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CTX-M-35 extended-spectrum β -lactamase conferring ceftazidime resistance in *Citrobacter koseri*

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Sir

CTX-M-type extended-spectrum β -lactamases (ESBLs) are a group of ESBLs that are becoming increasingly common worldwide. Over 90 CTX-M-type enzymes have been registered (<http://www.lahey.org/studies>), belonging to five clusters based on amino acid sequence homology. Unlike TEM- and SHV-type ESBLs, most CTX-M-type ESBLs have strong substrate preference for cefotaxime over ceftazidime. Here we report identification of a CTX-M-2 variant that preferentially hydrolyses ceftazidime, obtained from a clinical isolate in the USA.

The patient was a 98-year-old female who was admitted to the University of Pittsburgh Medical Center (Pittsburgh, PA) for exacerbation of congestive heart failure. She had not received any antimicrobials since her admission. During the hospital course she developed symptoms of cystitis, and urine taken on the seventh hospital day contained 182 leukocytes/high power field. Concurrent urine culture grew 10^5 colony-forming units (CFU)/mL of *Citrobacter koseri* and *Escherichia coli*. After 6 days of treatment with intravenous and then oral cefuroxime, to which *E. coli* but not *C. koseri* was susceptible, the patient became asymptomatic and leukocytes cleared from the urine. Repeat urine culture grew 80 000 CFU/mL of *C. koseri*. This was felt to represent colonisation and the patient was not further treated with antimicrobial therapy.

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Competing interests

YD receives research support from Merck.

Ethical approval

Not required.

The *C. koseri* strain (GB407) was resistant to all cephalosporins tested (Table 1). Polymerase chain reaction (PCR) analysis for this strain was positive for CTX-M- and TEM-type β -lactamase genes but negative for an SHV-type β -lactamase gene [1]. Upon sequencing, the TEM-type β -lactamase gene was identified as encoding TEM-1. When *E. coli* DH10B was transformed with plasmids purified from *C. koseri* GB407, transformants exhibiting high-level resistance to ceftazidime and intermediate resistance to cefotaxime were obtained. PCR analysis of one of the transformants was positive for the CTX-M-type β -lactamase gene only. Sequencing of the entire gene revealed it to encode CTX-M-35.

CTX-M-35 is a variant of CTX-M-2, with a proline to serine substitution at position 167 in the omega loop. CTX-M-35 has been generated experimentally by several investigators and shown to confer high-level resistance to ceftazidime through enhanced hydrolytic activity against this substrate [2,3]. We thus obtained *E. coli* DH10B isogenic clones harbouring pBCSK(-)-based recombinant plasmids encoding CTX-M-2 and CTX-M-35 using a previously described methodology [1]. Minimum inhibitory concentrations (MICs) of cephalosporins for these isogenic clones are shown in Table 1. Production of CTX-M-35 was associated with a 16-fold higher MIC of ceftazidime compared with CTX-M-2. On the other hand, the MIC of cefotaxime was 12-fold lower in the CTX-M-35-producing clone than in the CTX-M-2-producing clone. This finding was consistent with the results of aforementioned reports in which this enzyme was experimentally generated. Several other CTX-M variants with amino acid substitution at position 167 are known to exhibit the same phenomenon. These include CTX-M-19 (Pro167Ser) belonging to the CTX-M-9 group [4] and CTX-M-23 and -42 (Pro167Thr) [5,6] and CTX-M-54 (Pro167Gln) [7] belonging to the CTX-M-1 group. It has been speculated that these substitutions may enlarge the binding site for ceftazidime in the catalytic site [4]. The occurrence of CTX-M-35 has been documented in several Enterobacteriaceae species in Japan, although the details have not been described (GenBank accession nos. AB176532, AB176533 and AB176534).

To our knowledge, this is the first description of a CTX-M variant whose phenotype mimics that of TEM- and SHV-type ESBLs in the USA, where CTX-M-type ESBLs are increasingly recognised as the predominant ESBLs. The emergence of such a variant further adds to the already complex epidemiology of ESBLs.

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Table 1

Minimum inhibitory concentrations (MICs) of CTX-M-35-producing *Citrobacter koseri* and its transformant and isogenic clones producing CTX-M-35 and CTX-M-2^a

Antimicrobial	MIC (µg/mL)				
	<i>C. koseri</i> GB407	<i>E. coli</i> DH10B (pGB407) ^b	<i>E. coli</i> DH10B (pCTX-M-35) ^c	<i>E. coli</i> DH10B (pCTX-M-2) ^c	<i>E. coli</i> DH10B
PIP/TAZ	256	8	3	8	1.5
Cefoxitin	256	8	8	8	8
Ceftriaxone	32	32	32	32	0.047
Cefotaxime	256	24	4	48	0.064
Cefotaxime/clavulanic acid ^d	1.0	0.38	0.19	0.125	0.064
Ceftazidime	256	256	192	12	0.75
Ceftazidime/clavulanic acid ^d	4	4	1.5	0.38	0.25
Cefepime	64	3	1.5	6	0.064
Aztreonam	64	8	3	12	0.064
Imipenem	0.38	0.75	0.75	0.75	0.75

PIP/TAZ, piperacillin/tazobactam.

^a Susceptibility testing was performed by Etest (AB BIODISK, Solna, Sweden).

^b Transformant harbouring CTX-M-35-encoding plasmid from *C. koseri* GB407.

^c Transformants harbouring recombinant plasmids encoding CTX-M-35 and its parent CTX-M-2, respectively.

^d Clavulanic acid concentration was constant at 4 µg/mL.