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Prevalence of Quinolone Resistance in *Enterobacteriaceae* from Sierra Leone and the Detection of *qnrB* Pseudogenes and Modified LexA Binding Sites

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A collection of 74 *Enterobacteriaceae* isolates found in Bo, Sierra Leone, were tested for quinolone antibiotic susceptibility and resistance mechanisms. The majority of isolates (62%) were resistant to quinolones, and 61% harbored chromosomal *gyrA* and/or *parC* mutations. Plasmid-mediated quinolone resistance genes were ubiquitous, with *qnrB* and *aac(6′)-Ib-cr* being the most prevalent. Mutated LexA binding sites were found in all *qnrB1* genes, and truncated *qnrB* pseudogenes were found in the majority of *Citrobacter* isolates.

Quinolone and fluoroquinolone compounds are synthetic antibiotics whose antimicrobial activity occurs via concentration-dependent inhibition of type II topoisomerases (1, 2). Fluoroquinolones are currently used to treat a variety of human infections caused by both Gram-positive and Gram-negative bacteria due to their broad-spectrum antimicrobial activity (3–5). Their widespread and often indiscriminate use, however, has resulted in ubiquitous resistance, especially among members of the *Enterobacteriaceae* (3, 4, 6).

The most common causes for high-level quinolone resistance in Gram-negative bacteria are mutations in quinolone resistance determinant regions (QRDRs) of the type II topoisomerase genes *gyrA* and *parC* (5, 7). Another mechanism for quinolone resistance depends on the maintenance of low intracellular drug concentrations via decreased uptake of the drug and/or active efflux using efflux pumps. When upregulated, some of these multidrug efflux pumps confer cross-resistance to multiple classes of antimicrobials (4, 5).

Plasmid-mediated quinolone resistance (PMQR) is conferred by extrinsic resistance determinants that encode efflux pumps (*qepA* and *oqxAB*) (8), proteins that protect DNA gyrase and topoisomerase IV through specific binding (*qnr* genes), or quinolone-inactivating enzymes [*aac(6′)-Ib-cr*] (9). PMQR genes generally confer low-level resistance, with their MICs falling below Clinical and Laboratory Standards Institute (CLSI) breakpoints for intermediate resistance; therefore, their contribution to quinolone resistance can be masked in strains also harboring QRDR mutations in *gyrA* and *parC*. However, their clinical significance stems from the fact that they greatly facilitate the selection of more highly quinolone-resistant strains (10).

An increasing prevalence of quinolone resistance has been reported in West Africa (11–13), with several mechanistic surveys of quinolone nonsusceptibility within this region recently published (13–19). However, no such data are available for Sierra Leone (11). Here, we analyzed 70 *Enterobacteriaceae* urine sample isolates and four fomite isolates from the indoor environment from a small private hospital located in Bo, Sierra Leone (20). The collection included *Citrobacter freundii* ($n = 22$), *Enterobacter cloacae* ($n = 16$), *Klebsiella pneumoniae* ($n = 17$), *Escherichia coli* ($n = 13$), *Enterobacter* sp./*Leclercia* sp. ($n = 4$), *Escherichia hermannii* ($n = 1$), and *Pantoea dispersa* ($n = 1$). Each isolate in the collection was tested for susceptibility to nalidixic acid, ciprofloxacin, and moxifloxacin using Etest strips (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's recommendations using CLSI interpretative criteria (for nalidixic acid and ciprofloxacin) (21) and FDA recommendations (for moxifloxacin) (22) for phenotype classification.

We sequenced the QRDRs of the chromosomal *gyrA* and *parC* genes to identify potential resistance-conferring mutations and screened the isolates by PCR for the following PMQR genes: *aac(6′)-Ib-cr*, *qepA*, *oqxAB*, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, and *qnrVC* (see Table S1 in the supplemental material). Detected *qnr* genes were fully sequenced.

Overall, 62% of the tested isolates were clinically resistant to at least one quinolone antibiotic, while 41% were resistant to all three. The prevalence of resistance was highest in *C. freundii* and *Enterobacter* sp./*Leclercia* sp. and lowest (or absent) in *E. cloacae*, *E. hermannii*, and *P. dispersa* (Table 1). Resistance correlated strongly with the presence of *gyrA* and *parC* gene QRDR mutations (5, 7). While a single *gyrA* mutation was correlated with high-level resistance to nalidixic acid and elevated MICs (not

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TABLE 1 Quinolone resistance, PMQR genes, and QRDR mutation prevalence

