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

S Stephenson[1], PD Brown[1], A Holness[2], M Wilks[3]

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

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RESUMEN

Objetivo: *La resistencia a la quinolona es generalmente causada por varias mutaciones cromosomáticas, 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 plásmido 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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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

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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 *qnrA* 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.

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'-ATTTCTCACGCC AGGATTTG-3' and 5'-GATCGGCAAAGGTTAGGTCA-3' for *qnrA* yielding a 516-bp product, 5'-GATCGTGAAAGCC AGAAAGG-3' and 5'-ACGATGCCTGGTAGTTGTCC-3' for *qnrB* yielding a 469-bp product, and 5'-ACGACATT CGTCAACTGCAA-3' and 5'-TAAATTGGCACCCTGTA GGC-3' for *qnrB* 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 *QnrA*1+), *K pneumoniae* B1 *QnrB*1+ 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.

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

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

***qnr* locus**	**No of isolates with locus/total no of isolates (%)**		
	E coli	***Klebsiella* spp**	***Enterobacter* spp**
A	33/232 (14.2)	4/20 (20)	1/3 (33.3)
B	1/232 (0.4)	0/20	0/3
S	8/232 (3.4)	0/20	0/3
A and B	2/232 (0.8)	0/20	0/3
A and S	23/232 (9.9)	6/20 (30)	2/3 (66.7)
B and S	1/232 (0.4)	0/20	0/3
A, B and S	2/232 (0.8)	0/20	0/3
Total	70 (30.2)	10 (50)	3 (100)

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,

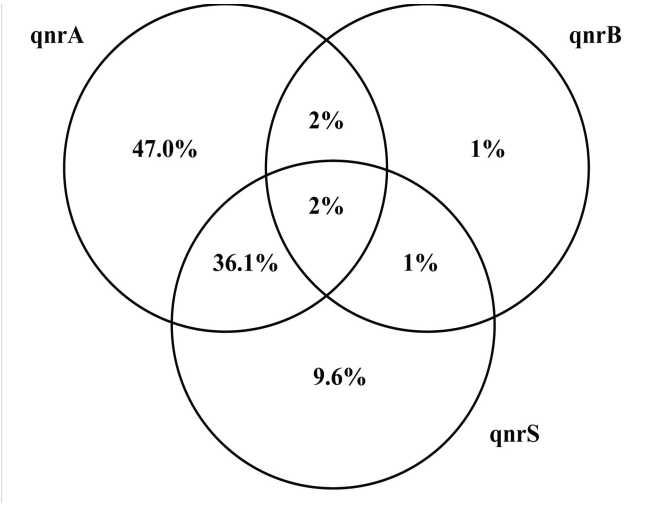

Fig. 1: Diagram illustrating the proportion of the various *qnr* genes and co-determinants found in *Enterobacteriaceae* from Jamaica.

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.

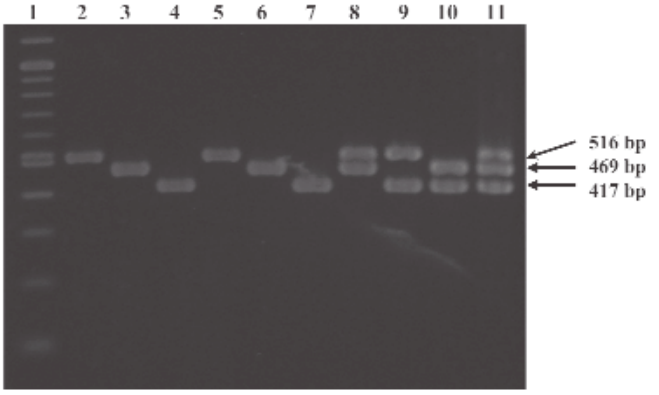

Fig. 2: Agarose gel electrophoresis (1%) used for the separation of multiplex PCR products. Lane 1, 100 bp molecular weight marker; Lane 2, *E coli* Lo *qnrA*+ve control; Lane 3, *K pneumoniae QnrB*+ve control; Lane 4, *E coli QnrS*+ve control; Lane 5, clinical isolate A345; Lane 6, clinical isolate A620b; Lane 7, clinical isolate A436; Lane 8, clinical isolate D032; Lane 9, clinical isolate A132; Lane 10, clinical isolate A496; Lane 11, clinical isolate U839.

Qnr genes were found in isolates from all age groups (neonates to the elderly) and from various specimens and were not associated with a particular clinical diagnosis. The *qnrB* gene was detected in an *E coli* isolate that was obtained from a 67-year old male from Mandeville, Manchester, who was admitted with presumed urosepsis. He was started on 1 g ceftriaxone intravenously, once daily. His fever resolved and the remainder of his stay in hospital was uneventful.

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), Canada (14), 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.

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