragmentation pattern analysis, (iii) hybridization with an *spvCD-orfE* virulence probe, and (iv) replicon typing. In addition, isolates of *S. typhimurium* were genotypically compared by pulsed-field gel electrophoresis of *Xba*I-macrorestricted chromosomal DNA. Altogether, these methods showed that 40 of the 41 PSE-1 producers were actually the progeny of a single epidemic *S. typhimurium* strain lysotype DT104. Isolates of that strain were found to harbor RepFIC virulence plasmids with somewhat different restriction profiles, but which all carried the bla_{PSE-1} gene. Of these virulence/resistance plasmids, 15 were transmissible to *Escherichia coli*. TEM-1-producing *S. typhimurium* displayed much greater genotypic and plasmidic diversities, suggesting the acquisition of the bla_{TEM-1} gene from multiple bacterial sources by individual strains. In agreement with this, 32 of the 35 *S. typhimurium* plasmids encoding TEM-1 were found to be conjugative. These data show that development of amoxicillin resistance among *Salmonella*, especially in serovar Typhimurium, results from both gene transfers and strain dissemination.

β-Lactam antibiotics are widely used in the treatment of salmonellosis. Recently, alarming reports have pointed out the rapid development of resistance to these agents, involving *Salmonella* serovars such as Enteritidis (28, 44), Typhimurium (28, 41, 43), Panama (6), and Typhi (13) in several countries. Clinical strains of *Salmonella* spp. producing large-spectrum β-lactamases and which are resistant to penicillins (44), or *Salmonella* spp. producing extended-spectrum β-lactamases and which are resistant to cephalosporins such as cefotaxime, ceftazidime, or ceftriaxone (1, 43), have been isolated from large outbreaks as well as from sporadic cases.

DNA-based typing methods have provided very useful information on the dissemination of resistant *Salmonella* to epidemiological investigations (39). On some occasions, the relatedness of R plasmids harbored by strains of various origins could be demonstrated by restriction fragmentation pattern analysis (RFP), allowing a better understanding of how resistant strains or R factors may propagate (3, 40, 44). More recently, methods such as random amplified polymorphic DNA fingerprinting analysis (17), IS*200* fingerprinting (33), ribotyping, and restriction fragment length polymorphism analysis (24) have been evaluated and found to be valuable tools for tracing large outbreaks due to the circulation of single epidemic clones (45). R plasmid characterization and strain genotyping have, however, rarely been combined to compare resistant isolates over long periods of time or to study the diffusion of resistance determinants among bacterial populations (13, 36).

From 1987 to 1994, we witnessed a tremendous increase in the prevalence of amoxicillin resistance among the *Salmonella* spp. isolated at the University Hospital of Besançon, France. To establish if such an increase was due to the dissemination of a few resistant clones in the community or whether it resulted from the acquisition of resistance determinants from multiple bacterial sources by *Salmonella*, we carried out the comparison of the R plasmids harbored by the resistant isolates, as well as the isolates themselves, by applying various typing methods.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. From 1987 to 1994, 489 *Salmonella* spp. were isolated from blood cultures (3%), stool samples (87%), blood and stool samples (1%), urine samples (3%), urine and stool samples (4%), and biopsies (2%) of patients hospitalized at the University Hospital of Besançon, France. All isolates were biochemically (API 20E strip; BioMérieux) and serotypically characterized (Sanofi Pasteur). Cultures were routinely performed at 37°C on Mueller-Hinton (MH) agar plates (Sanofi Pasteur) or in brain heart (BH) infusion agar (Sanofi Pasteur), supplemented with 300 μg of rifampicin per ml (Sigma) and/or 50 μg of amoxicillin per ml (SmithKline-Beecham), as required. Transferability of amoxicillin resistance was assessed by conjugational matings, with a mutant of *Escherichia coli* K-12 resistant to rifampicin as a recipient. Conjugations were carried out in BH infusion agar for 4 h at 37°C or, alternatively, on 0.45-μm-pore-size nitrocellulose filters (Millipore) for 18 h at 37°C (22). Transconjugants were selected on MH agar medium containing rifampicin and amoxicillin.

***Salmonella* plasmids.** Plasmids harbored by *Salmonella* were extracted by the method of Kieser (15) and were visualized by electrophoresis in a horizontal 0.8% (wt/vol) agarose gel calibrated with reference plasmids from *E. coli* V517 (18). Total plasmid DNA of *Salmonella* was purified by the method of Birnboim and Doly (4), cleaved with *Eco*RI, *Bam*HI, or *Hin*dIII (Boehringer Mannheim Biochemicals) according to the manufacturer's recommendations, and subsequently electrophoresed in a 0.8% (wt/vol) agarose gel.

