

ISEcp1-mediated transposition of *qnrB*-like gene in

Escherichia coli

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1 A novel QnrB-like plasmid-mediated resistance determinant QnrB19 was
2 identified from an *Escherichia coli* clinical isolate from Colombia. Its gene was
3 associated with an *ISEcp1*-like insertion element that did not act as a promoter for its
4 expression. Using an in-vitro model of transposition, we showed that *ISEcp1*-like
5 element was able to mobilize the *qnrB19* gene.

6 Word count: 54

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1 Resistance to quinolones in *Enterobacteriaceae* most commonly arises stepwise as
2 a result of chromosomal mutations responsible for modification of target enzymes (DNA
3 gyrase and topoisomerase IV) or decreased intracellular drug accumulation by upregulation
4 of efflux pumps and/or modified outer-membrane porins (27). Since the discovery of the
5 first plasmid-mediated quinolone resistance (PMQR) determinant QnrA in 1998 (15), four
6 other PMQR determinants have been identified to date: QnrB (12) and QnrS (10) proteins,
7 the aminoglycoside acetyltransferase AAC(6')-Ib-cr (25), and the efflux pump QepA
8 (19,30). Resistance due to Qnr determinants is increasingly reported worldwide in
9 enterobacterial isolates (18,26), and has been recently identified outside
10 *Enterobacteriaceae* in environmental *Aeromonas* isolates from France (3). The three types
11 of Qnr determinants, QnrA, QnrB, and QnrS, belong to the pentapeptide repeat family of
12 proteins (18,26). By protecting DNA gyrase and topoisomerase IV from the inhibitory
13 activity of quinolones, Qnr proteins confer resistance to quinolones (e.g. nalidixic acid)
14 and decreased susceptibility to fluoroquinolones, therefore facilitating recovery of
15 chromosome-encoded target mutants with higher level of resistance to fluoroquinolones
16 (18,26). Whereas *qnrA*-like genes have only been identified as part of complex *sull*-type
17 integrons in association with the *orf513* transposase gene (18,26) which is part of a region

1 redefined as ISCR1 (28), *qnrB*-like genes have been found associated with either the
2 *orf1005* gene encoding a putative transposase (12), or the ISCR1 element (8,24).

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

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

1 QnrB19 accordingly to the recent *qnr* gene nomenclature (11). It differed by a single
2 amino acid substitution at position 212 from QnrB5 (GenBank accession no. DQ303919)
3 previously identified in a non-Typhi *Salmonella* isolate from the US (9). Minimal
4 inhibitory concentrations (MICs) were determined on Mueller-Hinton solid agar plates and
5 results interpreted according to the Clinical and Laboratory Standards Institute guidelines
6 (6). *E. coli* R4525 was resistant to aminoglycosides (except amikacin), chloramphenicol,
7 tetracycline, sulphonamides and trimethoprim (data not shown). It was resistant to
8 nalidixic acid (MIC >32 µg/ml) and fluoroquinolones (MICs >32 µg/ml) (Table 1).
9 Sequence analysis of the quinolone-resistance determining regions (QRDRs) of *gyrA* and
10 *parC* genes by using primers previously described (2) showed that *E. coli* R4525 possessed
11 two amino acid substitutions both in GyrA (Ser83Leu and Asp87Tyr) and ParC (Ser80Ile
12 and Glu84Gly), as compared to wild-type QRDRs of *E. coli* (Table 1), and known to
13 confer resistance to quinolones and fluoroquinolones (27). Plasmid analysis of the *E. coli*
14 J53 transconjugant using the Kieser technique (13) identified a single 40-kb plasmid
15 (pR4525) shown to carry by PCR both *bla*_{CTX-M-12}, *bla*_{SHV-12} and *qnrB19* genes (data not
16 shown). As previously reported for QnrB-like proteins, QnrB19 expressed in *E. coli*
17 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 *ISEcp1*-like element (Fig. 1). The transposase of the *ISEcp1*-like-element (termed *ISEcp1C*) differed by one and two amino acids from those of *ISEcp1* (Genbank accession no. AJ242809) and *ISEcp1B* (GenBank accession no. AF458080) respectively, whereas its imperfect inverted repeat left (IRL) and right (IRR1) sequences (two bp mismatches) were identical to those of *ISEcp1* and *ISEcp1B* (Fig. 1). *ISEcp1*-like elements have been identified at the 5'-end of several β -lactamase genes, such as the *bla*_{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).

