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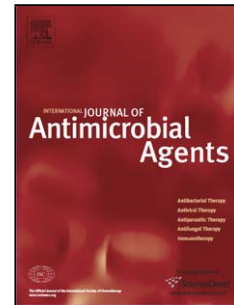
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**Prevalence of *qnr* determinants among extended-spectrum  $\beta$ -lactamase-positive Enterobacteriaceae clinical isolates in southern Stockholm, Sweden**

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**Abstract**

Three hundred and nineteen extended-spectrum  $\beta$ -lactamase-positive Enterobacteriaceae clinical isolates were screened for *qnr* genes. Twelve isolates were positive for *qnr*, including one *qnrA1*, two *qnrB1*, three *qnrB2*, one *qnrB4*, one *qnrB6* and four *qnrS1*. No *qnr*-positive strains were identified among the isolates recovered before 2006. The first *qnr*-positive *Escherichia coli* was detected from a patient in 2006. *qnr* genes remained rare in *E. coli* (6/288; 2.1%), but appeared to be more prevalent in *Klebsiella pneumoniae* (4/25; 16%) and *Enterobacter cloacae* (2/3; 66.7%). All *qnr*-positive isolates were resistant to nalidixic acid while presenting varied susceptibilities to fluoroquinolones. Isolates harbouring *qnrB4* or *qnrB6* were highly resistant to all the fluoroquinolones tested. Their high-level resistance is associated with multiple chromosomal substitutions in *gyrA* and *parC*. Alterations at codons Ser-83 and Asp-87 in GyrA and at codons Ser-80 and Glu-84 in ParC were observed in these isolates.

## 1. Introduction

Quinolone resistance typically arises as a result of chromosomal mutations in genes coding for DNA gyrase and topoisomerase IV as well as changes in drug entry and efflux [1]. Resistance to quinolones can also be mediated by plasmid-borne genes such as *qnr*, which protects the quinolone targets from inhibition [1,2]. Although *Qnr* by itself produces only low-level resistance, its presence facilitates the selection of higher-level resistance mutations, thus contributing to the alarming increase in resistance to quinolones [1].

*qnrA* was the first plasmid-mediated gene that conferred resistance to quinolones, initially reported in a *Klebsiella pneumoniae* isolate in the USA in 1994 [3].

Subsequently, plasmid-mediated quinolone resistance associated with *qnrA*, *qnrB* and *qnrS* has been reported among enterobacterial species in Asia, the USA, South America and several countries in Europe [2,4–6]. Recently, the *qnrC* gene was identified from a *Proteus mirabilis* clinical isolate from China [7]. Moreover, it has been frequently reported that the *qnr* genes have been detected among isolates producing extended-spectrum  $\beta$ -lactamases (ESBLs) [5,8–11].

The present study aimed to investigate the prevalence of *qnr* determinants in ESBL-positive Enterobacteriaceae clinical isolates in southern Stockholm, Sweden, as well as the relationship between quinolone resistance mechanisms and the degree of resistance.

## 2. Materials and methods

### 2.1. Bacterial isolates

The study included 319 non-duplicate ESBL-positive Enterobacteriaceae clinical isolates recovered from January 2001 to January 2008 in Karolinska University Hospital Huddinge, Sweden, which serves all healthcare centres and hospitals located in southern Stockholm. The isolates consisted of 288 *Escherichia coli*, 25 *K. pneumoniae*, 3 *P. mirabilis* and 3 *Enterobacter cloacae*.

### 2.2. Detection of extended-spectrum $\beta$ -lactamases

The presence of ESBLs was first screened for by cefotaxime, ceftazidime and cefpodoxime disks and was then confirmed by the double-disk method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [12] or by Etest (AB BIODISK, Solna, Sweden). All the isolates were also screened for *bla* genes (SHV, TEM, CTX-M or OXA) using a multiplex polymerase chain reaction (PCR) assay as described previously [13].

