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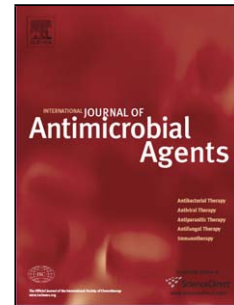
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**Characterisation of *qnr* plasmid-mediated quinolone resistance in Enterobacteriaceae from Italy: association of the *qnrB19* allele with the integron element *ISCR1* in *Escherichia coli***

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## ABSTRACT

The spread of plasmid-mediated quinolone resistance determinants (*qnr*-like determinants) was evaluated in a collection of 232 ciprofloxacin-resistant or extended-spectrum  $\beta$ -lactamase (ESBL)-producing enterobacterial isolates recovered between November 2007 and May 2008 at Padua University Hospital, Italy. *qnr* genes were mainly found in *Klebsiella pneumoniae* (68%) and to a lesser extent in *Escherichia coli* (5.1%). Among the *qnrA1*, *qnrS1* and *qnrB19* alleles found, the latter was by far the most frequent. Genetic environment analysis revealed that one *qnrB19* gene in *E. coli* was embedded in an ISCR1 complex class 1 integron. All other *qnrB19* genes were flanked by an ISEcp1C region as part of the Tn2012 transposon. *qnrA1*- and *qnrS1*-containing strains were not clonally related. Both topoisomerase II mutations and ESBL (mainly SHV-12, TEM-1 and TEM-150 types) were present in most of the *qnr*-positive strains. *qnrB19* is extremely frequent in *K. pneumoniae* isolates from Italy. In addition, association of *qnrB19* with the ISCR1 mobile element in *E. coli* suggests a broad distribution of this resistance gene in the near future.

## 1. Introduction

Fluoroquinolones are among the most commonly prescribed antimicrobials because of their broad-spectrum antimicrobial activity. However, this intense usage of fluoroquinolones has boosted the emergence of resistance worldwide, especially in Gram-negative bacterial species. Classic quinolone resistance in Enterobacteriaceae is mediated by chromosomally encoded point mutations in the quinolone resistance-determining regions (QRDRs) of the DNA gyrase and topoisomerase IV genes or by mutation of genes regulating the expression of efflux pumps [1]. More recently, plasmid-mediated quinolone resistance has been reported, with three known mechanisms to date: gyrase and topoisomerase IV protection encoded by the *qnr* genes [2,3]; enzymatic drug detoxification encoded by *aac(6′)-Ib-cr* [4]; and specific efflux mediated by the *qepA* product [5]. The latter two mechanisms affect only fluoroquinolones with specific features (i.e. hydrophilicity and free piperazinyl amine, e.g. ciprofloxacin and norfloxacin) and hence treatment with other compounds of the same antimicrobial class (e.g. levofloxacin and ofloxacin) remains an option. In contrast, *qnr*-mediated resistance is effective against all fluoroquinolones and hence requires treatment shift to a different class of antimicrobials. The observation that *qnr* is often found in association with genes that confer resistance to other classes of antibiotics [6] (e.g.  $\beta$ -lactams and aminoglycosides) implies that Enterobacteriaceae strains presenting *qnr* resistance may represent a serious concern in the clinical environment.

Three major groups of Qnr determinants have been described (QnrA, QnrB and QnrS), which share between 40% and 60% similarity [3]. More recently, two other groups have been described, *qnrC* [7] and *qnrD* [8].

In this paper, the dissemination of *qnr* plasmid-mediated quinolone resistance was assayed among clinical Enterobacteriaceae isolates from Italy. *qnr* genes were found mainly in *Klebsiella pneumoniae* and to a lesser extent in *Escherichia coli*. Among the *qnrA1*, *qnrS1* and *qnrB19* alleles found, the latter was by far the most frequent. Genetic environment analysis revealed that one *qnrB19* gene was embedded in an ISCR1 complex class 1 integron, whilst all other *qnrB19* genes were flanked by an ISEcp1C region as part of the Tn2012 transposon.

