The first report of the qnrB19, qnrS1 and aac(6')-Ib-cr genes in urinary isolates of ciprofloxacin-resistant Escherichia coli in Brazil

Magna Cristina Paiva¹, Andréa Maria Amaral Nascimento², Ilana Lopes Baratella Cunha Camargo³, Cláudia Iracema Lima-Bittencourt², Regina Maria Drummond Nardi¹/+¹

¹Departamento de Microbiologia ²Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, 31270-901 Belo Horizonte, MG, Brasil ³Laboratório de Epidemiologia e Microbiologia Molecular, Grupo de Cristalografia, Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, SP, Brasil

In this study, we investigated the presence of plasmid-mediated quinolone resistance (PMQR) genes among 101 ciprofloxacin-resistant urinary Escherichia coli isolates and searched for mutations in the quinolone-resistance-determining regions (QRDRs) of the DNA gyrase and topoisomerase IV genes in PMQR-carrying isolates. Eight isolates harboured the qnr and aac(6')-Ib-cr genes (3 qnrS1, 1 qnrB19 and 4 aac(6')-Ib-cr). A mutational analysis of the QRDRs in qnr and aac(6')-Ib-cr-positive isolates revealed mutations in gyrA, parC and parE that might be associated with high levels of resistance to quinolones. No mutation was detected in gyrB. Rare gyrA, parC and parE mutations were detected outside of the QRDRs. This is the first report of qnrB19, qnrS1 and aac(6')-Ib-cr-carrying E. coli isolates in Brazil.

Key words: qnrS1 - qnrB19 - aac(6')-Ib-cr

Escherichia coli is a common cause of community-acquired urinary tract infections (CA-UTIs). Quinolones have become the most frequently prescribed antimicrobials worldwide due to their broad-spectrum antimicrobial activity (Yang et al. 2010). However, in the last few decades, an increase in quinolone resistance has been documented among human and veterinary isolates of E. coli. Quinolone resistance among Enterobacteriaceae originally occurred due to chromosomal mutations in the quinolone-resistance-determining regions (QRDRs) of the gyrA and gyrB genes, which encode the gyrase A and B subunits, respectively and the parC and parE genes, which encode topoisomerase IV subunits (Hernández et al. 2011). In addition to the chromosomal mutations in the QRDRs, plasmid-mediated quinolone resistance (PMQR) determinants may also reduce the levels of quinolone susceptibility; these elements were first described in 1998 (Martinez-Martinez et al. 1998, Robicsek et al. 2006a, Hernández et al. 2011). Over the last several years, different PMQR gene variants have been described in different locations worldwide. Nevertheless, few studies of PMQR genes have been conducted in Brazil. The first PMQR determinants were described by Castanheira et al. (2007) (qnrA) and by Minarini et al. (2008) (qnrB). The overuse of quinolones in the clinical setting may lead to treatment failure and a public health risk; consequently, a better understanding of PMQR genes is of fundamental importance. Therefore, in this study, we investigated the presence of the qnrA, qnrB, qnrS and aac(6')-Ib-cr genes in a sample of ciprofloxacin-resistant E. coli isolates from women with CA-UTIs.

A total of 101 ciprofloxacin-resistant E. coli isolates collected from the urine of women with clinical and laboratory diagnoses of CA-UTI between May-November 2009 in Belo Horizonte, Minas Gerais, Brazil, were investigated. Only one isolate per patient was included. These isolates were identified using an automated VITEK 2 microbial identification system, version 04.02 (bioMérieux), according to the manufacturer’s instructions. This study was approved by the Ethical Committee of the Federal University of Minas Gerais (178/09). The minimum inhibitory concentration (MICs) of nalidixic acid, ciprofloxacin, ofloxacin, norfloxacin and levofloxacin (Sigma-Aldrich) for all aac(6')-Ib-cr and qnr-positive isolates were determined using the agar dilution method (CLSI 2009). Screening for extended-spectrum beta-lactamase (ESBL) production was performed with the broth microdilution method using an automated VITEK 2 system, version 04.02 (bioMérieux), according to the CLSI (2009) guidelines.

All isolates were screened for the presence of the qnrA, qnrB, qnrS and aac(6')-Ib-cr genes using previously described primers and amplification conditions (Park et al. 2006, Robicsek et al. 2006b). The QRDRs of the chromosomal genes were amplified only from the aac(6')-Ib-cr and qnr-positive isolates using previously described primers and conditions (Park et al. 2006, Morgan-Linnell et al. 2009). The PCR products were sequenced with a MegaBACE 1000 capillary sequencer. The predicted amino acid sequences of GyrA, GyrB, ParC and ParE were analysed to identify putative amino acid changes with respect to the wild-type protein sequences from E. coli K12 MG 1655 (GenBank accession 49175990). The nucleotide sequences obtained have been...
deposited in GenBank under the following accessions: qnrB19 (JF923528), aac(6')-Ib-cr (JF923529-JF923532), qnrSI (JF923533-JF923535), gyrA (JN565698-JN565702 and JN415087-JN415089), gyrB (JN565703-JN565710), parC (JN565711-JN565713 and JN565712-JN565716) and parE (JN565714-JN565721).

