Research letters

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Plasmid-mediated quinolone resistance in nalidixic-acid-susceptible strains of Salmonella enterica isolated in Scotland

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Sir,

Chromosomal mutations in the topoisomerase II gene gyrA and altered drug accumulation were considered to be the only mechanisms of quinolone resistance until 1998, when the plasmid-mediated quinolone resistant gene, qnrA, was described in an isolate of Klebsiella pneumoniae.1 Since then, other plasmid-borne resistance genes, qnrB, qnrS (and their variants), aac(6’)-Ib-cr and qepA, have been identified.2,3 The clinical importance of these qnr genes is that, although not conferring detectable resistance according to the criteria set by the CLSI or BSAC (currently ≥4 mg/L for ciprofloxacin), their presence facilitates the recovery of mutants with elevated levels of resistance to quinolones.2 The presence of qnr has frequently been detected in strains producing extended-spectrum β-lactamasases (ESBLs).2

We investigated a total of 70 isolates of Salmonella enterica that were selected based on reduced susceptibility to ciprofloxacin (≥0.125 mg/L) for the presence of qnr genes. Duplicate samples were excluded, and only a single representative isolate from each outbreak was included. Of these 70 isolates, 53 were susceptible to nalidixic acid (<40 mg/L). The remaining 17 isolates exhibited additional resistance to cefotaxime (1 mg/L).

Antimicrobial resistances were determined using an in-house breakpoint method. Isolates were screened for qnr genes by a touchdown multiplex PCR using primers described previously.4 Transconjugants of Escherichia coli J53 harbouring plasmids pMG252, pMG298 and pMG306 were used as positive controls for qnrA1, qnrB1 and qnrS1, respectively.

The results of the study are summarized in Table 1. Of the 34 isolates positive for one of the qnr genes, 30 were of human origin; the remaining 4 were isolated from ovine (2), bovine (1) and environmental (1) sources. qnr genes were identified in 12 different serotypes. Salmonella Typhimurium was the most common with 13 strains accounting for 38%, followed by 9 Salmonella Stanley. qnrB1 was identified in Salmonella Colindale, with qnrB2 being identified in Salmonella Agona and Salmonella Haifa. qnrS5 was identified in Salmonella Gaminara. With the exception of these six strains, all other qnr genes were identified as qnrS1. These results are similar to those of other laboratories. Scientists in Denmark identified qnrS1 in every one of the 23 strains of Salmonella Corvallis they examined,5 while scientists in England identified the qnrS1 gene in all but 2 of the 39 isolates confirmed as carrying the qnr genes.6

Of the 18 strains isolated from patients with a history of foreign travel, 16 were identified as harbouring qnrS1, one, qnrA1 and another, qnrB2. With the exception of two strains from South America and Africa, all qnrS1-positive strains originated from the Far East.

Reports on the identification of qnr genes have been increasingly frequent around the world;7 however, this is the first report of their presence in strains of Salmonella isolated in Scotland. The 34 qnr-positive strains belonged to 12 serotypes, including Salmonella Gaminara, Salmonella Rissen, Salmonella Agona, Salmonella Haifa, Salmonella Blockley, Salmonella Colindale and a monophasic strain of Salmonella Java. To the best of our knowledge, there have been no previous reports of the identification of qnr genes in these serotypes.

Plasmid-mediated quinolone resistance has serious implications for the use of this group of antimicrobials in the future. More importantly, the presence of these genes on integrons together with ESBLs further supports the necessity for ongoing surveillance of resistance. Surveillance will be problematic, in that routine diagnostic laboratories rarely test for reduced susceptibility to fluoroquinolones. This problem was addressed by the recommendation that nalidixic acid disc screening was indicative of fluoroquinolone susceptibility.7 However, as this study has shown, isolates that are nalidixic-acid-susceptible can possess qnr genes and have an MIC of ciprofloxacin as high as 0.75 mg/L. However, two cefotaxime-resistant strains with an MIC of ciprofloxacin of 1 mg/L were cross-resistant to nalidixic acid. The elevated MIC may indicate the presence of gyrase mutations in these strains and warrants further investigation. Although these strains are not considered resistant by CLSI or BSAC criteria, elevated MICs of ciprofloxacin have been shown to contribute to treatment failures in the past.8

This new mechanism of reduced susceptibility in Enterobacteriaceae again raises the question of the appropriateness of the current breakpoints for fluoroquinolones.9