Hybridization experiments. DNA probes were prepared from purified plasmids (Quiagen plasmid kit) by digestion with appropriate restriction enzymes or by amplification by PCR with oligonucleotide primers, as specified in Table 1. The DNA fragments were separated by agarose gel electrophoresis, purified with the Bio-Rad Prep-a-gene kit, and labelled by random priming (Random Primed Labelling kit; Boehringer-Mannheim) with [α^{32}P]dCTP. Colony and Southern

TABLE 1. DNA probes used in this study

Probe	Characteristics of probes			
	Plasmidic origin	Probe size (bp)	Restriction sites or primers	Reference
Replicon typing				
RepFIC	pULB2440	967	*Eco*RI-*Hin*dIII	7
RepFIIA	pULB2401	543	*Pst*I	7
RepHI1	pULB2436	2,250	*Eco*RI-*Hin*dIII	7
RepHI2	pULB2433	1,800	*Eco*RI	7
RepI1	pULB2428	1,100	*Eco*RI-*Pst*I	7
RepP	pULB2420	750	*Hae*II	7
RepX	pULB2405	942	*Hin*dIII	7
Virulence identification				
H6	pIP1350	3,500	*Hin*dIII	12
β-Lactamase determination				
TEM-1/2[a]	pBR322	489	5′GAGTACTCACCAGTCACAGAAAAGC3′ 5′GACTTCCCGTCGTGTAGATAAC3′	35
PSE-1	RPL11	586	5′AATGGCAATCAGCGCTTCCC3′ 5′GGGGCTTGATGCTCACTCCA3′	2, 16

[a] Oligonucleotides and probe TEM-1/2 are homologous to TEM-1 and TEM-2 β-lactamases. IEF allowed us to distinguish between both enzymes.

blot hybridizations were performed under highly stringent conditions. Plasmids R1 (21), RP1 (11), and RPL11 (16) were used as positive controls for the identification of TEM-1 (pI = 5.4), TEM-2 (pI = 5.6), and PSE-1 (pI = 5.7) β-lactamases, respectively. Replicon typing allows the classification of plasmids into replicon groups which, in some cases, match incompatibility groups (7). In this work, we used nine replicon-specific probes, known to be representative of *Salmonella* plasmids RepFIC, RepFIIA, RepHI1, RepHI2, RepI1, RepA/C, RepP, RepQ, and RepX (14, 37). Plasmids were individually typed by Southern hybridization.

PCR conditions and DNA sequencing. The PCR mixtures (25 μl) contained 1 μl of bacterial lysate (obtained by heating bacterial colonies to 100°C for 15 min) or 25 to 50 ng of purified DNA, 0.2 U of *Taq* DNA polymerase (Goldstar; Eurogentec), 1× PCR Goldstar buffer, 0.3 μM each primer (Table 1), and 0.2 mM each deoxynucleoside triphosphate. The amplification step was performed for 30 cycles in a Crocodile II thermal cycler (Appligène). Each amplification cycle consisted of 1 min at 92°C, 2 min at 50°C, and 3 min at 72°C. A final extension was performed at 72°C for 10 min. PCR products obtained after amplification with the PSE primers were purified by using the Wizard PCR Preps kit (Promega) and were sequenced by an ABI 373A automatic sequencer (Perkin-Elmer, Applied Biosystems). Their nucleotide sequences were analyzed with the GeneStream align program (22a).

Macrorestriction analysis. Preparation of whole cell DNA for pulsed-field gel electrophoresis (PFGE) was as described by Godard et al. (10). DNA-containing agarose plugs were incubated overnight in the presence of 50 U of *Xba*I (Boehringer-Mannheim) and underwent PFGE as reported previously. The restriction banding patterns of the isolates were compared by means of the Taxotron package (P. Grimont, Pasteur Institute), using *Sma*I restriction fragments of the *Staphylococcus aureus* NCTC 8325 genome for intergel calibration. Major restriction patterns were defined as differing by more than three bands and with similarity coefficients less than 85%, according to Struelens et al. (34). The Dice distance coefficient of macrorestriction analysis was calculated to be 1.

