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Dissemination of an Extended-Spectrum-β-Lactamase bla_{TEM-52} Gene-Carrying IncI1 Plasmid in Various *Salmonella enterica* Serovars Isolated from Poultry and Humans in Belgium and France between 2001 and 2005▽

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We report here the dissemination of a conjugative IncI1 plasmid carrying bla_{TEM-52} on a Tn*3* transposon conferring resistance to extended-spectrum cephalosporins in *Salmonella enterica* serovar Agona, Derby, Infantis, Paratyphi B dT$^+$, and Typhimurium isolates from poultry and humans in Belgium and France from 2001 to 2005. The most prevalent serovar spreading this resistance was serovar Infantis.

Food-producing animals are the primary reservoir of zoonotic pathogens, and the rate of detection of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Salmonella* strains has increased in recent years. ESBLs are widely detected in various human medical institutions, but they are not so frequently reported in bacterial populations circulating in animals. In Belgium and France the emergence of resistance to extended-spectrum cephalosporins, such as ceftriaxone and ceftiofur, has been recently reported in *Salmonella enterica* serovar Virchow isolates from poultry and humans (1, 15). Resistance was due to the ESBL genes $bla_{CTX-M-2}$ or $bla_{CTX-M-9}$ carried on large conjugative plasmids.

Since 2001 a large number of strains have been isolated from poultry (more than 150 in Belgium) and a more limited number from humans ($n = 15$) in Belgium and France showing resistance to extended-spectrum cephalosporins by production of an ESBL not belonging to the CTX-M family and with various additional resistances to other antibiotic families. The serovars concerned were Agona, Derby, Infantis, Paratyphi B dT$^+$, and Typhimurium. In particular, the emergence of extended-spectrum cephalosporin-resistant serovar Infantis with more than 80 strains isolated from poultry and 4 strains from humans caused some concern. The purpose of the present study was to identify the ESBL gene and its location in these strains.

Strains studied are shown in Table 1. Antibiotic susceptibility testing was done by the disk diffusion method, and the MICs of ceftriaxone and ceftiofur were determined as described previously (1, 14, 15). Resistance to extended-spectrum cephalosporins from all *Salmonella* strains was transferred to an *E. coli* recipient strain by conjugation as previously described (1, 14, 15), and all *E. coli* transconjugant strains showed the same antibiotic resistance profile (Table 1). Other resistances from multidrug-resistant strains were not transferred by conjugation. According to the MICs, the levels of resistance to ceftiofur and ceftriaxone were lower in the transconjugant strains than in the parental strains, but this was also observed in a previous study (14). PCR assays to detect ESBL genes (TEM, SHV, and CTX-M) were performed on parental and transconjugant strains using previously described primers (1, 14, 15), and nucleotide sequencing of the amplicons identified the bla_{TEM-52} resistance gene in all strains. Plasmids extracted from the transconjugants were further characterized by PstI restriction analysis showing that they were all identical and greater than 100 kb in size (Fig. 1). Southern blot hybridization experiment with a bla_{TEM-52} gene probe was performed as described previously (12). It revealed two PstI fragments of 2.9 and 2.75 kb. In fact, this PstI restriction profile corresponded exactly to that of bla_{TEM-52}-carrying plasmids isolated in 2002 and 2003 from four isolates of *S. enterica* serovars Typhimurium, Enteritidis, and Panama from French patients with gastroenteritis (14). A study performed in 2001 and 2002 on *Salmonella* isolated from poultry, poultry products, and human patients in The Netherlands revealed that the TEM-52 variant was the most common ESBL detected in this bacterial collection (6). In particular, TEM-52-producing salmonellae of the serovars Blockley, Virchow, Typhimurium, and Paratyphi B were identified from poultry, and strains of the serovars Thompson, London, Enteritidis, and Blockley were identified

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TABLE 1. Characteristics of the *Salmonella* strains and their transconjugants producing TEM-52 used in this study

