Nosocomial spread of ESBL-positive *Enterobacter cloacae* co-expressing plasmid-mediated quinolone resistance Qnr determinants in one hospital in France

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Although resistance to quinolones is mainly due to chromosome-encoded mechanisms, it may also result from the expression of plasmid-mediated determinants such as *aac(6')-Ib-cr* encoding an aminoglycoside acetyltransferase that is also capable of acetylating some fluoroquinolones, *qepA* encoding an efflux pump and *qnr* genes. Qnr proteins protect target enzymes (DNA gyrase and type IV topoisomerases) from quinolone inhibition. Five types of Qnr have been reported: QnrA, QnrB, QnrC, QnrD and QnrS.¹ In addition, the co-expression of extended-spectrum β-lactamases (ESBLs) and Qnr determinants has been frequently observed.² To date, two nosocomial outbreaks of Qnr- and ESBL-positive enterobacterial isolates have been reported, corresponding to the spread of QnrA¹- and CTX-M-9-positive *Enterobacter cloacae* isolates in a Dutch hospital³ and of QnrA¹- and VEB-1-positive *Enterobacter sakazakii* in a French hospital.⁴ There is also a recent report of co-expression of ESBL and QnrA¹ in *E. cloacae* in the UK.⁵ The aim of this work was to explore the mechanisms of resistance and the molecular epidemiology of clinical isolates of *E. cloacae* collected in 2006 in the Medical Intensive Care Unit (MICU) of the Bicêtre Hospital, France.

In 2006, 19 isolates of *E. cloacae* were collected from patients hospitalized in the MICU. These isolates were identified using the API20E system (bioMérieux, Marcy l’Étoile, France). Susceptibility testing was performed by disc diffusion assays and Etests as previously described,⁵ and results were interpreted according to the guidelines of the CLSI. Out of the 19 *E. cloacae* clinical isolates, 7 showed an ESBL phenotype associated with resistance to nalidixic acid and decreased susceptibility to fluoroquinolones (Table 1) and were further

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**Table 1.** Features of *E. cloacae* isolates and MICS of tigecycline, quinolones and fluoroquinolones for clinical isolates, corresponding transconjugants (Tc) and the *E. coli* J53 recipient strain

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Date of isolation</th>
<th>Source of isolation</th>
<th>β-Lactamase(s)</th>
<th>Qnr determinant(s)</th>
<th>Plasmid size(s) (kb)</th>
<th>MICS (mg/L) tigecycline</th>
<th>nalidixic acid</th>
<th>norfloxacin</th>
<th>ciprofloxacin</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>05-01-06</td>
<td>arterial catheter</td>
<td>SHV-12/TEM-1</td>
<td>QnrB4</td>
<td>160, 120</td>
<td>4</td>
<td>256</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>28-02-06</td>
<td>blood culture</td>
<td>SHV-12</td>
<td>QnrB4</td>
<td>160</td>
<td>4</td>
<td>256</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>06-05-06</td>
<td>urine</td>
<td>SHV-12/TEM-1/L-AP-2</td>
<td>QnrB4/QnrS1</td>
<td>160, 120, 100</td>
<td>1</td>
<td>&gt;256</td>
<td>8</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>19-09-06</td>
<td>bronchoalveolar lavage</td>
<td>SHV-12/TEM-1/L-AP-2</td>
<td>QnrB4/QnrS1</td>
<td>160, 120, 100</td>
<td>1</td>
<td>&gt;256</td>
<td>8</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>03-12-06</td>
<td>arterial catheter</td>
<td>SHV-12/TEM-1/L-AP-2</td>
<td>QnrB4/QnrS1</td>
<td>160, 120, 100</td>
<td>1</td>
<td>&gt;256</td>
<td>8</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>06-04-06</td>
<td>urine</td>
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<td>QnrB4/QnrS1</td>
<td>160, 120, 100</td>
<td>1</td>
<td>&gt;256</td>
<td>8</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>07-07-06</td>
<td>E. coli J53 Tc5-1</td>
<td>SHV-12</td>
<td>QnrB4</td>
<td>160</td>
<td>8</td>
<td>&gt;256</td>
<td>0.5</td>
<td>0.125</td>
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<tr>
<td>8</td>
<td>04-08-06</td>
<td>E. coli J53 Tc5-2</td>
<td>SHV-12</td>
<td>QnrB4</td>
<td>160</td>
<td>8</td>
<td>&gt;256</td>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>9</td>
<td>06-09-06</td>
<td>E. coli J53 Tc5-7</td>
<td>SHV-12</td>
<td>QnrB4</td>
<td>160</td>
<td>8</td>
<td>&gt;256</td>
<td>0.5</td>
<td>0.125</td>
</tr>
</tbody>
</table>