Antibiotic susceptibility. Routine drug susceptibility tests were performed by using the agar diffusion method (disks from Sanofi Pasteur), according to the guidelines of the National Committee for Clinical Laboratory Standards (23). MICs were determined more precisely on MH agar plates containing serial twofold dilutions of the following antibiotics: amoxicillin, amoxicillin-clavulanate (SmithKline-Beecham), piperacillin, piperacillin-tazobactam (Wyeth-Lederlé), cefoperazone (Pfizer), cefuroxime (Glaxo-Wellcome), and cefotaxime (Roussel-Uclaf). An inoculum of 10^4 bacteria per spot was deposited by a Steers inoculator (30). Isolates were also screened for resistance to chloramphenicol (8 μg/ml), streptomycin (16 μg/ml), spectinomycin (16 μg/ml), tetracycline (8 μg/ml), trimethoprim (2 μg/ml), sulfadiazine (64 μg/ml), and nalidixic acid (16 μg/ml).

IEF of β-lactamases. Analytical isoelectric focusing (IEF) of β-lactamases (19) produced by *Salmonella* was performed in precast polyacrylamide gels (Ampholine PAG Plate, pH 4.0 to 6.5 or pH 3.5 to 9.5; Pharmacia Biotech) using an LKB Multiphor 2117 apparatus (Pharmacia), with bacterial suspensions subjected to three cycles of freezing and thawing (5). β-Lactamase activity was revealed in gels by spreading 2 ml of a 0.05% (wt/vol) solution of nitrocefin (Glaxo-Wellcome).

RESULTS

Antimicrobial resistance of *Salmonella*. A dramatic increase in the prevalence of amoxicillin resistance was observed among the *Salmonella* serovars (n = 489) isolated at the University Hospital of Besançon between 1987 (0%) and 1994 (42.5%). Most of the resistant *Salmonella* isolates belonged to the serovar Typhimurium (n = 77). Other *Salmonella* serovars were each represented by less than seven resistant isolates: six *S. saint-paul*, three *S. enteritidis*, three *S. virchow*, one *S. agona*, one *S. blockley*, one *S. brandenburg*, one *S. heidelberg*, one *S. kedougou*, one *S. wien*, and one *Salmonella* sp.

As shown in Table 2, most of the amoxicillin-resistant isolates (74 of 96) were also resistant to piperacillin (MIC at which 90% of the isolates are inhibited [MIC_{90}] = 256 μg/ml). All of these isolates, however, were susceptible to the combination of piperacillin and tazobactam. Similarly, MICs of amoxicillin (MIC_{90} = > 2,048 μg/ml) were strongly reduced in the presence of the β-lactamase inhibitor clavulanic acid (MIC_{90} = 16 μg/ml), but seven isolates remained resistant to the combination of both drugs. No resistance to the expanded-spectrum cephalosporin cefotaxime was noted among the selected isolates.

Characterization of β-lactamases. As evidenced by IEF and Southern blot hybridizations with specific nucleic acid probes, 94 of 96 isolates were found to produce a single β-lactamase (54 produced TEM-1 and 40 produced PSE-1), and 1 of the 96 isolates produced two enzymes (PSE-1 and TEM-2). All PSE-1 (CARB-2) producers belonged to the serovar Typhimurium. Direct sequencing of the *pse-1* PCR products from 13 randomly chosen *S. typhimurium* isolates revealed that the amplified gene sequences shared 98% (and the derived amino acid sequence, more than 99%) identity with those of the PSE-1 gene carried by plasmid RPL11 in *Pseudomonas aeruginosa* (16). Finally, 1 of the 96 isolates expressed a β-lactamase of pI 7.4, tentatively identified as an OXA-1 enzyme (33a).

Identification and transferability of R plasmids. The resistant isolates were found to individually contain one (n = 38), two (n = 28), three (n = 15), four (n = 10), five (n = 3), or six (n = 2) plasmids, with molecular lengths ranging from 1 to 82 kb. Conjugational transfer of the amoxicillin resistance pheno-

TABLE 2. Susceptibility levels of PSE-1 and TEM-1 *Salmonella* isolates to β-lactam antibiotics[a]