### 2.3. Identification of *qnr* genes

The PCR assay for *qnr* genes (*qnrA*, *qnrB* and *qnrS*) was performed according to Robicsek et al. [11]. Positive results for *qnr* were further confirmed by sequencing of PCR products.

#### 2.4. Analysis of quinolone resistance-determining regions (QRDRs)

Isolates positive for *qnr* genes were further analysed by QRDR sequencing. QRDRs of the *gyrA* and *parC* genes were amplified by PCR followed by sequencing with the same primers [14].

#### 2.5. Effect of the efflux pump inhibitor Phe-Arg- $\beta$ -naphthylamide (PAN)

Synergy experiments were performed using ciprofloxacin and the efflux pump inhibitor PAN (Sigma-Aldrich, St Louis MO) to investigate the contribution of efflux pump overexpression to ciprofloxacin resistance.

#### 2.6. Antimicrobial susceptibility testing

The *qnr*-positive isolates were subject to susceptibility testing for ciprofloxacin, levofloxacin, norfloxacin and moxifloxacin by Etest (AB BIODISK) and for nalidixic acid by the disk diffusion method.

### 3. Results

Of the 319 ESBL-positive clinical isolates investigated, 12 isolates were positive for *qnr* genes, including 1 *qnrA1*, 2 *qnrB1*, 3 *qnrB2*, 1 *qnrB4*, 1 *qnrB6* and 4 *qnrS1* isolates (Table 1). No *qnr*-positive strains were identified among the isolates detected before 2006. The first *qnr*-positive *E. coli* was recovered from the urine of a 64-year-old female patient in 2006. Of the 12 *qnr*-positive isolates detected, 6 were *E. coli* (6/288; 2.1%), 4 were *K. pneumoniae* (4/25; 16%) and 2 were *E. cloacae* (2/3; 66.7%).

Among the 12 *qnr*-harbouring isolates, *bla*<sub>TEM</sub> was present in 10 of the isolates and genes coding for CTX-M, SHV and OXA were carried by 6, 8 and 3 of the isolates, respectively (Table 1).

All *qnr*-positive isolates were resistant to nalidixic acid while presenting varied susceptibilities to fluoroquinolones. Isolates harbouring *qnrB4* or *qnrB6* were highly resistant to all the fluoroquinolones tested (Table 1).

The high-level resistant isolates possessed multiple mutations in the QRDRs of *gyrA* and *parC* resulting in alterations at codons Ser-83 and Asp-87 in GyrA and codons Ser-80 and Glu-84 in ParC (Table 1).

In the presence of the efflux inhibitor PAN, there was a four-fold decrease in the ciprofloxacin minimum inhibitory concentration (MIC) for strain *E. cloacae* 07194. However, the effect of the inhibitor was not apparent with other isolates tested.

Regarding strain *E. cloacae* 07194, in addition to the presence of the *qnr* gene, a single mutation in the GyrA protein and efflux pump overexpression were also observed. Nevertheless, these resistance mechanisms are not enough to confer a ciprofloxacin-resistant phenotype in this strain.

#### 4. Discussion

In the present study, seven *qnrB*, four *qnrS* and one *qnrA* isolates were identified among 319 ESBL-positive Enterobacteriaceae clinical isolates. Genetic diversity was



observed in the *qnrB* allele in which four variants, i.e. *qnrB1*, *qnrB2*, *qnrB4* and *qnrB6*, were present, whilst only *qnrS1* was identified in *qnrS*-carrying isolates. *qnrB2* was spread in various species, i.e. *E. coli*, *K. pneumoniae* and *E. cloacae*. The prevalence of *qnr* genes (3.8%) in ESBL-positive Enterobacteriaceae clinical isolates from this study is comparable with that of a Spanish study [5] where 4.9% of 305 enterobacterial clinical isolates carrying ESBLs were positive for *qnr*. However, our findings differ from those of the Spanish study where *qnrA1* was the most prevalent; only one *qnrS* but no *qnrB* were detected. Similar to our findings, mainly *qnrB* and *qnrS* were present in a Taiwanese study [9] where 2035 *E. coli* and 1147 *K. pneumoniae* isolates were investigated. Our results regarding *qnrS1* prevalence (1.3%) are similar to those of a report from France [15] which showed that 1.6% of 186 ESBL-producing isolates carried *qnrS1*.

