Emergence of Ertapenem Resistance in an Escherichia coli Clinical Isolate Producing Extended-Spectrum β-Lactamase AmpC

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Escherichia coli isolate MEV, responsible for a bloodstream infection, was resistant to penicillins, cephalosporins, and ertapenem. Molecular and biochemical characterization revealed the production of a novel, chromosome-borne, extended-spectrum AmpC (ESAC) β-lactamase with a Ser-282 duplication and increased carbapenemase activity. This study demonstrates for the first time that chromosome-borne ESAC β-lactamases can contribute to the emergence of ertapenem resistance in E. coli clinical isolates.

Escherichia coli produces a chromosomal AmpC β-lactamase at a very low level because of a weak promoter (4). Spontaneous mutations affecting the promoter region can induce overproduction of this enzyme, conferring resistance to narrow-spectrum cephalosporins (20). Moreover, structural alterations in the R2 binding site, which accommodates the R2 lateral side chain of β-lactams, can broaden the hydrolysis spectrum of AmpC β-lactamases toward extended-spectrum cephalosporins (ESCs), including cefepime (14, 28). Extended-spectrum AmpC (ESAC) β-lactamases, which constitute novel group 1e in the updated functional classification of Bush and Jacoby (3), also exhibited increased catalytic efficiency against carbapenemase compared to that of the parental enzymes (28).

However, at that time, the phenotypic expression of this weak carbapenemase activity was detected only in porin-deficient E. coli recombinant clones (24).

We described here for the first time the emergence of ertapenem resistance in an ESAC-producing E. coli clinical isolate. E. coli MEV was recovered in two blood cultures from a 50-year-old patient suffering from myeloma at the Morvan hospital (Brest, France) in April 2008. The patient had been hospitalized multiple times in the previous 6 months and had received multiple courses of antibacterials. On admission, the patient was empirically treated with vancomycin, ceftazidime, and ciprofloxacin. Once the bacteriological documentation became available, the treatment was changed to imipenem and

<table>
<thead>
<tr>
<th>β-Lactam</th>
<th>E. coli MEV</th>
<th>E. coli MEV + CLOXA</th>
<th>E. coli MEV + PAβN</th>
<th>E. coli TOP10(pMEV)</th>
<th>E. coli TOP10(pAmpC-A)</th>
<th>E. coli TOP10</th>
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<tr>
<td>Amoxicillin</td>
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<td>&gt;512</td>
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<td>Amoxicillin-CLA</td>
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<td>Piperacillin</td>
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<td>Ceftazidime</td>
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<tr>
<td>Cefepime</td>
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<td>2</td>
<td>0.06</td>
<td>0.06</td>
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<tr>
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<td>0.25</td>
<td>0.5</td>
<td>0.25</td>
<td>0.06</td>
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<tr>
<td>Ertapenem</td>
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<td>0.5</td>
<td>0.125</td>
<td>0.016</td>
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<td>Meropenem</td>
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<td>0.25</td>
<td>0.032</td>
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</tr>
</tbody>
</table>

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vancomycin for 13 days combined with amikacin for the first 5 days. The course of the infection was favorable.

Susceptibility testing, which was performed as previously described (26), showed that *E. coli* MEV was resistant to penicillins, cephalosporins, and ertapenem but remained susceptible to imipenem according to the revised CLSI criteria (7, 21, 25). This insertion led to a duplication of the serine residue located at position 282 in the H-9 helix (Fig. 1) (9, 20, 28). This structural alteration is reported here for the first time.

The coding regions of the bla<sub>AmpC-MEV</sub> and bla<sub>AmpC-A</sub> genes were cloned into pCR-BluntII-Topo (Invitrogen, Cergy-Pontoise, France), and the recombinant plasmids were subsequently transformed into *E. coli* strain TOP10, giving rise to *E. coli* TOP10(pMEV) and TOP10(pAmpC-A) recombinant clones, respectively (26). In all of the recombinant plasmids, the orientation of the clone insert was the same, with the ampC gene under the transcriptional control of the lacZ promoter flanking the cloning site.

The AmpC-MEV and AmpC-A β-lactamases were extracted from the *E. coli* TOP10 recombinant strains and purified as described previously (26), yielding two extracts containing the proteins at 0.38 mg/ml and 1.52 mg/ml, respectively. The homogeneity of the purified extracts (>99%) was assessed by SDS-PAGE analysis (15). The specific activities, determined with 100 μM cephalothin as the substrate, were 60 and 490

