ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2002, p. 2821–2828 Vol. 46, No. 9
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.9.2821–2828.2002

Antibiotic Resistance Genes and *Salmonella* Genomic Island 1 in *Salmonella enterica* Serovar Typhimurium Isolated in Italy

Alessandra Carattoli,[1]* Emma Filetici,[1] Laura Villa,[1] Anna Maria Dionisi,[1] Antonia Ricci,[2] and Ida Luzzi[1]

Laboratory of Bacteriology and Medical Mycology, Istituto Superiore di Sanità, Rome,[1] and National Reference Laboratory for Salmonella, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro,[2] Italy

Received 4 September 2001/Returned for modification 20 October 2001/Accepted 14 June 2002

Fifty-four epidemiologically unrelated multidrug-resistant *Salmonella enterica* serovar Typhimurium isolates, collected between 1992 and 2000 in Italy, were analyzed for the presence of integrons. Strains were also tested for *Salmonella* genomic island 1 (SGI1), carrying antibiotic resistance genes in DT104 strains. A complete SGI1 was found in the majority of the DT104 strains. Two DT104 strains, showing resistance to streptomycin-spectinomycin and sulfonamides, carried a partially deleted SGI1 lacking the *flo*$_{st}$, *tetR*, and *tetA* genes, conferring chloramphenicol-florfenicol and tetracycline resistance, and the integron harboring the *pse-1* gene cassette, conferring ampicillin resistance. The presence of SGI1 was also observed in serovar Typhimurium strains belonging to other phage types, suggesting either the potential mobility of this genomic island or changes in the phage-related phenotype of DT104 strains.

Salmonella enterica is one of the most common pathogens causing food-borne infections in Italy (10). The epidemic strain serovar Typhimurium definitive phage type 104 (DT104) has been identified as a major cause of salmonellosis in humans and animals, in both Europe (13, 21, 32) and the United States (1, 12, 30).

The majority of the DT104 isolates are characterized by resistance to six drugs: ampicillin (A), chloramphenicol (C), streptomycin (S), spectinomycin (Sp), sulfonamides (Su), and tetracycline (T) (23, 31). Between 80 and 90% of isolates of DT104 show this resistance type (33).

The resistance mechanism in ACSSpSuT-type DT104 has been recently elucidated, and resistance genes have been described, all located within the same chromosomal locus, designated *Salmonella* genomic island 1 (SGI1) (2, 3). SGI1 is a 43-kb genomic island, showing 44 coding sequences, most of them encoding hypothetical proteins. SGI1 shows two 18-bp direct repeats at the external boundaries, which strongly supports the hypothesis that site-specific recombination events may have driven the insertion of the island within the serovar Typhimurium chromosome (2, 3).

The ACSSpSuT resistance genes have been identified within a 14-kb region in SGI1, carried by two class 1 integrons, InC and InD, encoding the aminoglycoside resistance gene *aadA2* and the β-lactamase *pse-1* gene, respectively (4). The intervening region, encompassing the two integrons, contains the *floR* gene, conferring resistance to florfenicol-chloramphenicol, and the *tetR* and *tetA* (class G) genes, conferring tetracycline resistance (2, 3, 4). InC and InD show a peculiar structure with respect to other class 1 integrons (19, 20, 29). InC carries the *qacEΔ1* gene, conferring resistance to disinfectants, and a truncated, nonfunctional *sul1* gene in the 3′-conserved segment (3′-CS). InD shows a deletion of the integrase gene (*intI1*) which is linked to the *groEL* gene in the 5′-conserved segment (5′-CS) (2, 3, 4); the *qacEΔ1* gene, the *sul1* gene, conferring sulfonamide resistance, and the two open reading frames of unknown function, ORF5 and ORF6, are contained in the 3′-CS followed by the IS*6100* element (20). The multidrug resistance region in SGI1 is bounded by characteristic 25-bp inverted repeats and flanked by a direct duplication of 5 bp of the target sequence, indicating that it was inserted by a transpositional mechanism (EMBL accession no. AF261825) (19, 20).

The aim of our study was to analyze the molecular basis of antibiotic resistance by searching for, identifying, and characterizing integrons and other resistance genes in multidrug-resistant serovar Typhimurium isolated in Italy from humans and food animals. Strains were also analyzed for the presence and conservation of SGI1.

MATERIALS AND METHODS

Bacterial strains. Fifty-four apparently epidemiologically unrelated serovar Typhimurium isolates of human and animal origin, representative of frequent phage types and recurrent multidrug resistance profiles in Italy (10), were chosen from the collections of the Istituto Zooprofilattico delle Venezie (Padua, Italy) and of the Istituto Superiore di Sanità (Rome, Italy). Serotypes were determined with anti-O and anti-H antisera obtained from Behringwerke AG (Marburg, Germany). Phage types were determined according to the work of Callow (5), and antibiotic resistance was determined by the disk diffusion assay on Mueller-Hinton agar with commercial antimicrobial susceptibility disks (Oxoid, Basingstoke, United Kingdom; Becton Dickinson Microbiological Systems, Cockeysville, Md.), according to the recommendations of the National Committee for Clinical Laboratory Standards (18). The following antibiotics were tested: ampicillin, ceftazidime, chloramphenicol, streptomycin, spectinomycin, sulfonamides, tetracycline, trimethoprim (Tp), kanamycin (K), gentamicin (G), tobramycin (N), amikacin, nalidixic acid, and ciprofloxacin. Strain ST30 was assigned to PT (phage type) U302 by John E. Threlfall (Public Health Laboratory Service, Colindale, London, United Kingdom).

Preparation of total DNA and Southern blot hybridization. Small-scale DNA preparations were made with 3 ml of bacterial liquid cultures grown overnight, as described by Ezaki and colleagues (9). Four micrograms of total DNA was digested with *Pvu*II-*Bam*HI or *Xba*I restriction enzymes. Restricted fragments

* Corresponding author. Mailing address: Laboratory of Bacteriology and Medical Mycology, Istituto Superiore di Sanità, v.le Regina Elena 299, 00161 Rome, Italy. Phone: 39-06-49903128. Fax: 39-06-49387112. E-mail: alecara@iss.it.

