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IS*Ecp1*-mediated transposition of *qnrB*-like gene in *Escherichia coli*

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A novel QnrB-like plasmid-mediated resistance determinant QnrB19 was identified from an *Escherichia coli* clinical isolate from Colombia. Its gene was associated with an IS*Ecp1*-like insertion element that did not act as a promoter for its expression. Using an in-vitro model of transposition, we showed that IS*Ecp1*-like element was able to mobilize the *qnrB19* gene.

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Resistance to quinolones in *Enterobacteriaceae* most commonly arises stepwise as a result of chromosomal mutations responsible for modification of target enzymes (DNA gyrase and topoisomerase IV) or decreased intracellular drug accumulation by upregulation of efflux pumps and/or modified outer-membrane porins (27). Since the discovery of the first plasmid-mediated quinolone resistance (PMQR) determinant QnrA in 1998 (15), four other PMQR determinants have been identified to date: QnrB (12) and QnrS (10) proteins, the aminoglycoside acetyltransferase AAC(6')-Ib-cr (25), and the efflux pump QepA (19,30). Resistance due to Qnr determinants is increasingly reported worldwide in enterobacterial isolates (18,26), and has been recently identified outside *Enterobacteriaceae* in environmental *Aeromonas* isolates from France (3). The three types of Qnr determinants, QnrA, QnrB, and QnrS, belong to the pentapeptide repeat family of proteins (18,26). By protecting DNA gyrase and topoisomerase IV from the inhibitory activity of quinolones, Qnr proteins confer resistance to quinolones (e.g. nalidixic acid) and decreased susceptibility to fluoroquinolones, therefore facilitating recovery of chromosome-encoded target mutants with higher level of resistance to fluoroquinolones (18,26). Whereas *qnrA*-like genes have only been identified as part of complex *sul1*-type integrons in association with the *orf513* transposase gene (18,26) which is part of a region

redefined as IS*CR1* (28), *qnrB*-like genes have been found associated with either the *orf1005* gene encoding a putative transposase (12), or the IS*CR1* element (8,24).

The aim of this study was (i) to investigate the genetic environment of a *qnrB*-like gene from an *Escherichia coli* clinical isolate, (ii) to evaluate experimentally the mobility of that putative transposon in *E. coli*, and (iii) to determine the promoter sequences of the *qnrB*-like gene responsible for the expression of that gene.

E. coli R4525 expressing an extended-spectrum β-lactamase (ESBL) phenotype had been isolated in 2002 from a wound culture of a patient hospitalized at the Hospital San Jeronimo in Monteria, Colombia. In the course of studying the genetic support of the ESBL determinant in *E. coli* R4525, conjugation experiments followed by selection with sodium azide (100 µg/ml) and amoxicillin (50 µg/ml) as previously described (3) gave *E. coli* J53 transconjugants displaying an ESBL phenotype and decreased susceptibility to fluoroquinolones. This result prompted us to search for the presence of PMQR determinants. Screening of *qnr* genes using a multiplex strategy (5) identified a *qnrB*-like gene in both *E. coli* R4525 and its transconjugant. By contrast, PCR screening performed as described (16) did not detect any AAC(6')-Ib-*cr*- or QepA-encoding gene. The *qnrB*-like gene was sequenced and found to encode a novel determinant, which was termed

QnrB19 accordingly to the recent *qnr* gene nomenclature (11). It differed by a single amino acid substitution at position 212 from QnrB5 (GenBank accession no. DQ303919) previously identified in a non-Typhi *Salmonella* isolate from the US (9). Minimal inhibitory concentrations (MICs) were determined on Mueller-Hinton solid agar plates and results interpreted according to the Clinical and Laboratory Standards Institute guidelines (6). *E. coli* R4525 was resistant to aminoglycosides (except amikacin), chloramphenicol, tetracycline, sulphonamides and trimethoprim (data not shown). It was resistant to nalidixic acid (MIC >32 µg/ml) and fluoroquinolones (MICs >32 µg/ml) (Table 1). Sequence analysis of the quinolone-resistance determining regions (QRDRs) of *gyrA* and *parC* genes by using primers previously described (2) showed that *E. coli* R4525 possessed two amino acid substitutions both in GyrA (Ser83Leu and Asp87Tyr) and ParC (Ser80Ile and Glu84Gly), as compared to wild-type QRDRs of *E. coli* (Table 1), and known to confer resistance to quinolones and fluoroquinolones (27). Plasmid analysis of the *E. coli* J53 transconjugant using the Kieser technique (13) identified a single 40-kb plasmid (pR4525) shown to carry by PCR both $bla_{\text{CTX-M-12}}$, $bla_{\text{SHV-12}}$ and *qnrB19* genes (data not shown). As previously reported for QnrB-like proteins, QnrB19 expressed in *E. coli* transconjugant conferred increased MIC values of quinolones and fluoroquinolones (Table

1) (9,12,24).