Strain[a]	Serovar	Geographic origin	Animal or human origin	Yr of isolation	Antibiotic resistance profile[b]	MIC (μg/ml)[c]		SGI1 variant[d]
						Xnl	Cro	
777SA01	Agona	Belgium	Poultry	2001	Ap(Caz)CfCm(Cro)FfSmSpSuTcTm(Xnl)	16	16	SGI1-A
777SA01TC1					Ap(Caz)Cf(Cro)(Xnl)	4	2	
260SA04	Agona	Belgium	Poultry	2004	ApCazCfCmCroFfSmSuTcTmXnl	64	256	—
260SA04TC1					Ap(Caz)Cf(Cro)(Xnl)	2	1	
833SA04	Agona	Belgium	Poultry	2004	ApCazCfCroXnl	32	64	—
833SA04TC1					Ap(Caz)Cf(Cro)(Xnl)	2	1	
977SA01	Derby	Belgium	Poultry	2001	ApCazCfCroXnl	32	16	—
977SA01TC1					Ap(Caz)Cf(Cro)(Xnl)	4	4	
988SA01	Infantis	Belgium	Poultry	2001	ApCazCfCroXnl	32	32	—
988SA01TC1					Ap(Caz)Cf(Cro)(Xnl)	2	1	
2004/10101	Infantis	Belgium	Poultry	2004	ApCazCfCroXnl	32	128	—
2004/10101TC1					Ap(Caz)Cf(Cro)(Xnl)	2	4	
2004/10256	Infantis	Belgium	Poultry	2004	ApCazCfCroXnl	32	64	—
2004/10256TC1					Ap(Caz)Cf(Cro)(Xnl)	2	2	
05-00001	Infantis	Belgium	Human	2005	ApCazCfCroXnl	32	64	—
05-00001TC1					Ap(Caz)Cf(Cro)(Xnl)	4	4	
05-00590	Infantis	Belgium	Human	2005	ApCazCfCroXnl	32	64	—
05-00590TC1					Ap(Caz)Cf(Cro)(Xnl)	2	4	
05-00838	Infantis	Belgium	Human	2005	ApCazCfCroXnl	16	64	—
05-00838TC1					Ap(Caz)Cf(Cro)(Xnl)	4	4	
1043SA04	Paratyphi B	Belgium	Poultry	2004	ApCazCfCroSmSpSuTmXnl	32	64	—
1043SA04TC1					Ap(Caz)Cf(Cro)(Xnl)	4	4	
153SA02	Typhimurium	Belgium	Poultry	2002	ApCazCfCroSmSpSuTmXnl	32	32	—
153SA02TC1					Ap(Caz)Cf(Cro)(Xnl)	2	1	
04-3486	Typhimurium	France	Human	2004	ApCazCfCm(Cro)FfSmSpSuTc(Xnl)	16	32	SGI1
04-3486TC1					Ap(Caz)Cf(Cro)(Xnl)	2	2	

[a] Strains labeled with TC1 are *E. coli* transconjugant strains.
[b] Antibiotics: Ap, ampicillin; Caz, ceftazidime; Cf, cefalothin; Cm, chloramphenicol; Cro, ceftriaxone; Ff, florfenicol; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamide; Tc, tetracycline; Tm, trimethoprim; Xnl, ceftiofur. Parentheses indicate intermediate resistance according to the breakpoints of the CA-SFM (Comité de l'Antibiogramme de la Société Française de Microbiologie) for *Enterobacteriaceae* (i.e., susceptible, >20 mm; resistant, <15 mm).
[c] Cro, ceftriaxone; Xnl, ceftiofur.
[d] —, negative for SGI1.