1 This potential transposon (named Tn2012), comprising *ISEcp1C* and *qnrB19*, was inserted
2 inside the *orf1* gene of the transposon Tn1721 (Fig. 1). Tn1721 is a 11.1-kb
3 nonconjugative transposon belonging to the Tn21 subgroup of the Tn3 family of bacterial
4 transposons that confers inducible tetracycline resistance (1). The novel transposon
5 Tn2012 was bracketed by two imperfect 14-bp IR sequences (7 mismatches), namely the
6 IRL of *ISEcp1C* and an IRR named IRR2 (Fig. 1). This IRR2 sequence shared 7 out of 14
7 bp with the original IRR1 of *ISEcp1C*. This observation is in accordance with previous
8 studies (14,22,23) showing that *ISEcp1B* was able to use as IRR, sequences sharing weak
9 identity with its original IRR1, corresponding to a one-ended transposition mechanism.

10 Transposition experiments were performed as previously described (14,22) in order
11 to determine whether *ISEcp1C* was able to mobilize the *qnrB19* gene. Briefly, sequence of
12 the entire transposon Tn2012 was amplified by PCR using primers *orf1*-A (5'-
13 CGACAACGGATATTCAAAGC-3') and *orf1*-B (5'-ACTTTGCAAATTATTCTGCCC-
14 3') and then cloned into the kanamycin-resistant pCR-BluntII-TOPO plasmid (Invitrogen).
15 This recombinant plasmid, first transferred and selected in *E. coli* TOP10 (Invitrogen), was
16 used for electrotransformation of *E. coli* RZ211 (pOX38-Gm). Plasmid pOX38-Gm is a
17 self-conjugative and IS-free plasmid carrying a gentamicin resistance marker.

1 Transposition of Tn2012 from the recombinant pCR-BluntII-TOPO derivative to plasmid
2 pOX38-Gm was investigated after a 24-h growth in TS broth, by mating RZ211 with
3 azide-resistant *E. coli* J53 and selecting for transconjugants growing on agar plates
4 containing 8 µg of gentamicin per ml, 6 µg of nalidixic acid per ml and 100 µg of azide per
5 ml. Plasmid DNA of several nalidixic acid-resistant transconjugants was extracted and
6 pOX38-Gm sequencing confirmed the *ISEcp1C*-mediated transposition of *qnrB19*. The
7 transposition frequency, calculated by dividing the number of transconjugants by the
8 number of donors, was estimated to be 10^{-6} - 10^{-7} per donor cell. Moreover, analysis of five
9 insertion events of *ISEcp1C-qnrB19* sequences in plasmid pOX38-Gm showed that
10 transposition had occurred into five different sites distantly located on the recipient
11 plasmid. Alignment of the target sites revealed variable sequences (ATTAT, ATTAC,
12 TCATA, TACAT, TTCAT), exhibiting an AT-rich content as previously observed (14,22).

13 It has been shown that *ISEcp1*-like elements may bring promoter sequences for high
14 level expression of downstream-located β-lactamase genes (17,23,29). However, as
15 opposed to what has been reported for those genes, the *qnrB19* gene was located in an
16 opposite orientation, respect to the transposase gene of *ISEcp1C* (Fig. 1). The promoter
17 sequences for the *qnrB19* expression were determined by using the 5'RACE technique

