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Extended-Spectrum-β-Lactamase (TEM-52)-Producing Strains of *Salmonella enterica* of Various Serotypes Isolated in France

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From 2002 to 2003, four isolates of *Salmonella enterica* serotypes Typhimurium, Enteritidis, Blockley, and Panama, isolated in France from patients with gastroenteritis, were found to produce extended-spectrum β-lactamase TEM-52. The study showed the bla_{TEM-52} gene to be located in a Tn*3*-like structure and carried by 100- or 32-kb conjugative plasmids.

Salmonella enterica is a major food-borne enteric pathogen in humans worldwide. Wild-type strains of *Salmonella* are usually susceptible to β-lactams, but an increase in the rate of ampicillin resistance in the last decade with the emergence of *S. enterica* serotype Typhimurium phage type DT 104 has been observed in developed countries (7). More recently, the emergence of strains of serotype Newport that produce the plasmidic cephamycinase CMY-2 (known as Newport-MDRAmpC) and are resistant to extended-spectrum cephalosporins (ESC) has been reported in the United States (9). Extended-spectrum β-lactamases (ESBLs), which also inactivate the ESC, are very rare in the genus *Salmonella*. However the number of reported cases in the different serotypes of this organism has been increasing worldwide in recent years (1, 3, 4, 6, 10, 12–14, 18, 20–23). Most ESBLs reported in *Salmonella* derive from the common plasmid-mediated penicillinases TEM-1, TEM-2, and SHV-1, although there are some other unrelated enzyme groups including CTX-M and PER. We report here the characterization of four TEM-52-ESBL-producing *S. enterica* strains received at the French National Reference Center for *Salmonella* in 2002 and 2003. The four isolates described in Table 1 were recovered from stool samples of patients with gastroenteritis. They belonged to four different serotypes: Enteritidis, Typhimurium, Panama, and Blockley. Pulsed-field gel electrophoresis with XbaI used according to a protocol described previously (22) confirmed the isolates to be unrelated (Table 1).

Antibiotic susceptibility was determined by the disk diffusion method using Mueller-Hinton agar and 32 antibiotic disks (Bio-Rad, Marnes la Coquette, France) according to the recommendations of the French Society of Microbiology (19). MICs of the β-lactams were determined by Etest (AB Biodisk, Solna, Sweden). *Escherichia coli* ATCC 25922 was used as the control. The four isolates were resistant to ampicillin, ceftriaxone, and ceftazidime by the disk diffusion method. All four isolates were susceptible to cefoxitin, imipenem, aminoglycosides, quinolones, sulfonamides, trimethoprim, chloramphenicol, and tetracycline. The ESBL detection Etest strips and the double disk diffusion test (11) showed an ESBL phenotype for the four isolates. The MICs of the β-lactams are shown in Table 2. Isolates from serotypes Typhimurium (TYP) and Enteritidis (ENT) exhibited a higher level of resistance to ceftazidime and ceftriaxone (MIC > 256 mg/liter) than isolates from serotypes Panama (PAN) and Blockley (BLO) (MIC from 8 to 32 mg/liter).

Crude extracts of β-lactamases were obtained by sonication. Isoelectrofocusing was performed by using a PhastSystem apparatus with PhastGel IEF 3-9 gels (Amersham-Pharmacia Biotech, Freiburg, Germany) as described previously (22). The four isolates produced only one β-lactamase with a pI of 6.0.

Total DNA was extracted by using the InstaGene matrix kit (Bio-Rad). PCR analysis was performed with primers TEM-F (5′-ATAAAATTCTTGAAGACGAAA-3′) and TEM-R (5′-GACAGTTACCAATGCTTAATC-3′) to amplify a 1,080-bp fragment of the bla_{TEM} gene as described previously (22). DNA sequencing of PCR products and deduced amino acid sequence analysis revealed that the β-lactamase was TEM-52, which differed from TEM-1 by three point mutations, Glu104→Lys, Met182→Thr, and Gly238→Ser (Ambler numbering) (2). The DNA sequence of isolate BLO was 100% identical to that of $bla_{TEM-52b}$ (GenBank accession numbers AF12644 and AF027199), while the sequence of the three other isolates was identical to that of $bla_{TEM-52a}$ (GenBank accession number Y13612), except for a silent mutation located at the position Gly78 (GGC→GGT).