TABLE 1. Primers used in this study

Primer	DNA sequence	Amplicon in Fig. 1	EMBL accession no., nucleotide positions
DR-FW	5′-GGGCAAAGCGCAGCTATTAG-3′		AF261825, 13–32
S004-RV	5′-CCCGCAGGGTAAGTAATG-3′	PCR1	AF261825, 3275–3258
S011-FW	5′-CGCCGGCTCCAAAGGAAATGG-3′		AF261825, 11764–11784
S014-RV	5′-AATTTCCTCATCGTCTAGC-3′	PCR2	AF261825, 14859–14842
S014-FW	5′-AGTCTGTGGCATGAAGAA C-3′		AF261825, 14521–14539
S022-FW	5′-CGCTGCAAGCACAATGATGA-3′		AF261825, 18301–18320
S024-RV	5′-GGTACGGTATCGCCTAAGTG-3′	PCR3	AF261825, 21930–21911
S026-FW	5′-TCGGGTAATCTCAGCAGAGC-3′		AF261825, 25021–25040
int-RV	5′-GGGCATGGTGGCTGAAGGACC-3′	PCR4	AF261825, 27266–27246
5′-CS	5′-GGCATCCAAGCAGCAAG-3′		AF261825, 27892–27908
3′-CS	5′-AAGCAGACTTGACCTGA-3′		AF261825, 28900–28884
flo-FW	5′-ATGACCACCACACGCCCCG-3′		AF261825, 30482–30500
flo-RV	5′-CTAGACGACTGGCGACTTC-3′	PCR5	AF261825, 31696–31678
tetR-FW	5′-CTGCTGATCGTGGGTCT-3′		AF261825, 32657–32673
tetA-RV	5′-TTGCGAATGGTCTGCGT-3′	PCR6	AF261825, 333797–33781
aadA2-FW	5′-GAGCGCCATCTGGAATCAACG-3′		AF261825, 28058–28078
ORF5-RV	5′-CCGAACGTTCGGAGGCTCCT-3′	PCR7	AF261825, 39750–39731
S044-FW	5′-ACCAGAGAGAGTTATCGAGC-3′		AF261825, 41911–41930
DR-RV	5′-CACGAAAAGGAGACGATGAGA-3′	PCR8	AF261825, 44483–44463
strAF	5′-AGCAGAGCGCGCCTTCGCTG-3′		NC_001740.1, 761–780
strAR	5′-CCAAAGCCCACTTCACCGAC-3′		NC_001740.1, 1464–1445

were separated by 1% agarose gel electrophoresis and transferred onto positively charged nylon membranes (Roche Diagnostics, Monza, Italy) by standard methods (24). Southern blot hybridization was carried out under high-stringency conditions (24). A specific probe for the *intI1* gene was obtained as previously described (6). The S014 and the PCR8 probes were obtained by PCR amplification with S014-FW–S014-RV and S044-FW–DR-RV primer pairs, respectively (Table 1). DNA probes were [α-^{32}P]dCTP labeled with a random priming kit (Life Technologies, Milan, Italy).

PCR amplification, cloning, and sequencing. Standard PCR amplifications were performed with primers listed in Table 1 and 2.5 U of *Taq* DNA polymerase (Roche Diagnostics), according to the manufacturer's recommendations. All PCR amplifications were run at 94°C for 30 s, 55°C for 30 s, and 72°C for 3 min, for a total of 30 cycles. Amplification products were sequenced (26) with fluorescent dye-labeled dideoxynucleotides and a 373 automatic DNA sequencer (Perkin-Elmer, Foster City, Calif.). Comparative analysis of nucleotide sequences was performed by the advanced BLAST search program 2.0 within the QBLAST system at the National Center for Biotechnology Information site (www.ncbi.nlm.nih.gov/blast/).

Pulsed-field gel electrophoresis (PFGE). Preparation of total DNA was performed as described by R. K. Gautom (11) except that 0.5 mg of proteinase K per ml was dissolved in the bacterial suspension before addition of 1% melted agarose containing 1% sodium dodecyl sulfate. DNA plugs were digested with *Xba*I (Roche Diagnostics). Electrophoresis was performed in a CHEF-DRII electrophoresis system (Bio-Rad Laboratories) at 14°C with 0.5× Tris-borate-EDTA running buffer and 1% pulsed-field certified agarose (Bio-Rad Laboratories). Electrophoresis conditions were as follows: phase 1, initial switch time, 0.5 s; final switch time, 60 s for 24 h, with 6 V cm^{-1}. For visualization, gels were stained in ethidium bromide, destained in water, and photographed.

RESULTS

Detection of integrons in multidrug-resistant serovar Typhimurium isolated in Italy. Forty-one isolates of DT104, 33 of resistance type ACSSpSuT and 8 with different resistance profiles, and 13 resistant isolates of other phage types (Table 2) were analyzed for the presence of class 1 integrons. The integron search was performed by Southern blot hybridization with the *intI1* gene probe (6), on total DNA digested with *Pvu*II-*Bam*HI restriction enzymes. This hybridization is expected to produce, in DT104 strains carrying SGI1, two integrase-positive restriction fragments of 7,738 bp (band A) and 4,402 bp (band B) (Fig. 1A). Band A contains InC and the *floR*, *tetA*, and *tetR* resistance genes, from the *Pvu*II site at position 27276 to the *Pvu*II site at position 35014 (EMBL accession no. AF261825). Band B contains InD from the *Pvu*II site at position 35546 to the *Bam*HI site at position 39948 in the SGI1 DNA sequence (Fig. 1A). As expected, all ACSSpSuT DT104 strains showed both bands, indicating that the SGI1 region containing the two integrons was well conserved (Fig. 2). Interestingly, the A and B bands were also observed in a strain belonging to DT1 (ST9) and in a PT U302 strain (ST30), both showing the ACSSpSuT resistance profile (Fig. 2).

Three DT104 strains showing resistance types SSpSu (ST2 and ST22) and ASSpSuT (ST48) were positive for a *Pvu*II-*Bam*HI fragment of approximately 3,700 bp (Fig. 2). This band indicates the presence of an integron located within a different genetic structure from that of the DT104 SGI1. Integron-positive plasmids were not found in these strains, suggesting that integrons carried by ST2, ST22, and ST48 are located on the bacterial chromosome.

Strain ST4, showing the ASSpSuGKTp resistance type, was positive for two bands of approximately 4,300 and 800 bp, suggesting the presence of two integrons. All the other serovar Typhimurium strains were negative for the presence of integrons (Fig. 2).