### TABLE 2. Kinetic parameters of AmpC-MEV β-lactamase and the wild-type AmpC-A enzyme

<table>
<thead>
<tr>
<th>β-Lactams</th>
<th>AmpC-MEV</th>
<th></th>
<th>AmpC-A</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>(k_{\text{cat}}) (s(^{-1}))</td>
<td>(K_m) (μM)</td>
<td>(k_{\text{cat}}/K_m) (μM(^{-1})s(^{-1}))</td>
<td>(k_{\text{cat}}) (s(^{-1}))</td>
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<td>Cephalothin</td>
<td>135 ± 15</td>
<td>18 ± 2</td>
<td>7.5</td>
<td>500 ± 20</td>
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<td>Cephaloridine</td>
<td>160 ± 12</td>
<td>38 ± 4</td>
<td>4.2</td>
<td>230 ± 15</td>
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<tr>
<td>Cefotaxime&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.056 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.7</td>
<td>0.3 ± 0.02</td>
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<tr>
<td>Ceftriaxime&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25 ± 0.05</td>
<td>0.05 ± 0.01</td>
<td>5</td>
<td>0.2 ± 0.005</td>
</tr>
<tr>
<td>Cefepime&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78 ± 0.03</td>
<td>0.4 ± 0.08</td>
<td>1.95</td>
<td>0.5 ± 0.03</td>
</tr>
<tr>
<td>Imipenem&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5 ± 0.2</td>
<td>60 ± 4</td>
<td>0.025</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> For those compounds with a \(K_m\) value of <5 μM, the \(K_i\) was determined instead of the \(K_m\) with cephaloridine as the substrate.

<sup>b</sup> ND, not determinable.

<sup>c</sup> The values shown are the means ± the standard deviations of at least three independent experiments.
μmol/min/mg of protein, respectively. The kinetic parameters, which were determined as previously described (26), are presented in Table 2. The $k_{cat}$ and $K_m$ values of AmpC-MEV β-lactamase were, respectively, increased and decreased with respect to those of AmpC-A for all ESCs and imipenem, accounting for enhanced catalytic efficiencies ($k_{cat}/K_m$).

Surprisingly, hydrolysis of ertapenem by AmpC-MEV and AmpC-A extracts was not detectable. Such a discrepancy between phenotypic and biochemical results has already been reported (22) and might be attributable to the low but nonzero deacylation rate of AmpC β-lactamases for this compound (16).

MICs for the E. coli TOP10(pMEV) and TOP10(pAmpC-A) recombinant strains are presented in Table 1. AmpC-MEV β-lactamase conferred higher levels of resistance to all oximinocephalosporins than the AmpC-A enzyme, thus confirming that the Ser-282 insertion extended the hydrolysis spectrum. In contrast, AmpC-MEV-lactamase were, respectively, increased and decreased with respect to those of AmpC-A for all ESCs and imipenem, accounting for enhanced catalytic efficiencies ($k_{cat}/K_m$).

The AcrAB efflux transporter and the marA gene, which encodes a transcriptional activator of the AcrAB transporter, were determined as previously described (11, 13). This showed that the expression of the AcrAB transporter was significantly increased (12-fold) (Fig. 2). This suggested that the E. coli MEV isolate expressed an additional mechanism of resistance affecting its susceptibility to ertapenem.

The AcrAB efflux transporter and the marA gene, which encodes a transcriptional activator of the acrRAB operon, were determined by quantitative reverse transcription (RT)-PCR as previously described (11, 13). This showed that the expression of the AcrAB transporter was significantly increased (12-fold) (Fig. 2).

Amplification of the acrR gene, which encodes a transcriptional repressor of the acrA gene (19), was performed with primers AcrR-F (5′-GCTGCGTTTATATTATCGTGTCG-3′) and AcrR-R (5′-GTCAAAACGCAAGAATATCACGACG-3′) and a standard PCR protocol (denaturation for 10 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C; and a final extension step of 10 min at 72°C). This yielded an 815-bp amplicon for E. coli strain TOP10, whereas it was negative for E. coli MEV, suggesting that the AcrAB efflux overexpression seen in E. coli MEV is related to the deletion of the acrR gene.

Whole-cell DNA of E. coli MEV was extracted and transferred onto a nylon membrane (31). Hybridization of the membrane with a fluorescein-labeled probe that was made of the PCR product of the acrR gene of E. coli strain TOP10 (23) failed, thus confirming the deletion of the acrR gene in E. coli MEV. Nevertheless, further studies, such as homologous recombination, are needed to confirm whether AcrAB efflux transporter overexpression contributes to ertapenem resistance in E. coli.

The outer membrane protein (OMP) profiles of E. coli clinical isolate MEV and control strains expressing OmpC and/or OmpF porins (27) were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (30). Comparison of the OMP profiles showed weak expression of OmpC and lack of OmpF protein in E. coli MEV (Fig. 3), which might explain the additional resistance to ertapenem seen in E. coli clinical isolate MEV (10, 30).

Carbapenem resistance is an emerging phenomenon among E. coli clinical isolates. To date, it has been related to transmissible β-lactamases with strong carbapenemase activity, such as metallo-β-lactamases and KPC-type and OXA-48-type β-lactamases or transmissible β-lactamases with weak carbapenemase activity, such as CTX-M-type β-lactamases or some plasmid-mediated AmpC β-lactamases, in combination with a lack of outer membrane permeability (2, 12, 17, 18, 22, 30, 32). This study demonstrates for the first time that chromosome-borne ESAC β-lactamases can also contribute to the emergence of ertapenem resistance in E. coli clinical isolates.
Nucleotide sequence accession number. The nucleotide sequence of the bla\textsubscript{AMPC-MEV} gene of \textit{E. coli} MEV has been deposited in the EMBL nucleotide sequence database under accession number HQ419012.

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REFERENCES