A resistance transfer experiment was carried out in liquid medium by using *E. coli* C1a (*nalA*) as the recipient strain. Transconjugants were selected on Mueller-Hinton agar supplemented with ceftazidime (2 mg/liter) and nalidixic acid (68 mg/liter). The *E. coli* transconjugants pPAN-1, pTYP-2′, pENT-5′, and pBLO-1 were obtained for isolates PAN, TYP, ENT, and BLO, respectively, presenting a similar pattern (Table 2) and expressing a β-lactamase with a pI of 6.0. Additionally, transconjugants pTYP-1 and pENT-1 were obtained for isolates TYP and ENT, respectively, presenting a lower resistance to β-lactams (Table 2).

Plasmid DNA was purified by using a QIAGEN (Courtaboeuf, France) Plasmid Midi kit. Molecular sizes of plasmids were determined by using Taxotron software (Institut Pasteur,

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TABLE 1. Characteristics of TEM-52-producing *S. enterica* clinical strains under study

Isolate	Serotype	Antigenic formula	Date of isolation	Location of isolation	Sex/age (yr) of patient	Suspected contaminated food	Recent antecedent hospitalization (<1 mo)	PFGE type[a]	Size of plasmid(s) carrying bla_{TEM} (kb)
TYP	Typhimurium	1,4,12:i:1,2	Feb 2002	Seine-St-Denis	F/40	Indian restaurant	No	4	100, 9
ENT	Enteritidis	9,12:g,m:−	May 2003	Nord	M/>65	Unknown	No	2	100, 10
PAN	Panama	9,12:l,v:1,5	July 2003	Pas-de-Calais	M/45	Unknown	No	3	100
BLO	Blockley	6,8:k:1,5	July 2003	Nord	M/31	Unknown	Unknown	1	32

[a] PFGE, pulsed-field gel electrophoresis.

Paris, France) by comparing them to plasmids of known sizes. Southern hybridization with a PCR-generated probe for bla_{TEM} (1,080 bp) was performed as described previously (7). Extraction of plasmid DNA revealed a 100-kb common plasmid in the isolates PAN, TYP, and ENT and a 32-kb plasmid in isolate BLO, both of which hybridized with probe bla_{TEM} (Fig. 1). Additionally, hybridization signals were observed with 10- and 9-kb plasmids in the more resistant isolates ENT and TYP, respectively (Fig. 1). The 100-kb plasmid was detected in *E. coli* transconjugants pPAN-1, pTYP-1, pTYP-2′, pENT-1, and pENT-5′, and the 32-kb plasmid was detected in pBLO-1 (Fig. 1). The 10- and 9-kb plasmids were also observed in transconjugants pENT-5′ and pTYP-2′, respectively. The plasmid DNA from transconjugants pPAN-1, pTYP-1, and pENT-1 were compared by restriction endonuclease (EcoRI and PstI; Roche, Mannheim, Germany) and Southern blot (with probe bla_{TEM}) analyses. The plasmids had a very similar fingerprint (Fig. 2A). To study the 10- and 9-kb plasmids carrying additional bla_{TEM} genes, Southern hybridization with a bla_{TEM} probe after restriction with PstI was performed on plasmid DNA from transconjugants pTYP-2′ and pENT-5′ that were compared to pTYP-1 and pENT-1, respectively (Fig. 2B and C). The probe hybridized intensively to three bands of 5, 2.9, and 1.2 kb with pENT-5′ and two bands of 2.9 and 2.75 kb with pTYP-2′. Due to the relative amount of 9- or 10- and 100-kb plasmid DNA in pENT-5′ and in pTYP-2′ (Fig. 1A, lanes 4 and 7), these hybridization patterns have been attributed to 9- and 10-kb plasmids. As there is one internal site in bla_{TEM}, these data suggested the existence of two copies of bla_{TEM} in the 9-kb plasmid and three copies in the 10-kb plasmid.

