ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2005, p. 477–478 Vol. 49, No. 1
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.1.477–478.2005

First Description of CTX-M-15-Producing *Klebsiella pneumoniae* in Portugal

The first CTX-M-15 β-lactamase was found in several enterobacterial isolates from India in 1999 (4). Worldwide spread of CTX-M-15 β-lactamases is now well documented (1–5, 7, 8, 12). CTX-M β-lactamases confer high-level resistance to cefotaxime, ceftriaxone, and aztreonam and are well inhibited by clavulanate and tazobactam (10, 11).

Klebsiella pneumoniae 193KFFUL was isolated from a blood culture in September 2003 at the Hospital de Santa Maria. The clinical isolate was resistant to cefotaxime and cefepime (MIC, >256 μg/ml) and ceftazidime and aztreonam (MIC, 96 μg/ml) and susceptible to imipenem (MIC, 0.125 μg/ml). Tazobactam and clavulanate restored the activities of piperacillin, cefotaxime, ceftazidime, and cefepime as determined by E-test strips (Table 1). Genomic DNA was prepared as described elsewhere (6), and PCR experiments were performed using specific primers in order to amplify *bla* genes coding for CTX-M β-lactamases. The set of primers CTX1 (5′-SCS ATG TGC AGY ACC AGT AA-3′) and CTX2 (5′-CCG CRA TAT GRT TGG TGG TG-3′), designed in accordance with consensus sequences from the $bla_{\text{CTX-M}}$ genes available at GenBank, produced an amplicon with 544 bp. In order to perform sequencing of the entire gene, PCR was performed with primers CTX-M-1F (5′-ATG GTT AAA AAA TCA CTG CGY C-3′) and CTX-M-1R (5′-TTA CAA ACC GTC GGT G-3′). The amplicon of 876 bp was cloned into the pCR2.1-TOPO vector, resulting in plasmid p193K1, and introduced into *Escherichia coli* TOP10 chemically competent cells. The sequenced gene shared 100% homology with $bla_{\text{CTX-M-15}}$. *E. coli* 193K1 revealed MIC profiles similar to those of the parental strain, particularly for cefotaxime, whose MIC was >256 μg/ml. CTX-M-15 showed increased activity against ceftazidime because of a single nucleotide substitution (A-725→G) that has already been reported in CTX-M-16 (2, 4, 10).

To explore the surrounding regions of $bla_{\text{CTX-M-15}}$, PCR was performed with internal primers CTX1 and CTX2 and primers hybridizing to the ends of the insertion sequences IS*Ecp1* and IS*903* (2) and to the conserved regions of class 1 integrons, 5′-CS and 3′-CS (6). Positive PCR products were obtained with primers IS*Ecp1*F and CTX2 (911 bp) and primers CTX1 and IS*903*R (1,430 bp). Nucleotide sequence analysis indicated that $bla_{\text{CTX-M-15}}$ was flanked upstream by an IS*Ecp1*-like element and downstream by an IS*903*-like element. Insertion sequences such as IS*Ecp1* or IS*903* have already been described as flanking regions of $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-17}}$, and $bla_{\text{CTX-M-19}}$ (2, 4, 9).

A class 1 integron was identified containing an *aadA1* and an *aadA2* gene not associated with the $bla_{\text{CTX-M-15}}$ gene.

The −35 and −10 promoter sequences for $bla_{\text{CTX-M-15}}$ expression are located at the end of an IS*Ecp1*-like element upstream of its inverted repeat, which is 48 bp from the start codon ATG (data not shown), as already described for $bla_{\text{CTX-M-15}}$ from India and Turkey and different from the $bla_{\text{CTX-M-15}}$ gene described in Poland, in which the distance is 128 bp (4, 9). In addition, analysis of the downstream region of $bla_{\text{CTX-M-15}}$ showed that 685 nucleotides had 97% similarity to IS*903-C* from $bla_{\text{CTX-M-17}}$.

This is the first report identifying an IS*903*-like element downstream of the $bla_{\text{CTX-M-15}}$ gene in a *K. pneumoniae* isolate from a Portuguese hospital.

TABLE 1. MICs of β-lactam antibiotics alone or in association with β-lactam inhibitors for *K. pneumoniae* clinical isolate 193KFFUL and *E. coli* 193K1 harboring recombinant plasmid p193K1

β-Lactam	MIC (μg/ml)	
	K. pneumoniae 193KFFUL	*E. coli* 193K1
Amoxicillin	>256	>256
Amoxicillin + CL[a]	8	16
Piperacillin	>256	>256
Piperacillin + TZ[b]	32	32
Ceftazidime	96	24
Ceftazidime + CL	0.5	1.0
Cefepime	>256	16
Cefepime + CL	0.19	0.064
Cefotaxime	>256	>256
Cefotaxime + CL	0.125	0.094

[a] CL, clavulanic acid.
[b] TZ, tazobactam.

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