## 2. Materials and methods

### 2.1. Sample selection

In total, 232 non-duplicate clinical isolates recovered between November 2007 and May 2008 at Padua University Hospital (Italy) were selected on the basis of ciprofloxacin resistance [minimum inhibitory concentration (MIC)  $\geq 0.25$   $\mu\text{g/mL}$ ] or extended-spectrum  $\beta$ -lactamase (ESBL) production. In addition, ten samples fully susceptible to ciprofloxacin (MIC  $< 0.25$   $\mu\text{g/mL}$ ) and lacking ESBL production were also included in the study. Bacterial isolates included 157 *E. coli*, 47 *K. pneumoniae*, 2 *Klebsiella oxytoca*, 12 *Proteus mirabilis*, 5 *Enterobacter aerogenes*, 4 *Enterobacter cloacae*, 4 *Citrobacter freundii* and 1 *Citrobacter koseri*. Sample origin was mostly urine (175 samples); the remaining were from the respiratory tract (17), blood (11), skin/wounds (14), stools (2), pus/viscera (10) and the urogenital tract (3). Of samples were collected, 92% were from inpatients. Patients were equally distributed between females (48%) and males (52%).

## 2.2. Antimicrobial susceptibility testing

Exact MIC values were determined by the disk diffusion test according to Clinical and Laboratory Standards Institute (CLSI) methods [9] and were confirmed by means of Etest strips (AB BIODISK, Solna, Sweden). ESBL production was detected according to CLSI guidelines M2-A9 and M100-S19 [9,10].

## 2.3. Polymerase chain reaction (PCR) amplification and DNA sequencing

Screening of *qnrA*, *qnrB* and *qnrS* genes was carried out by multiplex PCR amplification [11]. *qnrC* and *qnrD* genes were not included in this study as they were not known when the study was planned. Positive controls were represented by DNA of known *qnr*-positive strains: *E. cloacae* HM04-477 (*qnrA1*) [12]; *E. cloacae* HM05-186 (*qnrB4*) [13]; and *E. coli* HM5-184 (*qnrS1*) [14].

Primers for detection of *aac(6')-Ib* and *qepA* were: *aacF*, 5'-ATGACTGAGCATGACCTTG-3'; *aacR*, 5'-AACCATGTACACGGCTGG-3'; *qepAF*, 5'-TGGTCTACGCCATGGACCTCA-3'; and *qepAR*, 5'-TGAATTCGGACACCGTCTCCG-3'.

The presence of  $\beta$ -lactamases genes (ESBLs *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CTX-M-1</sub>, *bla*<sub>CTX-M-2</sub>, *bla*<sub>CTX-M-8</sub> and *bla*<sub>CTX-M-9</sub> and AmpC enzymes *bla*<sub>MOX-1</sub>, *bla*<sub>MOX-2</sub>, *bla*<sub>CMY-1-11</sub>, *bla*<sub>LAT-1-4</sub>, *bla*<sub>BIL-1</sub>, *bla*<sub>DHA-1</sub>, *bla*<sub>DHA-2</sub>, *bla*<sub>ACC</sub>, *bla*<sub>MIR-1T</sub>, *bla*<sub>ACT-1</sub>, and *bla*<sub>FOX-1-5b</sub>) was assessed on the *qnr*-positive isolates using specific primers [15–18].

PCR amplification and sequencing of the QRDR of *gyrA* and *parC* genes was performed using universal primers [19].

Primers for sequencing the genetic environment of *qnrB19* genes were specific for the *ISEcp1* insertion sequence or 5'-conserved sequence (5'CS) (prF *ISEcp1*, 5'-CGACATGGCGGTGGGTCATC-3'; prR *ISEcp1*, 5'-CTGCTTGTACACATTGTAAC-3'; and pr5'CS, 5'-GGCATCCAAGCAGCAAG-3' [20]) or were specific and outward facing for the *qnrB19* gene (pr5'*qnrB19*, 5'-GCGAGTCCAGCCGTGGAC-3'; and pr3'*qnrB19*comp, 5'-CCGCAATTCAATCTGTGCGATGG-3').

Positive results were confirmed by direct sequencing of PCR products.