Antibiotic	MIC (μg/ml)			No. resistant[b]
	Range	50%	90%	
PSE-1 producers (n = 40)				
Amoxicillin	≥2,048	>2,048	>2,048	40
Amoxicillin-clavulanic acid[c]	16–32	16	16	4
Piperacillin	256–>2,048	256	256	40
Piperacillin-tazobactam[d]	4–8	4	4	0
Cefuroxime	4–8	8	8	0
Cefoperazone	16–512	32	32	1
Cefotaxime	0.06–0.125	0.06	0.06	0
TEM-1 producers (n = 54)				
Amoxicillin	1,024–>2,048	2,048	>2,048	54
Amoxicillin-clavulanic acid	8–32	16	16	1
Piperacillin	16–>2,048	128	256	32
Piperacillin-tazobactam	1–8	4	4	0
Cefuroxime	2–32	4	8	1
Cefoperazone	1–512	4	16	1
Cefotaxime	0.03–0.5	0.06	0.125	0

[a] The MICs for 2 of 96 strains (the PSE-1/TEM-2- and the OXA-4-producing strains) are not included in this table.
[b] According to the National Committee for Clinical Laboratory Standards breakpoints.
[c] Amoxicillin and clavulanic acid were associated in the ratio of 1/0.5. MIC values refer to the concentrations of amoxicillin.
[d] MIC values refer to the concentrations of piperacillin in the presence of a fixed amount of tazobactam (4 μg/ml).

type to a recipient *E. coli* strain was successful in 67 of 96 (70%) of the *Salmonella* isolates. Most of the *E. coli* transconjugants acquired additional resistances to tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, neomycin, streptomycin, spectinomycin, nalidixic acid, and/or nitrofuranes (data not shown). Hybridization of the transferred plasmids with TEM- and PSE-type probes after Southern blotting demonstrated that the β-lactamase genes were all carried by plasmids larger than 35 kb (43 to 82 kb).

Identification of virulence plasmids. Sixty of the 77 (78%) *S. typhimurium* isolates and two *S. enteritidis* isolates harbored large (48- to 82-kb) plasmids that hybridized positively with a *spvCD-orfE* virulence probe after Southern transfer (Fig. 1). Interestingly, these virulence plasmids were found in 100% of the *Salmonella* spp. isolated from blood and urine samples, and in 50 and 47% of those obtained from biopsies and stool samples, respectively. Genes homologous to *spvCD-orfE* were not detected in serovars other than Typhimurium and Enteritidis. It should be stressed here that nearly all of the virulence plasmids (58 of 60) detected in the *S. typhimurium* isolates also carried genes coding for PSE-1 (n = 41) or TEM-1 (n = 17) β-lactamase. The two virulence plasmids found in *S. enteritidis* were demonstrated to determine β-lactam resistance as well (TEM-1).

Replicon typing. Replicon typing was carried out with probes specific to RepFIC, RepFIIA, RepHI1, RepHI2, RepI1, RepA/C, RepP, RepQ, and RepX groups. Eighty-three of the 96 amoxicillin-resistant *Salmonella* isolates (86.5%) contained one or several plasmids hybridizing with at least one of the selected probes. Plasmids of the RepFIC group or the RepFIC subgroup B (cross-hybridization with RepFIC and RepI1 probes) were predominant among the isolates hybridizing with just one probe (59 of 68), especially in *S. typhimurium* (n = 34). Other replicon groups were confined in less frequently isolated serovars (e.g., RepP [three Saint-Paul, one Brandenburg, and one Typhimurium], RepHI2 [one Kedougou and one Virchow], and RepQ [one Agona and one Blockley]). Fifteen of 96 strains scored positive with two (n = 9), three (n = 4), or even four (n = 2) different replicon probes, while 16 of 96 contained undetermined replicons. No plasmid hybridized with the RepFIIA, RepHI1, RepA/C, or RepX probes.

Plasmid restriction fragmentation analysis. Plasmids of the resistant *Salmonella* isolates were finally compared on the basis of the restriction banding patterns produced after digestion with endonuclease *Eco*RI, *Bam*HI, or *Hin*dIII. We could thus identify 14 different plasmidic groups showing unique core fragment patterns and differing from strain to strain by less than three bands (Fig. 2). The most frequent restriction profile (RFP I) was present in 25 isolates of *S. typhimurium* recovered from 1988 to 1994. All isolates of this group harbored a RepFIC virulence plasmid encoding a PSE-1 β-lactamase (Table 3). Thirteen other plasmid patterns (II to XIV) grouped four isolates or less each (Tables 3 and 4).

