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A New Plasmid-Mediated Quinolone Resistance Gene, *qnrB88*, Is an Outlier of the *qnrB* Family

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BACKGROUND: The *qnr* genes encode proteins of the pentapeptide repeat family and protect type II topoisomerases from quinolone action. The clinical isolate *Klebsiella pneumoniae* Q1130 showed low-level quinolone resistance but had a wild-type quinolone resistance-determining region of *gyrA* and was negative in the PCRs for the 6 known *qnr* families (*qnrA, qnrB, qnrC, qnrD, qnrS, qnrVC*), *aac(6')-Ib-cr* and *qepA*. Our aim was to investigate the possible presence of an unknown plasmid-mediated quinolone resistance gene in *K. pneumoniae* Q1130.

METHODS: Antibiotic susceptibility was tested under CLSI guidelines. Biparental conjugation was already described. Plasmid content was analyzed by S1-PFGE. Plasmids were extracted with the Qiagen Large-Construct Kit and sequenced with the Illumina technology. *qnrB88* was cloned in *Escherichia coli* TOP10 using the CloneJet PCR Cloning Kit. Neighbor-Joining (NJ) and Maximum Likelihood (ML) phylogenetic trees were constructed with MEGA6 and the reliability of the tree topology was assessed by bootstrapping (1,000 replicates).

RESULTS: *K. pneumoniae* Q1130 harbored a new *qnr* gene, named *qnrB88*, located on a conjugative plasmid of ca. 185 kb. The MICs of nalidixic acid, ciprofloxacin and levofloxacin of *E. coli* TOP10 harboring *qnrB88* were 8-, 64- and 32-times higher, respectively, than those of the isogenic strain without this gene. *qnrB88* differed from the *qnrB* alleles previously known by an average of 25.0% (range: 23.6-26.8%). Comparing with the QnrB proteins, QnrB88 differed by 15.8% in average (range: 14.0-17.8%) and the loops A and B, essential for the quinolone-protective activity of QnrB1, showed the changes K52Q and S113C, respectively. NJ and ML phylogenetic trees of all the known *qnr* genes showed that *qnrB88* was separated by a distance of 0.4 nucleotide substitutions/site from a cluster that comprised all the *qnrB* alleles previously described. NJ and ML phylogenetic trees of the Qnr proteins showed equivalent results, with a distance of 0.2 amino acid substitutions/site between QnrB88 and the cluster of all the QnrB proteins previously known. The genetic surroundings of *qnrB88* were different from those previously described for *qnrB* genes, showing that this gene was bracketed by IS*Ecp1* (upstream) and *araJ* (downstream), a transporter of the major facilitator superfamily.

CONCLUSIONS: *qnrB88* is an outlier of the *qnrB* family. IS*Ecp1* could be involved in the mobilization of this gene.

Keywords: Quinolone resistance; PMQR; new qnrB

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