Antibiotic resistance genes. Integron-borne gene cassettes were amplified with the 5′-CS and 3′-CS primer pair (16). All DT104 strains showing the ACSSpSuT resistance profile produced two PCR products of 1,008 and 1,133 bp. By DNA sequencing, these bands corresponded to the well-characterized *aadA2* and *pse-1* gene cassettes of InC and InD as previously described (Table 2) (25). The same gene cassettes were amplified and sequenced from strains ST9 (DT1) and ST30 (U302).

Strains ST2, ST22, and ST48 produced an amplicon of about 1,000 bp. These amplicons were fully sequenced. The nucleotide sequence of the ST48 integron revealed the presence of the *aadA1* gene cassette, encoding streptomycin-spectinomycin resistance. This gene cassette was 99.9% identical, without

TABLE 2. Characteristics of the 54 serovar Typhimurium isolates tested in this study[a]

Source, yr	No.	Phage type	Antibiotic resistance	Integron-borne gene cassette(s)	*strA* gene	SGI1	Designation(s)
Human, 1992	2	DT104	ACSSpSuT	*aadA2, pse-1*	Neg	Pos	ST7, ST41
Human, 1993	4	DT104	ACSSpSuT	*aadA2, pse-1*	Neg	Pos	ST23, ST24, ST26, ST28
Human, 1994	3	DT104	ACSSpSuT	*aadA2, pse-1*	Neg	Pos	ST29, ST32, ST33
Human, 1994	1	DT104	ACSSpSuT	*aadA2, pse-1*	Neg	SGI1 partially deleted	ST37
Animal, 1994	2	DT104	ACSSpSuT	*aadA2, pse-1*	Neg	Pos	ST27, ST31
Human, 1995	3	DT104	ACSSpSuT	*aadA2, pse-1*	Neg	Pos	ST11, ST39, ST40
Human, 1996	6	DT104	ACSSpSuT	*aadA2, pse-1*	Pos	Pos	ST5, ST12, ST13, ST14, ST17, ST21
Human, 1997	1	DT104	ACSSpSuT	*aadA2, pse-1*	Neg	Pos	ST1
Animal, 1998	1	DT104	ACSSpSuT	*aadA2, pse-1*	Neg	Pos	ST6
Human, 1999	1	DT104	ACSSpSuT	*aadA2, pse-1*	Pos	Pos	ST43
Animal, 1999	3	DT104	ACSSpSuT	*aadA2, pse-1*	Pos	Pos	ST42, ST47, ST52
Human, 2000	2	DT104	ACSSpSuT	*aadA2, pse-1*	Pos	Pos	ST45, ST46
Animal, 2000	4	DT104	ACSSpSuT	*aadA2, pse-1*	Neg	Pos	ST50, ST51, ST53, ST54
Animal, 1999	1	DT104	ASSpSuT	*aadA1*	Pos	Neg	ST48
Human, 1997	1	DT104	SSpSu	*aadA2*	Pos	SGI1 partially deleted	ST2
Animal, 1998	1	DT104	SSpSu	*aadA2*	Pos	SGI1 partially deleted	ST22
Human, 1994	1	DT104	ASSuT	Neg	Pos	Neg	ST38
Animal, 1999	2	DT104	ASSuT	Neg	Pos	Neg	ST44, ST49
Human, 1995	1	DT104	AST	Neg	Pos	Neg	ST10
Animal, 1993	1	DT104	SSuTp	Neg	Pos	Neg	ST25
Animal, 1994	1	U302	ACSSpSuT	*aadA2, pse-1*	Neg	Pos	ST30
Human, 1995	1	DT1	ACSSpSuT	*aadA2, pse-1*	Pos	Pos	ST9
Human, 1997	1	RDNC	ASSpSuGKTp	*aadB, oxaI-aadAI*	Neg	Neg	ST4
Human, 1995	1	DT1	S	Neg	Neg	Neg	ST8
Human, 1996	1	DT12	ASSuTTp	Neg	Pos	Neg	ST15
Human, 1994	1	DT179	AST	Neg	Pos	Neg	ST35
Human, 1994	1	DT194	AST	Neg	Pos	Neg	ST34
Human, 1996	1	DT194	AST	Neg	Pos	Neg	ST20
Human, 1997	1	DT208	ASSuT	Neg	Pos	Neg	ST3
Human, 1996	1	DT208	AST	Neg	Pos	Neg	ST19
Human, 1994	1	DT208	ASTTp	Neg	Pos	Neg	ST36
Human, 1996	1	UT	AST	Neg	Pos	Neg	ST18
Human, 1996	1	UT	SGNK	Neg	Pos	Neg	ST16

[a] RDNC, reacts but does not conform to any standard phage type; UT, phage untypeable; Neg, negative; Pos, positive.

amino acid changes, to the *aadA1* gene cassette identified within the In2 integron carried by the Tn*21* transposon (EMBL accession no. AF071413) (17).

Nucleotide sequences of amplicons obtained from both ST2 and ST22 strains showed the presence of the *aadA2* gene cassette, encoding streptomycin-spectinomycin resistance. These gene cassettes were 100% (ST2) and 99.9% (ST22) identical to the *aadA2* gene cassette carried by InC in SGI1 (EMBL accession no. AF261825). In the ST22 DNA sequence, a single point mutation at position 28211 was identified, resulting in an amino acid change (from glutamic acid to lysine) in the deduced protein sequence. The same substitution was previously described for the DT104 strain H3380 (EMBL accession no. AF071555) (4).

Two PCR products of 2,000 and 850 bp were obtained from strain ST4. The DNA sequence of the 850-bp amplicon revealed the presence of the *aadB* gene cassette, conferring resistance to kanamycin and gentamicin, while the DNA sequence of the 2,000-bp amplicon revealed the integron-borne *oxa1* and *aadA1* gene cassettes, conferring ampicillin and streptomycin-spectinomycin resistance, respectively.

Serovar *Typhimurium* strains were then tested for *strA*, a streptomycin resistance gene that is an alternative to the integron-borne *aadA* gene cassette (28). Positive PCR amplifications with the strAF-strAR primer pair (Table 1) were obtained from 21 of the 54 strains (Table 2), including the integron-negative DT104 strains ST25, ST38, ST10, ST44, and ST49 and the ampicillin-, tetracycline-, and chloramphenicol-susceptible DT104 strains ST2, ST22, and ST48.

