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Tetracycline and Streptomycin Resistance Genes, Transposons, and Plasmids in *Salmonella enterica* Isolates from Animals in Italy

Cristina Pezzella,[1] Antonia Ricci,[2] Elisabetta DiGiannatale,[3] Ida Luzzi,[1] and Alessandra Carattoli[1]*

Laboratory of Bacteriology and Medical Mycology, Istituto Superiore di Sanità, Rome,[1] Istituto Zooprofilattico Sperimentale delle Venezie, Padua,[2] and Istituto Zooprofilattico Sperimentale di Abruzzo e Molise, Teramo,[3] Italy

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Fifty-eight multidrug-resistant *Salmonella enterica* strains of 20 serotypes, isolated from animal sources in Italy, were analyzed for *tet*(A) and *strA-strB*, conferring tetracycline and streptomycin resistance, respectively. The *strA* and *strB* genes were highly prevalent in *Salmonella* strains of our collection, being detected in 84% of the streptomycin-resistant strains. In many strains, the *strA* and *strB* genes were linked to a particular Tn*5393*-derivative transposon characterized by the presence of the insertion sequence IS*1133*, previously identified only in the plant pathogen *Erwinia amylovora*. Sixty-eight percent of the tetracycline-resistant strains were *tet*(A) positive, indicating that this gene is widely diffused in *Salmonella* strains circulating in animals in Italy. Most of the *tet*(A) genes were localized within a deleted Tn*1721* transposon variant. Two prevalent repN and repI1 resistance plasmids were identified in *Salmonella* isolates of our collection.

Tetracyclines are broad-spectrum agents, exhibiting activity against a wide range of gram-positive and gram-negative bacteria, and are currently used for therapy and prophylaxis for human infections and for the prevention and control of bacterial infections in veterinary medicine (17, 18, 21). Tetracyclines are also used in aquaculture and sprayed onto fruit trees and other plants to control bacterial infection (5). Streptomycin has only limited current usage in clinical medicine, but this antibiotic remains important for therapy of and growth promotion in animals and for bacterial disease control in plants (27).

The increasing incidence of resistance to streptomycin and tetracyclines in *Salmonella* spp. of human and animal origins has been reported worldwide (10, 31). Genes conferring streptomycin and tetracycline resistance in *Salmonella enterica* serotype Typhimurium definitive type 104 (DT104) have been extensively studied (3, 6), but little information on the mechanism responsible for the wide diffusion of these resistances in other phage types and serotypes is available.

Several different *tet* genes have been described as conferring resistance to tetracyclines in *Salmonella.* The most frequent types of *tet* genes belong to classes A, B, C, D, and G (5, 8). The *tet*(G) gene has been identified in salmonella genomic island 1, located within the *S. enterica* serotype Typhimurium DT104 chromosome (3, 6). The *tet* gene of class A was found on plasmids as well as on the chromosome, whereas the genes *tet*(B), *tet*(C), and *tet*(D) were detected on the chromosomes of *S. enterica* bacteria of different serotypes, including Typhimurium, Enteritidis, Hadar, Saintpaul, and Choleraesuis (10). The *tet*(A) gene is often part of transposon Tn*1721*, and a truncated version of Tn*1721* lacking a portion of the left arm has also been described to occur in *Salmonella* (1, 8, 9, 21). Recently, a new allele of *tet*(A), designated *tet*(A)-*1*, was identified on the pSSTA-1 plasmid in *Shigella* spp. (12).

A large number of genes can confer streptomycin resistance (22). Among them, the phosphotransferase *aph(6)-Ia* gene (also named *strA*) and the *aph(6)-Id* gene (also named *strB*) appear to be widely distributed in *Salmonella* and other gram-negative bacteria. *strA-strB* has been identified in bacteria circulating in humans, animals, and plants (4, 22, 25, 27, 28). These genes have been described as being part of transposon Tn*5393* and are frequently located on plasmids (26).

Integron-borne gene cassettes conferring resistance to aminoglycosides are also very diffused in gram-negative bacteria, and integrons have frequently been associated with the widely distributed transposon Tn*21* (15). The Tn*21* transposon encodes genes and sites required for transposition (including *tnpA*, *tnpR*, *tnpM*, *res*, and inverted repeats), and integrons are located in the left arm, adjacent to the *tnpM* gene. The Tn*21*-associated integrons often carry the *aadA1* gene cassette, known to confer resistance to streptomycin and spectinomycin (15).