Since preliminary experiments failed to identify genetic structures that had been associated with *qnrB*-like genes (8,12,24), cloning experiments were performed with EcoRI-restricted whole-cell DNA of *E. coli* R4525 isolate followed by ligation of DNA fragments into the EcoRI-site of cloning vector pBK-CMV (Stratagene, La Jolla, CA), as previously described (3). Analysis of a 3.2-kb DNA fragment carrying the *qnrB19* gene identified its location downstream of extremity of an IS*Ecp1*-like element (Fig. 1). The transposase of the IS*Ecp1*-like-element (termed IS*Ecp1C*) differed by one and two amino acids from those of IS*Ecp1* (Genbank accession no. AJ242809) and IS*Ecp1B* (GenBank accession no. AF458080) respectively, whereas its imperfect inverted repeat left (IRL) and right (IRR1) sequences (two bp mismatches) were identical to those of IS*Ecp1* and IS*Ecp1B* (Fig. 1). IS*Ecp1*-like elements have been identified at the 5'-end of several β-lactamase genes, such as the $bla_{\text{CTX-M}}$, bla_{CMY} or bla_{ACC} genes, and associated with the 16S rRNA methyltransferase gene *rmtC* enabling those genes to be transposed (7,14,17,22,23,29).

The *qnrB19* gene was part of a 2,739-bp potential transposon flanked by a 5-bp duplication of the target site (ATCAA), a likely evidence of a transposition event (Fig. 1).

This potential transposon (named Tn*2012*), comprising IS*Ecp1C* and *qnrB19*, was inserted inside the *orf1* gene of the transposon Tn*1721* (Fig. 1). Tn*1721* is a 11.1-kb nonconjugative transposon belonging to the Tn*21* subgroup of the Tn*3* family of bacterial transposons that confers inducible tetracycline resistance (1). The novel transposon Tn*2012* was bracketed by two imperfect 14-bp IR sequences (7 mismatches), namely the IRL of IS*Ecp1C* and an IRR named IRR2 (Fig. 1). This IRR2 sequence shared 7 out of 14 bp with the original IRR1 of IS*Ecp1C*. This observation is in accordance with previous studies (14,22,23) showing that IS*Ecp1B* was able to use as IRR, sequences sharing weak identity with its original IRR1, corresponding to a one-ended transposition mechanism.

Transposition experiments were performed as previously described (14,22) in order to determine whether IS*Ecp1C* was able to mobilize the *qnrB19* gene. Briefly, sequence of the entire transposon Tn*2012* was amplified by PCR using primers *orf1*-A (5'-CGACAACGGATATTCAAAGC-3') and *orf1*-B (5'-ACTTTGCAAATTATTCTGCCC-3') and then cloned into the kanamycin-resistant pCR-BluntII-TOPO plasmid (Invitrogen). This recombinant plasmid, first transferred and selected in *E. coli* TOP10 (Invitrogen), was used for electrotransformation of *E. coli* RZ211 (pOX38-Gm). Plasmid pOX38-Gm is a self-conjugative and IS-free plasmid carrying a gentamicin resistance marker.

Transposition of Tn*2012* from the recombinant pCR-BluntII-TOPO derivative to plasmid pOX38-Gm was investigated after a 24-h growth in TS broth, by mating RZ211 with azide-resistant *E. coli* J53 and selecting for transconjugants growing on agar plates containing 8 µg of gentamicin per ml, 6 µg of nalidixic acid per ml and 100 µg of azide per ml. Plasmid DNA of several nalidixic acid-resistant transconjugants was extracted and pOX38-Gm sequencing confirmed the IS*Ecp1C*-mediated transposition of *qnrB19*. The transposition frequency, calculated by dividing the number of transconjugants by the number of donors, was estimated to be 10^{-6}-10^{-7} per donor cell. Moreover, analysis of five insertion events of IS*Ecp1C-qnrB19* sequences in plasmid pOX38-Gm showed that transposition had occurred into five different sites distantly located on the recipient plasmid. Alignment of the target sites revealed variable sequences (ATTAT, ATTAC, TCATA, TACAT, TTCAT), exhibiting an AT-rich content as previously observed (14,22).