Analysis of SGI1. In order to gather more information on the presence and conservation of SGI1 in the DT104 strains, Southern blot hybridization and PCR amplification experiments were performed.

SGI1 was initially sought by using Southern blot hybridization with the *intI1* probe on total DNA restricted with *Xba*I, in DT104 strains ST2 and ST22 lacking the A and B *Pvu*II-*Bam*HI bands, and in the ST9 strain (DT1), which tested positive for the presence of SGI1. ST1 and ST13 were used as positive controls and ST25, ST38, and ST3 were used as negative controls in these experiments.

As shown in Fig. 3, ST1 and ST13 produced an integrase-positive band of 11,758 bp (band C in Fig. 1A), as expected from the *Xba*I sites located at positions 26038 and 37796 in the SGI1 DNA sequence. This band was also observed in ST9, while ST2 and ST22 showed a different band of approximately 7,000 bp (band E in Fig. 3). As expected, band C was missing in the three strains lacking integrons (ST25, ST38, and ST3).

The *Xba*I Southern blot was then hybridized with the S014 probe, located in the left arm of SGI1. This probe recognized the 8,921-bp *Xba*I restriction fragment (band D in Fig. 1A) in

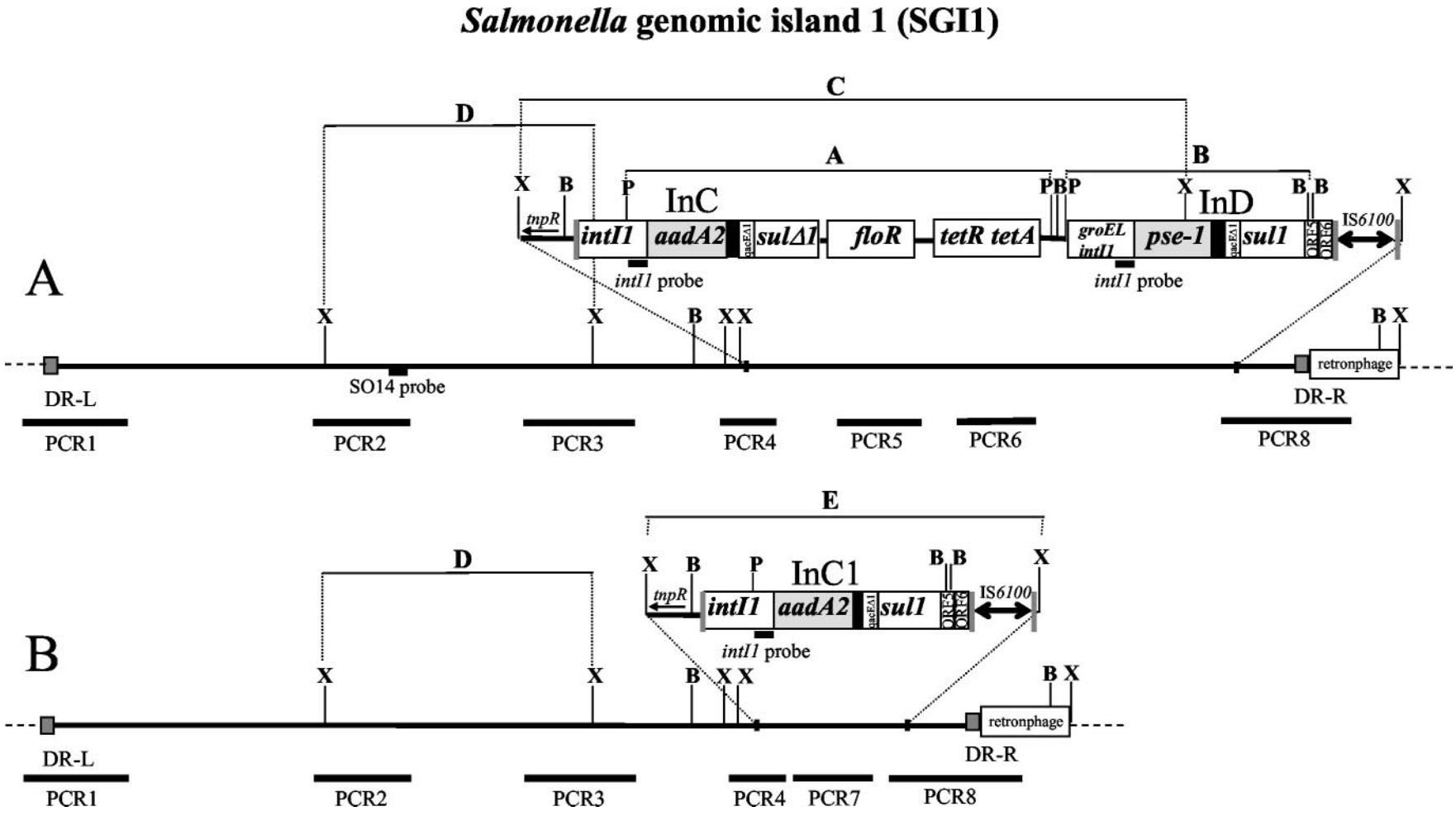

FIG. 1. Schematic representation of the entire and partially deleted SGI1. (A) *Xba*I (X), *Bam*HI (B), and *Pvu*II (P) restriction map was deduced from the DT104 DNA sequence released under EMBL accession no. AF261825. (B) Restriction map of the partially deleted SGI1 was experimentally determined. White boxes represent *intI1*, *qacEΔ1*, *sul1*, *floR*, *tetR*, *tetA*, ORF5, and ORF6 genes. The IS*6100* position is indicated between two arrows. The shaded boxes represent gene cassettes inserted into the InC and InD integrons. Black boxes are the 59-base element sites, and DR-R and DR-L boxes represent the direct repeat junctions of SGI1 in the *Salmonella* chromosome (dotted lines). Thin bars represent Southern blot hybridization bands A, B, C, and D. Heavy lines represent PCR amplicons (PCR1 to PCR8). The positions of the *intI1* gene and S014 probes are indicated.

