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Notes

High percentage of resistance to ciprofloxacin and *qnrB19* gene identified in urinary isolates of extended-spectrum β-lactamase–producing *Escherichia coli* in Madrid, Spain☆

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Abstract

The presence of *qnr* genes in 191 extended-spectrum β-lactamase–producing *Escherichia coli* from 2005, with 75% of resistance to ciprofloxacin, was evaluated. An SHV-12–producing *E. coli* carried *qnrB19*; both genes were transferred by conjugation. No *qnrA*- or *qnrS*-positive strains were detected. In addition, we identified 3 new *parC* mutations (S80W, E84R, and E84A).
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Keywords: *qnr*; *E. coli*; Fluoroquinolone; Resistance

Resistance to fluoroquinolones is caused mostly by the accumulation of point mutations in *gyrA* and/or *parC* genes (Chen and Lo, 2003; Lindgren et al., 2003).

Resistance to quinolones can also be mediated by plasmid-borne genes such as *qnr*, which protects the quinolone targets from inhibition. The first *qnr* gene detected was *qnrA* (Martinez-Martinez et al., 1998), followed by *qnrB* and *qnrS* (Robicsek et al., 2006a, 2006b). Recently, *qnrC* and *qnrD* have been described (Crémet et al., 2009; Fang et al., 2009). *qnr* has been detected in enterobacterial species from different countries (Jacoby et al., 2006; Lavilla et al., 2008). Moreover, *qnr* genes have been detected among isolates producing extended-spectrum β-lactamases (ESBL) (Fang et al., 2009; Lavilla et al., 2008Z; Martinez-Martinez, 2007).

In 2005, 191 nonduplicate clinically relevant ESBL-producing *Escherichia coli* strains were isolated from urine in the Hospital Clinico San Carlos, Madrid, Spain. These isolates were identified by Wider System (Soria Melguizo, SA, Madrid, Spain). The presence of ESBLs was confirmed using the agar dilution test with cefotaxime, cefotaxime–clavulanic, ceftazidime, and ceftazidime–clavulanic as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2007). All the isolates were screened for *bla* genes (SHV, TEM, and CTX-M) by polymerase chain reaction (PCR) (Table 1).

Susceptibility to ciprofloxacin and other antimicrobial agents was determined by agar dilution, following CLSI recommendations. Resistance to ciprofloxacin was defined as an MIC ≥4 mg/L.

All the 191 strains were screened for the presence of *qnr* genes (*qnrA*, *qnrB*, and *qnrS*) by PCR using specific primers (Table 1). *qnr*-positive strains were used as controls. Positive results were confirmed by DNA sequencing (Jacoby et al., 2008). Mating assays with *E. coli* K-12 as recipient strain were attempted to determine the transferability of *qnr* genes (Gerhardt et al., 1981). Transconjugants were selected on MacConkey plates supplemented with 100 mg/L rifampicin and 2 mg/L ceftazidime and/or 0.12 mg/L ciprofloxacin. PCR amplification and DNA sequencing of the *bla* and *qnr* genes of transconjugants were carried out with specific primers (Table 1).

We selected 30 of the 141 ciprofloxacin-resistant strains to represent the full range of ciprofloxacin resistance and classified them into 3 groups according to the ciprofloxacin

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Table 1
Primers used for PCR and DNA sequence determination

Gene	Primer name	Sequence (5′ to 3′)	Fragment size (bp)	Reference
gyrA	gyra6	CGACCTTGCGAGAGAAAT	626	McDonald et al. (2001)
	gyra631R	GTTCCATCAGCCCTTCAA		
parC	parCF43	AGCGCCTTGCGTACATGAAT	964	Lindgren et al. (2003)
	parCR981	GTGGTAGCGAAGAGGTGGTT		
qnrA	qnrA f	AGAGGATTTCTCACGCCAGG	580	Cattoir et al. (2007)
	qnrA r	TGCCAGGCACAGATCTTGAC		
qnrB	FQB1	ATGACGCCATTACTGTATAA	600	Jacoby et al. (2006)
	FQB2	GATCGCAATGTGTGAAGTTT		
qnrS	qnrS f	GCAAGTTCATTGAACAGGGT	428	Cattoir et al. (2007)
	qnrS r	TCTAAACCGTCGAGTTCGGCG		
bla_{TEM}	BLAT-A	ATAAAATTCTTGAAGACG	1076	Coque et al. (2002)
	BLAT-B	TTACCAATGCTTAATCA		
bla_{CTX-M}	CTX-M F	GTGACAAAGAGAGTGCAACGG	856	Coque et al. (2002)
	CTX-M R	ATGATTCTCGCCGCTGAAGCC		
bla_{SHV}	SHV-1	GGGTTATTCTTATTTGTCGC	930	Coque et al. (2002)
	SHV-2	TTAGCGTTGCCAGTGCTC		

MIC (Table 2). The *gyrA* and *parC* genes were sequenced in these isolates.

