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 ISEcp1 (upstream) and araJ (downstream), a transporter of the major facilitator superfamily.

CONCLUSIONS: qnrB88 is an outlier of the qnrB family. ISEcp1 could be involved in the mobilization of this gene.

Keywords: Quinolone resistance; PMQR; new qnrB

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