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

11 We demonstrated here that an *ISEcpI*-element was at the origin of acquisition of a
12 *qnrB*-like gene (*qnrB19*) and that it was not involved in the expression of that gene, as
13 opposed to what has been reported for other antibiotic resistance genes. Although several
14 *qnrB*-like genes have been reported to be associated with *ISCR1*-associated, we report here
15 a novel genetic structure responsible for *qnrB* acquisition and dissemination. It remains to
16 be determined what could be the natural reservoir of *ISEcpI*-like elements since it is
17 associated to structurally unrelated resistance determinants of various origins such as the

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

3

4 **Nucleotide sequence accession number.** The nucleotide sequence of the *qnrB19* gene and
5 that of Tn2012 shown in Figure 1 were submitted to the GenBank database and can be
6 found under accession no. EU432277 and EU523120, respectively.

7

8

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REFERENCES

14 1. Allmeier, H., B. Cresnar, M. Greck, and R. Schmitt. 1992. Complete nucleotide
15 sequence of Tn1721: gene organization and a novel gene product with features of a
16 chemotaxis protein. *Gene* **111**:11-20.

- 1 **2. Cattoir, V., P. Lesprit, C. Lascols, E. Denamur, P. Legrand, C. J. Soussy, and**
2 **E. Cambau.** 2006. In vivo selection during ofloxacin therapy of *Escherichia coli*
3 with combined topoisomerase mutations that confer high resistance to ofloxacin but
4 susceptibility to nalidixic acid. *J. Antimicrob. Chemother.* **58**:1054-1057.
- 5 **3. Cattoir, V., L. Poirel, C. Aubert, C. J. Soussy, and P. Nordmann.** 2008.
6 Unexpected occurrence of plasmid-mediated quinolone resistance determinants in
7 environmental *Aeromonas* spp. *Emerg. Infect. Dis.* **14**:231-237.
- 8 **4. Cattoir, V., L. Poirel, D. Mazel, C. J. Soussy, and P. Nordmann.** 2007. *Vibrio*
9 *splendidus* as the source of plasmid-mediated QnrS-like quinolone resistance
10 determinants. *Antimicrob. Agents Chemother.* **51**:2650-2651.
- 11 **5. Cattoir, V., L. Poirel, V. Rotimi, C. J. Soussy, and P. Nordmann.** 2007.
12 Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in
13 ESBL-producing enterobacterial isolates. *J. Antimicrob. Chemother.* **60**:394-397.
- 14 **6. Clinical and Laboratory Standards Institute.** 2006. Performance Standards for
15 Antimicrobial Disk Susceptibility Tests; Approved Standard, 9th ed. M2-A9.
16 Clinical and Laboratory Standards Institute, Wayne, Pa.

- 1 7. **Doloy, A., C. Verdet, V. Gautier, D. Decré, E. Ronco, A. Hammami, A.**
2 **Philippon, and G. Arlet.** 2006. Genetic environment of acquired *bla*_{ACC-1} β -
3 lactamase gene in *Enterobacteriaceae* isolates. *Antimicrob. Agents Chemother.*
4 **50:4177-4181.**
- 5 8. **Garnier, F., N. Raked, A. Gassama, F. Denis, and M. C. Ploy.** 2006. Genetic
6 environment of quinolone resistance gene *qnrB2* in a complex *sul1*-type integron in
7 the newly described *Salmonella enterica* serovar Keurmassar. *Antimicrob. Agents*
8 *Chemother.* **50:3200-3202.**
- 9 9. **Gay, K., A. Robicsek, J. Strahilevitz, C. H. Park, G. Jacoby, T. J. Barrett, F.**
10 **Medalla, T. M. Chiller, and D. C. Hooper.** 2006. Plasmid-mediated quinolone
11 resistance in non-Typhi serotypes of *Salmonella enterica*. *Clin. Infect. Dis.* **43:297-**
12 **304.**
- 13 10. **Hata, M., M. Suzuki, M. Matsumoto, M. Takahashi, K. Sato, S. Ibe, and K.**
14 **Sakae.** 2005. Cloning of a novel gene for quinolone resistance from a transferable
15 plasmid in *Shigella flexneri* 2b. *Antimicrob. Agents Chemother.* **49:801-803.**