#### 2.4. Plasmids isolation and analysis

Plasmids from *qnr*-positive bacterial isolates were purified according to the Kieser protocol [21]. Purified plasmids were run on a 0.7% Mb agarose gels at 4 °C for 5 h at 4.5 V/cm and were stained with ethidium bromide.

#### 2.5. Conjugation and transformation

Conjugation experiments were performed by a filter mating assay using a kanamycin-resistant *E. coli* J53<sup>Az<sup>R</sup></sup> strain as the recipient and selecting on Luria–Bertani (LB) agar supplemented with 100 µg/mL sodium azide, 50 µg/mL kanamycin and 30 µg/mL nalidixic acid.



For transformation experiments, plasmids purified and separated on agarose gels were electroporated into *E. coli* TOP10 (Invitrogen Ltd., Paisley, UK) using a Bio-Rad Gene Pulser<sup>®</sup> (Bio-Rad Laboratories, Milan, Italy). Transformants were selected on LB agar supplemented with 30 µg/mL nalidixic acid.

### 2.6. Molecular typing

The clonal relationship among strains was assessed based on the repetitive element PCR fingerprinting method, using the 'enterobacterial repetitive intergenic consensus' (ERIC) primers ERIC1 and ERIC2 [15].

## 3. Results

### 3.1. Phenotypic characterisation of isolates

*qnr* genes were detected in 40 (17%) of 232 unique clinical isolates of Enterobacteriaceae collected from Padua University Hospital between November 2007 and May 2008 (Table 1), comprising 32 (68%) of 47 *K. pneumoniae* and 8 (5.1%) of 157 *E. coli*. In particular, *qnrA* and *qnrB* were detected in 1 (2.1%) and 31 (66%) of 47 *K. pneumoniae* isolates, respectively, and *qnrB* and *qnrS* were detected in 5 (3.2%) and 3 (1.9%) of 157 *E. coli* isolates, respectively. No *qnr* gene was identified in other bacterial species and no isolate carried more than one *qnr* gene. All *qnrB* genes were identified as *qnrB19* alleles [22] by sequencing; *qnrA* and *qnrS* genes were identified as *qnrA1* and *qnrS1*, respectively. The genes *aac(6')-Ib-cr* and *qepA* were absent in all strains with the exception of five *qnrB19*-positive isolates that harboured *aac(6')-Ib* (samples #111, #137, #143, #144 and #176; Table 1).

Among the 40 *qnr*-positive isolates, 31/40 (78%), 3/40 (7.5%), 5/40 (13%), 3/28 (11%) and 3/40 (7.5%) isolates were nalidixic acid-, ciprofloxacin-, ofloxacin-, levofloxacin- and moxifloxacin-resistant, respectively, according to CLSI criteria (Table 2). However, when considering European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, the isolates were in general more resistant to fluoroquinolones (82%, 37%, 92%, 18% and 82%, respectively).

### 3.2. Mutations conferring quinolone resistance

Twenty-eight isolates were identified with a D87G GyrA mutation, one isolate with two point mutations in GyrA (S83L and D87N) and one mutation in ParC (S80I) (#9), and one isolate with S83I and S80I mutations in GyrA and ParC, respectively (#70). It is interesting to note that these latter two isolates were the only *qnr*-positive strains resistant to all tested fluoroquinolones. On the other hand, strains showing no mutation at the topoisomerase II level (i.e. the three *qnrS1*-positive *E. coli* strains #114, #191 and #282 and the *qnrB19*-positive *E. coli* #47, #50, #60 and #132 and *K. pneumoniae* #97, #204 and #229) were susceptible to all tested fluoroquinolones as well as to nalidixic acid.

### 3.3. Association between *qnr* and extended-spectrum $\beta$ -lactamase

Since *qnr* genes have been associated with plasmid-mediated  $\beta$ -lactamases [23], the presence of  $\beta$ -lactamases was assessed. In total, 30 of 40 samples contained *bla* genes (association of up to three different types was found in single isolates) (Table 1). Conversely, no sample carried plasmid-mediated AmpC  $\beta$ -lactamases. ESBL production was also

analysed phenotypically. On the whole, a clear association was found between *qnr* genes and ESBLs: whilst 33% of the initial 232 clinical isolates tested were ESBL-producers, this value increased to 72% (ESBL phenotypic testing) and 75% (ESBL genotypic analysis) in *qnr*-positive samples.