Acknowledgements

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Transparency declarations

None to declare.
### Table 1. Properties of *qnr*-positive *Salmonella* strains isolated in Scotland

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>Phage type</th>
<th>Number of isolates</th>
<th>Source</th>
<th>Foreign travel</th>
<th>Year of isolation</th>
<th>Range of CIP MICs (mg/L)</th>
<th>Additional resistances&lt;sup&gt;a&lt;/sup&gt;</th>
<th>qnr gene isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virchow</td>
<td>—</td>
<td>1</td>
<td>human</td>
<td>Oman</td>
<td>2001</td>
<td>0.125</td>
<td>AMP-FRZ-GEN-KAN-NET-SPT-STR-SMX-TET-TMP</td>
<td>A1</td>
</tr>
<tr>
<td>Corvallis</td>
<td>—</td>
<td>9</td>
<td>human</td>
<td>—</td>
<td>2004–2007</td>
<td>0.125–0.5</td>
<td>STR-SMX-TET</td>
<td>S1</td>
</tr>
<tr>
<td>Corvallis</td>
<td>—</td>
<td>3</td>
<td>human</td>
<td>Bali/Singapore (1)</td>
<td>2005</td>
<td>0.25</td>
<td>—</td>
<td>S1</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>13a</td>
<td>1</td>
<td>human</td>
<td>Thailand</td>
<td>2005</td>
<td>0.25</td>
<td>SMX</td>
<td>S1</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>RDNC</td>
<td>1</td>
<td>human</td>
<td>—</td>
<td>2004</td>
<td>0.25</td>
<td>AMP-GEN-SPT-STR-SMX-TMP-KAN</td>
<td>S1</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>RDNC</td>
<td>2</td>
<td>human</td>
<td>Malaysia (1)</td>
<td>2005</td>
<td>0.25–0.38</td>
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<td>S1</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>non-typeable</td>
<td>4</td>
<td>human, 2 ovine, bovine</td>
<td>—</td>
<td>2005</td>
<td>0.25–0.5</td>
<td>AMP-CHL-STR-SMX-TET-TMP</td>
<td>S1</td>
</tr>
<tr>
<td>Typhimurium</td>
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<td>1</td>
<td>human</td>
<td>Singapore</td>
<td>2007</td>
<td>0.38</td>
<td>STR-SMX-TET</td>
<td>S1</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>120</td>
<td>1</td>
<td>human</td>
<td>Thailand/Singapore</td>
<td>2006</td>
<td>0.75</td>
<td>AMP-STR-SMX-TET</td>
<td>S1</td>
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<tr>
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<td>1</td>
<td>human</td>
<td>—</td>
<td>2005</td>
<td>0.38</td>
<td>STR-SMX-TET</td>
<td>S1</td>
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<tr>
<td>Stanley</td>
<td>—</td>
<td>1</td>
<td>human</td>
<td>—</td>
<td>2005</td>
<td>0.125</td>
<td>SPT-STR-SMX-TET-TMP</td>
<td>A1</td>
</tr>
<tr>
<td>Stanley</td>
<td>—</td>
<td>1</td>
<td>human</td>
<td>Thailand</td>
<td>2005</td>
<td>0.19</td>
<td>CHL-GEN-KAN-SPT-STR-SMX-TET</td>
<td>S1</td>
</tr>
<tr>
<td>Gaminara</td>
<td>—</td>
<td>1</td>
<td>environment</td>
<td>—</td>
<td>2007</td>
<td>0.25</td>
<td>—</td>
<td>B5</td>
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<tr>
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<td>1</td>
<td>human</td>
<td>Nigeria/Angola</td>
<td>2007</td>
<td>0.5</td>
<td>AMP</td>
<td>S1</td>
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<tr>
<td>Rissen</td>
<td>—</td>
<td>1</td>
<td>human</td>
<td>Thailand</td>
<td>2007</td>
<td>0.125</td>
<td>AMP-CTX-CHL-GEN-SMX-TET-KAN</td>
<td>S1</td>
</tr>
<tr>
<td>Java</td>
<td>—</td>
<td>1</td>
<td>human</td>
<td>—</td>
<td>2007</td>
<td>0.19</td>
<td>AMP</td>
<td>S1</td>
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<tr>
<td>Colindale</td>
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<td>1</td>
<td>human</td>
<td>—</td>
<td>2007</td>
<td>0.19</td>
<td>AMP-CTX</td>
<td>B1</td>
</tr>
</tbody>
</table>

Antimicrobials in bold indicate intermediate susceptibility.