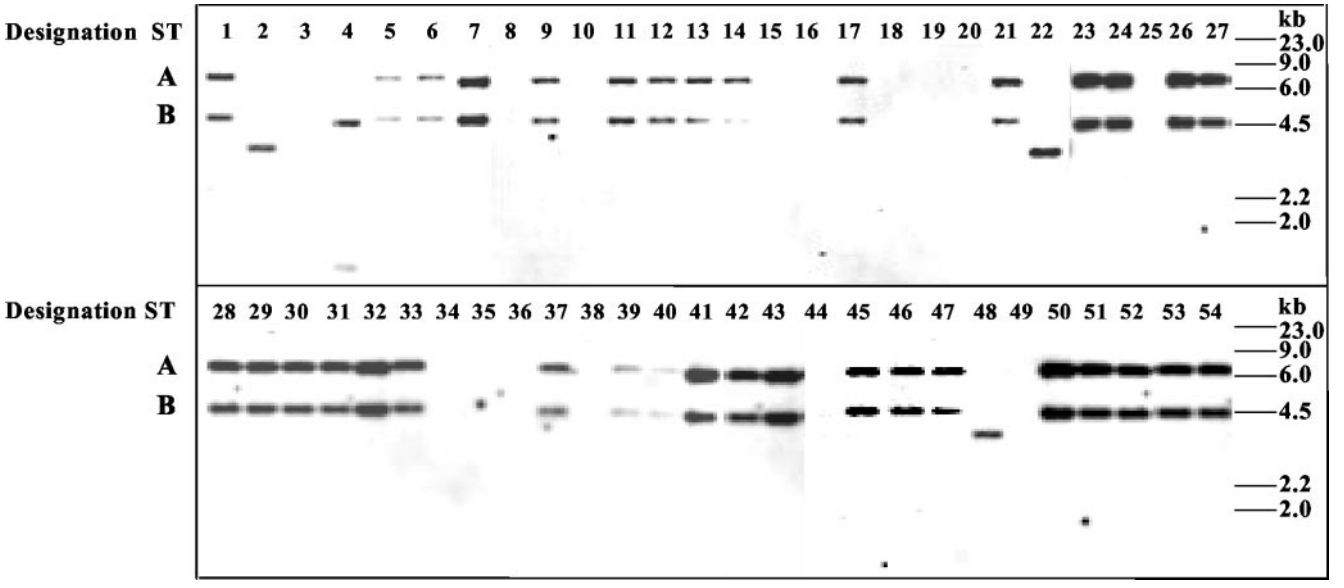

FIG. 2. Southern blot hybridization of serovar Typhimurium strains by the integrase gene. Total DNAs restricted with *Bam*HI-PvuII were separated by 1% agarose gel electrophoresis, blotted onto nylon membranes, and hybridized with an *intI1* gene-specific probe (6). Numbers above lanes indicate the designation number for each strain. The positions of bands A and B are indicated. Molecular size standards are shown on the right.

both ST2 and ST22 as well as in ST1, ST13, and ST9 (Fig. 3), revealing that this region of SGI1 was well conserved in these strains. Finally, ST2, ST22, and ST1 *Bam*HI-restricted DNAs were hybridized with the PCR8 amplification product as probe (Fig. 1). This probe recognized in both ST2 and ST22 as well as the ST1 positive control strain the 4,699-bp *Bam*HI restriction fragment containing ORF5, ORF6, IS*6100* of InD, and part of the retron phage (data not shown).

To fully characterize the conservation and structure of SGI1 in ST2 and ST22, PCR was performed with primer pairs listed in Table 1 (PCR amplifications relative to the SGI1 map are illustrated in Fig. 1). PCR experiments performed on the left (PCR1) and right (PCR8) direct repeat junctions of SGI1 and on other SGI1 regions (PCR2, PCR3, and PCR4) demonstrated that ST2 and ST22 produced indistinguishable bands with respect to the ST24 control strain (Fig. 4). In particular, PCR4, obtained with primers S026-FW and int-RV, demonstrated that integrons in ST2 and ST22 were located within SGI1, linked to the S026 DNA sequence. However, PCR amplifications with flo-FW–flo-RV and tetA-FW–tetR-RV primer pairs (PCR5 and PCR6) were negative in ST2 and ST22, demonstrating that these strains do not carry the *floR*

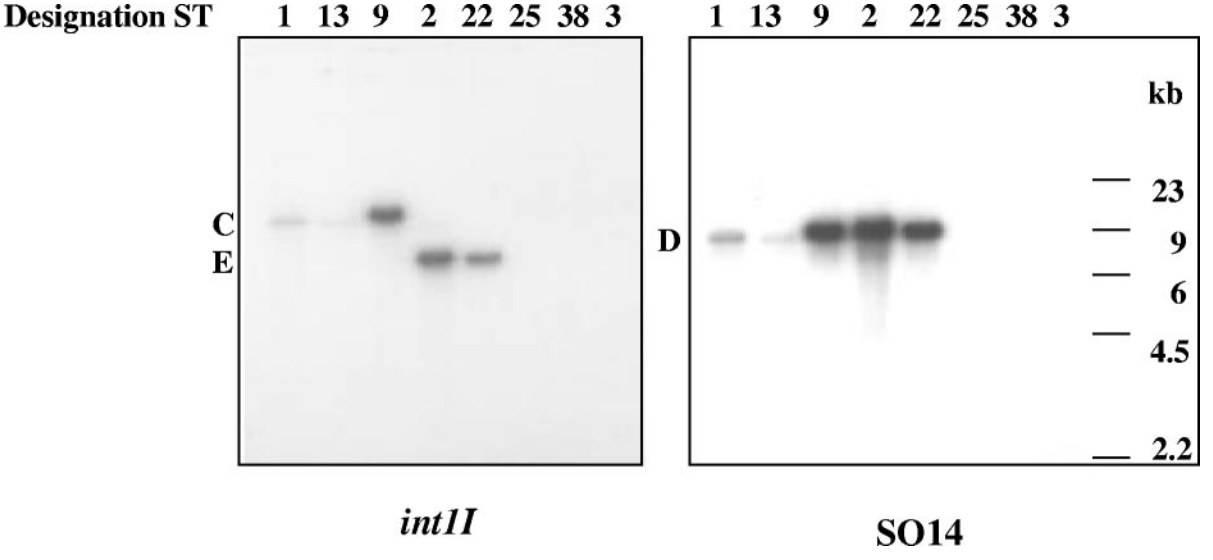

FIG. 3. Southern blot hybridization of the SGI1 island. Total DNAs restricted with *Xba*I were separated by 0.8% agarose gel electrophoresis, blotted onto nylon membranes, and hybridized with the *intI1* gene probe and with the S014 probe. Numbers above lanes indicate the designation number for each strain. The positions of bands C, D, and E are indicated. Molecular size standards are shown on the right of the figure.