Finally, *qnr*-positive isolates remained mostly susceptible to amikacin (80%), gentamicin (85%), tetracycline (86%), trimethoprim/sulfamethoxazole (90%) and imipenem (100%), indicating no significant increase in resistance to aminoglycosides, tetracyclines, co-trimoxazole or carbapenems associated with *qnr* genes. The *qnrA1*-positive sample was the only isolate displaying simultaneous resistance to aminoglycosides, tetracyclines and co-trimoxazole; in addition, *qnrS1*-positive *E. coli* isolate #282 was resistant to tetracyclines and co-trimoxazole (data not shown).

#### 3.4. Characterisation of the genetic environment of *qnr*

Analysing the plasmid electrophoretic profiles of *qnr*-positive isolates, all *qnrB19*-positive isolates showed identical profiles, with the exception of *qnrB19*-positive *E. coli* isolate #132. The plasmid pattern of *qnrA1*- and *qnrS1*-positive isolates was similar, but differed from both *qnrB19* patterns (Fig. 1).

To determine whether the *qnr* determinants were plasmid-encoded, *qnr*-containing electrophoretic bands from seven arbitrarily chosen samples (one *qnrA1*-, four *qnrB19*- and two *qnrS1*-positive samples) were successfully transformed into *E. coli* TOP10 electrocompetent cells, indicating a plasmid location for all tested *qnr* genes. The *qnr*-containing plasmid size in each case was >23 Kb. MICs of different fluoroquinolone drugs

against transformant strains were measured and were compared with those of the acceptor strain (i.e. *E. coli* TOP10). The MICs of ciprofloxacin and levofloxacin increased 27–80-fold and 80–115-fold, respectively, in the *qnr*-transformed strains. The MIC of nalidixic acid was the most variable, increasing from 16-fold to 128-fold in the transformant strains. When comparing transformant versus parent strains, MICs of the transformant strains decreased by at least 40-fold or 1–3-fold when the MIC of the parent strain was  $>1 \mu\text{g/mL}$  (i.e. strains #70, #71, #111 and #146) or  $<1 \mu\text{g/mL}$  (strains #132, #191 and #282), respectively (Table 1). In addition, *bla* genes were never co-transformed with *qnr*, indicating a different genetic location.

To determine whether the *qnr*-harbouring plasmid was conjugative, two *qnr*-positive *E. coli* isolates [#132 (*qnrB19*) and #191 (*qnrS1*)] were successfully used as donors in conjugation experiments. The MICs of ciprofloxacin, levofloxacin and nalidixic acid in the transconjugant strains were 14–20-, 7- and 5–10-fold higher, respectively, than those of the wild-type recipient, and 1–2-fold lower compared with those in the donor strains. Both transformed strains and transconjugants displayed no mutation at the *gyrA* and *parC* level.

The environment surrounding the *qnrB19* genes was first investigated using primers complementary to the *ISEcp1C* region of transposon Tn2012, previously reported to be associated with the *qnrB19* gene [22]. Of 36 *qnrB19*-positive samples, 35 contained the *ISEcp1C* transposase at the 3' end of the *qnrB19* gene. Sequence analysis confirmed that these *qnrB19* genes were all part of the Tn2012 transposon, with the *qnrB19* gene followed by the *ISEcp1C* element. Interestingly, these 35 isolates reported the exact same plasmid electrophoretic profile (see Fig. 1).

To assess the genetic environment of the remaining *qnrB19*-positive *E. coli* strain (#132), plasmid DNA was purified and subjected to direct sequencing. Analysis of a 1.3 kb *qnr*-containing DNA region indicated that the *qnrB19* gene was embedded in an ISCR1-containing mobile element integron complex class I. The integron was flanked by the 5'CS followed by the inverted repeat region 2 (IRR2). A truncated *psp* operon was immediately upstream of the *qnrB19* gene. Downstream, an *Int1* element with its putative recombination site was present, followed by the *sapA* gene disrupted by recombination events and a second recombination site (GenBank accession no. GU074393) (Fig. 2).