- 1 **11. Jacoby, G., V. Cattoir, D. Hooper, L. Martinez-Martinez, P. Nordmann, A.**
2 **Pascual, L. Poirel, and M. Wang.** 2008. *qnr* gene nomenclature. Antimicrob.
3 Agents Chemother. In press
- 4 **12. Jacoby, G. A., K. E. Walsh, D. M. Mills, V. J. Walker, H. Oh, A. Robicsek, and**
5 **D. C. Hooper.** 2006. *qnrB*, another plasmid-mediated gene for quinolone
6 resistance. Antimicrob. Agents Chemother. **50**:1178-82.
- 7 **13. Kieser, T.** 1984. Factors affecting the isolation of CCC DNA from *Streptomyces*
8 *lividans* and *Escherichia coli*. Plasmid **12**:19-36.
- 9 **14. Lartigue, M. F., L. Poirel, D. Aubert, and P. Nordmann.** 2006. In vitro analysis
10 of ISEcp1B-mediated mobilization of naturally occurring β -lactamase gene *bla*_{CTX-}
11 *M* of *Kluyvera ascorbata*. Antimicrob. Agents Chemother. **50**:1282-1286.
- 12 **15. Martinez-Martinez, L., A. Pascual, and G. A. Jacoby.** 1998. Quinolone
13 resistance from a transferable plasmid. Lancet **351**:797-799.
- 14 **16. Minarini, L. A., L. Poirel, V. Cattoir, A. L. Darini, and P. Nordmann.** 2008.
15 Plasmid-mediated quinolone resistance determinants among enterobacterial isolates
16 from outpatients in Brazil. J. Antimicrob. Chemother. In press.

- 1 **17. Nakano, R., R. Okamoto, N. Nagano, and M. Inoue.** 2007. Resistance to gram-
- 2 negative organisms due to high-level expression of plasmid-encoded *ampC* β -
- 3 lactamase *bla*_{CMY-4} promoted by insertion sequence *ISEcp1*. *J. Infect. Chemother.*
- 4 **13**:18-23.
- 5 **18. Nordmann, P., and L. Poirel.** 2005. Emergence of plasmid-mediated resistance to
- 6 quinolones in *Enterobacteriaceae*. *J. Antimicrob. Chemother.* **56**:463-469.
- 7 **19. Périchon, B., P. Courvalin, and M. Galimand.** 2007. Transferable resistance to
- 8 aminoglycosides by methylation of G1405 in 16S rRNA and to hydrophilic
- 9 fluoroquinolones by QepA-mediated efflux in *Escherichia coli*. *Antimicrob. Agents*
- 10 *Chemother.* **51**:2464-2469.
- 11 **20. Poirel, L., J. M. Rodriguez-Martinez, H. Mammeri, A. Liard, and P.**
- 12 **Nordmann.** 2005. Origin of plasmid-mediated quinolone resistance determinant
- 13 QnrA. *Antimicrob. Agents Chemother.* **49**:3523-3525.
- 14 **21. Poirel, L., A. Liard, J. M. Rodriguez-Martinez, and P. Nordmann.** 2005.
- 15 *Vibrionaceae* as a possible source of Qnr-like quinolone resistance determinants. *J.*
- 16 *Antimicrob. Chemother.* **56**:1118-1121.