Species	No. of isolates	Quinolone resistance prevalence (%)[a]			PMQR gene(s) detected and its prevalence (%)[b]	QRDR mutation prevalence (%)[c]			
						GyrA		ParC	
		NA	CIP	MOX		Ser83	Asp87	Ser80	Glu84
Citrobacter freundii	22	100	100	95	*aac(6′)-Ib-cr* (86), *qnrA1* (4), *qnrB1*a (10), *qnrB1*d (23), *qnrB6* (36), *qnrB12* (14), *qnrB81* (4), *qnrB82* (4), Δ*qnrB* (86), *qnrS1* (50)	100	14	59	0
Enterobacter sp./*Leclercia* sp.	4	100	100	25	*aac(6′)-Ib-cr* (100), *qnrB1*a (100)	100	0	100	0
Escherichia coli	13	61	23	23	*qepA1* (23)	61	23	31	8
Klebsiella pneumoniae	17	53	35	29	*aac(6′)-Ib-cr* (29), *qnrB1*d (23), *oqxAB* (100)	53	12	53	0
Enterobacter cloacae	16	6	0	19	*aac(6′)-Ib-cr* (81), *qnrB1*a (25), *qnrB1*i (50), *qnrB6*a (6), *qnrS1* (19), *oqxAB* (6)	12	0	6	0
Escherichia hermannii	1	0	0	0	*aac(6′)-Ib-cr*, *qnrB1*a, *qnrS1*	0	0	0	0
Pantoea dispersa	1	0	0	0	None detected	ND	ND	ND	ND

[a] Prevalence of clinically resistant isolates as measured using Etest assays. NA, nalidixic acid; CIP, ciprofloxacin; MOX, moxifloxacin.
[b] The presence of the following PMQR genes was tested: *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qnrVC*, *aac(6′)-Ib-cr*, *qepA*, and *oqxAB*. Numbers in parentheses indicate the prevalence (percent) of a particular PMQR gene among tested isolates (when more than one isolate was present).
[c] Presence of mutations was deduced via *in silico* translation of the obtained DNA sequences. The position numbers are based on *E. coli* GyrA and ParC protein sequences. ND, not done.

crossing the clinical resistance breakpoint) for ciprofloxacin and moxifloxacin ($P < 0.05$), additional mutations were correlated with high-level resistance to all three quinolones ($P < 0.00005$; see Table S2 in the supplemental material).

PMQR genes were ubiquitous among the analyzed *Enterobacteriaceae* (Table 1; for detailed information, see Table S2 in the supplemental material), consistent with previous reports of *Enterobacteriaceae* in West Africa (13–19). Observed in all species tested except *E. coli* and *P. dispersa*, various *qnr* genes and *aac(6′)-Ib-cr* were detected in 68% and 58% of the isolates, respectively. Other detected PMQR genes included *oqxAB* (found in all *K. pneumoniae* strains and one *E. cloacae* strain) and *qepA* (in three *E. coli* isolates). As indicated above, the phenotypic contributions of the PMQR genes were difficult to determine due to the presence of *gyrA* and *parC* QRDR mutations.

Among the PMQR genes, the *qnr* genes showed the highest diversity and widest distribution. Detected alleles belonged to the *qnrA*, *qnrB*, and *qnrS* families. *qnrA* and *qnrS* families were represented by only one allelic variant each (*qnrA1* and *qnrS1*, respectively). In contrast, the *qnrB* family was represented by three previously described full-length alleles (*qnrB1*, *qnrB6*, and *qnrB12*), two novel variants (*qnrB81* and *qnrB82*), and three variants of 5′ truncated *qnrB* pseudogenes (Δ*qnrB*). All of the *qnrB* gene variants (with the notable exception of *qnrB1*) and pseudogenes were found only in *C. freundii* isolates. The majority of the >80 *qnrB* gene variants described thus far have been found in this species, which is also postulated as the original source of the *qnrB* gene (23).

The complete DNA sequencing of all detected *qnrB1* genes showed that the coding regions were identical but revealed differences in the noncoding sequences upstream of the *qnrB1* open reading frame (ORF) within the LexA protein binding site. LexA is a transcriptional repressor that modulates the SOS regulon (24), and the expression of *qnrB* genes containing LexA binding sequences is induced as a part of the SOS response to a number of environmental stimuli, including quinolone exposure (25, 26). No analogous LexA binding sites are found within other *qnr* gene families.

Based on differences within the LexA binding motifs, three *qnrB1* subvariants could be distinguished: *qnrB1*a is identical to the prototype *qnrB1* sequence (accession number DQ351241) except for a single T→G substitution at position −23, *qnrB1*d harbors a single nucleotide deletion, and *qnrB1*i contains a single nucleotide insertion within the poly(A) sequence located between positions −12 and −18 of the prototype *qnrB1* sequence (Fig. 1). Of the remaining *qnrB* alleles identified in this study, only *qnrB6* in