### 3.5. Clonal relationships among isolates

Finally, the clonal relationship among the isolates in this study was assessed by studying the ERIC-PCR genomic DNA profiles. As shown in Table 1, *qnrB19*-positive samples were grouped in seven clonally related subgroups. In addition, 17 (47%) and 8 (22%) of 36 *qnrB19*-positive isolates belong to the same two most numerous subgroups, respectively. However, the three *qnrS1*-positive isolates were all clonally unrelated.

## 4. Discussion

In this work, we have detected for the first time the presence of *qnr* genes that confer mild resistance to quinolones among enterobacterial isolates collected in the Northeast region of Italy.

Interestingly, all *qnrB*-positive *K. pneumoniae* as well as some *E. coli* strains bore the *qnrB19* allele. Of these, all but one were embedded within a transposon Tn2012 structure associated

with an *ISEcp1* element and reported identical plasmid electrophoretic profiles. Tn2012 was reported for the first time in an *E. coli* clinical isolate from Colombia in 2008 [22]. Recently, *qnrB19* genes were reported in *Salmonella* strains from The Netherlands [24] and Italy [25]; all of them were transposon-associated.

In one of our analysed isolates, *qnrB19* was found in a completely different context. Genetic environment analysis revealed its association with an integron complex class I bearing an *ISCR1* mobile element. This is the first time a *qnrB19* gene has been reported in an *ISCR1* integron environment, whilst other *qnr* alleles have been previously described associated with this genetic element (i.e. *qnrA1*, *qnrB2*, *qnrB6* and *qnrB10*) [19,20,26–28]. Very recently, experimental data provided a likely mechanism for *ISCR1*-mediated *qnrB2* gene mobilisation [29]. *ISCR1* can transpose adjacent DNA sequences, mediated by a single copy of the element, in contrast to *ISEcp1* where two copies of the element are needed to flank the mobilised gene. In addition, the *ISCR1* element plays a role in the expression of nearby genes by providing a promoter [30]. Hence, *ISCR1* may be responsible for the mobilisation of virtually any DNA in an extremely efficient manner, and resistance genes found in *ISCR1* gene cassettes are potentially highly mobile [31]. The fact that only one isolate was found with *qnrB19* in this genetic background and that it did not present any further mutation increasing resistance to fluoroquinolones (i.e. topoisomerase II mutations) or to other classes of antibiotics (i.e. ESBL) indicates the rather recent acquisition of the *qnrB19* gene in this strain. The intrinsic properties of its genetic environment predict a high diffusion of this *qnrB19* embedded in an *ISCR1*-bearing mobile element and therefore we expect to find increased *qnrB19*-mediated resistance to fluoroquinolones in the near future.

In contrast, *ISEcp1 qnrB19* genes were probably acquired some time ago, since most of them displayed concurrent types of resistance mechanisms such as topoisomerase II mutations, high-level resistance to fluoroquinolones and intensive ESBL production, which are usually stimulated by and then become associated with the *qnr* gene [3]. It is possible that these *ISEcp1 qnrB19* genes were initially acquired from a single source in the initial location (i.e. Colombia where they were first reported [22]) through strain exchange by travellers and patients between South America and Europe. However, this gene now appears uniformly diffuse in our region since *qnrB19*-positive samples were collected both from inpatients and outpatients and were randomly distributed among different hospital units.

By contrast, non-*qnrB19* genes were found both in *K. pneumoniae* (*qnrA1*) and *E. coli* (*qnrS1*) isolates. Both their phenotypic and genetic profiles indicated that they were more diversified and were likely acquired from diverse sources.