- 1 **22. Poirel, L., M. F. Lartigue, J. W. Decousser, and P. Nordmann.** 2005. *ISEcp1B*-
2 mediated transposition of *bla*_{CTX-M} in *Escherichia coli*. *Antimicrob. Agents*
3 *Chemother.* **49**:447-450.
- 4 **23. Poirel, L., J. W. Decousser, and P. Nordmann.** 2003. Insertion sequence
5 *ISEcp1B* is involved in the expression and mobilization of a *bla*_{CTX-M} β -lactamase
6 gene. *Antimicrob. Agents Chemother.* **47**:2938-2945.
- 7 **24. Quiroga, M. P., P. Andres, A. Petroni, A. Soler-Bistue, L. Guerriero, L. Jorda**
8 **Vargas, A. Zorreguieta, M. Tokumoto, C. Quiroga, M. Tolmasky, M. Galas,**
9 **and D. Centron.** 2007. Complex class 1 integrons with diverse variable regions,
10 including *aac(6')-Ib-cr* and a novel allele, *qnrB10*, associated to *ISCR1* in clinical
11 enterobacterial isolates from Argentina. *Antimicrob. Agents Chemother.* **51**:4466-
12 4470.
- 13 **25. Robicsek, A., J. Strahilevitz, G. A. Jacoby, M. Macielag, D. Abbanat, C. H.**
14 **Park, K. Bush, and D. C. Hooper.** 2006. Fluoroquinolone-modifying enzyme: a
15 new adaptation of a common aminoglycoside acetyltransferase. *Nat. Med.* **12**:83-
16 88.

- 1 **26. Robicsek, A., G. A. Jacoby, and D. C. Hooper.** 2006. The worldwide emergence
2 of plasmid-mediated quinolone resistance. *Lancet Infect. Dis.* **6**:629-640.
- 3 **27. Ruiz, J.** 2003. Mechanisms of resistance to quinolones: target alterations,
4 decreased accumulation and DNA gyrase protection. *J. Antimicrob. Chemother.*
5 **51**:1109-1117.
- 6 **28. Toleman, M. A., P. M. Bennett, and T. R. Walsh.** 2006. ISCR elements: novel
7 gene-capturing systems of the 21st century? *Microbiol. Mol. Biol. Rev.* **70**:296-
8 316.
- 9 **29. Wachino, J., K. Yamane, K. Kimura, N. Shibata, S. Suzuki, Y. Ike, and Y.**
10 **Arakawa.** 2006. Mode of transposition and expression of 16S rRNA
11 methyltransferase gene *rmtC* accompanied by *ISEcp1*. *Antimicrob. Agents*
12 *Chemother.* **50**:3212-3215.
- 13 **30. Yamane, K., J. Wachino, S. Suzuki, K. Kimura, N. Shibata, H. Kato, K.**
14 **Shibayama, T. Konda, and Y. Arakawa.** 2007. New plasmid-mediated
15 fluoroquinolone efflux pump, QepA, found in an *Escherichia coli* clinical isolate.
16 *Antimicrob. Agents Chemother.* **51**:3354-3360.
- 17

FIGURES LEGEND

1

2 FIG. 1. Schematic map of structure of transposon Tn2012 identified on pR4525 natural
3 plasmid of *E. coli* R4525. Open reading frames (ORFs) are shown as arrows or horizontal
4 boxes with an arrow indicating the orientation of the coding sequence with the gene name
5 above the corresponding boxes. Inverted repeat left (IRL) or inverted repeat right (IRR1
6 and IRR2) motifs are indicated (blackened bp are identical whereas whitened bp are
7 different), and target site duplications (ATCAA) are represented by black bars.