Macrorestriction analysis. Isolates of the dominating serovar Typhimurium were analyzed by PFGE in order to study their genotypic relatedness. *Xba*I digestions resulted in approximately 15 fragments in the range of 10 to 675 kbp. A total of 19 different PFGE banding patterns were detected among the 77 isolates (representative patterns are shown in Fig. 3). With one exception, the 41 isolates producing PSE-1 β-lactamase could be grouped into a unique genotype, named A. Altogether, patterns C to H grouped 27 TEM-1-producing isolates, whereas eight isolates showed unique PFGE banding profiles. The isolate that produced OXA-1 β-lactamase showed a particular genotype named I (Table 3).

DISCUSSION

The dramatic increase in β-lactam resistance observed during the survey essentially concerned *S. typhimurium* (77 of 96 isolates), the most prevalent serovar isolated in our hospital (38%). In comparison, *S. enteritidis*, the second most prevalent serovar (26%), was only rarely resistant to amoxicillin (3 of 96 isolates). Analysis of the resistant *S. typhimurium* isolates by using various typing methods allowed the identification of two distinct groups of isolates, one producing PSE-1 β-lactamase and one producing TEM-1 enzyme (Table 3).

The PSE-1 group consisted of 40 isolates exhibiting very similar PFGE patterns, showing an identical resistance pheno-