<sup>a</sup>Additional resistances: CIP, ciprofloxacin; AMP, ampicillin; CTX, cefotaxime; CHL, chloramphenicol; FRZ, furazolidone; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; NET, netilmicin; SPT, spectinomycin; STR, streptomycin; SMX, sulfamethoxazole; TET, tetracycline; TMP, trimethoprim.
The current emergence and dissemination of clavulanic acid-inhibited extended-spectrum β-lactamases (ESBLs) represents a global threat, as they are difficult to trace and eradicate, with CTX-M-type ESBLs being of major concern in community-acquired clinical infections. Among β-lactam molecules, carbapenems (imipenem, ertapenem and meropenem) are the drugs of choice for treating infections caused by ESBL-producing Enterobacteriaceae. Ertapenem is a broad-spectrum carbapenem that is active against Enterobacteriaceae producing ESBLs. However, combinations of ESBL and porin deficiency have been reported to confer resistance to ertapenem. In a clinical microbiology laboratory, identification of an ESBL may be confirmed by the observation of a synergy image between an extended-spectrum cephalosporin and clavulanic acid. Surprisingly, a synergy image is sometimes observed between ertapenem- and clavulanic-acid-containing discs performed with CTX-M-producing Enterobacteriaceae. This observation suggests that ertapenem might be hydrolysed by CTX-M-type β-lactamases (Figure 1).

Therefore, we have compared the β-lactamase activity towards ertapenem from culture extracts of Escherichia coli producing the four most widespread CTX-Ms: CTX-M-2, CTX-M-3, CTX-M-9 and CTX-M-15. In order to express the blaCTX-M genes under the same promoter and in the same bacterial genetic context, the four blaCTX-M genes (blaCTX-M-2, blaCTX-M-3, blaCTX-M-9 and blaCTX-M-15) were amplified from our collection of reference E. coli strains without their promoter sequence and cloned into the low-copy-number pACYC184 plasmid (New England Biolabs, Ozyme, Saint-Quentin-en-Yvelines, France). The primers used in this study were CTX-M-2F (5′-AATGTATATTGAAAGCGAGG-3′), CTX-M-2R (5′-ATACCTGTCATTTGAC-3′), CTX-M-3F (5′-CTGTCTCTCTGAGAAATTG-3′), CTX-M-3R (5′-TAGCGCCGGATCCCA-3′), CTX-M-9F (5′-CTGATGTCACAGGATTGAC-3′), CTX-M-9R (5′-AGGCGGGCCCATATGAC-3′), CTX-M-15F (5′-CTCGTACGTCGAGGAGG-3′) and CTX-M-15R (also used for CTX-M-15 amplification). The PCR products were ligated into the pCRBluntII-TOPO plasmid (Invitrogen, Life Technologies, Cergy-Pontoise, France), excised from the recombinant plasmids pCTX-M were grown overnight at 37°C in 10 mL of Trypticase soy broth with 100 mg/L amoxicillin. After sonication and centrifugation, the recombinant vectors were introduced into electrocompetent cells of E. coli Wi, a wild-type clinical isolate recovered from urinary tract infection in our hospital (Hôpital Bicêtre, K. Bicêtre, France). Transformants were selected overnight at 37°C on nutrient agar containing 30 mg/L chloramphenicol. Cultures of E. coli harbouring the recombinant plasmids pCTX-M were grown overnight at 37°C in 10 mL of Trypticase soy broth with 100 mg/L amoxicillin. After sonication and centrifugation, β-lactamase activity of the supernatants was assayed by UV spectrophotometry, as described previously. One unit of enzyme activity was defined as the activity that hydrolysed 1 μmol of ertapenem/min. The total protein content was measured with bovine albumin as the standard (Bio-Rad DC Protein Assay Kit). Two other Ambler class A β-lactamases were used as controls in these enzymatic studies: KPC-2, a carbapenemase known to efficiently hydrolyse ertapenem, and TEM-3, an ESBL without any carbapenemase activity. β-Lactamase activities of the CTX-Ms towards ertapenem were very low.

References


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Do CTX-M β-lactamases hydrolyse ertapenem?

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