Prevalence of plasmid-mediated quinolone resistance determinants among oxyiminocephalosporin-resistant Enterobacteriaceae in Argentina

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High quinolone resistance rates were observed among oxyiminocephalosporin-resistant enterobacteria. In the present study, we searched for the prevalence of plasmid-mediated quinolone resistance (PMQR) genes within the 55 oxyiminocephalosporin-resistant enterobacteria collected in a previous survey. The main PMQR determinants were aac(6’)-Ib-cr and qnrB, which had prevalence rates of 42.4% and 33.3%, respectively. The aac(6’)-Ib-cr gene was more frequently found in CTX-M-15-producing isolates, while qnrB was homogeneously distributed among all CTX-M producers.

Key words: PMQR - ESBL-producing Enterobacteriaceae - fluoroquinolone

Quinolone resistance in Gram-negative bacilli is primarily related to mutations in the chromosomal genes encoding for type II topoisomerases, the target site of quinolones (Drlica & Zhao 1997). However, in 1998, the first plasmid-mediated quinolone resistance (PMQR) determinant, qnrA, was reported in a Klebsiella pneumoniae strain. Since then, four additional qnr determinants, qnrB, qnrC, qnrD and qnrS, have been identified in Enterobacteriaceae species and some of these determinants have several allelic variants (Rodriguez-Martinez et al. 2011). These determinants encode for a pentapeptide repeat protein that binds to DNA gyrase, protecting the DNA gyrase from quinolone-mediated inhibition and increasing the minimum inhibitory concentrations (MICs) of the quinolones by eight-64-fold (Rodriguez-Martinez et al. 2011).

In addition to the qnr genes, various new PMQR genes have been discovered during the past decade, including the modified acetyltransferase aac(6’)-Ib-cr and the efflux pumps qepA and oqxAB (Rodriguez-Martinez et al. 2011).

The association of PMQR genes with extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases is noteworthy (Canton & Coque 2006). Although a few studies describing PMQR determinants in selected isolates have been performed, these associations have not been previously studied in Argentina (Quiroga et al. 2007, Jacoby et al. 2009, Andres et al. 2013). This study aimed to investigate the prevalence of PMQR genes (qnrA, -B, -S, -C and -D, aac(6’)-Ib-cr and qepA) in oxyiminocephalosporin-resistant Enterobacteriaceae recovered during a recent multicentre survey conducted in Argentina (Sennati et al. 2012). In addition, we also examined the coexistence of these determinants with different ESBL and/or AmpC β-lactamases.

The surveillance study was performed during October 2010 in 15 community hospitals distributed in three different regions of Argentina. Samples from both inpatients and outpatients were included. From 1,586 consecutive and non-repetitive enterobacterial clinical isolates recovered during this period, 207 (13.05%) displayed reduced susceptibility to expanded-spectrum cephalosporins (ESC) (Sennati et al. 2012). Antimicrobial susceptibility tests were performed by dilution and diffusion methods according to the Clinical and Laboratory Standards Institute (CLSI) for ampicillin, amoxicillin-clavulanic acid, piperacillin/tazobactam, cephalothin, cefoxitin, cefotaxime, ceftazidime, cefotaxime/clavulanic acid, ceftazidime/clavulanic acid, cefepime, imipenem, meropenem, amikacin, gentamicin, tobramycin, nalidixic acid, ciprofloxacin, levofloxacin and gatifloxacin (CLSI/NCCLS 2010). Molecular epidemiology of PMQR determinants was conducted for all confirmed ESC-resistant isolates (n = 55) collected during the first week of the study (22 K. pneumoniae, 16 Escherichia coli, 6 Proteus mirabilis, 4 Klebsiella oxytoca, 3 Serratia spp, 3 Enterobacter spp and 1 Providencia sp.) (Sennati et al. 2012). This sample was considered to be representative of the entire study period because the relative frequency of the most prevalent species was similar throughout the study period.