Eight of the 101 isolates evaluated (7.9%) harboured PMQR genes. Among the qnr-positive isolates, three contained qnrSI and one contained qnrB19. It should be noted that no isolate harboured the qnrA gene, although the detection of this gene in Brazilian isolates was previously described by Castanheira et al. (2007). Few qnrSI and qnrB19-positive E. coli isolates have been reported since 2008 and most of these isolates were qnrB19-positive isolates from South America (Deepak et al. 2009). In Brazil, the detection of the qnrA1 gene in E. coli (Castanheira et al. 2007) and of qnrB2 and qnrB8 genes in other Enterobacteriaceae (Minarini et al. 2008) has been described previously and the qnrB19 gene was recently detected for the first time in Salmonella isolated from poultry (Ferrari et al. 2011). Furthermore, four isolates in our study were aac(6')-Ib-cr-positive. Although the aac(6')-Ib-cr gene appears to be more prevalent overall than any of the qnr genes (Morgan-Linnell et al. 2009), we detected these genes at the same frequency in the present study. These findings conflict with the results of with previous studies, which indicated that qnrB was the most widespread PMQR in Brazil (Minarini et al. 2008).

The analysis of the qnrSI, qnrB19 and aac(6')-Ib-cr sequences revealed 100% identity to the reported qnrSI sequence from the IncN plasmid from the Salmonella enterica subsp. enterica strain 382/03 (GenBank accession HQ214119.1); 99% identity to the qnrB19 sequence from the E. coli plasmid p013.1 IncR (GenBank accession HM146784.1) and 100% identity to the aac(6')-Ib-cr sequence from the Shigella dysenteriae strain DS-505 (GenBank accession HQ166949.1), respectively.

Sequence analysis of the chromosomal QRDR revealed mutations in the gyrA, parC and parE genes. No gyrB mutation was detected. Most isolates exhibited double mutations in GyrA and single mutations in ParC and ParE (Table). Our results are in agreement with those of previous works, which showed that substitutions at S83 and D87 in gyrA and S80 in parC are common and lead to a high level of quinolone resistance (Hopkins et al. 2005, Sorlozano et al. 2007). Additionally, rare mutations outside of the GyrA, ParC and ParE QRDRs were detected (Table); among these, only S458A in parE had been reported previously (Sorlozano et al. 2007, Moon et al. 2010, Bansal & Tandon 2011). This mutation was originally reported in Spain (Sorlozano et al. 2007) and was identified at a high frequency in E. coli isolates recovered in Delhi, India (Bansal & Tandon 2011). There have been few reports of uncommon mutations outside QRDRs (Friedman et al. 2001, Lindgren et al. 2003, Sorlozano et al. 2007) and the effects of such mutations on quinolone susceptibility should be studied further.

The high observed MICs for quinolones (Table) were likely a consequence of mutations in the chromosomal QRDRs associated with PMQR genes. Previous works also suggested that PMQR and chromosomal resistance
mechanisms are additive and can enhance the quinolone resistance of clinical isolates (Martinez-Martínez et al. 2003, Rodríguez-Martínez et al. 2011). Furthermore, it should be noted that PMQR genes may facilitate the emergence of quinolone resistance, which would have therapeutic implications (Rodríguez-Martínez et al. 2011).

Recently, the association of aac(6′)-Ib-cr with genes encoding the beta-lactamase CTX-M-15 or other ESBLs has been reported (Pitout et al. 2008). Therefore, we considered it important to analyse the ESBL production of PMQR-positive E. coli isolates. Only two of the eight PMQR-positive isolates screened produced ESBLs and both of these isolates were positive for aac(6′)-Ib-cr. To verify the horizontal mobility and potential capacity for the spreading of these genes, conjugation (Yang et al. 2008) and transformation (Sambrook & Russell 2001) experiments were conducted. However, the genes did not appear to be transferable by transformation or conjugation.

This article describes the first E. coli isolates in Brazil harbouring the qnrS1, qnrB19 and aac(6′)-Ib-cr genes. The aac(6′)-Ib-cr-positive isolates, which also produced ESBLs, are of particular clinical concern.

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REFERENCES