On the whole, *K. pneumoniae* species were more likely to present *qnr* genes than *E. coli* isolates (68% and 5% of the initial *K. pneumoniae* and *E. coli* isolates, respectively). To our knowledge, this is one of the widest spreading of *qnr* genes in *K. pneumoniae* isolates. In addition, no similar distribution for the *qnrB19* gene has ever been reported. Generally, *qnr* genes are largely associated with ESBLs: the prevalence of *bla* genes increased from 33% in all tested isolates to 75% in *qnr*-positive strains. Nevertheless, contrary to most samples reported in the literature [3,6,22], *qnr* and *bla* genes did not co-localise on the same plasmid. *qnr*-positive samples were mainly resistant to nalidixic acid and susceptible to other types of antibiotics. Resistance to nalidixic acid and the presence of ESBLs are two phenotypically measured properties that possibly indicate the presence of plasmid-mediated mechanisms of

resistance to fluoroquinolones. However, in our experience these two features did not make a predictive phenotype, since six of ten nalidixic acid-susceptible and ESBL-negative samples were *qnr*-positive. Hence, it would be interesting to analyse next the dissemination of plasmid-mediated resistance mechanisms on a larger set of 'susceptible' clinical isolates.

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### **Competing interests**

None declared.

### **Ethical approval**

Not required.



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**Fig. 1.** Plasmid electrophoretic profiles of *qnr*-positive samples. M, molecular weight marker. Samples are indicated according to their ID number (ID #) and their *qnr* allele (*B* stands for *qnrB19* and *S* for *qnrS1*).

**Fig. 2.** Genetic environment flanking the *qnrB19* gene in isolate #132. The *qnrB19* gene is embedded in an ISCR1-type mobile element integron complex class I (In1:ISCR1:QnrB19). The integron is flanked on one side by the 5'-conserved sequence (5'CS) followed by the inverted repeat region 2 (IRR2). The *psp* operon transcriptional activator (*psp* O-TA) is truncated and immediately upstream of the *qnrB19* gene. Downstream, an integrase 1 (*int1*) with its putative recombination site is present, followed by the *sapA* (a peptide transport system permease) gene disrupted by recombination events and a second recombination site (GenBank accession no. GU074393).

**Table 1**Properties of *qnr*-positive clinical isolates and transformant and transconjugant strains

Sample ID	Bacterial species	<i>qnr</i> gene	<i>aac(6')</i> - <i>lb</i> gene	MIC (mg/L)					GyrA	Par C	ESBL <sup>a</sup>		ERIC -PCR
				NA	CIP	OFX	LVF	MXF			Phenotypic	Genotypic	
5	<i>Klebsiella pneumoniae</i>	<i>qnrB1</i> <sub>9</sub>		>25 6	1	4	1.5	1.5	D87 G	–	X		B2
9	<i>Escherichia coli</i>	<i>qnrB1</i> <sub>9</sub>		>25 6	>32	>32	>32	>32	S83L , D87 N	S80I	X	TEM-1 + SHV-12	B7
16	<i>K. pneumoniae</i>	<i>qnrB1</i> <sub>9</sub>		>25 6	1.5	6	2	2	D87 G	–	X	TEM-150 + SHV- 12	B2
47	<i>E. coli</i>	<i>qnrB1</i> <sub>9</sub>		4	0.12 5	1	N/D	0.38	–	–	X	TEM-1	B7
50	<i>E. coli</i>	<i>qnrB1</i> <sub>9</sub>		8	0.19	0.75	N/D	0.5	–	–	X	SHV-12	B6
60	<i>E. coli</i>	<i>qnrB1</i> <sub>9</sub>		6	0.12 5	2	N/D	0.38	–	–	X	TEM-1	B7

70	<i>K. pneumoniae</i>	<i>qnrA1</i>	>25 6	>32	>32	>32	>32	S83I	S80I	X	TEM-1 + SHV-12 + CTX- M-1	A1
70T	<i>E. coli</i> TOP10	<i>qnrA1</i>	48 6	0.01	N/D	N/D	N/D	–	–			
71	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	>25 6	1.5	8	N/D	2	D87 G	–			B5
71T	<i>E. coli</i> TOP10	<i>qnrB1</i> 9	64 3	0.02	N/D	N/D	N/D	–	–			
97	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	16	0.75	3	N/D	1.5	–	–			B4
99	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	>25 6	1.5	6	N/D	2	D87 G	–		SHV-28	B5
107	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	>25 6	1.5	4	N/D	1.5	D87 G	–	X		B2
109	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	>25 6	1	4	N/D	1.5	D87 G	–	X		B2