In this study, we have examined the prevalence of the *strA*, *strB*, and *tet*(A) resistance genes and integrons conferring streptomycin and tetracycline resistance in a collection of unrelated multidrug-resistant *S. enterica* strains of different serotypes, isolated in Italy during the years 2000 and 2001.

MATERIALS AND METHODS

Bacterial strains. Totals of 392 and 386 multidrug-resistant *S. enterica* strains were isolated from animals and foods of animal origin at the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVE), located in northern Italy, during routine surveillance activity in 2000 and 2001, respectively. Fifty-seven multidrug-resistant strains were isolated at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise (IZSAM), located in central Italy, in 2001. Antibiotic susceptibility was established by the disk diffusion method (19, 20) by using 16 different antimicrobial drugs: colistin (10 μg), nalidixic acid (30 μg), ampicillin (10 μg), cefotaxime (30 μg), ciprofloxacin (5 μg), enrofloxacin (5 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), streptomycin (10 μg), sulfonamides (300 μg), tetracycline (30 μg), a combination of trimethoprim and sulfamethoxazole (1.25 and 23.75 μg, respectively), amoxicillin-clavulanic acid (30 μg), cephalothin (30 μg), and neomycin (30 μg). Neomycin breakpoints were defined according to the instructions of the manufacturer of the disk (BBL Sensi-Disk; Becton Dickinson).

All strains were serotyped by agglutination tests with specific O and H antisera

* Corresponding author. Mailing address: Laboratory of Bacteriology and Medical Mycology, Istituto Superiore di Sanità, viale Regina Elena 299, 00161 Rome, Italy. Phone: 39-06-4990-3128. Fax: 39-06-4938-7112. E-mail: alecara@iss.it.

(Staten Serum Institute, Copenhagen, Denmark) and classified according to the Kauffman-White scheme. Strains of serotype Typhimurium were phage typed according to a standard procedure (2).

Fifty-eight isolates were chosen from among the 835 multidrug-resistant strains of the collection by using the following criteria. To be included in the study, strains had to be resistant to at least three antimicrobial drugs from among aminoglycosides, tetracyclines, sulfonamides, trimethoprim, and β-lactams, and they had to be from different animal sources, representing both frequent and rare serotypes. Moreover, *Salmonella* serotype Typhimurium DT104 isolates were excluded, as were repeat isolates of the same serotype obtained in the course of an investigation or during monitoring activities.

The sources of isolation, serotypes, and patterns of resistance of the S*almonella* strains analyzed in this study are shown in Table 1.

Resistance plasmids. *Salmonella* strains were tested by conjugation with the use of E. *coli* CSH26Rif^R as the recipient strain (16). Pellets of overnight broth cultures of the donor and recipient strains were mixed at a ratio of 1:4 and incubated for 6 h at 37°C in nutrient agar. Transconjugants were cultured by plating bacteria on Luria-Bertani agar plates containing rifampin (100 μg/ml) along with streptomycin (30 μg/ml), tetracycline (30 μg/ml), or ampicillin (50 μg/ml). Transconjugant colonies were further purified by plating on selective agar media and tested for antimicrobial resistance patterns.

PCR amplification. Standard PCR amplifications were performed with the primer pairs listed in Table 2 and 2.5 U of *Taq* DNA polymerase (Roche Diagnostic, Mannheim, Germany) according to the manufacturer's recommendations. The *tet*(A) gene was searched by using the TAF-TAR, TAF-TetAR3, and TAF-TetAR2 primer pairs (Table 2). Primers TAF and TetAR2 are specific to the *tet*(A)-*1* gene variant described as occurring in the pSSTA-1 plasmid (12). All PCR amplifications consisted of a hot start cycle of 94°C for 5 min, followed by 30 cycles of a denaturation step at 94°C for 30 s, an annealing step at various temperatures for 1 min, and a polymerization step at 72°C for 1 min.

Replicon typing by PCR was performed for the repN and repI1 replicons by using the N1-N2 and I1-I2 primer pairs, respectively (Table 2).

The possibility of the presence of class 1 integrons was investigated by PCR amplification with the 5′CS and 3′CS primers as previously described (Table 2) (14). 5′CS-3′CS amplicons were purified with a PCR purification kit (QIAGEN, Milan, Italy) and sequenced by the dideoxy chain termination method with external primers. Plasmid DNA from the 17/1 and 17/19 strains were prepared with a Concert high-purity plasmid kit (Life Technologies, Milan, Italy) and directly sequenced by using primers 17/1F, 17/1R, smBF, and smAR (Table 2). DNA sequences were determined by using fluorescent-dye-labeled dideoxynucleotides and a model 373 automatic DNA sequencer (Perkin-Elmer, Foster City, Calif.).

