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. Antimicrobial susceptibility was tested by Etest and disc diffusion. The screening for the presence of aac(6′)-Ib 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 (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 monitorisation 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