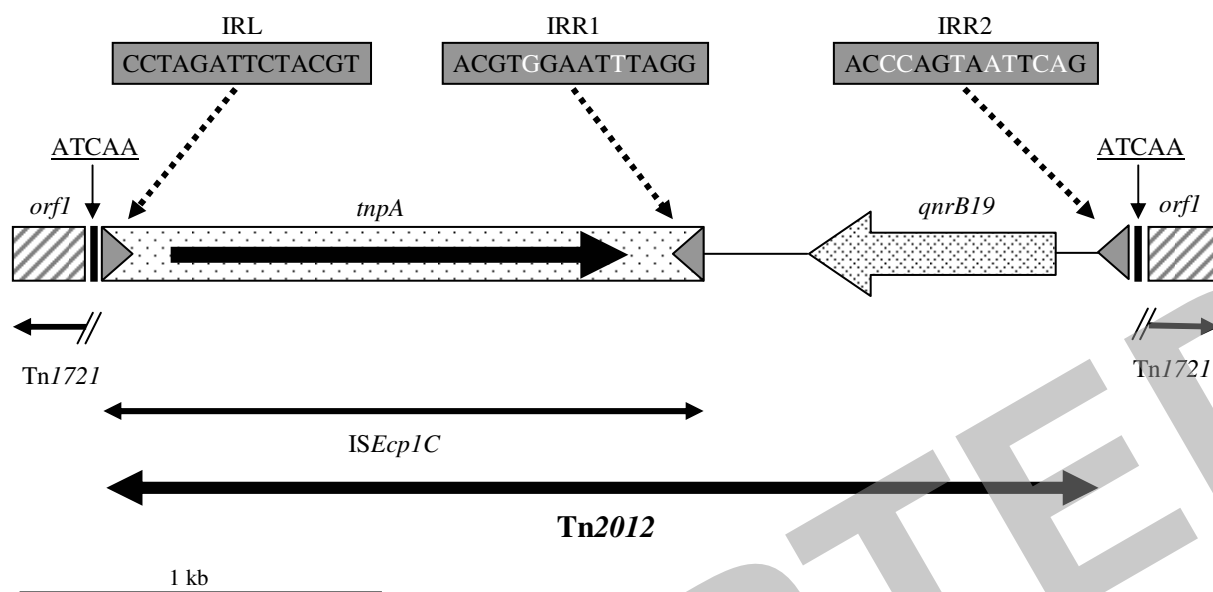
8

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

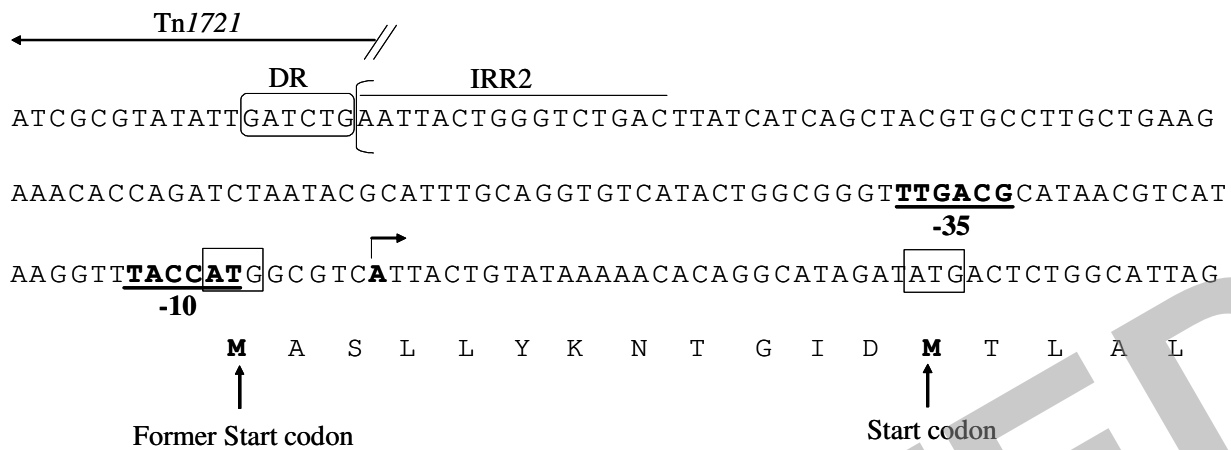
TABLE 1. MICs of quinolones for the *E. coli* clinical isolate R4525, its transconjugant, and reference strain *E. coli* J53 Az^R

Strain	Mutations in		MICs ($\mu\text{g/ml}$) ^a of:					
	QRDRs:		NAL	NOR	OFL	CIP	ENR	SPX
	<i>gyrA</i>	<i>parC</i>						
<i>E. coli</i> R4525	S83L+	S80I+	>256	>256	>32	>32	>32	>32
	D87Y	E84G						
Tc J53/R4525	- ^b	-	32	1	1	0.25	0.5	1
<i>E. coli</i> 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.



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