Comparative analysis of nucleotide sequences was performed by using the advanced BLAST search program, version 2.0, within the QBLAST system at the National Center for Biotechnology Information site (www.ncbi.nlm.nih.gov/blast/).

RESULTS

Streptomycin and tetracycline resistance genes and transposons. Fifty-eight multidrug-resistant *S. enterica* strains were analyzed for the presence of genetic determinants conferring streptomycin and tetracycline resistance. Thirty-six strains were obtained from the IZSVE collection (Table 1, strains beginning with "17"), and 22 strains were obtained from the IZSAM collection (Table 1, strains beginning with "27"). Twenty different serotypes were represented in the resulting collection. The serotypes occurring most frequently were Hadar (20.7%), Blockley (10.3%), Heidelberg (10.3%), Derby (8.6%), and Typhimurium (7.0%) (Table 1). *Salmonella* serotype Typhimurium DT104 was not included in the collection.

All strains showed resistance to at least three different antimicrobials; 98 and 95% of the strains were resistant to tetracycline and streptomycin, respectively. The prevalent (60%) resistance pattern in the collection was Sm^r Te^r Am^r (Table 1).

Strains were analyzed by PCR amplification for the presence of *tet*(A) and *strA-strB*.

Of the tetracycline-resistant strains, 68% tested positive for the *tet*(A) gene, indicating that this gene is widely diffused in *Salmonella* strains circulating in animals in both northern and southern Italy (Table 1). Thirty-seven of 39 *tet*(A) genes were found to be located within the Tn*1721* transposon by PCR with primers TAF and TetAR3 (12) (Table 2). Since a Tn*1721* deleted version (ΔTn*1721*) has been described previously (9), a different primer pair (LAF-LAR [Table 2]) was used to identify the left arm of the transposon. Only four strains were positive by this PCR assay, indicating that the deletion of the Tn*1721* transposon was found widely among the *Salmonella* strains of our collection (Table 1). Only two *tet*(A)-positive strains (isolates 17/31 and 27/27) were negative for Tn*1721*. One of them, an *S. enterica* London strain (isolate 17/31), carried the *tet*(A)-*1* gene variant, previously described to occur only on the *Shigella* spp. pSSTA-1 plasmid (12).

The *strA* and *strB* genes were also highly prevalent in *Salmonella* strains of our collection, being detected in 46 of 55 (84%) streptomycin-resistant strains.

Streptomycin resistance genes were also detected as cassette-borne genes within class 1 integrons. Seventeen (29%) strains were positive for integrons (Table 1). Six different integrons carrying one or a combination of two of the gene cassettes *dfrA12* and *dfrA1* (conferring resistance to trimethoprim) and *aadA1*, *aadA2*, and *aadB* (conferring aminoglycoside resistance) were observed. Twelve of the 17 integron-positive strains were positive for Tn*21* by PCR amplification with primers designed for the *tnpM* gene in the left arm of Tn*21* (Table 2).

Resistance plasmids. To better characterize *strA-strB* and *tet*(A), conjugation experiments were performed on 27 of 58 isolates from our collection (selected strains are indicated in Table 1). These strains represented 15 different serotypes and included integron-, *strA-strB*-, and *tet*(A)-positive and -negative strains. Nine (33%) transconjugants were obtained, and their features are shown in Table 3.

Plasmid DNA was purified from the transconjugants and analyzed by *Eco*RI and *Sal*I restriction and Southern blot hybridization (23), using the *tet*(A) and *strA-strB* PCR products as probes (data not shown). This analysis revealed the presence of a common plasmid in these strains; the 27/30, 17/32, 17/22, and 17/14 transconjugants carried very similar plasmids, positive by *strA-strB* hybridization, while strains 17/1 and 17/19 carried a different plasmid, producing a single fragment of ca. 20 kb by *Eco*RI digestion that was positive by both *strA-strB* and *tet*(A) gene hybridization. Strain 27/28 shows the simultaneous presence of the two plasmids observed in the 17/1 and 27/30 strains, respectively. Replicon typing of the resistance plasmids from the 17/1, 17/19, and 27/30 transconjugants was then performed by hybridization with each clone of the *inc*/*rep* plasmid bank, specific for the major incompatibility groups, as previously described (7). Plasmids from both the 17/1 and the 17/19 transconjugants were positive for the repN replicon, while a plasmid from the 27/30 transconjugant was positive for the repI1 replicon (data not shown).

