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 amoxicillin, 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′-TTACTGTAG CGCGTCGGAGGATATGG) and the internal primers TN2 (5′-TTCACCTGCGGTCGGTACGGT) and TN3 (5′-CGGATGAGG TCACGGATAAACGCC), 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 in the pCR2.1-TOPO vector with a TOPO TA cloning kit, resulting in the plasmid p2En1. The plasmid p2En1 transformant showed the same pI as the parental strain (pI 8.68), and the substrate profile of the enzyme EcloFFUL2En 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. 

To search for a possible chromosomal location of the blaAmpC 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).

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 TNS and TN2 (see above), consisting of a 576-bp fragment of blaAmpC and a 16S RNA gene probe amplified with universal primers described elsewhere (4). The blaAmpC probe hybridized only with the 630-kb fragment of E. cloacae FFUL2En. These data indicate the chromosomal location of the blaAmpC gene, coding for the AmpC β-lactamase EcloFFUL2En, in E. cloacae FFUL2En, which is closely related to the plasmidborne MIR-1 from Klebsiella pneumoniae.

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REFERENCES

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### 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

<table>
<thead>
<tr>
<th>β-Lactam</th>
<th>MIC (µg/ml)</th>
<th>E. cloacae FFUL2En</th>
<th>E. coli 2En1</th>
<th>E. coli TOP10(pBK-CMV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin + TZBa</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>3</td>
<td></td>
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<tr>
<td>Cefoxitin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>8</td>
<td></td>
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<tr>
<td>Cefuroxime</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;256</td>
<td>8</td>
<td>0.094</td>
<td></td>
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<tr>
<td>Ceftazidime</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Cefepine</td>
<td>0.5</td>
<td>0.38</td>
<td>0.064</td>
<td></td>
</tr>
<tr>
<td>Aztreonam</td>
<td>48</td>
<td>6</td>
<td>0.094</td>
<td></td>
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<tr>
<td>Imipenem</td>
<td>0.75</td>
<td>0.38</td>
<td>NDb</td>
<td></td>
</tr>
</tbody>
</table>

a TZB, tazobactam.
b ND, not determined.