To determine whether bla_{TEM-52} was located in Tn*3*, Tn*3*-specific PCR was performed on plasmid DNA with the following primers designed on the basis of the sequence of Tn*3* in *Salmonella* (GenBank accession number AB103092): forward primer 5′-CACGAATGAGGGCCGACAGGA-3′ (located at positions 4018 to 4038 in the *tnpR* gene) and reverse primer 5′-ACCCACTCGTGCACCCAACTG-3′ (located at positions 4492 to 4512 in bla_{TEM}). All transconjugants gave the expected PCR product of 500 bp (data not shown). These results indicated the presence in our isolates of bla_{TEM-52} in a Tn*3*-like structure carried by a self-conjugative plasmid. Transposition of one or more copies of bla_{TEM-52} to other plasmids within a bacterium may explain the higher resistance of isolates TYP and ENT.

TEM-52 ESBL was first reported in a *Klebsiella pneumoniae* strain isolated in 1996 in France, from hospitalized children originating from Athens, Greece (17). Since 1996, only one isolate of TEM-52-producing *E. coli* has been identified in France during a national hospital survey (8). In 1997 and 1999, two studies demonstrated TEM-52 to be the most prevalent TEM-type ESBL among *E. coli* strains in Korea and among

TABLE 2. MICs of β-lactams used in this study[f]

β-lactam(s)[a]	MIC (mg/liter)										*E. coli* C1a
	PAN	pPAN-1	TYP	pTYP-1	pTYP-2′	ENT	pENT-1	pENT-5′	BLO	pBLO-1	
Ampicillin	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	4
Amoxicillin-CLA[b]	4	4	8	4	8	8	4	8	4	4	4
Ticarcillin	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	2
Ticarcillin-CLA[c]	16	16	64	8	32	64	8	128	16	16	2
Piperacillin	>256	>256	>256	>256	>256	>256	256	>256	>256	>256	2
Piperacillin-TZB[d]	4	4	4	2	4	4	2	4	4	4	2
Cefoxitin	2	4	2	2	2	2	2	2	4	4	2
Ceftazidime	32	16	>256	16	>256	>256	32	>256	32	32	0.25
Ceftazidime-CLA[e]	0.25	0.5	0.5	0.125	0.25	1	0.125	0.5	0.5	0.5	NT
Ceftriaxone	8	8	128	8	128	128	8	256	32	32	0.06
Cefotaxime-CLA[e]	0.06	0.06	0.125	0.03	0.03	0.125	0.03	0.03	0.25	0.25	NT
Cefepime	2	1	>32	1	16	32	2	32	4	4	0.06
Aztreonam	4	4	32	2	16	16	4	16	4	4	0.06
Imipenem	0.25	0.25	0.25	0.25	0.25	0.25	0.125	0.25	0.25	0.25	0.25

[a] CLA, clavulanic acid; TZB, tazobactam; NT, not tested.
[b] Amoxicillin-CLA (2:1).
[c] CLA, 2 mg/liter.
[d] TZB, 4 mg/liter.
[e] CLA, 4 mg/liter.
[f] MICs of β-lactams (Etest) for TEM-52-producing *S. enterica* isolates (PAN, TYP, ENT, and BLO), their *E. coli* C1a transconjugants (pPAN-1, pTYP-1, pTYP-2′, pENT-1, pENT-5′, and pBLO-1), and *E. coli* C1a are shown.

