

MIC=0.38 µg/ml, carried a *qnrB* gene showing 99% of aminoacidic identity with the sequence of *qnrB10* and 98% of identity with *qnrB5* found in *S. Berta*. No mutation in the QRDRs was found. Conjugation experiments revealed that all resistances were successfully transferred, except trimethoprim/sulfamethoxazole. Detailed plasmid characterisation of 16 kb region conferring multidrug resistance, identified the *qnrB* gene flanked by *ISEcp1* and *IS26*, a *blaSHV-12* gene flanked by two open reading frame of unknown function and by *IS26*, a *blaTEM1* gene followed by truncated *class1* integron, containing *aadB* gene cassette.

Conclusion: The plasmid-mediated quinolone resistance of the *Qnr* type is emerging in Enterobacteriaceae in many different geographic areas, but it remains rare in *Salmonella* in Italy. However the presence of *qnrB* genes in *S. Typhimurium*, never been described before, deserves particular attention because of the high prevalence of human infections due to this ubiquitous serotype.

P1523 Prevalence of the quinolone-modifying enzyme *aac(6′)-Ib-cr* in extended-spectrum β-lactamase-producing enterobacterial isolates in Barcelona

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Background: Quinolone resistance usually results from mutations in chromosomal genes coding for type II topoisomerases, efflux pumps, and/or porins alterations. Recently, plasmid-mediated quinolone resistance mechanisms has been reported by the *qnr* and the *aac(6′)-Ib-cr* genes.

Objective: To evaluate the presence of *aac(6′)-Ib-cr* among enterobacterial isolates carrying ESBLs in Barcelona, determine the ESBL present in each isolate and establish if the *aac(6′)-Ib-cr* and the ESBL genes were located in the same plasmid.

Methods: A total of 305 non-duplicate clinically relevant ESBL-producing enterobacterial isolates, obtained at Hospital Vall d'Hebron (Barcelona), collected between 2003 and 2004 were analysed for the presence of *aac(6′)-Ib-cr*. Antimicrobial susceptibility was tested by Etest and disc diffusion. The screening for the presence of *aac(6′)-Ib-cr* was studied by PCR using specific primers. Positive amplicons were digested with *BstCI* and sequenced to identify the *aac(6′)-Ib-cr* variant. ESBLs of all *aac(6′)-Ib-cr*-positive isolates were determined by IEF and PCR using specific primers. The quinolonone-resistance-determining region (QRDR) of *gyrA* and *parC* genes was amplified and sequenced. Plasmid number and location of *aac(6′)-Ib-cr* and *bla* genes was performed on all *aac(6′)-Ib-cr*-positive isolates by *S1* nuclease digestion and hybridisation with specific probes.

Results: Nineteen isolates (6.2%) carried *aac(6′)-Ib-cr* (8 *Escherichia coli*, 7 *Klebsiella pneumoniae*, 3 *Enterobacter cloacae* and 1 *Klebsiella oxytoca*). Of these, 6 isolates (31.6%) carried the *aac(6′)-Ib-cr* variant (5 *E. coli* and 1 *K. pneumoniae*). Among the 6 *aac(6′)-Ib-cr*-positive isolates 4 were positive for CTX-M-1 group. The ESBLs of the remaining two isolates are currently been determined. The 5 *aac(6′)-Ib-cr*-positive *E. coli* isolates were resistant to nalidixic acid and ciprofloxacin and the *K. pneumoniae aac(6′)-Ib-cr*-positive isolate was susceptible. Sequencing of the QRDR of the *gyrA* and *parC* genes identified amino acid changes in *gyrA* (S83-L and D87-N) and *parC* (S80-I and E84-V) on the 5 *E. coli* isolates. In the *K. pneumoniae* isolate no aminoacid changes were detected. The 4 *aac(6′)-Ib-cr*-positive isolates positive for CTX-M-1 group harboured the ESBL gene and *aac(6′)-Ib-cr* located in the same plasmid.

Conclusion: The prevalence of *aac(6′)-Ib-cr*, responsible for low-level quinolone resistance, among enterobacterial clinical isolates carrying ESBLs between 2003 and 2004 in Barcelona was 1.9%.

