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Detection of QnrB54 and Its Novel Genetic Context in *Citrobacter freundii* Isolated from a Clinical Case

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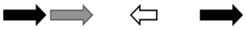
Quinolone resistance in *Enterobacteriaceae* is mediated by mutations in the quinolone resistance-determining regions (QRDR) of topoisomerase genes and/or by plasmid-mediated quinolone resistance determinants (PMQR) such as the *qnr* genes encoding pentapeptide repeat proteins (1). The *qnrB* family is represented by 80 different alleles (http://www.lahey.org/qnrStudies/); most of them originated from *Citrobacter* strains and spread to other *Enterobacteriaceae* species (2). This work describes the identification of a new allele of the quinolone resistance protein QnrB, QnrB54, in a human clinical isolate of *Citrobacter freundii* detected in Spain.

Isolate HLR20 was detected in October 2007, in a fecal sample of a 53-year-old man treated in the Hospital of Llerena. Identification of the isolate as *C. freundii* was performed by API 20E testing (bioMérieux), by sequencing its 16S rRNA gene (HG974539) and *dnaJ* gene (3) (LN624598) and by using a Vitek matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) system (bioMérieux). The bacterium showed resistance (4) to ciprofloxacin (CIP; MIC, 4 μg/ml) and nalidixic acid (NAL; MIC, >512 μg/ml), and, accordingly, it presented mutations T83I and S80I in the QRDR of GyrA (5) and ParC (6), respectively. In addition, the screening for PMQR determinants (7–12) detected a *qnrB* gene in a region that was amplified by PCR with primers FWD (5′-CGCGCGGACCTGCTGGATCGTCT-3′) and REV (5′-TTGCGGGTTGAACGTATTGACCT-3′) (AB734053), producing a 2,409-bp DNA fragment (HE820727). The sequence of *qnrB* corresponded to a new allele of QnrB, QnrB54, spanning variants G7S-S79A-I142M-A144T-V212I (http://www.lahey.org/qnrStudies/). The sequence downstream of *qnrB* (Table 1) encodes a protein with the CaMKIIAD signature (cl17504), a linkage that has been observed in *Klebsiella* plasmids (13) and *Citrobacter* chromosomes (2, 14). The *qnrB54* gene was shown to encode a functional protein by its amplification with primers FWD (5′-CATCAGCTTCGCGCTTTG-3′) and REV (5′-CCCGCTACACATTCACTTATGC-3′) (HE820727) and cloning in pGEM-Teasy vector (Promega) and *Escherichia coli* J53 cells; the transformants showed MICs for CIP and NAL of 0.25 μg/ml and 16 μg/ml, respectively. The conjugation potential of *qnrB* was attempted by mating performed between isolate HLR20 and *E. coli* J53, but quinolone resistance was not mobilized. In addition, pulsed-field gel electrophoresis (PFGE) (14) of the HLR20 DNA digested by the enzyme I-CeuI and hybridized to a digoxigenin (DIG)-labeled *qnrB* probe (Roche) produced a signal, among all the bands detected by a 23S rRNA gene probe, matching an I-ceuI band of 450 to 500 kb, a size much larger than that of any known plasmid of *Citrobacter*.

TABLE 1 Genetic context of *qnrB* sequences found between *pspF* and *sapA* genes in *Citobacter* sp.

Context	GenBank accession no.[a]	SL[b]	Allele[c]
pspF[e]-*qnrB*[f]-*sapA*[g]	JN173060	C	QnrB38
	JN173057	C	QnrB35
	AB734053	C	QnrB61
	AB734055	C	QnrB60
pspF-qnrB-orf-int[h]*-sapA*	AB734052	C	QnrBΔ
	AB734054	C	QnrBΔ
	JX440358	C	QnrB51
	JX440357	C	QnrB50
pspF-qnrB-sdr[i]*-cinA*[j]*-sapA*	JQ356870	P	QnrB4
	JN215524	P	QnrB4
	JN215523	P	QnrB4
pspF-qnrB-camKIIAD[k]*-sapA*	ADLG01000026	C	ND[d]
	HE820727	C	QnrB54

[a] All sequences correspond to *C. freundii* isolates, except AB734053, which is from *C. braakii*.
[b] Sequence location (SL): C, chromosome; P, plasmid.
[c] QnrBΔ, interrupted coding sequence.
[d] ND, not determined.
[e] *pspF*, phage shock protein F.
[f] *qnrB*, quinolone resistance protein.
[g] *sapA*, peptide transport periplasmic protein.
[h] *int*, integrase.
[i] *sdr*, short-chain dehydrogenase/reductase.
[j] *cinA*, competence damage-inducible protein A.
[k] *camKIIAD*, calcium/calmodulin-dependent protein kinase II association.

The description of the QnrB54 allele, expressed from the chromosome of *C. freundii*, might provide information of interest to trace the spread of quinolone resistance determinants between *Enterobacteriaceae*.

Nucleotide sequence accession numbers. The GenBank accession numbers for the sequences determined in this work are HG974539, LN624598, and HE820727.

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We declare that we have no conflicts of interest.

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