Specific replicon PCR assays were then applied for the detection of repN and repI1 replicons. repN typing was accomplished by PCR amplification with the N1-N2 primer pair as previously described (11). repI1 typing was performed with the I1-I2 primer pair, designed on the basis of the origin of replication (*oriT*) of the P64 plasmid, belonging to incompatibility

TABLE 1. Characteristics of *S. enterica* isolates tested in this study

Isolate[a]	Source	Serotype	Resistances[b]	Assay result for[c]:								
				tet(A)	Tn*1721*R	Tn*1721*L	*strA-strB*	*strA* IS*1133*	Integron-borne gene cassette(s)	Tn*21*	repN	repI
17/24	Animal	Agona	Sm^r Te^r SxT^r	+	+	−	+	−	*dfrA12-aadA2*	+	−	−
17/22*	Swine	Agona	Sm^r Te^r SxT^r	−	−	−	+	−	−	−	−	+
27/28*	Turkey liver	Agona	Sm^r Te^r Ap^r SxT^r Gm^r Km^r	+	+	−	+	+	*dfrA1-aadA1*	+	+	+
27/14	Food	Anatum	Sm^r Te^r SxT^r Gm^r Km^r Na^r Cm^r	+	+	+	+	−	*dfrA1-aadA1*	+	−	−
17/20	Bovine meat	Anatum	Sm^r Te^r SxT^r Na^r Gm^r	+	+	+	+	−	*dfrA1-aadA2*	+	−	−
17/7*	Turkey	Blockley	Sm^r Te^r Ap^r Amc^r Km^r Na^r Nm^r En^r	+	+	−	+	−	−	−	+	−
17/17	Turkey	Blockley	Sm^r Te^r Km^r Na^r Nm^r Cf^r	+	+	−	+	−	−	−	+	−
17/18*	Turkey stool	Blockley	Sm^r Te^r Km^r Na^r Nm^r En^r	+	+	−	+	−	−	−	+	−
17/34*	Meat food	Blockley	Sm^r Te^r Km^r Na^r Nm^r	+	+	−	+	−	−	−	+	−
17/5	Guinea fowl	Blockley	Sm^r Te^r Km^r Nm^r	+	+	−	+	−	−	−	+	−
17/41	Swine meat	Blockley	Sm^r Te^r Km^r Nm^r	+	+	−	+	−	−	−	−	−
27/7	Chicken	Bredeney	Sm^r Te^r Ap^r Amc^r Cf^r	+	+	−	+	−	−	−	−	−
17/8*	Turkey	Bredeney	Sm^r Te^r Ap^r Amc^r SxT^r Na^r Cm^r Cf^r En^r	+	+	+	+	+	*dfrA1-aadA1*	+	−	+
17/3	Dog stool	Bredeney	Sm^r Te^r Su^r Km^r Nm^r	−	−	−	+	−	−	−	−	−
27/5*	Swine	Colorado	Sm^r Te^r Ap^r Amc^r Cm^r Cf^r	+	+	−	−	−	−	−	+	−
27/8	Chicken	Derby	Sm^r Te^r Ap^r SxT^r	−	−	−	+	−	−	−	−	−
27/1*	Pork sausage	Derby	Sm^r Te^r Ap^r SxT^r Cp^r	−	−	−	+	−	−	−	−	−
17/11	Food	Derby	Sm^r Te^r Su^r	+	+	−	+	−	*aadA2*	−	−	+
17/10	Swine	Derby	Sm^r Te^r Su^r Km^r Na^r Nm^r	−	−	−	+	−	−	−	+	−
17/14*	Swine	Derby	Sm^r Te^r Su^r Km^r Nm^r	−	−	−	+	−	−	−	+	−
27/25*	Chicken	Enteritidis	Te^r Ap^r SxT^r Gm^r Km^r	+	+	−	−	−	*dfrA1-aadA1*	+	−	−
27/27*	Chicken	Enteritidis	Te^r Ap^r SxT^r Gm^r Km^r	+	−	−	−	−	*dfrA1-aadA1*	+	−	−
27/30*	Pork sausage	Give	Sm^r Te^r Ap^r SxT^r	+	+	−	+	+	*aadA1*, *dfrA1-aadA1*	+	−	+
27/4	Chicken	Hadar	Sm^r Te^r Ap^r Cf^r	+	+	−	+	−	−	−	−	−
27/9*	Chicken	Hadar	Sm^r Te^r Ap^r Amc^r Cf^r	−	−	−	−	−	−	−	−	−
27/11	Chicken	Hadar	Sm^r Te^r Ap^r Amc^r Cf^r	+	+	−	+	+	−	−	−	−