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Research letters
investigated. These isolates were resistant to most aminoglycosides, but remained susceptible to imipenem and colistin. Resistance to tigecycline (MIC > 2 mg/L) was detected in five isolates. Higher MIC values of quinolones were observed for E. cloacae isolates 1, 2, 3, 6 and 7, compared with E. cloacae 4 and 5 (Table 1), which might be explained by overexpression of efflux pumps, in accordance with observed MICs of tigecycline. The seven isolates were recovered from seven patients and their clonal relationship was assessed by PFGE. Five out of the seven patients were considered as being colonized with E. cloacae whereas two patients were infected (patients 5 and 7). Infection with multidrug-resistant E. cloacae was considered to have contributed to the death of patient 5 whose treatment combining imipenem and colistin failed. The other infected patient was successfully treated with imipenem and amikacin.

Identification of β-lactamase genes (bla<sub>SHV</sub>, bla<sub>TEM</sub>, bla<sub>CTX-M</sub>) and qnrA, qnrB and qnrS genes was performed as described previously using PCR-specific primers and sequencing. All isolates possessed the bla<sub>SHV</sub> gene and E. cloacae 1, 3, 5, 6 and 7 also harboured the bla<sub>TEM-1</sub> β-lactamase gene. PCR and sequencing identified the qnrB4 gene in the seven isolates. In addition, the qnrS1 gene was detected in isolates 5 and 6, indicating the co-occurrence of qnrB4 and qnrS1 in those isolates, as previously reported. Since an association between the qnrS1 gene and the gene encoding the narrow-spectrum β-lactamase LAP-1 had been found previously, PCR experiments using primers specific for the bla<sub>LAP</sub> gene were performed and gave positive results only for isolates 5 and 6. Sequencing revealed that the bla<sub>LAP</sub> gene encoded the β-lactamase LAP-2 determinant, which differs from LAP-1 by a single amino acid change at Ambler position 196. In addition, the seven isolates were resistant to quinolones with a single amino acid change in the quinolone resistance-determining region (QRDR) of gyrA (S83Y) and none in the QRDR of parC. The search for qepA and aac(6')-Ib-cr genes among the qnr-positive isolates remained negative.

Analysis of plasmid content in the seven isolates, using the Kieser technique, showed that all of the isolates possessed an ~160 kb plasmid. Additionally, isolates 1, 3, 5, 6 and 7 possessed an ~120 kb plasmid and isolates 5 and 6 also possessed an ~100 kb plasmid. Mating-out assays were performed as described previously for analysing the transferability of the quinolone resistance determinants. Transconjugants were obtained with isolates 5 and 7 used as donor, which showed resistance to expanded-spectrum cephalosporins and reduced susceptibility to quinolones (Escherichia coli J53 Tc5-1 and Tc7) (Table 1). Analysis of the plasmid content of the transconjugants identified a 160 kb plasmid carrying the bla<sub>SHV-12</sub> gene, together with the qnrB4 gene. In addition, a second phenotype of resistance was obtained using E. cloacae isolate 5 as donor. This E. coli transconjugant, Tc5-2, exhibited a narrow-spectrum resistance profile to β-lactams, associated with reduced susceptibility to fluoroquinolones, and harboured an ~100 kb plasmid, carrying the qnrS1 and bla<sub>LAP-2</sub> genes (Table 1).

We report here a nosocomial dissemination of ESBL- and Qnr-positive E. cloacae during a 12 month period and restricted to the MICU of the Bicêtre Hospital. Interestingly, this work points out that several Qnr resistance determinants may be acquired over time in given isolates. It has been reported that Qnr resistance determinants provide the first resistance step for further in vivo selection of high-level resistance to fluoroquinolones. Spread and persistence of Qnr determinants may become a favourable background for selection of fluoroquinolone resistance in the hospital setting where these antibiotics are largely used.

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Transparency declarations

None to declare.

References


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Only for substrate antibiotics are a functional AcrAB–ToLC efflux pump and RamA required to select multidrug-resistant Salmonella Typhimurium

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