The Emergence of Qnr-Mediated Quinolone Resistance among Enterobacteriaceae in Jamaica

S Stephenson¹, PD Brown¹, A Holness², M Wilks³

ABSTRACT

Objective: Quinolone resistance is usually caused by various chromosomal mutations, but has been more recently associated with plasmids which carry the qnr determinant. The aim of this study is to investigate the prevalence of qnr genes in clinical isolates of Enterobacteriaceae in Jamaica.

Methods: A total of 255 non-duplicate fluoroquinolone-resistant Enterobacteriaceae clinical isolates, comprising 232 Escherichia coli, 20 Klebsiella species and three Enterobacter spp were collected between October 2007 and November 2008 from hospitalized patients in Jamaica. The presence of the qnr gene was screened by PCR using specific primers for qnrA, qnrB and qnrS in extracted plasmid DNA.

Results: Eighty-three (32.5%) of these isolates were qnr-positive, of which 47.0% housed the qnrA gene only, 1.2% qnrB and 9.6% qnrS only. Another 36.1% possessed both qnrA and qnrS genes. Approximately 30% of the quinolone-resistant E coli isolates harboured the qnr gene while 50% Klebsiella spp and all Enterobacter spp were positive.

Conclusion: The emergence of qnr-mediated quinolone resistance among clinical Enterobacteriaceae isolates is described for the first time in Jamaica.

Keywords: Enterobacteriaceae, E coli, fluoroquinolone, Klebsiella, plasmid-mediated

Surgimiento de la Resistencia a las Quinolonas Mediada por Qnr entre las Enterobacteriaceae en Jamaica

S Stephenson¹, PD Brown¹, A Holness², M Wilks³

RESUMEN

Objetivo: La resistencia a la quinolona es generalmente causada por varias mutaciones cromosómicas, pero más recientemente ha sido asociada con plásmidos portadores del determinante qnr. El objetivo de este estudio fue investigar la prevalencia de genes qnr en los aislados clínicos de Enterobacteriaceae en Jamaica.

Métodos: Un total de 255 aislados clínicos no duplicados de Enterobacteriaceae resistentes a la fluoroquinolona, incluyendo 232 de Escherichia coli, 20 especies de Klebsiella y tres Enterobacter spp, fueron recogidos entre octubre de 2007 y noviembre de 2008, de pacientes hospitalizados en Jamaica. La presencia del gen qnr fue tamizada mediante marcadores PCR, usando primers específicos para qnrA, qnrB y qnrS en el ADN plasmídico extraído.

Resultados: Ochenta y tres (32.5%) de éstos aislados fueron qnr-positivos. De ellos, 47.0% alojaban solamente el gen qnrA, 1.2% el qnrB y 9.6% el qnrS solamente. Otro 36.1% poseía tanto genes qnrA cuanto genes qnrS. Aproximadamente 30% de los aislados E. coli resistentes a la quinolona, albergaban el gen qnr mientras que 50% de Klebsiella spp y todas las Enterobacter spp fueron positivas.

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**INTRODUCTION**

The *Enterobacteriaceae* group accounts for more than 50% of all nosocomial infections in the United States of America (USA) and has been associated with bacteraemia, gastrointestinal infections, urinary tract infections, respiratory infections, meningitis (in neonates or adults), sepsis, liver abscess and wound infections (1, 2). In fact, some hospital and medical laboratories in Jamaica have reported an increase in quinolone resistance among members of the *Enterobacteriaceae*. Quinolone resistance in *Enterobacteriaceae* results mainly from mutations in type II DNA topoisomerase genes (3) and/or changes in the expression of outer membrane and efflux pumps (4). Recent studies have shown that plasmid-mediated resistance mechanisms also play a significant role in fluoroquinolone resistance and its prevalence is increasing worldwide (5). The *qnr* gene is the plasmid-mediated quinolone resistance gene encoding a 218 amino acid protein of the pentapeptide family that protects DNA gyrase from quinolone inhibition (6). The new plasmid-mediated quinolone resistance genes, *qnrB* and *qnrS*, have been reported in clinical isolates (7, 8). The objective of this study was to screen for the presence of the *qnr* gene in clinical ciprofloxacin-or norfloxacin-resistant isolates of *Enterobacteriaceae* from hospitalized patients in Jamaica.