27/17*	Chicken	Hadar	Sm^r Te^r Ap^r Amc^r Cf^r	+	+	−	+	+	−	−	−	−
27/19	Chicken	Hadar	Sm^r Te^r Ap^r Amc^r	+	+	−	+	+	−	−	−	−
17/16*	Duck	Hadar	Sm^r Te^r Ap^r Amc^r Km^r Nm^r Cf^r	+	+	−	+	+	−	−	−	−
17/36	Chicken	Hadar	Sm^r Te^r Ap^r Na^r Cf^r En^r	+	+	−	+	+	−	−	−	−
17/37	Chicken	Hadar	Sm^r Te^r Ap^r Na^r Cf^r En^r	+	+	−	+	+	−	−	+	−
17/2*	Duck	Hadar	Sm^r Te^r Ap^r Amc^r Na^r Cf^r En^r	−	−	−	+	−	−	−	−	−
17/35	Chicken	Hadar	Sm^r Te^r Ap^r Amc^r Na^r Cf^r En^r	+	+	−	+	+	−	−	−	−
17/15	Chicken	Hadar	Sm^r Te^r Ap^r Amc^r Na^r Km^r Cf^r En^r	+	+	−	+	+	−	−	−	−
17/9	Duck	Hadar	Sm^r Te^r Ap^r SxT^r Nm^r	+	+	−	+	+	−	−	−	−
17/1*	Turkey	Heidelberg	Sm^r Te^r Ap^r Amc^r Gm^r Km^r Na^r Nm^r Cf^r En^r	+	+	−	+	+	−	−	+	−
17/4	Turkey	Heidelberg	Sm^r Te^r Ap^r Amc^r Na^r	+	+	−	+	−	−	−	+	−
17/19*	Food	Heidelberg	Sm^r Te^r Ap^r Su^r Na^r Gm^r Km^r Cf^r	+	+	−	+	+	*aadA1*	+	+	−
17/29	Swine meat	Heidelberg	Sm^r Te^r Ap^r SxT^r Cf^r	+	+	−	−	−	−	−	+	−
17/32*	Turkey	Heidelberg	Sm^r Te^r Su^r Km^r Na^r Gm^r	−	−	−	+	−	*aadB-aadA2*	+	−	+
17/6	Swine	Heidelberg	Sm^r Te^r SxT^r Gm^r Km^r Cm^r Nm^r	+	+	+	−	−	*dfrA1-aadA1*	+	−	−
17/23*	Chicken	Infantis	Sm^r Te^r Su^r Na^r En^r	+	+	−	−	−	*aadA1*	−	−	+
27/24	Minced meat	Kisii	Sm^r Te^r Ap^r Km^r	−	−	−	−	−	−	−	−	−
27/12	Chicken	Livingstone	Sm^r Te^r Na^r Cf^r	−	−	−	−	−	−	−	−	−
17/40	Chicken	London	Sm^r Te^r Ap^r Amc^r Su^r Na^r Cf^r En^r	+	+	−	+	+	−	−	−	−
17/31*	Swine	London	Sm^r Te^r Su^r	+[d]	−	−	+	−	−	−	−	−
27/22*	Pork sausage	Panama	Sm^r Te^r Ap^r Amc^r Gm^r Cm^r Cl^r Cf^r	−	−	−	−	−	−	−	−	−
17/25*	Turkey	Saintpaul	Sm^r Te^r Ap^r Amc^r Su^r Gm^r Km^r Na^r Nm^r Cf^r	−	−	−	+	−	*aadB-aadA2*	−	−	+
17/27*	Turkey	Saintpaul	Sm^r Te^r Ap^r Amc^r Su^r Gm^r Km^r Na^r Nm^r Cf^r	−	−	−	+	−	*aadB-aadA2*	−	−	−
17/21*	Turkey	Saintpaul	Sm^r Te^r Ap^r Amc^r SxT^r Gm^r Km^r Na^r Nm^r Cf^r En^r	−	−	−	+	−	*aadB-aadA2*	−	−	−
17/13	Chicken	Senftenberg	Sm^r Ap^r Amc^r SxT^r Ctx^r Cl^r Cf^r	−	−	−	+	−	−	−	−	−
17/26*	Chicken	Senftenberg	Sm^r Te^r Ap^r Amc^r SxT^r Na^r Cp^r Cf^r En^r	+	+	−	+	−	−	−	+	−
27/18	Chicken	Tshiongwe	Sm^r Te^r Ap^r Amc^r	+	+	−	+	+	−	−	−	−
27/2	Pork sausage	Typhimurium	Sm^r Te^r Ap^r Amc^r Gm^r Cm^r Cl^r Cf^r	−	−	−	−	−	*aadA1*	+	−	−
27/21	Swine	Typhimurium	Sm^r Te^r Ap^r Gm^r Cm^r	+	+	−	+	−	−	+	−	−
27/6	Swine	Typhimurium	Sm^r Te^r Ap^r SxT^r Cf^r	+	+	−	+	−	−	−	−	−
27/13	Pork sausage	Typhimurium	Sm^r Te^r SxT^r	−	−	−	+	−	−	−	−	−
17/12*	Hen egg	Virchow	Te^r Su^r Km^r Na^r Nm^r En^r	−	−	−	−	−	−	−	−	−

