Short Communication

Characterization of CTX-M-22 and TEM-141 Encoded by a Single Plasmid from a Clinical Isolate of *Enterobacter cloacae* in China

Gang Liu¹,²*, Bao-Dong Ling¹*, Yong-En Xie¹, Li Lin¹, Yun Zeng¹, Xiang Zhang¹ and Jun Lei¹

¹Pharmaceutical Research Institute and Department of Pharmacology, North Sichuan Medical College, and ²National Engineering Research Center for Biomaterials, Sichuan University, Sichuan, China

(Received July 27, 2006. Accepted May 9, 2007)

SUMMARY: We analyzed the resistance to expanded-spectrum cephalosporins of an *Enterobacter cloacae* clinical isolate, EC002, by transconjugation, isoelectric-focusing analysis, and cloning experiments. It produced two β-lactamases with isoelectric point values of 5.4 and 8.7, corresponding to TEM-141, a novel variant of TEM-1, and CTX-M-22, encoded by a transferable plasmid.

Resistance to expanded-spectrum cephalosporins commonly develops in *Enterobacter cloacae* during therapy due to the selection of mutants producing high levels of chromosomal AmpC (1,2). However, a high prevalence of plasmid-encoded expanded spectrum β-lactamase (ESBL) producers, in particular an unprecedented, rapid increase in the recognition of *E. cloacae* clinical isolates containing CTX-M-type β-lactamases, has been observed in China (3-7), which has been increasingly concerned about therapy for clinical infections. In an attempt to assess β-lactam resistance in *E. cloacae* isolated from our major teaching hospital in the northern region of Sichuan Province, China, we have initiated a characterization of the molecular mechanisms responsible for resistance to ESBL (5). Here we report the characterization of TEM-141, a novel variant of TEM-1, and CTX-M-22, encoded by a transferable plasmid from an *E. cloacae* clinical isolate EC002.

*E. cloacae* isolate EC002 was isolated from a sample obtained at the First Teaching Hospital of North Sichuan Medical College; the specimen was taken from the urine of an infected patient who was previously treated unsuccessfully with cefotaxime for 2 weeks in September 2004. The β-lactam resistance of strain EC002 was successfully transferred to strains of *Escherichia coli* JM109, by transconjugation (8). The analysis of the plasmid content of EC002 and its transconjugant revealed a plasmid, which was designated as pEC002. The clinical isolate EC002 and the transconjugants were resistant to all of the penicillins, as well as most of the cephalosporins tested (Table 1), which clearly demonstrated that pEC002 contributed to a β-lactam resistance phenotype similar to that observed in *E. cloacae* EC002. The pEC002 contained two *bla* genes, as determined by polymerase chain reaction (PCR) with previously described primers (5). Sequence analysis revealed that one of the nucleotide sequences (GenBank accession no. AY954529) was identical to that of the reported *Klebsiella pneumoniae* CTX-M-22 (AY080894), and the other was a novel TEM-type penicillinase, TEM-141 (AY956335). Compared to the *bla*(*CTX-M*) gene (X92506), the *bla* gene of CTX-M-22 had nine points nucleotide substitutions, while only three of these changes resulted in residue alteration (i.e., Val 80Ala, Asp 117Asn, and Ser143Ala). In comparison with TEM-1 (AY302260), the *bla*~TEM~ gene of pEC002 had three nucleotide substitutions, i.e., A → G at position 94, C → T at position 228, and G → T at position 396, yielding genetic code changes from AAA to GAA, GGC to GGT, and GCG to GCT, respectively. The first change led to a lysine-to-glutamic acid substitution at residue position 34 (on the basis of the standard β-lactam numbering, as previously suggested [9]); the others were silent and resulted in no residue alteration. This pEC002-encoded TEM-type β-lactamase was submitted to the β-lactamase database at the Lahey Clinics (http://www.lahey.org/Studies), and was assigned as TEM-141, which has not been previously reported.

In order to determine the phenotype and activity of the pEC002-encoded β-lactamase, the PCR products for TEM-1 (a laboratory collection), TEM-141, and CTX-M-22 were cloned into vector pBK-cmv (kanamycin resistant; Stratagene, La Jolla, Calif., USA) after appropriate restriction digestion, yielding plasmids pBK-TEM-1, pBK-TEM-141, and pBK-CTX-M-22. All of the cloning and the *bla* genes were confirmed by nucleotide sequencing. Each of these three plasmids was used to transform *E. coli* JM109, and the transformants were selected with kanamycin and ampicillin (50 µg/ml each). EC002 and its transconjugants expressed two β-lactamases with isoelectric point (pI) values of 5.4 and 8.7 by isoelectric focusing (IEF) (10), corresponding to TEM-141 and CTX-M-22. All of the cloning and the *bla* genes were confirmed by nucleotide sequencing. Each of these three plasmids was used to transform *E. coli* JM109, and the transformants were selected with kanamycin and ampicillin (50 µg/ml each).

EC002 and its transconjugants expressed two β-lactamases with isoelectric point values of 5.4 and 8.7, respectively. Both TEM-1 and TEM-141 showed similar substrate profiles that did not include oxyimino-cephalosporins (Table 2). Consistent with the MIC results (Table 1), CTX-M-22 hydrolyzed cefotaxime more efficiently than cefazidime (Table 2).

Plasmid-mediated ESBLs are becoming increasingly frequent among clinical isolates of the family *Enterobacteriaceae* worldwide (11-15). Among the ESBLs, the CTX-M-type β-lactamases constitute a rapidly growing cluster of enzymes that have disseminated geographically, and these enzymes have been found predominantly in *Enterobacteriaceae* (11,12). In China, the emerging family of the CTX-M-type β-lactamases was the most frequently observed of the ESBLs, accounting for 30-40% ESBLs examined in *E. cloacae* isolates (5-7). Recently, the coexistence of CTX-M-encoding genes with other *bla* genes on the same plasmids was emphasized (5,11).

The strains harboring such plasmids might exhibit a high MIC.
value against β-lactams, as E. coli JM109 (pBK-CTX-M-22) was found to have lower MIC values for piperacillin, ceftazolin, cefepime, and cefazolin, as expected for the CTX-M-derived ESBLs. However, it should be noted that the biochemical characteristics of CTX-M-22 were not confirmed using a crude extract from E. coli JM109. As previous studies have confirmed, the hydrolytic activity against oxyimino cephalosporins of the CTX-M-type-ESBLs was related to the Ω-loop and certain mutations such as 167Ser or 240Asp (11); however, further study is still needed to determine how and why the substitutions in CTX-M-22 influence its hydrolytic activity against oxyimino cephalosporins.

ACKNOWLEDGMENTS

We kindly thank Dr. Xian-Zhi Li for his contribution to this work. This work was supported by a key project (No. 2000J13-030) of Sichuan Province, China.

REFERENCES