Molecular detection of qnrA, qnrB, qnrC, qnrD and qnrS was carried out by polymerase chain reaction (PCR) amplification using total heat-extracted DNA as a template and primers previously described (Cattoir et al.
2007, Cavaco et al. 2009, Wang et al. 2009). For further characterisation of qnrB alleles, the following primers were designed (5′-3′): qnrBeF: GTRGCGAAAAAT-TRACAG, qnrBBeF: ATGTYGYCATATTATGATA and qnrBBeR: CCMATHAYMCGATRCCCAAG. All qnrB amplicons were sequenced on both strands using an ABI PRISM 3700 DNA sequencer. Screening for the aac(6′)-Ib gene was performed using the following primers (5′-3′): aac(6′)IbF: CGATCTCACATCGTGAGTG and aac(6′)IbR: TTAGGCTACCTGCGTTTC. Characterisation of the aac(6′)-Ib-cr variant was conducted by restriction fragment length polymorphism-PCR using BseGI (Fermentas, Thermo Fisher Scientific Inc, Massachusetts, USA) (Park et al. 2006) and sequencing. The presence of the qepA gene was investigated by PCR amplification using the following primers (5′-3′): qepAF: ACATCTACGGCTTCTTCGTCG and qepAR: AACGCTTGAAGCCCTAGATC.

The 55 ESC-resistant isolates investigated in this study included 50 ESBL producers and the remaining five isolates were strong producers of AmpC. Among the ESBL-positive isolates, 47 were CTX-M producers (94%), with the most prevalent enzymes produced being CTX-M-2 (44%) and CTX-M-15 (38%) and to a lesser extent CTX-M-14 (3/50), PER-2 (3/50), SHV-12 (2/50), SHV-5 (2/50), CTX-M-8 (1/50) and CTX-M-56 (1/50). Three isolates encoded two different ESBLs simultaneously. Susceptibility to nalidixic acid and ciprofloxacin was 7.3% and the susceptibility rate of isolates to either levofloxacin or gatifloxicin was 23.6%. Gentamicin, amikacin and tobramycin displayed susceptibility rates of 43.6%, 61.8% and 23.6%, respectively. The MIC50 and MIC90 values of the fluoroquinolones were higher for PMQR-positive K. pneumoniae isolates (data not shown). However, no differences in MIC values were observed within E. coli isolates.

High diversity of PMQR genes was found among these enterobacteria. Sixty-six percent (33/50) of ESBL-producing isolates had at least one PMQR determinant (Table). In contrast, no PMQR genes were detected in isolates that produced high levels of AmpC (2 E. coli and 1 P. mirabilis harbouring CMY-2 and 2 Enterobacter spp). Among the PMQR-positive isolates, 42.4% (14/33) and 33.3% (11/33) encoded either aac(6′)-Ib-cr or qnrB as a determinant of quinolone resistance respectively, while 24.3% (8/33) had both determinants. No isolates rendered a positive amplification of qnrA, qnrS, qnrC, qnrD or qepA.

Five qnrB variants were found in this study; qnrB2-like was the most prevalent (8/19), followed by qnrB19-like (6/19), qnrB16-like (3/19), qnrB1-like (1/19) and qnrB6-like (1/19). A homogeneous distribution of qnrB variants among CTX-M producers was observed (Table).

The aac(6′)-Ib-cr gene was detected in 44% (22/50) of the ESBL-producing isolates, displaying similar percentages for both E. coli (56.2%, 9/16) and K. pneumoniae (54.5%, 12/22). However, aac(6′)-Ib-cr was mainly associated with CTX-M-15-producing Enterobacteriaceae (15/19) and to a lesser extent with other CTX-Ms, including CTX-M-2 (5), CTX-M-15/CTX-M-2 (1), CTX-M-14 (1) and CTX-M-8 (1). One of these 22 aac(6′)-Ib-cr-harbouring isolates (K. pneumoniae CV1) also carried the wild-type aac(6′)-Ib gene coupled to qnrB19 and both CTX-M-2 and CTX-M-15.