It has been shown that IS*Ecp1*-like elements may bring promoter sequences for high level expression of downstream-located β-lactamase genes (17,23,29). However, as opposed to what has been reported for those genes, the *qnrB19* gene was located in an opposite orientation, respect to the transposase gene of IS*Ecp1C* (Fig. 1). The promoter sequences for the *qnrB19* expression were determined by using the 5'RACE technique

(Invitrogen). Total RNA was extracted from cultures of *E. coli* R4525 by using the RNeasy Protect Mini Kit (Qiagen, Courtaboeuf, France). The +1 transcription start site was identified 28-bp upstream of an ATG codon located inside the presumed *qnrB19* gene. This prompted us to consider the *qnrB19* gene to be only 645-bp long and the QnrB19 protein to be 214 amino-acid long and not 226 amino-acid long as previously considered for QnrB1 (11,12). This result is in accordance with other observations showing that the putative QnrB1 sequence was longer than QnrA and QnrS, but also than other chromosome-encoded Qnr-like from *Vibrionaceae* (11,21). The deduced promoter region based on the +1 start site identified a -35 box (TTGACG) and a -10 box (TACCAT) separated by a 17-bp sequence (Fig. 2).

We demonstrated here that an IS*Ecp1*-element was at the origin of acquisition of a *qnrB*-like gene (*qnrB19*) and that it was not involved in the expression of that gene, as opposed to what has been reported for other antibiotic resistance genes. Although several *qnrB*-like genes have been reported to be associated with IS*CR1*-associated, we report here a novel genetic structure responsible for *qnrB* acquisition and dissemination. It remains to be determined what could be the natural reservoir of IS*Ecp1*-like elements since it is associated to structurally unrelated resistance determinants of various origins such as the

bla$_{CTX-M}$ and *bla*$_{CMY}$ genes from *Enterobacteriaceae* and the *qnr*-like genes from *Vibrionaceae* and *Shewanellaceae* (4,20).

Nucleotide sequence accession number. The nucleotide sequence of the *qnrB19* gene and that of Tn*2012* shown in Figure 1 were submitted to the GenBank database and can be found under accession no. EU432277 and EU523120, respectively.

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FIGURES LEGEND

FIG. 1. Schematic map of structure of transposon Tn*2012* identified on pR4525 natural plasmid of *E. coli* R4525. Open reading frames (ORFs) are shown as arrows or horizontal boxes with an arrow indicating the orientation of the coding sequence with the gene name above the corresponding boxes. Inverted repeat left (IRL) or inverted repeat right (IRR1 and IRR2) motifs are indicated (blackened bp are identical whereas whitened bp are different), and target site duplications (ATCAA) are represented by black bars.

FIG. 2. Nucleotide sequence of the upstream region of *qnrB19* gene. The -35 and -10 promoter elements are indicated, and transcription start site is represented by an arrow. The former start codon corresponds to the first ATG that was initially proposed for QnrB1 (226 amino acid) (11,12).

TABLE 1. MICs of quinolones for the *E. coli* clinical isolate R4525, its transconjugant, and reference strain *E. coli* J53 Az^R

Strain	Mutations in QRDRs:		MICs (µg/ml)[a] of:					
	gyrA	*parC*	NAL	NOR	OFL	CIP	ENR	SPX
E. coli R4525	S83L+ D87Y	S80I+ E84G	>256	>256	>32	>32	>32	>32
Tc J53/R4525	-[b]	-	32	1	1	0.25	0.5	1
E. coli J53 Az^R	-	-	4	0.12	0.06	0.01	0.01	0.01

[a] NAL, nalidixic acid; NOR, norfloxacin; OFL, ofloxacin; CIP, ciprofloxacin; ENR, enrofloxacin; SPX, sparfloxacin. [b] -, no mutation.