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First Isolation of a CTX-M-3-Producing *Enterobacter cloacae* in France

At the beginning of the 1990s, a new class A extended-spectrum β-lactamase (ESBL), MEN-1 (CTX-M-1), was characterized in *Escherichia coli* strains isolated from Italian and German patients (1, 2). CTX-M-1 was the first member of the CTX-M β-lactamase family, which now comprises nine members: CTX-M-1 (MEN-1) (1, 2), CTX-M-2 (2), Toho-1 (7), CTX-M-3 (6), CTX-M-4 (6), CTX-M-5 (4), Toho-2 (8), CTX-M-6 (5), CTX-M-7 (5), and CTX-M-8 (3). These ESBLs conferred higher cefotaxime MICs than those of ceftazidime.

E. coli (1, 7–10) and *Salmonella typhimurium* (2, 5, 6, 11) strains are the species most frequently reported to produce CTX-M enzymes. These enzymes are reported mainly in three geographic areas: South America (2, 3), East Europe (4–7, 10, 11), and Japan (8, 9).

During a multicenter survey of ESBLs in France in 1998 (C. De Champs, D. Sirot, C. Chanal, J. Sirot, and the French Study Group, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 149.C2-1485, p. 169, 1999), an *Enterobacter cloacae* strain (Ver-1) was selected for its resistance to broad-spectrum cephalosporins and a positive double-disk synergy test. This strain was isolated from a 56-year-old man admitted to the Versailles Hospital (Le Chesnay, France) with unbalanced non-insulin-dependent diabetes and severe arteriopathy. His last hospitalization was in 1996 for noninfected urinary tract retention. He had osteitis and a foot ulcer infected by a *Staphylococcus aureus* strain. After 2 days of treatment with oxacillin, the *E. cloacae* strain, Ver-1, was isolated from a urine sample collected for dysuria. This colonization was not treated.

Ver-1 was also resistant to tetracycline, co-trimoxazole, gentamicin, and tobramycin. The ESBL phenotype was transferred to *E. coli* HB101, resistant to rifampin, at 37°C during an overnight mating assay on solid Mueller-Hinton medium containing rifampin (300 μg/ml). The *E. coli* transconjugant, designated TrVer-1, did not exhibit cotransferred resistance markers.

Table 1 shows the MICs of β-lactams, determined by the agar dilution method, for the strain *E. cloacae* Ver-1 and its *E. coli* transconjugant TrVer-1. These two strains were resistant to penicillins. MICs of cefotaxime (128 to 32 μg/ml) were 16- to 64-fold higher than those of ceftazidime (8 to 0.5 μg/ml). The β-lactam inhibitors clavulanate and tazobactam restored partially or totally the susceptibilities to piperacillin and cephalosporins.

Sonicates of the clinical strain and its *E. coli* transconjugant were subjected to analytical isoelectric focusing over the pH range of 3 to 10. Both *E. cloacae* and its *E. coli* transconjugant produced a β-lactamase of isoelectric point 8.4, associated with a β-lactamase of pI 5.4.

PCR and direct DNA sequencing identified the β-lactamase of pI 5.4 as TEM-1 penicillinase. No PCR products were obtained with primers specific for bla_{SHV}. In contrast, positive amplification was obtained with primers CTX-M-3A (5′-GGT TAAAAAATCGCG-3′) and CTX-M-3B (5′-TTACAAACCG TCGGTGA-3′), which amplified the complete sequence of open reading frame $bla_{CTX\text{-}M\text{-}3}$. The obtained DNA sequence of the PCR products exhibited 100% identity to the sequence $bla_{CTX\text{-}M\text{-}3}$ (8).

While only CTX-M-1 and -2 were characterized in 1990 and 1992 (1, 2), seven new CTX-M enzymes were described in 1998 and 1999, showing that the CTX-M family of ESBLs was small but rapidly growing. CTX-M-3 was first characterized in 1998 for *Citrobacter freundii* and *E. coli* strains at Praski Hospital in Poland (8). This enzyme has spread in other species of the *Enterobacteriaceae* (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *E. cloacae*, and *Morganella morganii*) and was the more frequently observed ESBL (11). Here, we report the first characterization of a CTX-M-3-producing strain isolated from a French patient with no history of travel the year before. The spread of the CTX-M-3 enzyme from East European countries cannot be excluded. Tassios et al. (11) have shown the probable spread of a CTX-M-4-producing *S. typhimurium* clone in Russia, Greece, and Hungary. However, the emergence of CTX-M enzymes from widespread environmental bacteria could also explain their spread.

During the study of ESBLs (De Champs et al., 39th ICAAC) which led to characterization of these CTX-M-3-producing *E. cloacae* strains, 79 ESBL-producing strains were isolated. Only one CTX-M-producing strain was observed. Thus, this CTX-M-3-producing strain seems to be a sporadic isolate. However, in view of the spread of CTX-M-producing strains in East European countries, emergence of CTX-M-producing strains could be observed in France. The characterization of this CTX-M-3-producing strain highlights the feasibility of this process and constitutes a forewarning of the probable existence of CTX-M-producing strains in France.

TABLE 1. MICs of β-lactams for *E. cloacae* isolate Ver-1 and its *E. coli* transconjugant TrVer-1 in comparison with wild-type *E. cloacae* and TEM-1-producing *E. coli*[a]

β-Lactam[b]	MIC			
	E. cloacae		*E. coli*	
	Ver-1 (5.4, 8.4, >8.6)	Ver-2[c] (>8.6)	TrVer-1 (5.4, 8.4)	Tr4[d] (5.4)
Amoxicillin	>512	>512	>512	>512
Amoxicillin + CA	512	>512	32	64
Ticarcillin	>512	8	>512	>512
Ticarcillin + CA	128	8	16	16
Piperacillin	>512	4	512	512
Piperacillin + TA	4	2	0.5	0.5
Cefoxitin	128	256	2	2
Cefotaxime	128	1	32	<0.06
Cefotaxime + CA	<0.06	1	0.5	<0.06
Ceftazidime	8	0.5	0.5	0.25
Ceftazidime + CA	0.5	0.5	0.12	0.12
Cefpirome	32	0.12	4	0.12
Cefpirome + CA	<0.06	0.12	<0.06	0.06
Cefepime	16	0.12	2	0.12
Cefepime + CA	0.06	0.06	<0.06	<0.06
Aztreonam	16	0.06	2	0.06
Aztreonam + CA	0.125	0.12	<0.06	<0.06
Imipenem	0.5	0.5	0.25	0.12

[a] β-Lactamase pIs are indicated in parentheses.
[b] CA, clavulanate at a fixed concentration of 2 μg/ml; TA, tazobactam at a fixed concentration of 4 μg/ml.
[c] Clinical isolate, which produces only a chromosomal cephalosporinase.
[d] *E. coli* HB101 transconjugant, which produces only the penicillinase TEM-1.

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