We focused on the relationship between the isolates that harboured both the aac(6′)-Ib-cr and blaCTX-M-15 determinants. Different clones were observed among the E. coli (7) and K. pneumoniae (8) isolates (Table). Phylogenetic analysis (Clermont et al. 2000) grouped the E. coli isolates into groups A (2) and B2 (5). Isolates belonging to the phylogenetic group B2 displayed a similar banding profile by REP-PCR and were characterised as ST131 according to the MLST Database (mlst.ucc.ie/MLST/dbs/Ecoli), corresponding with the worldwide pandemic clone known to cause nosocomial and community-acquired infections. Additionally, four/eight K. pneumoniae isolates were grouped into the same cluster (Kp1) and two of these isolates also possessed the qnrB2 allele. According to MLST analysis (Diancourt et al. 2005), seven/eight K. pneumoniae isolates were typed as ST11 (Table).

The true prevalence of PMQR genes is underestimated because there are no reliable phenotypic methods to detect their presence; however, previous surveillance reports have shown the prevalence of PMQR determinants among ESBL producers (Cremet et al. 2011, Walsh & Rogers 2012). Reports on contemporary isolates in Latin American countries displayed conflicting results. Nevertheless, comparisons between these studies should be performed carefully due to the different bacterial selection criteria used. In concordance with a multicentre study performed in Mexico (Silva-Sanchez et al. 2011), we observed a high frequency of qnrB and aac(6′)-Ib-cr genes amongst ESBL-producing isolates. However, a very low proportion of these markers were detected in Enterobacteriaceae isolated in a paediatric hospital in Uruguay (Garcia-Fulgueiras et al. 2011). Furthermore, these PMQR genes have also been detected in clinical enterobacteria, with unusual phenotypes of quinolone susceptibility collected in Argentina. Compared to this study, another study reported a different distribution in the qnrB allelic variants and the presence of different determinants (Andres et al. 2013).

The present study highlights a putative association between aac(6′)-Ib-cr and blaCTX-M-15, and a more homogeneous distribution of qnrB alleles amongst ESBL-produces E. coli and K. pneumoniae.

Notably, some PMQR determinants have been described in multiresistant clones with worldwide distribution (Woodford et al. 2011), such as E. coli ST131 and K. pneumoniae ST11, which were also detected in the present study, further underscoring the ability of these resistance mechanisms to disseminate.

In conclusion, this study is the first report the prevalence of PMQR genes in ESBL-producing Enterobacteriaceae in Argentina and suggests that the qnrB and aac(6′)-Ib-cr genes are widely dispersed among Enterobacteriaceae, as found in many other countries. These isolates showed high-level quinolone resistance ESC resistance that was mediated by ESBLs; therefore, this study demonstrates the importance of understanding the potential risk associated with empirical treatment using these antibiotic families.
**TABLE**

Main features of the plasmid-mediated quinolone resistance-harbouring enterobacteria isolated in this study

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate</th>
<th>City</th>
<th>Hospital</th>
<th>qnrB-like allele</th>
<th>aac(6')-Ib allele</th>
<th>ESBL genes</th>
<th>Phylogenetic group/clone</th>
<th>ST</th>
<th>MIC (μg/mL)</th>
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</table>

AMK: amikacin; BA: Buenos Aires; CAB: Ciudad Autónoma de Buenos Aires; CH: Chubut; CIP: ciprofloxacin; ESBL: extended-spectrum β-lactamases; GAT: gatifloxacin; GEN: gentamicin; H1: Hospital de Clínicas, Universidad de Buenos Aires; H2: Corporación médica San Martín; H3: Hospital Alemán; H4: Hospital Británico; H5: Hospital巡视; H6: CEMIC; H7: Sanatorio Mater Dei; H8: Hospital Eva Perón; H9: Hospital Cullen; H10: Hospital de Trelew; LEV: levofloxacin; MIC: minimum inhibitory concentration; NAL: nalidixic acid; NC: not correspond; ND: not determined; SF: Santa Fe; TOB: tobramycin.
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