[a] Strains with asterisks were chosen for conjugation experiments.

[b] AP, ampicillin; Amc, amoxicillin-clavulanic acid; Cf, cephalothin; Cl, colistin; Cm, chloramphenicol; Cp, ciprofloxacin; En, enrofloxacin; Sm, streptomycin; Su, sulfonamides; Te, tetracycline; SxT, trimethoprim-sulfamethoxazole; Km, kanamycin; Gm, gentamicin; Na, nalidixic acid; Nm, neomycin; Ctx, cefotaxime. Strains showing both Su^r and SxT^r phenotypes are reported as SxT^r.

[c] Tn*1721*R and Tn*1721*L, right and left arms of Tn*1721*, respectively;. +, present; −, absent.

[d] *tet*(A)-*1* gene variant (12).

group I1 (IncI1) (EMBL accession no. AP005147). The specificity of the repI1 PCR was tested by using R144 and JR66a IncI1 as reference plasmids (7) and the TP114 (IncI2), R16 (IncB/O), and R387 (IncK) plasmids as negative controls. All transconjugants and their relative *Salmonella* donor strains were tested by both repN and repI1 PCR assays. Strains 17/14, 17/1, and 17/19 were repN positive, and strains 17/25, 17/32, 17/22, and 27/30 were repI1 positive (Table 3). The simultaneous presence of two plasmids in the 27/28 transconjugant was confirmed, as this transconjugant tested positive in both

TABLE 2. Primers used in PCR amplification and DNA sequencing

Primer	DNA sequence (5′ to 3′)	Target gene(s)	Nucleotide positions	EMBL accession no.	Amplicon size (bp)	Reference
TAF	GTAATTCTGAGCACTGTCGC	*tet*(A)	6718–6737	X61367		33
TAR	CTGCCTGGACAACATTGCTT	*tet*(A)	7674–7655	X61367	956	33
TetAR3	GGCATAGGCCTATCGTTTCCA	*tet*(A) of Tn*1721*	7917–7897	X61367	1,199	12
TetAR2	GTGCAACGGGAATTTGAAG	*tet*(A)-1 in pSSTA-1	2255–2237	AF502943	1,168	12
SAF	AGCAGAGCGCGCCTTCGCTG	*strA* of RSF1010	761–780	NC_001740		4
SBR	CCAAAGCCCACTTCACCGAC	*strB* of RSF1010	1464–1445	NC_001740	703	4
LAF	GTTCGGGTCAGCAGCTTTGAC	Left arm of Tn*1721* (*tnpR*)	2101–2121	X61367		This study
LAR	GAGGGTTTCCCGGCTGATGT	Left arm of Tn*1721* (*tnpR*)	2591–2610	X61367	509	This study
5′ CS	GGCATCCAAGCAGCAAG	Integron variable region	1190–1206	M73819	Varied	15
3′ CS	AAGCAGACTTGACCTGA	Integron variable region	1342–1326	M73819		15
TnpMF	TCAACCTGACGGCGGCGA	*tnpM* of Tn*21*	3689–3706	AF071413	348	32
TnpMR	GGAGGTGGTAGCCGAGG	*tnpM* of Tn*21*	4037–4021	AF071413		32
N1	AGTTCACCACCTACTCGCTCCG	IncN *repA*	32164–32185	AY046276	163	11
N2	CAAGTTCTTCTGTTGGGATTCCG	IncN *repA*	32327–32305	AY046276		11
11	CGGGACAGGATGTGCAA	IncI *oriT*	66951–66967	AP005147	97	This study
12	ACTTCAGGCTCCTTACGGG	IncI *oriT*	67048–67031	AP005147		This study
IS1133F	GATTGGCTGGGCAACAGGTGA	*tnpA* of IS*1133*	4465–4486	M95402	715	This study
smAR	TCCTCCTGCCAGTTGATCAC	*strA* of Tn*5393*	5180–5161	M95402		This study
17/1F[a]	GCCCACTGGGACGACATCC	Δ*tnpA* of Tn*1721*	10570–10588	X61367		This study
17/1R[a]	CGATCCCCAATACATTGAATA	*strA* of RF1010	804–784	NC_001740		This study
smBF[a]	GCGGCCGCGATCAAGCAGGT	*strB* of Tn*5393*	6561–6580	M95402		This study

