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New Chromosomal AmpC β-Lactamase in *Enterobacter cloacae*

Several members of the *Enterobacteriaceae*, including *Enterobacter* spp., are naturally resistant to amoxicillin and cephalosporins. *Enterobacter cloacae* produces chromosomally encoded β-lactamases, also called cephalosporinases (1), and is a serious nosocomial pathogen, the third most prevalent bacterium isolated in intensive care settings (5, 8). We report here the study of a new chromosomal AmpC β-lactamase produced by *E. cloacae* FFUL2En isolated from the blood culture of a patient hospitalized in a medicine ward of Hospital de Santa Maria, Lisbon, Portugal. The antibiogram revealed resistance to aminopenicillins, aztreonam, and broad-spectrum cephalosporins, except imipenem, aminoglycosides, and quinolones. By isoelectrofocusing, the sonicate extracts expressed a pI of 8.68, suggesting the presence of a presumed AmpC enzyme.

A total DNA preparation from *E. cloacae* FFUL2En was used in PCR experiments with two sets of primers, TN5 (5′-CGTTT GTCAGGCACAGTCAAATCCA) and TN4 (5′-TTACTGTAG CGCGTCGAGGATATGG) and the internal primers TN2 (5′-TTCCACTGCGGCTGCCAGT) and TN3 (5′-CGGATGAGG TCACGGATAACGCC), designed in accordance with consensus sequences from the *ampC* genes described for *E. cloacae* and available at GenBank. The amplicon with 1,234 bp was cloned into the SmaI site of the pBK-CMV vector (6) with a TOPO TA cloning kit, resulting in the plasmid p2En1. The β-lactam susceptibility pattern of *Escherichia coli* 2En1, harboring the recombinant plasmid p2En1, displayed cefoxitin, cefuroxime, ceftazidime, and piperacillin plus tazobactam MICs of >256 μg/ml and a cefepime MIC of 0.5 μg/ml. The MICs of cefotaxime and aztreonam were lower than those for the parental strain (Table 1). The *E. coli* 2En1 transformant showed the same pI as the parental strain (pI 8.68), and the substrate profile of the enzyme EcloFFUL2En was determined with the transformant crude enzymatic extract (7). The V_{max} values indicate that cephalothin, with a V_{max} of 3,000.1 μM/min, is hydrolyzed more quickly than cefoxitin (V_{max} = 3.7 μM/min). Ceftazidime and cefotaxime are not hydrolyzed at detectable levels (V_{max} = <0.1 μM/min).

In order to perform the sequencing reactions, the amplicon of 1,234 bp was cloned in the pCR2.1-TOPO vector with a TOPO TA cloning kit, resulting in the plasmid p2En2. The sequence with 382 amino acids has an 86% identity with the AmpC of *E. cloacae* P99 and 98% identity with the plasmid-borne MIR-1 β-lactamase gene product (2).

To search for a possible chromosomal location of the bla_{AmpC} gene, whole-cell DNA of *E. cloacae* FFUL2En was restricted with I-CeuI endonuclease (New England Biolabs), which recognizes a 26-bp sequence in *rrn* genes coding for the 23S large-subunit rRNA. After digestion, separation of the resulting fragments was performed on a contour-clamped homogeneous electric field-DRII apparatus, as described previously (3).

TABLE 1. MICs of β-lactams for *E. cloacae* FFUL2En clinical isolate, *E. coli* 2En1 harboring recombinant plasmid p2En1, and reference strain *E. coli* TOP10 harboring the pBK-CMV plasmid

β-Lactam	MIC (μg/ml)		
	E. cloacae FFUL2En	*E. coli* 2En1	*E. coli* TOP10(pBK-CMV)
Piperacillin + TZB[a]	>256	>256	3
Cefoxitin	>256	>256	8
Cefuroxime	>256	>256	4
Cefotaxime	>256	8	0.094
Ceftazidime	>256	>256	0.5
Cefepime	0.5	0.38	0.064
Aztreonam	48	6	0.094
Imipenem	0.75	0.38	ND[b]

[a] TZB, tazobactam.
[b] ND, not determined.

The restricted fragments of *E. cloacae* FFUL2En DNA were transferred to a nylon membrane by Southern blotting (9) and were hybridized by using a nonradioactive labeling and detection kit (Roche) with a PCR-obtained probe with primers TN5 and TN2 (see above), consisting of a 576-bp fragment of bla_{AmpC} and a 16S rRNA gene probe amplified with universal primers described elsewhere (4). The bla_{AmpC} probe hybridized only with the 630-kb fragment of *E. cloacae* FFUL2En. These data indicate the chromosomal location of the bla_{AmpC} gene, coding for the AmpC β-lactamase EcloFFUL2En, in *E. cloacae* FFUL2En, which is closely related to the plasmid-borne MIR-1 from *Klebsiella pneumoniae*.

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REFERENCES

1. **Bush, K., G. A. Jacoby, and A. A. Medeiros.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211–1233.
2. **Jacoby, G. A., and J. Tran.** 1999. Sequence of the MIR-1 β-lactamase gene. Antimicrob. Agents Chemother. **43:**1759–1760.
3. **Liu, S. L., A. Hessel, and K. E. Sanderson.** 1993. Genomic mapping with I-Ceu-I, an intron-encoded endonuclease specific for ribosomal RNA, in *Salmonella* spp., *Escherichia coli*, and other bacteria. Proc. Natl. Acad. Sci. USA **90:**6874–6878.
4. **Mammeri, H., L. Poirel, N. Mangeney, and P. Nordmann.** 2003. Chromosomal integration of a cephalosporinase gene from *Acinetobacter baumannii* into *Oligella urethralis* as a source of acquired resistance to β-lactams. Antimicrob. Agents Chemother. **47:**1536–1542.
5. **Rottman, M., Y. Benzerara, B. Hanau-Berçot, C. Bizet, A. Philippon, and G. Arlet.** 2002. Chromosomal *ampC* genes in *Enterobacter* species other than *Enterobacter cloacae*, and ancestral association of the ACT-1 plasmid-encoded cephalosporinase to *Enterobacter asburiae.* FEMS Microbiol. Lett. **210:**87–92.
6. **Sambrook, J., and D. W. Russell.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
7. **Samuni, A.** 1975. A direct spectrophotometric assay and determination of Michaelis constants for the β-lactamase reaction. Anal. Biochem. **63:**17–26.
8. **Sanders, W. E., Jr., and C. C. Sanders.** 1997. *Enterobacter* spp.: pathogens poised to flourish at the turn of the century. Clin. Microbiol. Rev. **10:**220–241.
9. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.

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