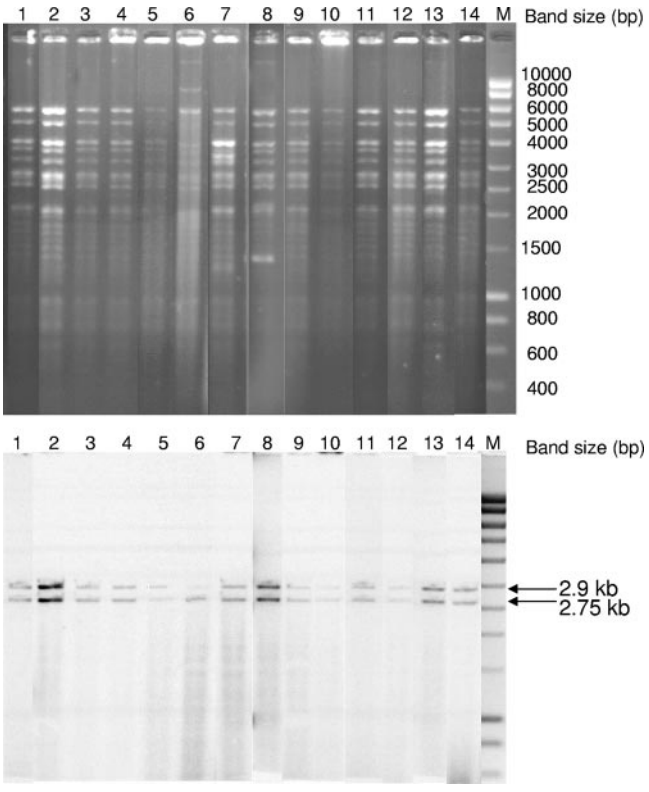

FIG. 1. Restriction analysis (PstI) (upper panel) and Southern blot hybridization with a bla_{TEM-52} probe (lower panel) of plasmid DNAs isolated from *E. coli* transconjugants. Lane 1, *E. coli* transconjugant 988SA01TC1; lane 2, *E. coli* transconjugant 2004/10101TC1; lane 3, *E. coli* transconjugant 2004/10256TC1; lane 4, *E. coli* transconjugant 05-00001TC1; lane 5, *E. coli* transconjugant 05-00590TC1; lane 6, *E. coli* transconjugant 05-00838TC1; lane 7, *E. coli* transconjugant 1043SA 04TC1; lane 8, *E. coli* transconjugant 777SA01TC1; lane 9, *E. coli* transconjugant 260SA04TC1; lane 10, *E. coli* transconjugant 833SA 04TC1; lane 11, *E. coli* transconjugant 153SA02TC1; lane 12, *E. coli* transconjugant 04-3486TC1; lane 13, *E. coli* transconjugant 977SA 01TC1; lane 14, *E. coli* transconjugant pPAN-1 (the latter strain served as control and was previously published (14); lane M, markers (Smartladder; Eurogentec, Seraing, Belgium).

from human patients (6). Several sporadic cases of *E. coli* TEM-52 producers were reported in animals: dogs in Portugal, rabbits in Spain, and beef meat in Denmark (2, 4, 8). These findings suggest a wide dissemination of this ESBLs in Europe in animals and humans. The presence of the bla_{TEM-52} gene in *E. coli*, as well as in different *Salmonella* serovars, strongly indicated that it is not due to the spread of a single clone but to the horizontal transmission of this resistance trait. To better identify the molecular mechanism of dissemination of this ESBL, the bla_{TEM-52}-positive plasmids were typed by the PCR-based replicon typing as previously described (3), demonstrating that they all belong to the IncI1 incompatibility group. IncI1 plasmids were recently described in *E. coli* and *Salmonella* strains of different serovars isolated in the United King-