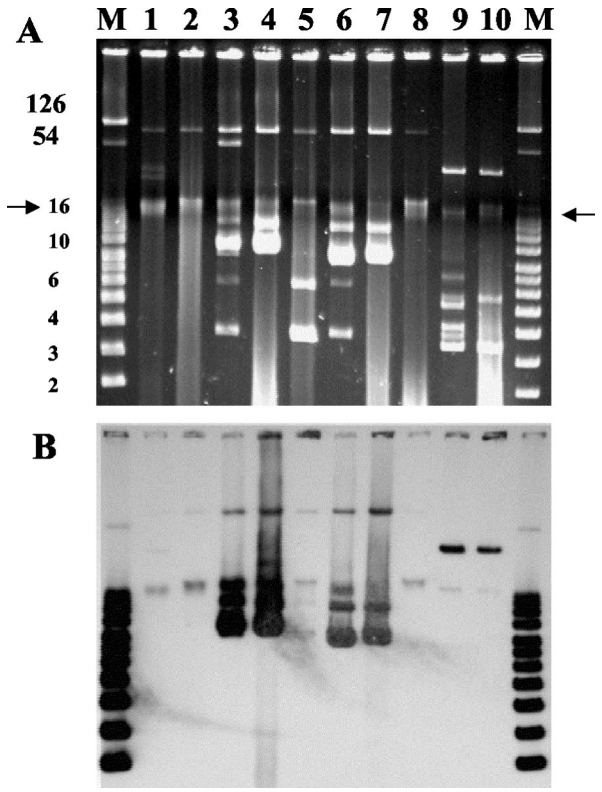

FIG. 1. (A) Agarose (0.8%) electrophoresis of plasmid DNA from the four *Salmonella* isolates TYP, ENT, PAN, and BLO and their *E. coli* transconjugants. (B) Hybridization of plasmid content with a bla_{TEM} probe. The chromosome position is indicated by arrows. A supercoiled DNA ladder (Invitrogen, Groningen, The Netherlands), plus RP4 and pIP173 plasmids which served as the molecular size marker (M) (band sizes are in kilobase pairs), is shown. Lane 1, isolate PAN; lane 2, *E. coli* transconjugant pPAN-1; lane 3, isolate ENT; lane 4, *E. coli* transconjugant pENT-5′; lane 5, *E. coli* transconjugant pENT-1; lane 6, isolate TYP; lane 7, *E. coli* transconjugant pTYP-2′; lane 8, *E. coli* transconjugant pTYP-1; lane 9 isolate BLO; lane 10, *E. coli* transconjugant pBLO-1.

