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Further Identification of Plasmid-Mediated Quinolone Resistance Determinant in *Enterobacteriaceae* in Turkey

Quinolone resistance arises mostly from chromosomal mutations in genes coding for DNA gyrase (topoisomerase II) and genes coding for outer membrane proteins in members of the family *Enterobacteriaceae* (6). However, plasmid-mediated resistance to quinolones has been reported in 1998 from a *Klebsiella pneumoniae* strain isolated from Birmingham, Ala. (3). This determinant, named Qnr (and recently renamed QnrA according to G. A. Jacoby's suggestion), is a 218-amino-acid protein that binds to subunits of the DNA gyrase, preventing further binding of quinolones (7, 8). QnrA confers resistance to nalidixic acid and increases the MICs of fluoroquinolones by four- to eightfold (10). Published data report the spread of *qnr*-positive enterobacterial isolates mostly from the United States, Southeast Asia, and more recently from Europe (1–3, 9, 11). Several *qnrA*-like-positive enterobacterial isolates produced clavulanic acid-inhibited extended-spectrum β-lactamases (ESBLs) (1, 2, 9, 11). Thus, we have screened for *qnrA*-like genes in nalidixic acid-resistant and ESBL-positive enterobacterial isolates from the university hospital of Istanbul in Turkey, a country located between Europe and Asia.

Screening was performed among nonclonally strains distributed as follows: six *Escherichia coli* isolates and one *Klebsiella pneumoniae* isolate from 2002; two *E. coli* isolates and one *K. pneumoniae* isolate from 2003; and 28 *E. coli* isolates, five *K. pneumoniae* isolates, four *Enterobacter cloacae* isolates, and two *Citrobacter freundii* isolates from 2004. Out of a total of 49 ESBL-positive strains, two strains (*E. cloacae* 14300 and C. *freundii* Lut) (4%) were positive for *qnrA*-like genes using PCR technique and detection primers, as reported previously (2). *E. cloacae* 14300 was from a skin abscess of a 33-year-old injured male, whereas *C. freundii* LUT was from an urinary tract infection of a 63-year-old male after 12 days of treatment with ciprofloxacin. A quinolone resistance determinant from fluoroquinolone-resistant clinical strains was transferred by conjugation using *E. coli* strain J53 that was resistant to azide, as detailed previously (2). According to disk diffusion susceptibility testing and MIC determinations (4), *E. cloacae* transconjugants were resistant to nalidixic acid, chloramphenicol, tetracycline, kanamycin, tobramycin, streptomycin, sulfamides, and trimethoprim, whereas *C. freundii* transconjugants were also resistant to gentamicin and rifampin. Transconjugants and clinical isolates had an ESBL-positive resistance profile that caused us to analyze their β-lactamase and plasmid content also using a series of techniques reported elsewhere (2, 5). *E. cloacae* 14300 harbored the QnrA determinant as previously reported (7) located on a 50-kb conjugative plasmid in association with the $bla_{\text{SHV-5}}$ gene, whereas *C. freundii* Lut harbored two plasmids of 170 and 80 kb. In the latter case, the 170-kb conjugative plasmid harbored a *qnrA* gene, a $bla_{\text{VEB-1}}$ ESBL gene, whereas the nonconjugative 80-kb plasmid harbored the $bla_{\text{OXA-48}}$ β-lactamase gene. In both cases, the *qnrA* gene was preceded by the CR1 element containing the Orf513 and providing sequence promoters for its expression as demonstrated previously (2).

Identification of QnrA associated with SHV-5-like ESBL (SHV-7) was performed previously (9), whereas the ESBL VEB-1 has been identified recently from a QnrA-positive *E. coli* isolate from France (2). The carbapenem resistance of *C. freundii* Lut was explained by the production of a carbapenem-hydrolyzing β-lactamase OXA-48 as reported initially in a *K. pneumoniae* isolate isolated from the same city (5).

From a general point of view, this report underlines the spread of a plasmid-mediated quinolone resistance determinant in *Enterobacteriaceae* with its peculiar association with ESBLs. It also identified ESBL VEB-1 from another part of the world. Interestingly, association of QnrA now with the powerful carbapenemase OXA-48 gives rise to an unknown level of multidrug resistance.

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Hasan Nazic
Department of Microbiology
Istanbul Faculty of Medicine
Istanbul, Turkey

Laurent Poirel
Patrice Nordmann*
Service de Bactériologie-Virologie
Hôpital de Bicêtre
Assistance Publique/Hôpitaux de Paris
Faculté de Médecine Paris-Sud
Université Paris XI
94275 Le Kremlin-Bicêtre, France

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