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Novel Extended-Spectrum TEM-Type β-Lactamase from an *Escherichia coli* Isolate Resistant to Ceftazidime and Susceptible to Cephalothin

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A novel extended-spectrum TEM-type β-lactamase was detected in an *Escherichia coli* isolate which was resistant to ceftazidime and susceptible to cephalothin. The corresponding *bla* gene was sequenced. The deduced amino acid sequence showed the following three amino acid replacements with respect to the TEM-2 sequence: Glu→Lys-104, Arg→Ser-164, and Glu→Lys-240. Since it confers a ceftazidimase-type resistance phenotype, we propose for this novel enzyme the designation CAZ-9, corresponding to TEM-46 in the sequential numbering scheme of TEM β-lactamases.

Escherichia coli strains are usually very susceptible to extended-spectrum cephalosporins. However, three mechanisms can be responsible for resistance to extended-spectrum cephalosporins in *E. coli* isolates (15): alterations in outer membrane proteins, overproduction of the chromosomal cephalosporinase, or production of an extended-spectrum β-lactamase.

We describe here a novel extended-spectrum β-lactamase produced by a clinical isolate of *E. coli* (CF 1702) recovered from the urine specimens of a patient with a urinary tract catheter. The isolate was highly resistant to ceftazidime and aztreonam but remained susceptible to cephalothin, cefamandole, and cefotaxime. The double-disk synergy test (7) was positive between clavulanic acid and ceftazidime or aztreonam. This unusual β-lactam resistance phenotype was transferred to *E. coli* HB101, which was resistant to rifampin.

The β-lactamase described in this report is a new extended-spectrum TEM-type β-lactamase. For this novel ceftazidimase-type extended-spectrum β-lactamase, we propose the denomination CAZ-9, corresponding to TEM-46 in the sequential numbering scheme of TEM β-lactamases.

The MICs of β-lactams were determined on Mueller-Hinton agar (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) by a dilution method with an inoculum of 10^4 CFU per spot. Table 1 lists the MICs of cephalothin, cefotaxime, ceftazidime, and aztreonam alone or combined with clavulanic acid (2 μg/ml) for the *E. coli* isolate (CF 1702) and its transconjugant (CF 1802) compared with those for the *E. coli* transconjugant producing CAZ-6/TEM-24 (CF 1202).

The *E. coli* isolate CF 1702 and its transconjugant CF 1802 were highly resistant to ceftazidime (MICs, 512 and 128 μg/ml, respectively) and to aztreonam (MICs, 256 and 32 μg/ml, respectively). The MICs of cefotaxime remained low (0.5 and 0.12 μg/ml, respectively). Moreover, the MICs of cephalothin were identical to that for the recipient strain. Conversely, the previously described extended-spectrum β-lactamase CAZ-6/TEM-24 conferred high levels of resistance to cephalothin as well as to broad-spectrum cephalosporins.

The novel enzyme CAZ-9/TEM-46 was inhibited by clavulanic acid, which restored the impaired activities of ceftazidime and aztreonam.

Analytical isoelectric focusing was performed with polyacrylamide gels containing ampholines (pH range, 3.5 to 10) as previously described (3). The *E. coli* clinical strain and its transconjugant produced a β-lactamase with a pI of 6.5.

Kinetic constants were obtained by the computerized microacidimetric method as previously described (9). K_m and relative V_{max} values of this novel β-lactamase compared with those of TEM-24 (pI 6.5) are reported in Table 2. Compared to TEM-24, TEM-46 showed a decrease in V_{max}/K_m (efficiency of hydrolysis) values for all cephalosporins and aztreonam. Nevertheless, this effect was more drastic for cephalothin and cefotaxime (greater than 10-fold decrease) and was moderate for ceftazidime and aztreonam (2- to 3-fold decrease).

The concentrations of inhibitors required to inhibit 50% of the β-lactamase activity were measured after 10 min of preincubation of the enzyme with the inhibitor and with penicillin G as substrate. These concentrations of clavulanic acid, sulbactam, and tazobactam with regard to TEM-46 were 20, 50, and 15 nM, respectively.