**MATERIALS AND METHODS**

**Bacterial Isolates**

A total of 255 non-duplicate fluoroquinolone-resistant *Enterobacteriaceae* isolates (232 *Escherichia coli*, 20 *Klebsiella* species and three *Enterobacter* species) were analysed in this study. The isolates were collected from inpatients at hospitals in the eastern half of Jamaica including Kingston metropolis and surrounding parishes of St Catherine, St Ann, St Mary, Clarendon, Portland and the southern parish of Manchester, between October 2007 and November 2008. These isolates were screened for ciprofloxacin or norfloxacin resistance by the disk diffusion method according to the criteria of NCCLS (9). Isolates were conventionally identified by colony growth on MacConkey agar, Simmon’s citrate agar, Urea agar, Triple-iron agar, and Motility-indole-lysine media incubated at 37°C and compared to the standard *E coli* strain, ATCC 25922. Isolates were routinely cultured on Luria-Bertani agar (LB; Sigma-Aldrich Inc, MO, USA) and stored on Tryptic Soy agar (EMD Chemicals, USA) slants or in LB broth with 80% (v/v) molecular-grade glycerol (Promega Corp., Madison, WI, USA) at -80°C.

**RESULTS**

A total of 83 (32.5 %) fluoroquinolone-resistant clinical isolates, including 70 *E coli* and 10 *Klebsiella spp* (included *K pneumoniae* and *K oxytoca*) were positive for the *qnr* gene (Table). Approximately 30% of the *E coli* isolates harboured plasmid-extraction and *qnr* amplification

The isolates were screened by single- or multiplex PCR amplification of *qnrA*, *qnrB* and *qnrS*, using the Promega GoTaq® Green Master Mix (Promega, Madison, WI, USA) as previously described (10, 11). Plasmid DNA was extracted from isolates previously grown in 4 ml LB broth at 37°C with constant shaking for 18 hours. The primers used for *qnrA*, *qnrB*, and *qnrS* were as follows: 5’-ATTTCCTCACGCC AGGATTTG-3’ and 5’-GATCGGCAAAGGTAGGTC-3’ for *qnrA* yielding a 516-bp product, 5’-GATCGTGAAAGGCC AGAAAGG-3’ and 5’-ACGATGTCCTGTAAGTTGTC-3’ for *qnrB* yielding a 469-bp product, and 5’-ACGACATT CGTCAACTGCAA-3’ and 5’-TAAATTGACCCCTGTA GGC-3’ for *qnrS* yielding a 417-bp product. Amplification was carried out in a GeneAmp 9700 Thermal Cycler (Applied Biosystems, USA) using the following protocol: 94°C for 4 min; 32 cycles at 94°C for 45 sec, 53°C for 45 sec, and 72°C for 60 sec and a final extension at 72°C for 5 min. *Qnr*-positive control strains *E coli* Lo *QnrA*+, *E coli* J53 pMG252 (also *QnrA1*), *K pneumoniae* B1 *QnrB1*+ and *E coli* 57 *QnrS*+ (kind gifts of P Nordmann and G Jacoby) were used as positive controls and tubes without DNA template served as negative controls in each run. PCR products were separated by electrophoresis in a 1% agarose gel for one hour at 100 V, stained with ethidium bromide and detected by UV transillumination. Amplified genes were identified on the basis of fragment size compared to the positive controls and Kb marker.

**Table:** Prevalence of *qnr* genes in *Enterobacteriaceae* isolates from Jamaica

<table>
<thead>
<tr>
<th><em>qnr</em> locus</th>
<th>No of isolates with locus/total no of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E coli</em></td>
<td><em>Klebsiella spp</em></td>
</tr>
<tr>
<td>A</td>
<td>33/232 (14.2)</td>
</tr>
<tr>
<td>B</td>
<td>1/232 (0.4)</td>
</tr>
<tr>
<td>S</td>
<td>8/232 (3.4)</td>
</tr>
<tr>
<td>A and B</td>
<td>2/232 (0.8)</td>
</tr>
<tr>
<td>A and S</td>
<td>1/232 (0.4)</td>
</tr>
<tr>
<td>A, B and S</td>
<td>2/232 (0.8)</td>
</tr>
<tr>
<td>Total</td>
<td>70 (30.2)</td>
</tr>
</tbody>
</table>

**Conclusión:** Se describe por primera vez el surgimiento de la resistencia a las quinolonas mediada por *qnr* en Jamaica.