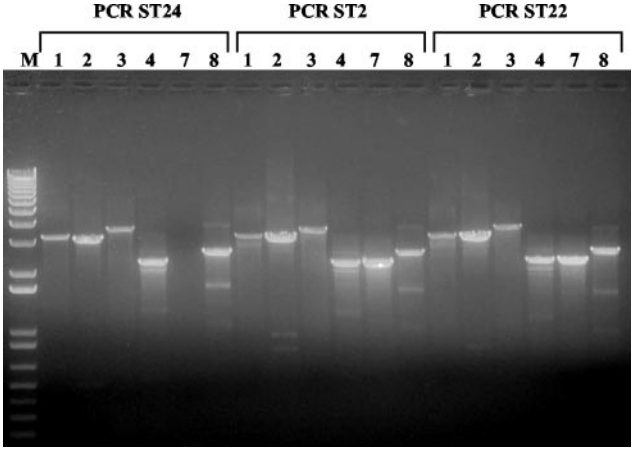

FIG. 4. PCR analysis of SGI1. PCR1 to PCR8 were performed on the ST2 and ST22 strains. Strain ST24 was used as DT104 positive control. The amplification product PCR7 (ca. 12 kb) was not obtained from the ST24 DT104 control strains, since no long-run PCR conditions were used in these experiments. A 1-kb marker (KiloBase DNA marker; Pharmacia Biotech, Milan, Italy) was used as a standard.

and the *tetA* and *tetR* genes, respectively (data not shown). Finally, ST2 and ST22 produced a PCR product of approximately 2,000 bp (Fig. 4) with the aadA2-FW–ORF5-RV primer pairs designed on the *aadA2* gene cassette and on the ORF5 DNA sequence, respectively (PCR7, Fig. 1B). This amplicon is about 10 kb shorter than the expected amplicon for complete SGI1 (ca. 12 kb). Southern blot hybridization and PCR amplification results demonstrate that ST2 and ST22 harbor only one of the two integrons of SGI1; this integron (InC1 in Fig. 1) carries the same *aadA2* gene cassette of InC and shows a 3′-CS identical to that of InD, carrying *qacEΔ1*, conferring resistance to disinfectants; the entire *sul1* gene, conferring sulfonamide resistance; ORF5; ORF6; and the IS*6100* element (Fig. 1B).

PCR amplification (PCR1 to PCR8) was then performed on the remaining isolates, demonstrating that, with the exception of strain ST37, all ACSSpSuT DT104 strains carried an intact SGI1 (Table 2). Strain ST37 was negative for PCR1 and PCR2 but positive for the other PCR amplifications performed on SGI1, suggesting the partial deletion of the left arm of the genomic island.

An intact SGI1 was identified in ST9 (DT1) and in ST30 (U302), while it was completely absent in ST48 (DT104), which harbors the *aadA1* integron-borne gene cassette.

PFGE of macrorestricted genomic DNA. Strains ST2 (DT104), ST22 (DT104), ST9 (DT1), and ST30 (U302) were analyzed by PFGE following digestion with *Xba*I. Strain ST24 was used as the DT104 reference control strain. The results showed that the three DT104 strains were highly related at the chromosomal level. ST30 (U302) showed a DT104-related PFGE pattern differing by three bands (Fig. 5). PT U302 has been previously shown to be closely related to DT104, and phage conversion of DT104 to U302 has been observed, possibly by the acquisition of plasmids (15, 34). The presence of a plasmid in ST30 was revealed by PFGE of undigested DNA (Fig. 5), suggesting that this plasmid could have caused a DT104-U302 phage conversion in this strain. In contrast, ST9 (DT1) was divergent at the chromosomal level from DT104, differing by at least four bands in the PFGE pattern (Fig. 5). In this case the presence of the DT104 resistance traits could be explained by the horizontal transfer of SGI1.

DISCUSSION

Several studies have focused on resistance genes in DT104 strains, and new molecular methods for detection and analysis of the DT104 clone have been proposed elsewhere (8, 14). Most of these methods are based on PCR amplification of DT104-related traits, such as the 1.0- and 1.2-kb integrons or the *floR* resistance gene located within the resistance gene cluster (22).

More recently, the cloning and sequencing of the entire genomic island from multidrug-resistant DT104 have opened new possibilities for investigating genetic characteristics of this important and widely diffused *Salmonella* clone. However, complete information is not available for the conservation of SGI1 among strains circulating in animals and humans, and the frequency of transfer of the genomic island to serovar Typhimurium strains of other phage types has not been quantified. This information could be important to understanding the origin and evolution of the genomic island and potential horizontal mobility of SGI1.

To study antibiotic resistance genes carried by integrons and to investigate the presence of SGI1, multidrug-resistant serovar Typhimurium strains of different phage types of both human and animal origin were analyzed by both PCR amplification and Southern blot hybridization. All DT104 strains showing the ACSSpSuT resistance profile harbor the two integrons InC and InD, located within SGI1. Two DT104 strains, isolated in Italy in 1997 (ST2) and in 1998 (ST22) and showing the SSpSu resistance profile, revealed the presence of a partially deleted SGI1. These isolates lack the region of the island encoding resistance to chloramphenicol-florphenicol and tetracycline and also the InD integron carrying the *pse-1* ampicillin resistance gene. These two DT104 strains carried the InC1 integron, which shows a 3′-CS, including the *qacEΔ1*, *sul1*, ORF5, ORF6, and IS*6100* DNA sequences. This integron was found in an *Xba*I integrase gene-positive band smaller than that observed in DT104 control strains.

The ST2 and ST22 resistance island may represent the precursor of the DT104 resistance gene cluster in which InC1 is the unique integron harbored by the genomic island. Our findings suggest that the assembly of the resistance gene cluster within SGI1 could be due to sequential acquisition of resistance determinants. In fact, InC could derive from InC1 by deletion of part of the 3′-CS caused by the insertion of the *floR* and *tet* genes. The second integron, InD, could also have been acquired through an independent integration event, leading to the full assembly of the resistance gene cluster. The deleted SGI1 could then represent the precursor of the genomic island before the assembly of InD, *floR*, and *tet* genes. However, the genetic structure observed in ST2 and ST22 could also be explained by homologous recombination between InC and InD in the 3′-CS. Recombination between the two *sul1* genes could lead to the deletion of a 10-kb DNA region containing the *floR*, *tet*, *intI1*, and *pse-1* genes and to the reconstruction of InC1 carrying the 3′-CS of InD.