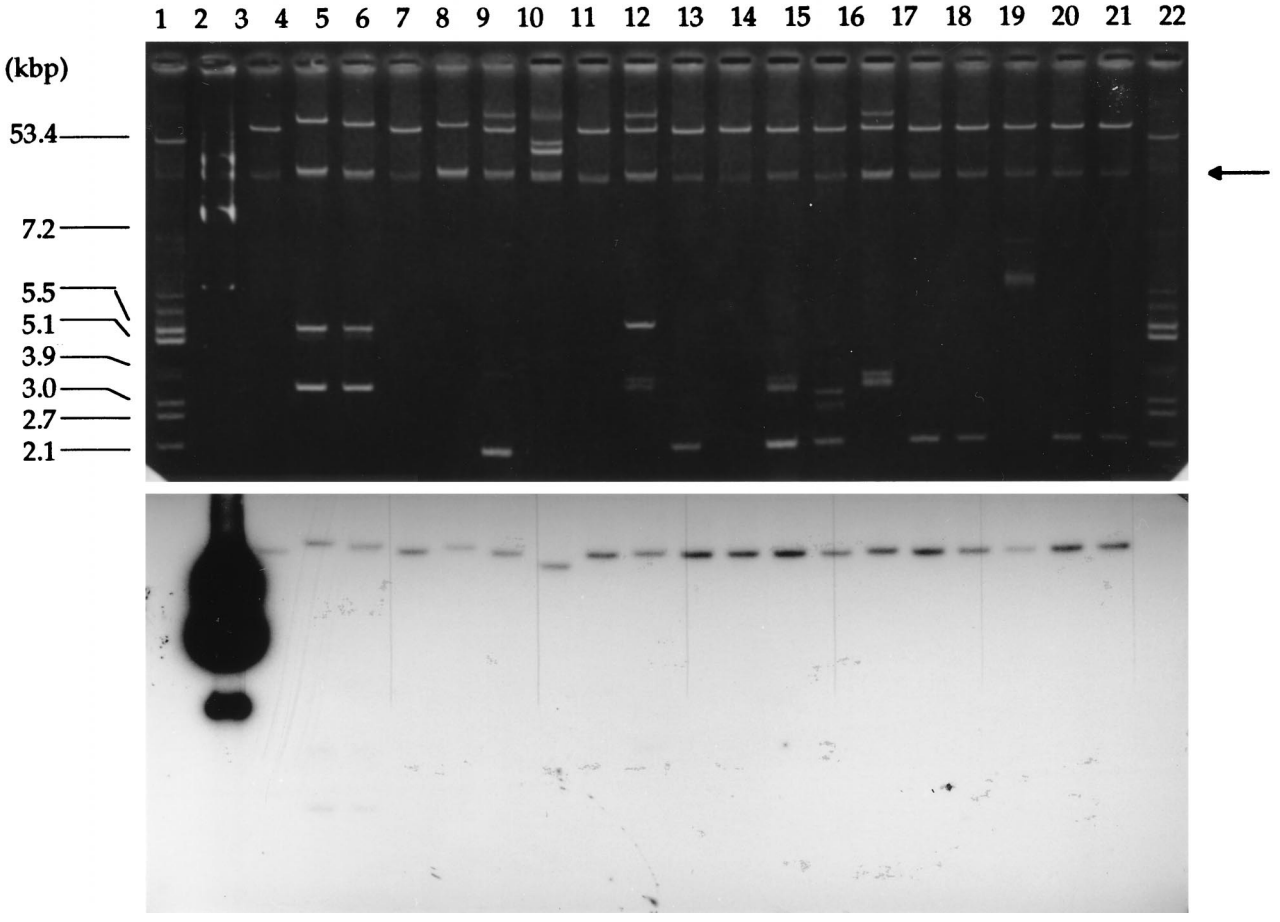

FIG. 1. Identification of the virulence plasmids in *Salmonella typhimurium*. (Upper panel) Visualization of *Salmonella* plasmids by agarose gel electrophoresis after extraction by the method of Kieser (15). Lanes 1 and 22, *E. coli* V517 plasmids used as molecular size standards (kbp are indicated on the left of the gel); lane 2, plasmid pIP1350 containing the *spvCD-orfE* virulence determinant was used as a positive control; lanes 3 to 21, selected virulent *Salmonella* isolates S1, S3, S7, S15, S16, S22, S39, S40, S41, S42, S43, S49, S50, S51, S53, S54, S55, S57, and S58, respectively. Chromosomal DNA bands (arrow) are seen in each lane. (Lower panel) Autoradiogram of the plasmids after Southern transfer and hybridization with the *spvCD-orfE* probe.

type (Ap^r Cm^r Sp^r Sm^r Su^r Tc^r), and belonging to the DT104 phage type (as determined from five randomly chosen isolates) (data not presented). These results strongly suggest that all the PSE-1 producers (except one that displayed a unique PFGE profile) actually are the progeny of a single epidemic strain that spread through France beginning in 1988. The observation that all these bacteria shared the rather unusual feature of having RepFIC virulence plasmids carrying the bla_{PSE-1} gene reinforces this hypothesis. This is also consistent with recent epidemiological data indicating that multiresistant DT104 *S. typhimurium* is increasing in incidence worldwide mostly because of the transmission of the pathogens from cattle to humans via food (27).

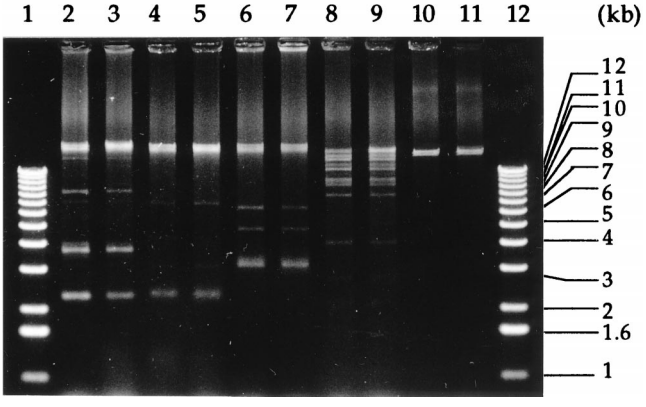

FIG. 2. Restriction banding patterns of total plasmid DNA from selected *Salmonella* isolates. Lanes 1 and 12, DNA marker fragments (sizes in kbp are indicated on the right edge of the gel); lanes 2 to 7, *Bam*HI digestion of DNA from isolates S3 and S5 (RFP II), S78 and S80 (RFP I), and S13 and S14 (RFP III); lanes 8 and 9, *Eco*RI digestion of S16 and S18 (RFP IV); lanes 10 and 11, *Hin*dIII digestion of S27 and S28 (RFP V).

While our isolates appeared to be closely related with respect to the markers cited above, their RepFIC virulence/resistance plasmids showed a somewhat greater diversity. Differences in the size and transferability of the plasmids were indeed noted among the isolates. Furthermore, restriction banding pattern analysis demonstrated major differences between some of the RepFIC virulence/resistance replicons in bacteria harboring single plasmids (as shown by the different plasmidic groups in Table 3). This tends to indicate that variations occurred in the RepFIC virulence/resistance plasmid of the DT104 epidemic strain over time. In support of this speculation, it has been shown that *Salmonella* plasmids are frequently subjected to molecular rearrangements by homologous or illegitimate recombinations (37). Although the plasmidic profile analysis has been described as a useful epidemiological tool for the differentiation of epidemic from nonepidemic