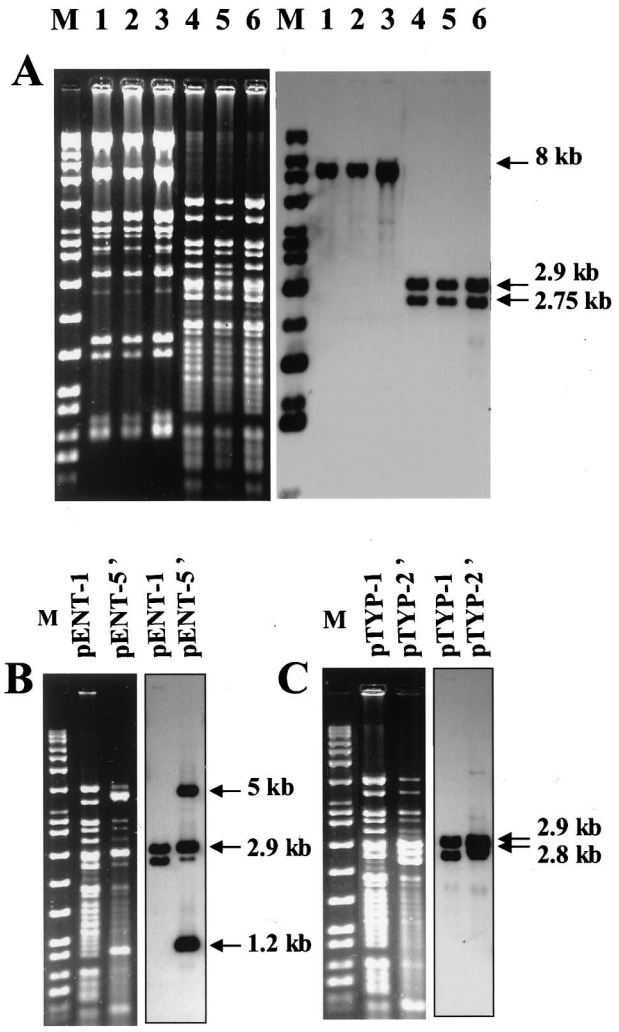

FIG. 2. Restriction analysis (left panels) and bla_{TEM} hybridization (right panels) of plasmids isolated from *E. coli* transconjugants. (A) Lane 1, EcoRI-restricted pPAN-1; lane 2, EcoRI-restricted pTYP-1; lane 3, EcoRI-restricted pENT-1; lane 4, PstI-restricted pPAN-1; lane 5, PstI-restricted pTYP-1; lane 6, PstI-restricted pENT-1. (B) PstI-digested plasmids extracted from pENT-1 and pENT-5′. (C) PstI-digested plasmids extracted from pTYP-1, and pTYP-2′. Marker Raoul I (Appligene, Illkirch, France) was used as a molecular size marker.

enterobacterial species in Italy (15, 16). The first report of TEM-52 in the genus *Salmonella* described two strains isolated in 1998 from a hospitalized Yugoslavian infant (20). Both strains exhibited different resistance phenotypes attributed to different copy numbers of bla_{TEM-52}. As resistance was not self-transmissible, the authors suggested the location of the ESBL gene on a transposon without experiment. A second report described five isolates of *S. enterica* serotypes Saintpaul, Stanley, Agona, and Enteritidis producing TEM-52 and recovered between 1995 and 1997 in Korea that came mostly from hospitalized patients (12). The bla_{TEM-52} genes were carried on conjugative plasmids greater than 180 kb in length with diverse genetic characteristics. The last report concerned TEM-52-producing isolates of serotype Enteritidis from a hospital outbreak (five cases) in Scotland in 2001 and 2002 (23). The bla_{TEM-52} gene was carried on a 95-kb conjugative plasmid. The emergence of ESBL-producing *Salmonella* strains in France is a new phenomenon. No ESBLs were detected among human *Salmonella* isolates in multicenter surveys conducted by a hospital-based network in 1994 (n = 2,622) and 1997 (n = 2,464) (5). More recently, two surveys conducted by the French National Reference Center for *Salmonella* in 2000 (n = 1,066) and in 2002 (n = 1,140) identified only one human ESBL-producing *Salmonella* isolate (isolate TYP, this study) in 2002. One hypothesis for the emergence of TEM-52-producing *Salmonella* is the nosocomial acquisition by the exchange of ESBL genes among enteric bacteria frequently encountered in hospitals and selected by traces of ESC used in humans. An alternative hypothesis that has been suggested in a report of ESBL (SHV-12)-producing *Salmonella*, and with the emergence of Newport MDRAmpC, is the transmission through the food chain and consequently to antibiotic selection pressure in livestock (6, 9). In our study, the isolates were generally not acquired from a hospital, and we suspected the second hypothesis but we didn't have evidence for it. This hypothesis was

strengthened by the detection of six TEM-52-producing *Salmonella* strains belonging to four different serotypes and isolated in poultry in Belgium during the period of 2001 to 2002 (A. Cloeckaert, personal communication).

This study is the first report of bla_{TEM-52} in *S. enterica* serotypes Panama and Blockley and describes TEM-52-producing isolates of *Salmonella* in France. The study shows that the bla_{TEM-52} gene is located in a Tn*3*-like structure and carried by a conjugative plasmid. Both features explain the high level of resistance of some isolates and the spreading of bla_{TEM-52} among *Salmonella* of various serotypes.

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