110	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	>25 6	0.75	3	N/D	1.5	D87 G	–	X	TEM-150	B1	
111	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	<i>aac(6')-Ib</i> 6	>25 6	1.5	4	N/D	2 D87 G	–	X	TEM-1 + SHV-12	B2	
111T	<i>E. coli</i> TOP10	<i>qnrB1</i> 9		128 3	0.02	N/D	N/D N/D	–	–				
112	<i>K. pneumoniae</i>	<i>qnrB1</i> 9		>25 6	1	4	N/D	1.5 D87 G	–	X		B1	
114	<i>E. coli</i>	<i>qnrS1</i>		16	0.5	2	N/D	0.75	–	–	X	TEM-150 + SHV-12 + CTX-M-1	S3
129	<i>K. pneumoniae</i>	<i>qnrB1</i> 9		>25 6	12	≥8	32	≥8 D87 G	–	X	TEM-150 + SHV-12	B1	
132	<i>E. coli</i>	<i>qnrB1</i> 9		16	0.32	1.5	0.38	0.5	–	–		B6	
132T	<i>E. coli</i> TOP10	<i>qnrB1</i> 9		16	0.16	N/D	0.23	N/D	–	–			



132C	<i>E. coli</i> J53 <sup>AzKanNa</sup> R	<i>qnrB1</i> 9		16	0.32	N/D	0.32	N/D	–	–			
137	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	<i>aac(6')</i> -lb	>25 6	1	4	1.5	1.5	D87 G	–	X	TEM-1	B2
138	<i>K. pneumoniae</i>	<i>qnrB1</i> 9		>25 6	1.5	6	1.5	2	D87 G	–	X	TEM-150 + SHV- 12	B1
143	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	<i>aac(6')</i> -lb	>25 6	1.5	4	1.5	1.5	D87 G	–		TEM-150 + SHV- 12	B2
144	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	<i>aac(6')</i> -lb	>25 6	1.5	6	1.5	1.5	D87 G	–	X	TEM-150	B2
146	<i>K. pneumoniae</i>	<i>qnrB1</i> 9		>25 6	1	4	1.5	2	D87 G	–	X	TEM-150 + SHV- 12	B1
146T	<i>E. coli</i> TOP10	<i>qnrB1</i> 9		128	0.02 3	N/D	N/D	N/D	–	–			
147	<i>K. pneumoniae</i>	<i>qnrB1</i> 9		>25 6	1	6	2	2	D87 G	–	X	TEM-1	B1

150	<i>K. pneumoniae</i>	<i>qnrB1</i>	>25 6	1	6	1.5	1.5	D87 G	-	X	TEM-1 + SHV-12	B1
151	<i>K. pneumoniae</i>	<i>qnrB1</i>	>25 6	1	6	1.5	2	D87 G	-	X	TEM-1 + SHV-12	B1
169	<i>K. pneumoniae</i>	<i>qnrB1</i>	>25 6	1	3	1.5	2	D87 G	-	X		B1
172	<i>K. pneumoniae</i>	<i>qnrB1</i>	>25 6	0.75	2	1	1.5	D87 G	-	X	TEM-1 + SHV-12	B1
176	<i>K. pneumoniae</i>	<i>qnrB1</i>	<i>aac(6')</i> <i>-lb</i>	>25 6	1	2	1.5	1.5	D87 G	-	TEM-150	B1
191	<i>E. coli</i>	<i>qnrS1</i>	64	0.50	1.5	0.75	1.5	-	-			S1
191T	<i>E. coli</i>	<i>qnrS1</i>	32	0.16	N/D	0.32	N/D	-	-			
	TOP10											
191C	<i>E. coli</i>	<i>qnrS1</i>	32	0.23	N/D	0.32	N/D	-	-			
	J53 <sup>AzKanNa</sup>											
	R											

