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Plasmid-Mediated Quinolone Resistance Determinant QnrB4 Identified in France in an *Enterobacter cloacae* Clinical Isolate Coexpressing a QnrS1 Determinant▽

Plasmid-mediated quinolone resistance is increasingly reported worldwide for *Enterobacteriaceae* (4). This resistance is related to Qnr-like proteins belonging to the pentapeptide repeat family that protects DNA from quinolone binding to type II topoisomerases (9, 10). The three main groups of Qnr determinants, QnrA, QnrB, and QnrS, are known. QnrA (six variants) has been identified worldwide, whereas QnrB (six variants) and QnrS (two variants) have been reported in a few countries (7) and QnrB has not yet been identified in Europe. The aim of this study was to determine the prevalence of QnrB in extended-spectrum β-lactamase (ESBL)-producing enterobacterial isolates already tested for QnrA and QnrS (6).

(The results of this study were presented in part at the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2006.)

A total of 186 ESBL-producing enterobacterial isolates were collected from the Bicêtre hospital, in a suburb of Paris, France, from January to June 2005 (5). The presence of the *qnrB* gene was investigated by a PCR assay as previously described (2). A single *Enterobacter cloacae* isolate, S1, recovered from an ascites of a 2.5-year-old child in May 2005, was positive for *qnrB*. This isolate was shown to be *qnrS1* positive also (5). *E. cloacae* S1 was resistant to β-lactams via production of the ESBL SHV-12, the penicillinase TEM-1, and the novel penicillinase LAP-1 (5). In addition, this isolate was resistant to fluoroquinolones with a single amino acid change in the quinolone resistance-determining region (QRDR) of *gyrA* (S83F) and none in the QRDR of *parC*. QnrS1-mediated quinolone and LAP-1-mediated β-lactam resistance determinants were self-transferable and located onto a 100-kb conjugative plasmid termed pS1A.

Conjugation and transformation experiments using azide-resistant *Escherichia coli* J53 and *E. coli* TOP10 as recipient strains, respectively, were performed as previously described (5). QnrB-like protein-mediated quinolone resistance and SHV-12-mediated β-lactam resistance determinants were transferred only by transformation. Analysis of plasmid content in *E. cloacae* S1 and its transformants performed by using the Kieser technique (3) identified a 160-kb plasmid (termed pS1B) that hybridized to a *qnrB*-specific probe. Antibiotic susceptibility testing was carried out according to the guidelines of the CLSI (1). MICs of quinolones and fluoroquinolones were determined for *E. coli* TOP10 and the corresponding *qnrB4*- and *qnrS1*-positive transformants by using the E-test technique according to the manufacturer's recommendations. The *qnrB*-positive transformant expressed an ESBL phenotype (the $bla_{\text{SHV-12}}{}^{+}$ phenotype), together with resistance to aminoglycosides (except amikacin), chloramphenicol, and rifampin. In addition, it showed reduced susceptibilities to quinolones and fluoroquinolones, as observed for the *qnrS*-positive transformant (Table 1).

TABLE 1. MICs of different quinolones and fluoroquinolones for *E. cloacae* S1, its *E. coli* TOP10 transformants harboring pS1A and pS1B (expressing QnrB4 and QnrS1 determinants, respectively), and reference strain *E. coli* TOP10

Antibiotic	MIC (μg/ml) of:			
	E. cloacae S1	*E. coli* TOP10 (pS1A)	*E. coli* TOP10 (pS1B)	*E. coli* TOP10
Nalidixic acid	>256	2	4	1
Norfloxacin	>32	0.25	0.5	0.03
Ofloxacin	>32	0.12	0.25	0.01
Ciprofloxacin	>32	0.06	0.12	<0.01
Moxifloxacin	>32	0.12	0.25	<0.01
Sparfloxacin	>32	0.03	0.25	<0.01

Cloning experiments with total DNA of *E. cloacae* S1 allowed the sequence of the *qnrB*-like gene, which had perfect nucleotide identity with the *qnrB4* variant reported for an *E. coli* isolate from the United States, to be determined (8). The genetic environment of the *qnrB4* gene was bracketed at its 5′ extremity by a *psp* operon coding for putative phage shock proteins and at its 3′ extremity by a *sap* operon coding for a putative peptide transport system permease, sharing 87% and 80% amino acid identities, respectively, with similar proteins identified in *E. coli* K-12 (GenBank accession no. U00096).

This report identified the first QnrB-like determinant from Europe and the first coproduction of two Qnr-like determinants in a single isolate. This finding underlines that these plasmid-mediated quinolone resistance determinants may accumulate in enterobacterial clinical isolates, which could very likely further increase resistance to quinolones.

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Vincent Cattoir
Service de Bactériologie-Virologie-Hygiène
Hôpital Henri Mondor
Assistance Publique/Hôpitaux de Paris
Faculté de Médecine de Créteil
Université Paris XII
Créteil, France

Laurent Poirel
Patrice Nordmann*
Service de Bactériologie-Virologie
Hôpital de Bicêtre
Assistance Publique/Hôpitaux de Paris
Faculté de Médecine Paris-Sud
Université Paris XI
K.-Bicêtre, France

*Phone: 33-1-45-21-36-32
Fax: 33-1-45-21-63-40
E-mail: nordmann.patrice@bct.aphp.fr

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