Single-stranded DNA templates for sequencing were generated by PCR performed with an asymmetric ratio of amplification primers A and B (2). Nucleotide sequences were determined by direct sequencing of the amplification products as

TABLE 1. MICs of β-lactams for *E. coli* clinical isolate CF 1702 and *E. coli* transconjugants

Strain (enzyme)	MIC (μg/ml)							
	Cephalothin		Cefotaxime		Ceftazidime		Aztreonam	
	Alone	+CA[a]	Alone	+CA[a]	Alone	+CA[a]	Alone	+CA[a]
E. coli CF 1702 (CAZ-9/TEM-46)	8	4	0.5	≤0.06	512	2	256	0.25
E. coli CF 1802[b] (CAZ-9/TEM-46)	4	4	0.12	≤0.06	128	0.5	32	0.06
E. coli CF 1202[b] (CAZ-6/TEM-24)	256	32	4	0.12	256	1	32	0.12
E. coli HB101[c]	4	4	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06

[a] CA, clavulanic acid (2 μg/ml).
[b] *E. coli* HB101 transconjugant.
[c] Recipient strain.

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TABLE 2. Kinetic constants of CAZ-9/TEM-46 and CAZ-6/TEM-24 from *E. coli* transconjugants

Drug	CAZ-9/TEM-46[a]			CAZ-6/TEM-24[b]		
	K_m (μM)	Relative V_{max} (%)[c]	Relative V_{max}/K_m[c]	K_m (μM)	Relative V_{max} (%)[c]	Relative V_{max}/K_m[c]
Benzylpenicillin	5	100	100	5.5	100	100
Amoxicillin	18	76	21	43	62	7.9
Ticarcillin	<5	69	69	<5	62	68
Cephalothin	100	46	2.3	43	281	36
Cefotaxime	50	13	1.3	50	130	14.3
Ceftazidime	159	185	5.8	377	1,410	20.6
Aztreonam	12	35	9.2	42	126	16.5

[a] Specific activity obtained with benzylpenicillin as substrate, 30 mU/mg.
[b] Specific activity obtained with benzylpenicillin as substrate, 30 mU/mg.
[c] Values are relative to those for benzylpenicillin (taken as 100%).

previously described (2). As shown in Table 3, analysis of nucleotide sequences reveals that the gene encoding TEM-46 is identical to bla_{TEM-2} at all positions except one (A→G-925) which are known to allow discrimination of the bla_{TEM-1a}, bla_{TEM-1b}, and bla_{TEM-2} genes (4). In addition to this silent mutation, the gene encoding TEM-46 differs from bla_{TEM-2} by three mutations (nucleotides 512, 692, and 917) leading to the following amino acid substitutions: Glu→Lys-104, Arg→Ser-164, and Glu→Lys-240.

The role of the Glu→Lys-104 change, which has been observed for many extended-spectrum β-lactamases, remains unclear. Recent mutagenesis work (12) has shown that this change may perturb the SDN loop and its interaction with substrate but is probably not directly involved in the extension of the substrate range of mutant enzymes.

The substitution Arg→Ser-164 was suggested to increase the omega loop flexibility without a specific effect on ceftazidime compared to cefotaxime (8, 11).

The Glu→Lys-240 change is known to significantly increase levels of resistance to ceftazidime and aztreonam. It has been suggested (10, 13) that the amino group of Lys-240 could establish electrostatic interactions with the oxime acid group of ceftazidime or aztreonam. These interactions could not be established with cefotaxime, which has no carboxyl group on the side chain.

The three substitutions observed for TEM-46 have been previously described for TEM-24 in association with the substitution Ala→Thr-237. Since it had previously been reported (5, 6) that the presence of a threonine residue at position 237 increased catalytic efficiency against cephems, we suppose that the lack of the substitution Ala→Thr-237 in TEM-46 is responsible for its hydrolytic activity being lower than that of TEM-24 and especially for its low efficiency of hydrolysis with regard to cephalothin and cefotaxime.

TABLE 3. Nucleotide and amino acid substitutions in bla_{TEM} genes

Nucleotide no.[a]	Nucleotide (amino acid)[b] in:		
	bla_{TEM-2}	bla_{TEM-46}	bla_{TEM-24}
226	C (Phe)	C	C
317	A (Lys-39)	A	A
346	G (Glu)	G	G
436	T (Gly)	T	T
512	G (Glu-104)	A (Lys)	A (Lys)
604	G (Ala)	G	G
682	C (Thr)	C	T
692	C (Arg-164)	A (Ser)	A (Ser)
911	G (Ala-237)	G	A (Thr)
917	G (Glu-240)	A (Lys)	A (Lys)
925	A (Gly)	G	G

[a] Nucleotide numbering is according to that given by Sutcliffe (14).
[b] The amino acid is indicated for cases in which a point mutation leads to an amino acid substitution compared with the sequence of TEM-2 (4). Numbering is according to that given by Ambler et al. (1).

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