196	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	>25 6	1	3	1.5	1.5	D87 G	-	X	TEM-1	B1
204	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	32	0.75	4	1.5	2	-	-		SHV-12	B4
229	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	32	0.5	2	1	1.5	-	-		TEM-1	B4
282	<i>E. coli</i>	<i>qnrS1</i>	16	0.5	1.5	0.75	0.5	-	-		TEM-1	S2
282T	<i>E. coli</i> TOP10	<i>qnrS1</i>	16	0.23	N/D	0.32	N/D	-	-			
290	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	>25 6	2	6	3	3	D87 G	-	X	TEM-150 + SHV-12	B3
307	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	>25 6	1	3	1.5	2	D87 G	-	X		B1
328	<i>K. pneumoniae</i>	<i>qnrB1</i> 9	>25 6	2	12	3	3	D87 G	-	X	TEM-150 + SHV-12	B1

370	<i>K. pneumoniae</i>	<i>qnrB1</i>	>25 6	1.5	6	2	3	D87 G	–	X	TEM-1 + SHV-12	B1
374	<i>K. pneumoniae</i>	<i>qnrB1</i>	>25 6	1.5	4	1.5	2	D87 G	–	X	SHV-12	B1
450	<i>K. pneumoniae</i>	<i>qnrB1</i>	>25 6	0.25	0.5	0.38	0.38	D87 G	–		SHV-28	B1
	<i>E. coli</i>		1	0.00	0.01	0.00	0.00	–	–			
	TOP10			2	6	4	6					
	J53 <sup>AzKanR</sup>		3	0.01	0.04	0.04	0.02	–	–			
				6	7	7	3					

MIC, minimum inhibitory concentration; NA, nalidixic acid; CIP, ciprofloxacin; OFX, ofloxacin; LVF, levofloxacin; MXF, moxifloxacin; ESBL, extended-spectrum  $\beta$ -lactamase; ERIC-PCR, 'enterobacterial repetitive intergenic consensus' polymerase chain reaction; T, transformant strain; C, transconjugant strain; N/D, not determined.

<sup>a</sup> ESBL phenotypic determination (X = ESBL-producer) and genotypic determination (ESBL type).

**Table 2**

Susceptibility (no. of isolates) of *qnr*-positive isolates to quinolone drugs according to Clinical and Laboratory Standards Institute (CLSI)<sup>a</sup> and European Committee on Antimicrobial Susceptibility Testing (EUCAST)<sup>b</sup> breakpoints

Susceptibility	NA		CIP		OFX		LVF		MXF	
	CL	EUCAS	CLS	EUCAS	CLS	EUCAS	CLS	EUCAS	CLS	EUCAS
	SI	T	I	T	I	T	I	T	I	T
R	31	33	3	15	5	37	3	5	3	33
I	2	0	12	16	24	2	2	17	3	1
S	7	7	25	9	11	1	23	6	34	6

NA, nalidixic acid; CIP, ciprofloxacin; OFX, ofloxacin; LVF, levofloxacin; MXF, moxifloxacin; R, resistant; I, intermediate; S, susceptible.

<sup>a</sup> Resistance ranges according to CLSI breakpoints (mg/L): nalidixic acid,  $S \leq 16$ ,  $R \geq 32$ ; ciprofloxacin,  $S \leq 1$ ,  $I = 2$ ,  $R \geq 4$ ; and ofloxacin, levofloxacin and moxifloxacin,  $S \leq 2$ ,  $I = 4$ ,  $R \geq 8$ .

<sup>b</sup> Resistance ranges according to EUCAST breakpoints (mg/L): nalidixic acid,  $S \leq 16$ ,  $R > 16$ ; ciprofloxacin, ofloxacin and moxifloxacin,  $S \leq 0.5$ ,  $R > 1$ ; and levofloxacin,  $S \leq 1$ ,  $R > 2$ .

**S S S B B B** *qnr*  
**282 114 191 132 M 111 146 370** sample  
ID #