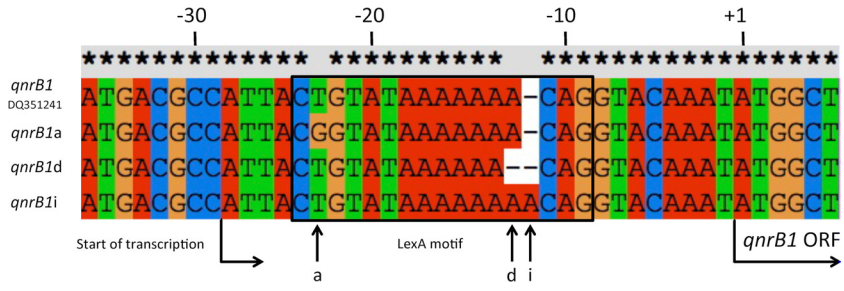

FIG 1 Mutations within the LexA binding site directly upstream of the *qnrB1* gene. The sequences flanking the 5′ end of the *qnrB1* ORF from the three detected *qnrB1* subvariants (*qnrB1*a, *qnrB1*d, and *qnrB1*i) were aligned with the analogous region from the prototype *qnrB1* gene (GenBank accession number DQ351241). The nucleotide position numbers are based on the prototype *qnrB1* gene sequence and denote the distance from the first nucleotide of the translation initiation codon (labeled +1). The sequences enclosed in the black box indicate the LexA binding motif. The vertical arrows indicate the point mutation sites characteristic of the particular *qnrB1* gene variants (a, *qnrB1*a [*qnrB6*a]; d, *qnrB1*d; i, *qnrB1*i).

E. cloacae harbored mutations within this upstream region; as this mutation was identical to that found in *qnrB1*a, this allele was thus designated *qnrB6*a. The mutation in *qnrB1*a (and *qnrB6*a) changes the highly conserved 5′ CTGT of the LexA binding palindrome consensus CTGT-N_8-ACAG characteristic of *Gammaproteobacteria* (24), while the LexA binding site mutations in *qnrB1*i and *qnrB1*d affect the length of this motif. This is the first report of mutated LexA binding sites identified in *qnrB* genes from clinical isolates. Although we did not explore the effect of these mutations on LexA binding or phenotype, it has been previously shown that the loss of LexA binding results in increased quinolone MICs (25–27). The *qnrB1* subvariants were unevenly distributed among the analyzed species (see Table S2 in the supplemental material).

In addition to the full-length alleles, three truncated *qnrB* pseudogenes (Δ*qnrB*) were also identified in the majority (91%) of the *C. freundii* isolates, and most of the *C. freundii* isolates contained these pseudogenes together with full-length *qnrB* genes. To our knowledge, this is the first report in which both full-length *qnrB* genes and truncated *qnrB* pseudogenes were found to coexist in the same isolates. While the presence of truncated *qnrB* pseudogenes in *C. freundii* has been previously reported, it has never been observed in such a high prevalence as documented in this study (23, 28–30). The three distinct *qnrB* pseudogene variants (Δ^1*qnrB*, Δ^2*qnrB*, and Δ^3*qnrB* [see Table S2 in the supplemental material]) all encompassed a 283-bp remnant of the ORF that resulted from the deletion of the 360 bp at the 5′ end of the gene.

Flanking region sequences indicated that all of the *qnrB* pseudogenes and the newly described *qnrB* variants (*qnrB81* and *qnrB82*) were located within the context of genetic platform GP3 while all other *qnrB* alleles had flanking sequences consistent with the GP2 environment (30). In addition, the finished genome sequencing of the *C. freundii* isolate SL151 (T. A. Leski, C. R. Taitt, U. Bangura, R. Ansumana, D. A. Stenger, Z. Wang, and G. Vora, submitted for publication; accession numbers CP016952 [genome], CP017058 [larger plasmid], and CP017059 [smaller plasmid]) revealed that the pseudogene variant Δ^2*qnrB* was located on the chromosome while the other two *qnr* genes (*qnrB6* and *qnrS1*) as well as *aac(6′)-Ib-cr* were located on two distinct plasmids. The wide distribution of the *qnrB1* allele in five of the seven analyzed species (Table 1) suggests that it is likely located on a transferable plasmid. Overall, the variety of *qnrB* genes found in the tested *C. freundii* isolates, the detection of novel variants, and the presence of truncated pseudogenes are consistent with the postulated origin of the *qnrB* gene family as mobilized chromosomal genes from the *C. freundii* complex (23, 29).

In summary, the high prevalence and diversity of the PMQR genes make the multidrug-resistant *Enterobacteriaceae* strains circulating in the population served by Mercy Hospital a potent reservoir of quinolone resistance genes that threaten the continued usefulness of this class of antibiotics in this community.

Accession number(s). Novel gene sequences obtained in this study were deposited in GenBank under the following accession numbers: new *qnrB* variants, *qnrB81* (SL157) and *qnrB82* (SL156), KX372671 and KX372672, respectively; *qnrB1* subvariants, *qnrB1*a (SL166), *qnrB1*i (SL174), and *qnrB1*d (SL185), KX372673 to KX372675; and truncated *qnrB* pseudogenes, Δ^1*qnrB* (SL129), Δ^2*qnrB* (SL151), and Δ^3*qnrB* (SL157), KX372668 to KX372670.

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