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A Novel Extended-Spectrum TEM-Type β-Lactamase, TEM-138, from *Salmonella enterica* Serovar Infantis

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A novel natural TEM β-lactamase with extended-spectrum activity, TEM-138, was identified in a ceftazidime-resistant clinical isolate of *Salmonella enterica* serovar Infantis. Compared to TEM-1, TEM-138 contains the following mutations: E104K, N175I, and G238S. The $bla_{\text{TEM-138}}$ gene was located on a 50-kb transferable plasmid. Expression studies with *Escherichia coli* revealed efficient ceftazidimase and cefotaximase activities for TEM-138.

Class A β-lactamases are the most widespread enzymes among gram-negative bacteria. The extensive use of cephalosporins in clinical practice has selected a large number of natural variants of the parental plasmid-encoded β-lactamases as TEM-1/2 and SHV-1, with an extended-spectrum activity towards oxyiminocephalosporins such as cefotaxime, ceftazidime, and ceftriaxone and monobactams such as aztreonam but not to cephamycins or carbapenems (7, 15). These extended-spectrum β-lactamases (ESBLs) are frequently associated with nosocomial outbreaks (21), with production detected most commonly in *Klebsiella pneumoniae* (3, 21) in addition to other members of the *Enterobacteriaceae* family (14) and *Pseudomonas aeruginosa* (5, 23). They arose through mutations that introduce one or more amino acid substitutions that alter the configuration or binding characteristics of the active site (7, 13), resulting in an expansion of the substrate range of the enzymes (G. A. Jacoby and K. Bush website [http://www.lahey.org/studies/]) (8, 9).

Early studies reported an increasing frequency of nosocomial infections caused by ESBL-producing species in Tunisia, with a particular prevalence of the SHV-2 type (6, 16). A recent investigation showed the presence of SHV-2a and TEM-4 in isolates of *Salmonella enterica* serotype Mbandaka from a Tunisian hospital (19). More-recent studies revealed the involvement of SHV-12- and SHV-2a-encoding plasmids in outbreaks of *Klebsiella pneumoniae* in a Tunisian neonatal ward (4).

Salmonella enterica serovar Infantis CN2310 was isolated from a stool culture of a patient in the pediatric unit of the La-Rabta Hospital (Tunisia). MICs for CN2310 (22) indicated that this strain was resistant (CLSI documents M2-A9 and M100-S16 [11, 12]) to all β-lactams tested except cefoxitin and imipenem (Table 1) and also to chloramphenicol and tetracycline. Synergies between amoxicillin-clavulanic acid and expanded-spectrum cephalosporins, such as cefotaxime and ceftazidime, were observed in a double disk diffusion test (22), suggesting ESBL production.

A 50-kb plasmid DNA (pCN2310) was extracted from *S. enterica* CN2310 by the alkaline lysis method (24). Electroporation of the plasmid pCN2310 (200 ng of DNA) into *Escherichia coli* DH10B (Invitrogen/Life Technologies) was carried out using a Bio-Rad gene pulser apparatus as recommended by the manufacturer. Transformants were selected by plating on LB agar plates containing 100 μg/ml ampicillin. In addition, conjugation experiments were carried out in liquid LB medium with *E. coli* HB101 as the recipient, as described previously (4). Transconjugants were selected on LB agar containing ampicillin (100 μg/ml) and streptomycin (100 μg/ml). The frequency of conjugational transfer is 0.45×10^{-5}/donor.

Analytical isoelectric focusing of crude extract of *S. enterica* CN2310 and its transconjugants and transformants, performed as previously described (2), revealed the presence of an identical β-lactamase band of approximately pI 5.8. The *E. coli* transformants and transconjugants were resistant to penicillins and expanded-spectrum cephalosporins but were susceptible to cefoxitin and imipenem (Table 1). These results confirmed the plasmid-mediated production of an ESBL enzyme which exhibits notable activity against ceftazidime and cefotaxime.

Amplification of the bla_{TEM} gene by PCR was carried out using the plasmid pCN2310 as the template, the primers TEM-D (5′-ATAAAATTCTTGAAGACGAAAG-3′) and TEM-R (5′-TTACCAATGCTTAATCAGTGA-3′), and *Taq* DNA polymerase (Promega), as described previously (1). The 1,080-bp PCR product was cloned into pGEM-T Easy cloning vector to yield the recombinant plasmid pGEM-138, which was used to transform *E. coli* DH5α. pGEM-138 was isolated and the sequence of the PCR-generated insert was determined on both strands (25). The complete sequence of the insert revealed an open reading frame that was identical to $bla_{\text{TEM-1}}$ except for three point mutations. The authenticity of this sequence was confirmed by sequencing, on both strands, the bla_{TEM} gene of the original plasmid.