Although the antimicrobial coresistance with quinolones in ESBL-producing isolates has been previously reported (Crémet et al., 2009; Fang et al., 2009; Lavilla et al., 2008; Martinez-Martinez, 2007; Poirel et al., 2006a, 2006b; Robisek et al., 2006a, 2006b; Schwaber et al., 2005), our percentage of resistant strains (75%) is higher than those observed elsewhere—62% to 68% in Spain (de Cueto et al., 2006; Hernandez et al., 2005), 44% in Italy (Mugnaioli et al., 2006), and 30% in France (Lavigne et al., 2004).

In the present study, *qnr* genes were rare (0.5%), no *qnrA* or *qnrS* were detected, and we have found only 1 isolate possessing *qnrB*. It was recovered from the urine of a 93-year-old male patient with pneumonia treated with levofloxacin. The low prevalence of *qnr* genes among our *E. coli* isolates is comparable with the results of previous studies, where *qnr* genes were more prevalent in other enterobacterial species (Fang et al., 2009; Jacoby et al., 2006; Lavilla et al., 2008; Poirel et al., 2006a, 2006b; Robisek et al., 2006a, 2006b). The presence of *qnrB* in *E. coli* has been found previously (Fang et al., 2009; Kim et al., 2009); other authors have identified *qnrA* and *qnrS*, but not *qnrB* in *E. coli* (Lavilla et al., 2008; Rodriguez-Martinez, 2005).

Table 2
Distribution of *gyrA* and *parC* genes in the 30 selected isolates

Group MIC range (mg/L)	No. of strains	*gyrA*		*parC*	
		S83	D87	S80	E84
1 (4–8)	5	L	N	I	
	2	L	G	I	
	1	L	N	W	
	1	L	Y	I	
	1	L			R
2 (16–32)	3	L	N	I	
	2	L	N	I	V
	1	L	N	I	G
	1	L	N	I	A
	1	L	N		K
	1		N	I	
3 (≥64)	6	L	N	I	V
	3	L	N	I	
	1	V	T	I	V
	1	L	N	R	

Sequencing of the *qnrB* gene of our strain indicated the variant. To date, only a few *qnrB19*-positive *E. coli* isolates have been reported, all of them from South America. Qnr B19 determinant was identified in a clinical isolate from Colombia (Cattoir et al., 2008). Pallecchi et al. (2009) have already reported the presence of *qnrB19* in commensal *E. coli* from Peru and Bolivia.

Our *qnrB19*-positive *E. coli* also carried a bla_{SHV-12} gene; the association between *qnr* and bla_{SHV} genes has also been observed by other investigators (Fang et al., 2009; Jacoby et al., 2006; Martinez-Martinez, 2007; Poirel et al., 2006a; 2006b; Wang et al., 2003). The presence of both these genes on the same plasmid could explain this relationship; *qnr* is in an integron structure upstream of *qacEΔ1* and *sul1*, and has been found in conjugative plasmids (Cattoir et al., 2008; Rodriguez-Martinez, 2005). In *Klebsiella pneumoniae*, *qnrB19* has been described along with blaKPC in the “KQ element” (Rice et al., 2008). In our work, the transfer of these genes by conjugation was successful; the transconjugant carried *qnrB19* and bla_{SHV-12} genes.

In the conjugation experiment, we tried also to investigate the role of *qnr* genes in fluoroquinolone resistance. The *qnr*-positive donor exhibited ciprofloxacin resistance (MIC, 16 mg/L), and the transconjugant carrying *qnrB19* showed a ciprofloxacin MIC ≤0.03 mg/L. Therefore, it seems likely that the resistance of the donor strain is caused by 3 mutations found, 2 in *gyrA* (S83L and D87N) and 1 in *parC* (S80I). Our findings agree with previous reports demonstrating that *qnr* alone did not ensure resistance to fluoroquinolones; however, its presence may facilitate the selection of additional chromosomally encoded quinolone resistance mechanisms (Mammeri et al., 2005; Poirel et al.,

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