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

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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

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deposited in GenBank under the following accessions: *qnrB19* (JF923528), *aac(6')Ib-cr* (JF923529-JF923532), *qnrS1* (JF923533-JF923535), *gyrA* (JN565698-JN565702 and JN415087-JN415089), *gyrB* (JN565703-JN565710), *parC* (JN565711-JN565713 and JN657512-JN657516) and *parE* (JN565714-JN565721).

Eight of the 101 isolates evaluated (7.9%) harboured PMQR genes. Among the *qnr*-positive isolates, three contained *qnrS1* 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 *qnrS1* 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 *qnrS1*, *qnrB19* and *aac(6')-Ib-cr* sequences revealed 100% identity to the reported *qnrS1* 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 S458 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

TABLE

Molecular characterization of the quinolone resistance mechanisms, extended-spectrum beta-lactamase (ESBL)-producing, resistance profile and minimal inhibitory concentrations (MICs) of quinolones in ciprofloxacin-resistant *Escherichia coli* isolates

Sample	Mutation			Quinolone resistance gene	ESBL production	Resistance profile	MIC (µg/mL)				
	GyrA	ParC	ParE				NAL	OFX	NOR	CIP	LVX
7A	S83L/D87N	S80L	S458A	*aac(6')-Ib-cr*	-	AMP	> 1024	128	512	>1024	64
8A	S83L/D87N	S80L	S458A	*aac(6')-Ib-cr*	+	AMP, CFL, CAZ, CTX, CFL, AZM	512	64	256	128	32
19A	S83L/D87N/G56C	S80L/A117E/M118V	S458A/ H509Q/E413D	*aac(6')-Ib-cr*	-	AMP	> 1024	64	256	256	32
24A	S83L/D87N	S80L	S458A	*aac(6')-Ib-cr*	+	AMP, CFL, CAZ, CTX, CFL, AZM	> 1024	256	256	128	256
34A	S83L/D87N/A175T	S80L	ND	*qnrB19*	-	AMP	> 1024	64	256	64	128
26A	S83L/D87N/K154N	S80L	S458A/C404R	*qnrS1*	-	ND	> 1024	64	256	64	64
56A	S83L/D87N	S80L	ND	*qnrS1*	-	AMP	> 1024	16	32	08	16
90A	S83L/D87N	S80L	ND	*qnrS1*	-	AMP	> 1024	16	32	08	16

AMP: ampicillin; AZM: aztreonam; CAZ: ceftazidime; CFL: cefepime; CIP: ciprofloxacin; CTX: cefotaxime; LVX: levofloxacin; NAL: nalidixic acid; ND: not detected; NOR: norfloxacin; OFX: ofloxacin; +: ESBL-producer isolate; -: no ESBL-producer isolate.

mechanisms are additive and can enhance the quinolone resistance of clinical isolates (Martínez-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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