

Identification of the new variant QepA3, a plasmid-mediated quinolone resistance determinant, collected in a CMY-2-producing *Escherichia coli*

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The efflux pump QepA confers decreased susceptibility to hydrophilic fluoroquinolones (e.g., norfloxacin, ciprofloxacin, and enrofloxacin). In this study, we characterized the third variant, named qepA3, collected from an *Escherichia coli* isolate in Portugal.

INSRA6015 was isolated in 2005 from the urine of a 77-year-old female patient hospitalized at the Hospital Fernando Fonseca, Portugal. Susceptibility testing was performed by disk diffusion and MIC methods, (SFM and EUCAST guidelines, respectively). PCR and sequencing were used to screen and identify *bla* (*bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA}, *bla*_{CTX-M} and plasmid-mediated *ampC*) genes, as well as plasmid-mediated quinolone resistance (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA* and *aac(6')Ib-cr*), and the quinolone resistance-determining regions (QRDR: *gyrA*, *gyrB*, *parC*, and *parE*) genes. PCR-mapping was used to characterize the genetic environment of the new *qepA3* gene. Transfer of resistance of the QepA3 determinant, was performed through electroporation, using the *E. coli* TOP10 as recipient. Plasmid content was characterized by PCR-based replicon typing.

Molecular characterization of INSRA6015 showed the presence of *bla*_{TEM-1}, *bla*_{CMY-2} and a new variant of *qepA* possessing two nucleotide substitutions, leading to Phe85Leu and Val134Ile changes. This variant, named QepA3, conferred a similar phenotype to that of the QepA1 and QepA2 determinants. Sequencing of the QRDR detected substitutions Ser83Leu and Asp87Asn in the GyrA subunit and Glu84Lys in the ParC subunit, which are consistent with the high resistance to ciprofloxacin observed in the MICs. Sequence analysis of *qepA3* genetic environment revealed that the gene was located inside a genetic structure identical to that of previously described for *qepA1* and *qepA2*. It is noteworthy that *qepA3* gene, as *qepA2*, was not associated with the *rmtB* gene encoding an aminoglycoside ribosomal methylase, contrarily to *qepA1*. PCR-based replicon typing indicated the presence of the IncF plasmid.

We have identified and characterized a new variant of the plasmid-mediated efflux pump QepA, which is responsible for the increased levels of resistance to several clinically important quinolones, such as ciprofloxacin, and norfloxacin. This is, at our knowledge, the first description of the co-production of QepA and CMY-2. The study highlights the need of surveillance of this resistance mechanism and reinforces a more careful use of quinolones.