Conclusion: A high prevalence of PMQR among clinical isolates of Enterobacteriaceae producing DHA-type-pAmpC and those producing VIM-1 was observed. A correlation between qnrB4 and DHA-type-production was also observed as well as between qnrD and P. mirabilis producing CMY-2.

P1737 Beta-lactams and trimethoprim induce the expression of qnrB and smaqnr genes by SOS depending regulation

A. Briales, J.M. Rodríguez-Martínez, P. Diaz de Alba, C. Velasco, J. Machuca, J. Blázquez, A. Pascual (Seville, Madrid, ES)*

Objectives: Direct SOS-depending regulation, mediated by LexA, of qnrB genes by fluoroquinolones (FQ) has been recently reported. smaqnr gene, from the chromosomal of *Serratia marcescens*, contains a putative LexA box. The aim of this study was to evaluate whether smaqnr gene is induced via a SOS depending mechanism, and to investigate whether others antimicrobial agents induce the expression of qnrB and smaqnr leading to low-level of FQ resistance.
Methods: *Serratia marcescens* 257 reference strain (Institute Pasteur) and *Escherichia coli* J53 carrying a natural plasmid that harboured qnrB1 were used for RT-PCR assays. Expression of smaqnr and qnrB genes were studied by real time RT-PCR and quantified compared to rpoB gene for *S. marcescens* and mdH gene for *E. coli*. Ceftazidime (CAZ) at MIC concentration was tested as possible inducer. Strains were grown to exponential phase at 37°C and the inducer was added during 45 minute, leaving one culture as control. Additionally, promoter regions of qnrB and smaqnr were cloned in pMS201, fused to GPF protein and used for gene report assays. Three *E. coli* wild-type strains (MG1655, ATCC25922 and DR1) and *E. coli* HB101 (recA deficient and isogenic with DR1) were used for these assays. Controls using the promoter region of recA and the empty vector were included. Disk diffusion and E-test were used to evidence the induction with ciprofloxacin (CIP), betalactams, trimethoprim (TMP), imipenem (IP) and colistin (CS). Mytomycin C was used as positive control for induction.
Results: RT-PCR assays showed that both qnrB and smaqnr were induced at MIC of CAZ increasing transcription 2- and 3.5-fold compared to the basal expression, in *E. coli* and *S. marcescens*, respectively. Gene report assays showed that qnrB and smaqnr genes were induced by CIP, as expected, but also by (CAZ), cefepime (FEP), ampicillin or TMP in the *E. coli* wild-type strains, but not in the recA-deficient *E. coli* HB101. Induction was not evident for IP or CS in any case. Fluorescent quantification showed that CIP, CAZ and FEP increased the level of expression 55%, 65% and 74% for qnrB and 44%, 60% and 68% for smaqnr, compared to recA expression.
Conclusions: Betalactams and trimethoprim induce the transcription of qnrB and smaqnr genes by a SOS depending regulation. These results show a direct SOS-dependent regulation of a low-level FQ-resistance mechanism in response to others antimicrobials. Its consequences in terms of cross resistance are currently unknown.

P1738 Identification of the new variant QepA3, a plasmid-mediated quinolone resistance determinant, collected in a CMY-2-producing *Escherichia coli*

M. Caniça, V. Manageiro, D. Jones-Dias, D. Félix, E. Ferreira (Lisbon, PT)*

Objectives: 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.
Methods: 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 (blaTEM, blaSHV, blaOXA, blaCTX-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.
Results: Molecular characterization of INSRA6015 showed the presence of blaTEM-1, blaCMY-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 QDRD 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.
Conclusion: 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.

P1739 High prevalence of fluoroquinolone efflux pump OqxAB in ESBL-producing *Klebsiella pneumoniae* in Spain

J.M. Rodríguez-Martínez, P. Diaz de Alba, J. Machuca, A. Briales, J. Rodriguez-Baño, L. Martínez-Martínez, A. Pascual (Seville, Santander, ES)*

Objectives: Plasmid-mediated quinolone resistance is an emergent phenomenon, especially in Enterobacteriaceae, and includes mechanisms of target protection (qnr), antimicrobial inactivation (aac(6′)-Ib-cr) or active efflux systems (qepA and oqxAB). The efflux pump OqxAB-TolC was originally described in *E. coli* and later in *K. pneumoniae*. The aims of this study were (i) to analyze the presence of oqxA and oqxB genes in a collection of ESBL-producing *K. pneumoniae* strains, (ii) to determine its chromosomal and/or plasmidic location and (iii) to analyze their expression levels in relation to susceptibility or resistance to quinolones.
Methods: A collection of 114 non-repetitive isolates of ESBL-producing *K. pneumoniae* from a multicenter study in Spain was used. 37.8% of isolates were susceptible to ciprofloxacin (CIP), while 62.2% were resistant or intermediate. Detection of oqxA and oqxB genes was performed by PCR. Chromosomal and/or plasmidic location was performed using plasmid DNA (Kieser technique) and subsequent hybridization. oqxA gene expression was analyzed by real time RT-PCR. Normalized expression levels of the target gene transcripts were calculated in comparison to the expression of rpoB using the 2 -DDCT method. *K. pneumoniae* ATCC 27799 and *K. pneumoniae* ATCC 700603 were included in the study.
Results: Both oqxA and oqxB were detected in *K. pneumoniae* at high prevalence, 77% and 75% respectively. Ten amplicons were sequenced and showed 100% homology with previously described genes showing a high degree of conservation. Hybridization assays showed the simultaneous presence of oqxA (16%) and oqxB (13%) in both chromosome and large size plasmids locations. These plasmids were not transferable by transformation into *E. coli*. RT-PCR assays showed a higher expression (four-folds) in strains with reduced susceptibility to quinolones compared to that of susceptible strains. Interestingly, *K. pneumoniae* ATCC 700603 showed an 18-folds higher expression than *K. pneumoniae* ATCC 27799. These differences were in accordance to the MICs of CIP (0.5 and 0.125 mg/L, respectively).