These mutations were reflected in a change of three amino acids. The mutations consisted of a replacement of the glu-

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TABLE 1. MICs of various antimicrobial agents for the clinical isolate *S. enterica* serovar Infantis CN2310, its transconjugants and transformants, and *E. coli* recipients

Antibiotic	MIC for:				
	S. enterica serovar Infantis CN2310	*E. coli*			
		DH10B/pCN2310	DH10B	CN2310 × HB101[a]	HB101
Ampicillin	>256	128	2	>256	4
Oxacillin	>256	>256	2	>256	<1
Ticarcillin	>256	>256	2	>256	2
Benzylpenicillin	>256	>256	<1	>256	<1
Imipenem	<1	<1	1	2	1
Cephalothin	>256	>256	<1	>256	2
Cephaloridine	>156	>256	1	128	1
Cefotaxime	>256	>256	4	>256	1
Ceftazidime	256	128	1	128	<1
Ceftriaxone	>256	>256	1	>256	<1
Cefuroxime	>256	>256	2	>256	2
Cefpirome	256	256	<1	256	2
Cefoxitin	<1	<1	4	<1	2
Streptomycin	8	2	1	>256	>256
Chloramphenicol	>256	<1	2	<1	<1
Nalidixic acid	16	2	2	2	1
Tetracycline	16	<1	<1	<1	2

[a] CN2310 conjugated with HB101 as the recipient.

tamic acid residue at position 104 (codon GAG) by lysine (codon AAG), of the asparagine residue at position 175 (codon AAC) by isoleucine (codon ATC), and of the glycine residue at position 238 (codon GGT) by serine (codon AGT).

TEM-138 is closely related to TEM-15 (18). The same critical substitutions involved in the extension of the β-lactamase spectrum were present in this enzyme at positions 104 and 238, but TEM-138 differed from TEM-15 and all other TEM variants by an Asn-to-Ile change at position 175. TEM-138 differed also from the cefotaximase TEM-3 by a Lys-to-Gln change at position 39 (27).

E. coli DH10B/pCN2310 was grown overnight at 37°C in Trypto-Casein soya broth medium (Sanofi Diagnostics, Pasteur France) supplemented with cephaloridine, 100 μg/ml, resuspended in 50 mM sodium phosphate buffer, pH 7.0 (buffer A). After sonication (45 s on ice) and centrifugation (10,000 rpm for 20 min at 4°C), the supernatant was loaded onto a Sephadex G-75 column (95 by 2 cm; Pharmacia, Sweden) equilibrated with buffer A. Eluted fractions were collected and tested spectrophotometrically for β-lactamase activity with 50 μM cephaloridine as the substrate at 255 nm. Active fractions were pooled, dialyzed against 25 mM Tris-HCl buffer at pH 7.5 (buffer B), and then applied to a DEAE-Sepharose anion-exchange column (10.2 by 1.2 cm), equilibrated with buffer B. The TEM-138 β-lactamase was eluted at 250 mM NaCl and subsequently dialyzed overnight against buffer A containing 150 mM NaCl. After 98-fold purification, TEM-138 enzyme was found, with a specific activity of 399 U/mg of proteins. One unit of enzyme activity was defined as the amount of enzyme that hydrolyzed 1 μmol of cephaloridine per min per mg of proteins at room temperature. The Michaelis constant (K_m) and the maximal hydrolysis rate (V_{max}) were determined with the partially purified enzyme by a computerized microacidimetry assay with buffer A (17).

Kinetic analysis showed that TEM-138 was able to hydrolyze ceftazidime and cefotaxime with a relative efficiency similar to that for benzylpenicillin. The kinetic parameters (K_m and V_{max}) of the enzyme are compared to those of TEM-1, TEM-3 (22), and TEM-15 (20) are in Table 2. The TEM-138 β-lactamase had a lower K_m for ceftazidime than TEM-3 and TEM-15 β-lactamases. However, the K_m values of the TEM-138 enzyme were higher for benzylpenicillin, cephaloridine, and cefotaxime than those of TEM-3. The 50% inhibitory concentration values of clavulanic acid for TEM-138, TEM-1, and TEM-3 were 0.8 ± 0.06, 0.08, and 0.6 μM, respectively (26). The presence of the Glu104Lys and Gly238Ser mutations induces a lower inhibition efficiency of clavulanic acid (26). In addition, the 50% inhibitory concentration values of sulbactam increased for TEM-138 (80 ± 9.7 μM) compared to TEM-1 (10 μM).

The Asn175Ile substitution, not previously detected in other TEM-type variants, might be involved in the high affinity of the TEM-138 enzyme for ceftazidime. Structural characterization of the enzyme will be interesting to clarify the role of this mutation.

This report extends the list of ESBLs produced by *S. enterica* serovar Infantis, one of the two major serotypes of nontyphoidal *Salmonella*. The increasing number of *Salmonella* serotypes involved in ESBL production, the variety of the ESBLs, the high stability of the genetic determinants, and the cotransfer of resistance to antibiotics recommended for the treatment of systemic nontyphoidal *Salmonella* infection and typhoid fever are serious problems, especially in developing countries where these infections are still endemic (1, 10).

Nucleotide sequence accession number. The nucleotide sequence determined in this work was submitted to the GenBank database and assigned accession number AY853593.

TABLE 2. Kinetic parameters of TEM-1, TEM-3, TEM-15, and TEM-138[a]

Antibiotic	TEM-1		TEM-3		TEM-15		TEM-138	
	K_m (μM)	Rel V_{max} (%)	K_m (μM)	Rel V_{max} (%)	K_m (μM)	Rel V_{max} (%)	K_m (μM)[b]	Rel V_{max} (%)
Benzylpenicillin	26	100	3.8	100	6	100	4.9 ± 1.2	100
Ampicillin	40	122	8.2	126			6.8 ± 0.75	108
Cephaloridine	244	25	19	120	32	189	31 ± 6.8	48
Ceftazidime			350	65	257	19	187 ± 32.2	79
Cefotaxime			35	252	59	292	56 ± 11.6	65

[a] See reference 22 for TEM-1 and TEM-3 and reference 20 for TEM-15. Relative (Rel) V_{max} values were obtained by normalizing the value for each antibiotic to that for benzylpenicillin (taken as 100).
[b] Standard deviations are from triplicate determinations.

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