TABLE 3. Comparison of *S. typhimurium* isolates by using various phenotypic and genotypic markers

β-Lactamase	PFGE group	R plasmid characterization					Isolates (*n*)
		Virulence[a,b]	Replicon type	Transfer[b,c]	Length (kbp)	Restriction profile	
PSE-1	A	+	FIC	+/−	60–72	I	25[d]
			FIC	+	60–72	VIII	4
			FIC	+/−	60–72	NR[e]	9
			FIC/Q	−	72	NR	2
	B	+	FIC	−	60	NR	1
TEM-1	C	+	FIC	+	78–82	II	3
	D	+	FIC/P/Q	+	72–82	NR	3[f]
	E	−	ND[g]	+	43	V	3
		−	FIC-B[h]	+	43	VI	2
		−	ND	+	35	VII	2
		−	ND	+	43	NR	4
		−	FIC/P/HI2	+	75	NR	1
	F	−	ND	+	43	XIII	2
	G	+	FIC	+	75	IV	2
		+	FIC	+	67–82	XII	2
		+	FIC	+	67	NR	1
	H	+	FIC	+/−	82	XIV	2
	Ungrouped	+/−	Various	+/−	45–82	NR	8
OXA-1	I	−	FIC	−	78	NR	1

[a] Hybridization with *spvCD-orfE* probe.
[b] +, positive; −, negative for the character tested.
[c] Conjugational transfer of amoxicillin resistance.
[d] One of these isolates also produces a TEM-2 β-lactamase.
[e] NR, different by more than three electrophoretic bands.
[f] Two of these isolates harbored two distinct plasmids, one coding for virulence and another coding for β-lactamase; the replicon typing data concern the R plasmid.
[g] ND, not determined.
[h] RepFIC subgroup B.

strains of *Salmonella* in outbreaks (39), this marker appears to be inappropriate to ascertain the epidemiological relatedness of strains isolated over long periods of time.

DT104 isolates resistant to amoxicillin by production of PSE-1 β-lactamase have been reported to be involved in large outbreaks (9, 41). In contrast to the DT104 epidemic strain isolated in our hospital, French, Danish, and British isolates were found to contain the bla_{PSE-1} gene integrated into the bacterial chromosome (26, 29, 32). In these latter strains, the bla_{PSE-1} and *aadA2* genes (encoding streptomycin and spectinomycin resistance, respectively) were present on two distinct integrons, while the genetic determinants responsible for

TABLE 4. Comparison of the resistant *Salmonella* serovars other than Typhimurium

Serovars	β-Lactamase	R plasmid characterization					Isolates (*n*)
		Virulence[a,b]	Replicon type	Transfer[b,c]	Length (kbp)	Restriction profile	
Agona	TEM-1	−	Q	+	54	NR[d]	1
Blockley	TEM-1	−	Q	+	43	NR	1
Brandenburg	TEM-1	−	P	+	43	NR	1
Enteritidis	TEM-1	+	FIC	+	52	IX	2
		−	FIC-B/P/HI2	+	67	NR	1
Heidelberg	TEM-1	−	FICA-B[f]	+	75	NR	1
Kedougou	TEM-1	−	HI2	+	43	NR	1
Saint-Paul	TEM-1	−	P	+/−	66	III	2
		−	P	−	43	NR	1
		−	P/HI2	+	57	NR	1
		−	P/HI2	+	43	X	2
Virchow	TEM-1	−	ND[e]	+	48	NR	1
		−	HI2	+	72	NR	1
		−	P/HI2	+	82	NR	1
Wien	TEM-1	−	FICA-B	+	82	NR	1
Salmonella sp.	TEM-1	−	P/HI2	+	82	NR	1

[a] Hybridization with *spvCD-orfE* probe.
[b] +, positive; −, negative for the character tested.
[c] Conjugational transfer of amoxicillin resistance.
[d] NR, different by more than three electrophoretic bands.
[e] ND, not determined.
[f] RepFIC subgroup A or B.