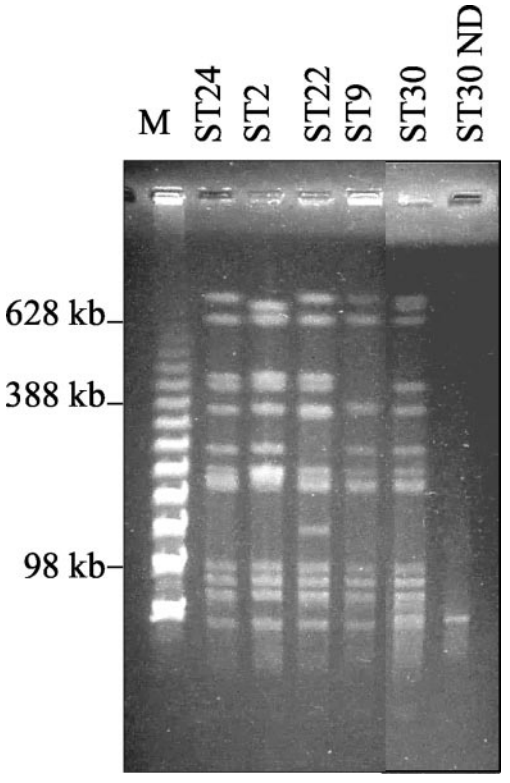

FIG. 5. Analysis by PFGE. Serovar Typhimurium DNAs were digested by *Xba*I and separated by PFGE on a 1% agarose gel. Designation numbers of tested strains are reported above each lane. Lane M, the molecular size lambda ladder ranged from 48.5 to 1,000 kb (Roche Diagnostics). ND, not digested.

A different deletion in the genomic island was observed in the ST37 ACSSpSuT DT104 strain, lacking the left arm of SGI1. These results demonstrate that several rearrangements could occur in SGI1, suggesting a constant and dynamic evolution of this genetic trait.

In another DT104 strain (ST48) an integron that carried the *aadA1* gene cassette was found to be located in the chromosome. This strain lacked SGI1, demonstrating that integrons other than InC and InD can be acquired by DT104 strains. Most of the DT104 strains showing different resistance profiles than the ACSSpSuT resistance type lack SGI1 but contain the *strA* resistance gene, conferring streptomycin resistance. ST2, ST22, and ST48, as well as many DT104 strains harboring the entire SGI1, also carried the *strA* gene, suggesting that multiple mechanisms of resistance can be simultaneously present in these strains.

Of note, the entire SGI1 was found in the chromosome of ST9 (DT1) and in the ST30 (U302) isolate, suggesting the potential capacity of mobilization of SGI1. However, recent studies have demonstrated that strains of U302, DT120, and DT12 of the ACSSpSuT resistance type are probably derived from DT104 by a change of phage sensitivity (15). This possibility should be taken into consideration. The presence of the DT104 resistance genes has also been previously reported for several serovar Agona strains (2, 7). However, the multiresistant serovar Agona strains lacked the retron phage at the DR-R boundary of SGI1, while results with serovar Typhimurium strains ST2 and ST22 indicate that in these strains SGI1 is located between the *thdf* gene and the retron phage, a situation similar to that described for the multiresistant DT104 strain. There is no experimental evidence demonstrating the molecular mechanism of the SGI1 horizontal transfer among *Salmonella* strains, although transduction experiments with a P22-like phage demonstrated a facilitated transduction of resistance genes to a susceptible *Salmonella* strain (27).

Multidrug resistance in serovar Typhimurium is now a cause of great concern in both clinical and veterinary medicine. Studies of evolution and dissemination of resistance determinants may help us to better understand the origin of such strains and the transmission from food animals to humans.

ACKNOWLEDGMENTS

We are grateful to John E. Threlfall for encouraging discussions, helpful suggestions, and characterization of the ST30 strain. We thank Paul D. Fey for critical reading of the manuscript; Sergio Arena, Susanna Mariotti, and Maria Giovanna Daga for technical assistance; and F. Riccobono for DNA sequencing.

This research was supported by grants to Alessandra Carattoli on the "Progetto Antibiotico Resistenza 2000" and to Ida Luzzi and Antonia Ricci on the "Ricerca Corrente 1999 e 2000" from the Italian Ministry of Health.