P1524 Emergence and dissemination of plasmid-borne fluoroquinolone resistance genes and their association with *blaCTX-M* genes in Enterobacteriaceae isolates from Portugal

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Objectives: Considering the increasing resistance to fluoroquinolones among the Enterobacteriaceae and the alarming mobility of some of the genes responsible, we aimed to understand the level of association between different plasmid-mediated fluoroquinolone resistance (PMFR) genes in Portuguese isolates and the correlation between these genes and a number of β-lactamase genes.

Methods: We screened two collections of Enterobacteriaceae isolates for the presence of *qnrA*, *qnrB*, *qnrS* and *aac(6′)-Ib-cr*. The first collection consisted of 104 isolates gathered in 1999 from 16 different Portuguese hospitals (namely 57 *Klebsiella pneumoniae*, 42 *Escherichia coli*, 4 *Kluyvera* spp. and 1 *Hafnia alvei*) and the second collection consisted of 467 isolates gathered from 15 hospitals in the period 2004–2006 (namely 314 *E. coli*, 88 *K. pneumoniae*, 41 *Enterobacter* spp., 6 *Klebsiella oxytoca*, 6 *Serratia marcescens*, 6 *Morganella morganii*, 3 *Citrobacter freundii*, 2 *Proteus mirabilis* and 1 *Kluyvera* spp.). All isolates from 2004–2006 were identified as extended-spectrum β-lactamase (ESBL) producers. PCR and nucleotide sequencing were used with specific primers for the PMFR genes and for the β-lactamase genes (*blaTEM*, *blaSHV*, *blaOXA*, *blaCTX-M* and *ampC*).

Results: We observed an emergence of *qnrB* (from 0% to 10%), *qnrS* (from 0% to 8%) and *aac(6′)-Ib-cr* (from 14% to 65%) from 1999 to 2004–2006, the latter's more evident in *E. coli* (from 19% to 79%); *qnrB* was mainly found in *K. pneumoniae* isolates (36%). We also observed that, while the other genes seem to be evenly distributed across the country, the frequency of *qnrS* is 17% in the North region, 5% in the Centre region and 1% in the Lisbon and Tagus Valley region. The frequency of *blaCTX-M* and *blaOXA* in isolates positive for *aac(6′)-Ib-cr* was 80% and 76%, respectively; in isolates negative for *aac(6′)-Ib-cr* this frequency was only 32% and 17%, respectively. The gene *blaSHV* was more frequent in positive isolates for *qnrB* (69%) than in negative (14%). There was also a higher frequency of *qnrB* in *qnrS* positive isolates (22%) than in *qnrS* negative ones (10%).

Conclusion: The emergence of PMFR genes over time contradicts previous reports and the uneven distribution of *qnrS* further implies a dissemination process. There seems to be a close association between *aac(6′)-Ib-cr* and the ESBL gene *blaCTX-M*. These findings reinforce a more careful use of fluoroquinolones and an increased monitoring of their related resistance mechanisms.

P1525 Aquatic environment contamination with an epidemic *Escherichia coli* clone harbouring *blaCTX-M-15*, *blaOXA-1*, *blaTEM-1*, and *aac(6′)-Ib-cr* in Portugal

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Objectives: The presence of CTX-M-15-producing isolates in non-clinical compartments has been scarcely investigated. In this study we describe the presence of

CTX-M-15-producing *Escherichia coli* isolates in aquatic environments in Portugal. We also compared its clonal relationship with CTX-M-15-producing isolates recovered from clinical settings in the same geographic area.

Methods: We studied two CTX-M-15-producing *E. coli* isolates recovered from sea and streams reaching the shore, in Porto area, Portugal, during 2006. Water samples were plated on MacConkey agar with and without 1 mg/L ceftazidime or cefotaxime after filtration. Species identification, susceptibility testing and conjugation assays were performed by standard methods. ESBL characterisation included synergy test, IEF, and identification of known *bla* genes by PCR and sequencing. Clonal relatedness was established by RAPD-PCR. The RAPD patterns