Our study showed that the *qnr* gene was present in 16.0% of *K. pneumoniae* isolates, 2.1% of *E. coli* isolates and two (66.7%) of three *E. cloacae* isolates, which is in accordance with the findings from the USA by Robicsek et al. [11].

All 12 *qnr*-positive isolates were resistant to nalidixic acid. Regarding ciprofloxacin, four isolates were susceptible, five showed decreased susceptibility, one presented low-level resistance (MIC = 6 mg/L) and two exhibited high-level resistance (MIC > 32 mg/L). The high-level fluoroquinolone resistance exhibited by these strains is presumably due to the associated multiple mutations in the QRDR of *gyrA* and *parC*. These data agree with previous reports demonstrating that *qnr* determinants confer resistance to quinolones, e.g. nalidixic acid, and reduced susceptibility to

fluoroquinolones, and that the presence of *qnr* does not necessarily lead to MICs above CLSI breakpoints for resistance to ciprofloxacin [3,8].

In conclusion, the first *qnr*-positive *E. coli* in southern Stockholm emerged in 2006, and *qnr* genes remained rare in *E. coli* but appeared to be more prevalent in *K. pneumoniae* and *E. cloacae*. Varied *qnrB* alleles and uniform *qnrS*, i.e. *qnrS1*, were identified among the strains investigated. High-level fluoroquinolone resistance is associated with multiple chromosomal substitutions in *gyrA* and *parC*.

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**Competing interests:** None declared.

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Table 1

Characteristics of *qnr*-positive clinical isolates of Enterobacteriaceae species detected in southern Stockholm, Sweden

Strain	Species	Source	<i>qnr</i> gene	$\beta$ -Lactamase gene	MIC (mg/L)				QRDR mutation(s)	
					CIP	MXF	NOR	LVX	GyrA	ParC
06011	<i>Escherichia coli</i>	Urine	B2	SHV, TEM	0.38	1	1	0.5	–	–
07082	<i>Klebsiella pneumoniae</i>	Urine	S1	SHV, TEM	1.5	2	4	1.5	–	–
07111	<i>E. coli</i>	Urine	S1	TEM, CTX-M	1	1.5	4	1	–	–
07139	<i>K. pneumoniae</i>	Wound	B2	SHV, TEM	1.5	2	4	2	S83T	–
07151	<i>E. coli</i>	Faeces	B1	TEM, CTX-M, OXA	2.5	1.5	4	0.75	–	–
07156	<i>E. coli</i>	Perineum	B6	TEM, CTX-M, OXA	>32	>32	>256	>32	S83L, D87N	S80I
07159	<i>K. pneumoniae</i>	Urine	B1	SHV, TEM, CTX-M, OXA	6	2	12	1.5	–	–
07169	<i>E. coli</i>	Bronchi	S1	TEM, CTX-M	0.38	1.5	2	0.75	–	–
07194	<i>Enterobacter cloacae</i>	Wound	B2	SHV, TEM	2	2	4	2	S83Y	–
07216	<i>E. coli</i>	Urine	B4	SHV, TEM, CTX-M	>32	>32	>256	>32	S83L, D87N	S80I, E84G
07228	<i>K. pneumoniae</i>	Faeces	S1	SHV	2	2	6	2	–	–
08019	<i>E. cloacae</i>	Abscess	A1	SHV	0.38	1	1	0.75	–	–

MIC, minimum inhibitory concentration; CIP, ciprofloxacin; MXF, moxifloxacin; NOR, norfloxacin; LVX, levofloxacin; QRDR,

quinolone resistance-determining region; –, no alteration.