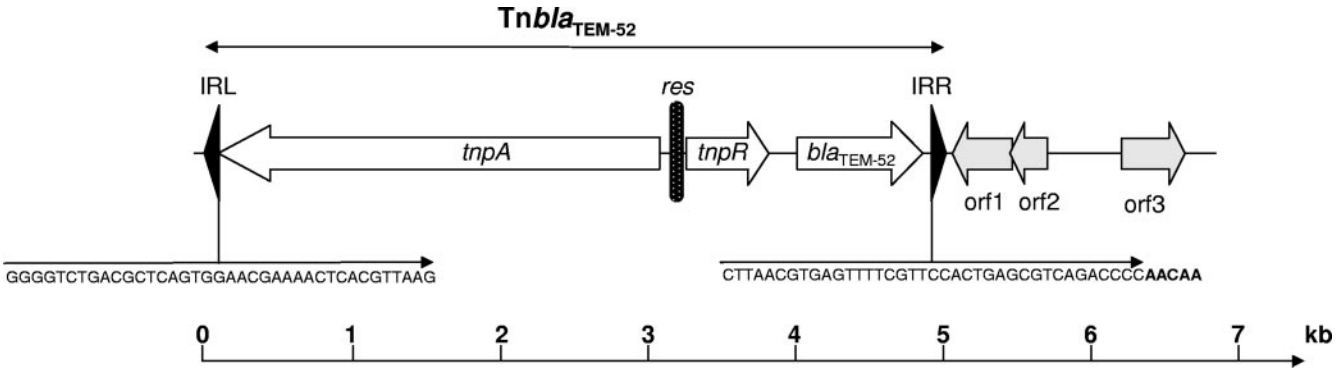

FIG. 2. Genetic organization of the bla_{TEM-52} carrying transposon on a conjugative IncI1 plasmid from serovar Typhimurium strain 04-3486. The position and orientation of the genes are indicated by arrows. Gray arrows correspond to plasmid genes flanking the transposon. IRL and IRR correspond, respectively, to left and right terminal inverted repeats of Tn*3* and are indicated as black arrowheads. The sequences of IRL and IRR are also indicated. The 5-bp direct repeat at the integration site of Tn*3* downstream IRR is indicated in boldface letters. The black box between *tnpA* and *tnpR* indicates the resolution site of Tn*3*. The nucleotide sequence of Tnbla_{TEM-52} has been deposited under GenBank accession number EF141186. A distance scale in kilobase pairs is given above the map.

dom associated with relevant β-lactamases such as CMY-2, CMY-7, and CTX-M-15, suggesting a large prevalence of IncI1 plasmids in Europe (7).

To identify the mobile genetic element carrying the bla_{TEM-52} gene, the plasmid DNA of *E. coli* transconjugant 04-3486TC1 extracted with a QIAfilter Midi kit (QIAGEN, Courtaboeuf, France) was digested with ClaI and ligated into the ClaI-restricted phagemid pBK-CMV (Stratagene). Recombinant plasmids were introduced into *E. coli* DH10B by electroporation (Bio-Rad Gene Pulser II; Bio-Rad, Marnes-La-Coquette, France) and selected on Mueller-Hinton (MH) agar (Bio-Rad) containing kanamycin (30 μg/ml) and ceftazidime (2 μg/ml). Recombinant plasmids that possessed a 4.7-kb insert were selected. Nucleotide sequencing of the insert indicated that the bla_{TEM-52} gene was located on a Tn*3* transposon. To complete the transposon sequence, nucleotide sequencing was further performed by genome walking on the native plasmid. This Tn*3* transposon structure is shown in Fig. 2. Its nucleotide sequence is deposited in GenBank under accession number EF141186. Very few sequences of complete Tn*3* elements from *Salmonella* are currently available (10). Complete plasmid-borne Tn*3* elements, however, specifying non-ESBLs have recently been described in serovar Typhimurium from a rabbit and in serovar Infantis from poultry and shown to be linked to either the tetracycline resistance gene *tet*(A) or the quinolone resistance gene *qnrS* (9, 13).

Among the extended-spectrum cephalosporin-resistant *Salmonella* strains studied, five isolates belonging to serovars Agona, Paratyphi B dT^+, and Typhimurium, showed an additional multidrug resistance profile with resistances to chloramphenicol, florfenicol, streptomycin, spectinomycin, sulfonamide, tetracycline, and trimethoprim (Table 1). This multidrug resistance profile is characteristic of SGI1 antibiotic resistance gene clusters, which were previously identified in these serovars (11). Identification of SGI1 and mapping of its antibiotic resistance gene cluster performed as described previously (5) showed that two of the five isolates possessed SGI1 and the SGI1-A variant in serovar Typhimurium strain 04-3486 and serovar Agona strain 777SA01, respectively (Table 1). Serovar Agona strains with SGI1-A are frequently isolated from poultry in Belgium (5). The serovar Typhimurium isolate carrying SGI1 was further shown to be of phage type DT104, a dominant multidrug-resistant clone that has spread all over the world (11). To our knowledge, this is the first time that multidrug-resistant strains carrying SGI1 together with a plasmid-borne ESBL gene have been reported, and further surveillance of such strains is thus warranted.

Since most of the strains showing extended-spectrum cephalosporin resistance were of serovar Infantis, these were further investigated for clonality by XbaI and BlnI macrorestriction pulsed-field gel electrophoresis analysis. The Infantis isolates showing the same resistance profile and whatever their origin, poultry or human, showed identical XbaI and BlnI macrorestriction profiles, indicating that these were clonal (data not shown).

In conclusion, the present study showed the spread of an IncI1 plasmid carrying the bla_{TEM-52} gene among *S. enterica* serovars Agona, Derby, Infantis, Paratyphi B dT^+, and Typhimurium, as well as the spread of a single Infantis clone carrying this plasmid mainly in poultry. It is thus likely that humans infected with these strains were contaminated by ingestion of undercooked poultry products. The further spread of such plasmids in multidrug-resistant strains carrying SGI1 is of concern.

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