[a] Primer used for DNA sequencing.

the repN and the repI1 PCR assays. The 17/31 transconjugant was negative for the two replicon systems tested (Table 3).

The repN and repI PCR assays were then performed with total DNA extracted from all of the *Salmonella* strains in our collection. It was interesting that repN and repI1 replicons were identified in 15 and 7 *Salmonella* strains from our collection, respectively (Table 1). In particular, five of six *S. enterica* serotype Blockley strains and four of six serotype Heidelberg strains carried repN-positive plasmids, suggesting the frequent presence of this kind of plasmid in these *Salmonella* serotypes (Table 1).

The geographical distribution of the plasmids revealed that most (18 of 22) of the repN- and repI1-positive strains were from the IZSVE, suggesting that these plasmids were largely distributed in *Salmonella* strains from northern Italy.

It is interesting that all of the repI1-positive strains were also integron positive but that the repN-positive strains were not associated with the presence of integrons (the integron found in strain 17/19 was not transferred by conjugation with the repN plasmid [Table 3]).

Characterization of the repN plasmid. Since the repN replicons, diffused in the isolates of our collection, were difficult to analyze by restriction fragment length polymorphism analysis, plasmids purified from the 17/1 and 17/19 transconjugants were further analyzed by DNA sequencing of regions flanking *strA-strB* and *tet*(A). The results of this analysis confirmed the pres-

TABLE 3. Characteristics of transconjugants

Strain	Resistance[a]	Trans-conjugant	Transferred resistance(s)[a]	Assay result for[b]:				
				Transferred *strA-strB*	Transferred *tet*(A)	Transferred integron	repN	repI
27/28	Sm^r Te^r Ap^r SxT^r Gm^r Km^r	T27/28	Sm^r Te^r Ap^r SxT^r	+	+	*dfrA1-aadA1*	+	+
17/25	Sm^r Te^r Ap^r Amc^r Su^r Gm^r Km^r Na^r Nm^r Cf^r	T17/25	Sm^r Te^r Ap^r Su^r Gm^r Km^r Nm^r	−	−	*aadB-aadA2*	−	+
17/32	Sm^r Te^r Su^r Gm^r Km^r Na^r	T17/32	Sm^r Su^r Gm^r Km^r	+	−	*aadB-aadA2*	−	+
27/30	Sm^r Te^r Ap^r SxT^r	T27/30	Sm^r SxT^r	+	−	*dfrA1-aadA1*	−	+
17/1	Sm^r Te^r Ap^r Amc^r Gm^r Km^r Na^r Nm^r Cf^r En^r	T17/1	Sm^r Te^r Ap^r Gm^r Km^r Nm^r	+	+	−	+	−
17/14	Sm^r Te^r Su^r Km^r Nm^r	T17/14	Sm^r Te^r Su^r Km^r Nm^r	+	−	−	+	−
17/19	Sm^r Te^r Ap^r Su^r Na^r Gm^r Km^r Cf^r	T17/19	Sm^r Te^r Ap^r	+	+	−	+	−
17/22	Sm^r Te^r SxT^r	T17/22	Sm^r	+	−	−	−	+
17/31	Sm^r Te^r Su^r	T17/31	Sm^r Te^r Su^r	+	+[c]	−	−	−