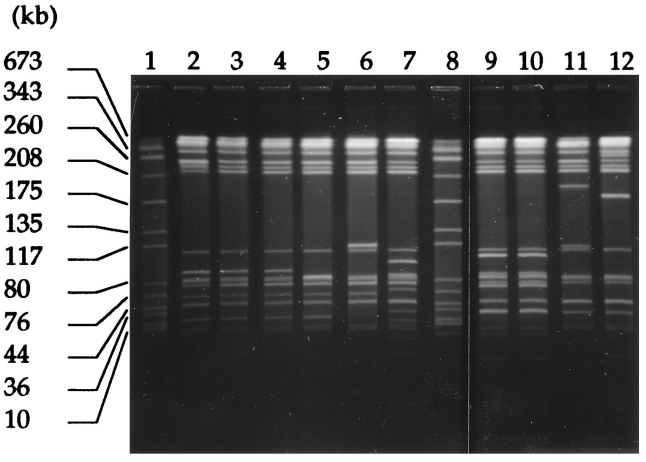

FIG. 3. *Xba*I macrorestriction patterns of selected *S. typhimurium* isolates. Lanes 1 and 8, DNA marker fragments of *S. aureus* NCTC 8325 (sizes in kbp are indicated on the left of the gel); lanes 2 to 5, pulsotypes F (isolates S88, S16, S18, and S23); lanes 6, 7, 11, and 12, unrelated pulsotypes (S82, S22, S72, and S5); lanes 9 and 10, pulsotypes C (S41 and S51).

chloramphenicol and tetracycline resistance were suspected to reside on transposons. As shown by conjugational transfers (in 15 of 41 isolates), the RepFIC virulence/resistance plasmids characterized in this work confered multiresistance to chloramphenicol, spectinomycin, streptomycin, sulfadiazine, and tetracycline, in addition to β-lactam resistance. Therefore, it is tempting to assume that the plasmidic genes that determine these resistances are carried by integrons and/or transposons able to jump from the chromosome to resident plasmids and vice versa (29). There is increasing evidence that integrons are responsible for the acquisition and dissemination of resistance genes among *Salmonella* serovars through plasmid transfers. According to Tosini et al. (42), RepFI (including RepFIC) plasmids are frequent vehicles of class 1 integrons, which possibly explains their molecular evolution. The fact that the virulence plasmid of *S. typhimurium* may serve as a carrier for resistance genes is, however, unprecedented in DT104 isolates. The implications of this finding are not clear, but this finding raises the question of what role antibiotics play as selective agents in the possible dissemination of such virulence/resistance plasmids among gram-negative enteric bacteria. Interestingly, the locations of resistance genes on virulence plasmids in *S. typhimurium* isolate phage type 193 (40) and *Shigella dysenteriae* type 1 have recently been described (8).

The bacterial reservoir of the $bla_{\text{PSE-1}}$ gene (equivalent to $bla_{\text{CARB-2}}$) also remains unclear, since PSE-1/CARB-2 producers are infrequently encountered among enteric gram-negative bacteria such as *E. coli* (38) and *Shigella* sp. (20), in contrast to *P. aeruginosa* (2). Our sequencing results demonstrate that the β-lactamase gene carried by the RepFIC virulence/resistance plasmids of *S. typhimurium* is highly homologous to that previously detected in *P. aeruginosa* plasmid RPL11. Possible transfers of plasmids between *Salmonella* serovars and *P. aeruginosa* must be confirmed, since these species do not occur in similar ecological niches (20).

The second group of amoxicillin-resistant *S. typhimurium* isolates ($n = 54$) produced TEM-1 enzyme. In striking contrast to the PSE-1-producing isolates, members of the TEM-1 group showed a great genomic diversity when examined by PFGE (Table 3). Furthermore, the $bla_{\text{TEM-1}}$ gene was detected on transferable plasmids (in 32 of 35 isolates) with dissimilar phenotypic and genotypic features. The transferability of R plasmids coding for TEM-1 β-lactamase was also a common feature for amoxicillin-resistant *Salmonella* other than *S. typhimurium* (17 of 19) (Table 4). The observation that TEM-1 β-lactamase is met with increasing frequencies in other gram-negative enteric species, such as *E. coli* (31), suggests that *Salmonella* may inherit the $bla_{\text{TEM-1}}$ gene from the intestinal flora of humans (25) or animals. Indeed, transfers of amoxicillin resistance between *S. enteritidis* and *E. coli* have recently been demonstrated to occur in vivo (3).

Altogether, our results reinforce the notion that *Salmonella* serovars may efficiently acquire β-lactamase genes from various bacterial sources and that the increasing prevalence of amoxicillin resistance in these bacteria is part of a global trend that involves many other gram-negative species producing TEM-1 enzyme. In contrast, the development of β-lactam resistance due to the production of PSE-1 enzyme results from the dissemination of a few epidemic clones into the population.

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