REFERENCES

1. **Besser, T. E., M. Goldoft, L. C. Pritchett, R. Khakhria, D. D. Hankock, D. H. Rices, J. M. Gay, W. Johnson, and C. C. Gay.** 2000. Multiresistant *Salmonella* Typhimurium DT104 infections of humans and domestic animals in the Pacific Northwest of the United States. Epidemiol. Infect. **124:**193–200.
2. **Boyd, D., G. A. Peters, A. Cloeckaert, K. S. Boumedine, E. Chaslus-Dancla, H. Imberechts, and M. R. Mulvey.** 2001. Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar Typhimurium DT104 and its identification in phage type DT120 and serovar Agona. J. Bacteriol. **183:**5725–5732.
3. **Boyd, D. A., G. A. Peters, L. K. Ng, and M. R. Mulvery.** 2000. Partial characterisation of a genomic island associated with the multidrug resistant region of *Salmonella enterica* Typhimurium DT104. FEMS Microb. Lett. **189:**285–291.
4. **Briggs, C. E., and P. M. Fratamico.** 1999. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella typhimurium* DT104. Antimicrob. Agents Chemother. **43:**846–849.
5. **Callow, B.** 1959. A new phage typing scheme for *Salmonella typhimurium*. J. Hyg. (Cambridge) **57:**346–359.
6. **Carattoli, A., L. Villa, C. Pezzella, E. Bordi, and P. Visca.** 2001. Expanding drug-resistance through integron acquisition by IncFI plasmids of *Salmonella enterica* serotype Typhimurium. Emerg. Infect. Dis. **7:**444–447.
7. **Cloeckaert, A., K. S. Boumedine, G. Flaujac, H. Imberechts, I. D'Hooghe, and E. Chaslus-Dancla.** 2000. Occurrence of a *Salmonella enterica* serovar Typhimurium DT104-like antibiotic resistance gene cluster including the *floR* gene in *S. enterica* serovar Agona. Antimicrob. Agents Chemother. **44:**1359–1361.
8. **Ebner, P. D., and A. G. Mathew.** 2001. Three molecular methods to identify *Salmonella enterica* serotype Typhimurium DT104: PCR fingerprinting, multiplex PCR and rapid PFGE. FEMS Microbiol. Lett. **205:**25–29.
9. **Ezaki, T., N. Takeuchi, S. L. Liu, A. Kai, H. Yamamoto, and E. Yabuuchi.** 1988. Small-scale DNA preparation for rapid genetic identification of *Campylobacter* species without radioisotope. Microbiol. Immunol. **32:**141–150.
10. **Fantasia, M., E. Filetici, S. Arena, and S. Mariotti.** 1998. Serotype and phage type distribution of salmonellas from humans and non-human sources in Italy in the period 1973–1995. Eur. J. Epidemiol. **14:**701–710.
11. **Gautom, R. K.** 1997. Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other gram-negative organisms in 1 day. J. Clin. Microbiol. **35:**2977–2980.
12. **Glynn, M. K., C. Bopp, W. Dewitt, P. Dabney, M. Mokhtar, and F. J. Angulo.** 1998. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. N. Engl. J. Med. **338:**1333–1338.
13. **Hancock D., T. Besser, J. Gay, D. Rice, M. Davis, and C. Gay.** 2000. The global epidemiology of multiresistant *Salmonella enterica* serovar Typhimurium DT104, p. 217–243. *In* C. Brown and C. Bolin (ed.), Emerging diseases of animals. ASM Press, Washington, D.C.

14. **Khan, A. A., M. S. Nawaz, S. A. Khan, and C. E. Cerniglia.** 2000. Detection of multidrug-resistant *Salmonella typhimurium* DT104 by multiplex polymerase chain reaction. FEMS Microbiol. Lett. **182:**355–360.
15. **Lawson, A. J., M. U. Dassama, L. R. Ward, and E. J. Threlfall.** 2002. Multiply resistant (MR) *Salmonella enterica* serotype Typhimurium DT 12 and DT 120: a case of MR DT 104 in disguise? Emerg. Infect. Dis. **8:**434–436.
16. **Levesque, C., L. Pichè, C. Larose, and P. Roy.** 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother. **39:**185–191.
17. **Liebert, C. A., R. M. Hall, and A. O. Summers.** 1999. Transposon Tn*21*, flagship of the floating genome. Microbiol. Mol. Biol. Rev. **63:**507–522.
18. **National Committee for Clinical Laboratory Standards.** 1992. Performance standards for antimicrobial disc susceptibility tests. M2A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
19. **Partridge, S. R., H. J. Brown, H. W. Stokes, and R. M. Hall.** 2001. Transposons Tn*1696* and Tn*21* and their integrons In4 and In2 have independent origins. Antimicrob. Agents Chemother. **45:**1263–1270.
20. **Partridge, S. R., G. D. Recchia, H. W. Stokes, and R. M. Hall.** 2001. Family of class 1 integrons related to In4 from Tn*1696*. Antimicrob. Agents Chemother. **45:**3014–3020.
21. **Poppe, C., N. Smart, R. Khakhria, W. Johnson, J. Spika, and J. Prescot.** 1998. *Salmonella typhimurium* DT104: a virulent and drug-resistant pathogen. Can. Vet. J. **39:**559–565.
22. **Pritchett, L. C., M. E. Konkel, J. M. Gay, and T. E. Besser.** 2000. Identification of DT104 and U302 phage types among *Salmonella enterica* serotype Typhimurium isolates by PCR. J. Clin. Microbiol. **38:**3484–3488.
23. **Ridley, A., and E. J. Threlfall.** 1998. Molecular epidemiology of antibiotic resistance genes in multiresistant epidemic *Salmonella typhimurium* DT104. Microb. Drug Resist. **4:**113–118.
24. **Sambrook, J. E., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
25. **Sandvang, D., F. M. Aarestrup, and L. B. Jensen.** 1997. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica* Typhimurium DT104. FEMS Microbiol. Lett. **157:**177–181.
26. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
27. **Schmieger, H., and P. Schicklmaier.** 1999. Transduction of multiple drug resistance of *Salmonella enterica* serovar Typhimurium DT104. FEMS Microbiol. Lett. **170:**251–256.
28. **Scholz, P., V. Haring, B. Wittmann-Liebold, K. Ashman, M. Bagdasarian, and E. Scherzinger.** 1989. Complete nucleotide sequence and gene organization of the broad-host-range plasmid RSF1010. Gene **75:**271–288.
29. **Stokes, H. W., and R. M. Hall.** 1989. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol. Microbiol. **3:**1669–1683.
30. **Tauxe, R. V.** 1999. *Salmonella* Enteritidis and *Salmonella* Typhimurium DT104: successful subtypes in the modern world, p. 37–52. *In* W. M. Scheld, W. A. Craig, and J. M. Hughes (ed.), Emerging infections 3. ASM Press, Washington, D.C.
31. **Threlfall, E. J., L. R. Ward, and B. Rowe.** 1998. Multiresistant *Salmonella typhimurium* DT104 and *Salmonella* bacteraemia. Lancet **352:**287–288.
32. **Threlfall, E. J., J. A. Frost, L. R. Ward, and B. Rowe.** 1994. Epidemic in cattle and humans of *Salmonella typhimurium* DT 104 with chromosomally integrated multiple drug resistance. Vet. Rec. **134:**577.
33. **Threlfall, E. J., J. A. Skinner, A. Graham, L. R. Ward, and H. R. Smith.** 2000. Resistance to ceftriaxone and cefotaxime in non-typhoidal *Salmonella enterica* in England and Wales, 1998–99. J. Antimicrob. Chemother. **46:**860–862.
34. **Walker, R. A., E. Lindsay, M. J. Woodward, L. R. Ward, and E. J. Threlfall.** 2001. Variation in clonality and antibiotic-resistance genes among multiresistant *Salmonella enterica* serotype Typhimurium phage-type U302 (MR U302) from humans, animals, and foods. Microb. Drug Resist. **7:**13–21.