[a] For abbreviations, see Table 1, footnote *b*.
[b] +, present; −, absent.
[c] *tet*(A)-*1* gene variant.

ence of the ΔTn*1721* plasmid in both the 17/1 and the 17/19 strains and demonstrated that *strA-strB* was located within a particular Tn*5393*-derived transposon, previously identified only in the plant pathogen *Erwinia amylovora* (97% homology to the DNA sequence released under EMBL accession no. M95402). In this transposon variant, the *strA* gene is located adjacent to the insertion sequence IS*1133* (24). A PCR assay specific for the detection of the *strA* gene linked to the IS*1133* element was then used to identify the prevalence of this resistance determinant in the isolates from our collection (Table 2, primers IS1133F and smAR). Sixteen strains were positive for the Tn*5393*-IS*1133*::*strA-strB* transposon, but only four strains (strains 17/1, 17/19, 17/37, and 27/28) carried a repN plasmid; furthermore, most of the strains carrying this element were also negative in the repI1 PCR assay.

The Tn*5393*-IS*1133*::*strA-strB* transposon was found in *Salmonella* strains of different serotypes, including serotypes Hadar, Agona, Bredeney, Give, Heidelberg, London, and Tshiongwe (Table 1).

DISCUSSION

A wide diffusion of the *strA-strB* and *tet*(A) resistance genes was observed in epidemiologically unrelated *Salmonella* strains of animal origin isolated in Italy.

Most of the *tet*(A) genes were located within the truncated version of transposon Tn*1721.* This variant was previously described to occur in two isolates of *Salmonella choleraesuis* and *S. enterica* serotype Typhimurium variant Copenhagen isolated from animals in Germany (9, 10). Many strains also carried integrons encoding resistance to trimethoprim, kanamycin, sulfonamides, and streptomycin, with most of these integrons being associated with the Tn*21* transposon. Recurrent repN- and repI1-positive plasmids were identified in *Salmonella* strains of different serotypes isolated from distant geographical areas.

The *strA-strB* gene pair has previously been described for bacteria isolated from humans and animals. These genes are often located on small broad-host-range nonconjugative plasmids, such as RSF1010 and pBP1, while in isolates of vegetable origin they are carried on large conjugative plasmids characterized by the presence of transposon Tn*5393* (24, 25, 26, 27, 29). In the Tn*5393* transposon, the *strA* and *strB* genes are located downstream of the *tnpA* (transposase) and *tnpR* (resolvase) genes. This transposon has been described to occur in the apple tree pathogen *E. amylovora*; in *Aeromonas salmonicida*, a fish pathogen bacterium, in Norway (13); in *Campylobacter jejuni* and *Pseudomonas aeruginosa* (24); and in a multiresistance plasmid, pTP10, from *Corynebacterium striatum* (30). It is important to note that the two Tn*5393* variants containing the insertion sequences IS*1133* and IS*6100* were described for the plant pathogens *E. amylovora* and *Xanthomonas campestris*, respectively (26). The insertion of these IS elements within the transposon has the consequence of increasing the expression of the *strA* and *strB* genes (29). In particular, the IS*1133* variant was previously described only for *E. amylovora* strains from plant sources. Our findings demonstrate the presence of the IS*1133* variant in several *Salmonella* strains of animal origin. Most of the strains carrying the Tn*5393*::IS*1133* element were from poultry sources (chicken, duck, and turkey). This genetic determinant was localized on repN- and repI1-positive plasmids, but it was also detected in several strains that did not transfer the resistance by conjugation, suggesting a chromosomal localization of the transposon. To our knowledge, this is the first time that this resistance determinant has been identified in *Salmonella* isolates. The identification of the Tn*5393*::IS*1133* element in *Salmonella* isolates suggests novel scenarios of resistance transmission among zoonotic and plant pathogens; it may be hypothesized that *Salmonella* imported this genetic element from plant pathogens, probably through the contamination of animal feeds.

Since tetracycline and streptomycin are among the most used antimicrobials in veterinary medicine in the European Union (European Federation for Animal Health data, available at http://www.fedesa.be/Europe/Topics/antibio/Kit3.htm), the extensive use of such drugs may have contributed to the successful spread of these genetic determinants in zoonotic pathogens.

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