**Palabras claves:** Enterobacteriaceae, *E coli*, fluoroquinolona, *Klebsiella*, mediada por plásmidos
the qnr gene while all three Enterobacter spp isolates (included *E. cloacae* and *gergoviae*) were positive.

As shown in Fig. 1, the sole *qnrA*, *qnrB* and *qnrS* gene locus was identified in 48%, 1% and 10% of the isolates, respectively. Another 36% of the isolates were positive for *qnrA* and *qnrS*, and two isolates (both *E. coli*) had all three determinants. A typical gel of multiplex PCR results is shown in Fig. 2.

**DISCUSSION**

This study is the first report of detection of *qnr*-mediated fluoroquinolone resistance in clinical *Enterobacteriaceae* isolates in Jamaica and the English-speaking Caribbean. A considerably high frequency of *qnr* detection (32.5%) was observed in this study, with *E. coli* isolates accounting for the majority of this. This is similar to the prevalence reported in the UK (12) but is much higher than reports from the USA (10, 11, 13), Australia (15), China (16) and continental Europe (17–21). Frequency rates of 48% in Thailand (22) and 86% in Vietnam (23) have been reported. Because the numbers of *Klebsiella* and *Enterobacter* spp were so small, no comparisons can be made with other studies. However, any comparison should be taken with caution since different criteria for selecting the bacterial isolates were used in these studies. For instance, resistance to tobramycin was used as a selection criterion in the Spanish study which probably under-estimated the real prevalence (24). In the present study, the criterion used for selecting these isolates was resistance or reduced susceptibility to ciprofloxacin or norfloxacin based on NCCLS criteria. However, it is noteworthy that several *qnr*-positive isolates have increasingly been detected in nalidixic acid-susceptible isolates (17, 18, 25) and so the true prevalence of *qnr* determinants could be underestimated in this study. Such isolates could be an additional reservoir for undetected spread of plasmid-mediated quinolone resistance (3).

*qnrA* (as the sole determinant) was detected in a majority of the isolates in this study, compared to *qnrB* or *qnrS*. The dominance of the *qnrA* gene in this collection is similar to studies from the UK (12) and Spain (24) but contrasts with other studies from Europe (26, 21). The fact that about 90% of clinical diagnoses involved urinary tract infections is not surprising as urinary tract infections are one of the most frequent infections affecting people (150 million per annum), particularly women, with *E. coli* being the most frequent culprit (27, 28). As has been noted in previous studies (5, 14), we found on some occasions more than one *qnr* gene in the same organism.

Because *qnr*-mediated quinolone resistance is usually associated with integrons and multidrug resistance (5, 29), this combination has the additional effect of genetically linking low-level quinolone resistance with other antibiotic resistances and thus may promote co-selection upon exposure to other antimicrobials with resistance also encoded on the integron. The emergence of plasmid-determined quinolone resistance thus may contribute by several means to the rapid increase in bacterial resistance to quinolones, and may explain why an increase has been noted among *Enterobacteriaceae* in Jamaica. Consequently, and based on the results of this study, fluoroquinolone usage and prescription should be restricted among community and hospitalized patients, mainly among populations with an increased risk of developing this resistance phenotype. Further, additional surveillance studies are necessary to understand the dis-
semination of quinolone resistance in Jamaica, to help implement appropriate control measures, as well as to guide the adequate use of antimicrobial agents in the local hospitals. We are in the process of assessing the genetic structure of the \( qnr \) loci to understand the environment in which these genes exist in these diverse strains.

**CONCLUSIONS**

This study showed that the multiplex PCR technique is a fast and reliable tool for rapid screening of \( qnr \)-positive strains and can be used in an epidemiological setting to identify all so-far known \( qnr \) genes. This is the first report of the emergence and identification of \( qnr \)-mediated quinolone resistance and \( qnr \) co-determinants among Enterobacteriaceae isolates in Jamaica. However, because fluoroquinolones are second line antibiotics, it is of concern that such a high prevalence was noted in these isolates.

**ACKNOWLEDGEMENTS**

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