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qnrB19 Gene Bracketed by IS*26* on a 40-Kilobase IncR Plasmid from an *Escherichia coli* Isolate from a Veal Calf[▽]

qnrB19 genes have been reported in *Escherichia coli*, *Escherichia hermannii*, *Salmonella enterica*, and *Klebsiella* spp., located on IncN, IncL/M (human isolates), and ColE-like (both human and chicken isolates) plasmids (2, 6, 8, 9, 11, 13, 14, 16). This study describes the characterization of the genetic environment of a plasmid-mediated *qnrB19* gene identified in *E. coli* isolated from a veal calf in the Netherlands.

E. coli strain 013.1 was selected from a fecal sample grown on a MacConkey agar plate supplemented with 0.125 mg/liter ciprofloxacin. This strain showed MIC values of 0.25 mg/liter and 16 mg/liter for ciprofloxacin and nalidixic acid, respectively, suggesting the presence of a plasmid-mediated quinolone resistance mechanism (10, 18).

PCR as described for *qnrA* and *qnrS* (4), *qnrB* (3), *qnrC* (20), *qnrD* (5), *qepA* (17), and *aac*(*6′*)-*Ib-cr* (15) and sequence analysis revealed a *qnrB19* gene (12), whereas chromosomal mutations in the topoisomerase genes *gyrA* and/or *gyrB* and *parC* and/or *parE* were absent (7).

Conjugation experiments using standard broth mating experiments with rifampin-resistant *E. coli* K-12 as the recipient were not successful. However, DH10B (Gibco Invitrogen) transformants (designated 013.1-T) could be selected on Luria-Bertani agar plates supplemented with 0.03 mg/liter ciprofloxacin (Sigma) using plasmids isolated from strain 013.1, indicating that the *qnrB19* gene was located on the plasmid. Strain 013.1-T showed MIC values for ciprofloxacin and nalidixic acid of 0.12 mg/liter and ≤4 mg/liter, respectively. Untransformed DH10B cells were susceptible to ciprofloxacin (MIC ≤ 0.008 mg/liter) and nalidixic acid (MIC ≤ 4 mg/liter). Strain 013.1-T did not harbor the *qepA* or *aac*(*6′*)-*Ib-cr* gene or mutations in the chromosomally located *gyrA*, *gyrB*, *parC*, or *parE* genes.

Plasmid sizes were determined by S1 pulsed-field gel electrophoresis (PFGE) (19), as well as by S1 gel electrophoresis (GE) (running at 80 V for 4 h). Strain 013.1 harbored a 40-kb plasmid and a 2.6-kb plasmid. Strain 013.1-T harbored a 40-kb plasmid only. Subsequently, the plasmids were identified by PCR-based replicon typing (PBRT) (1, 9) as replicon type IncR and as ColE in strain 013.1. Transformant 013.1-T harbored only an IncR-type plasmid, designated p013.1IncR.

Sequence analysis of the *qnr* gene and its flanking regions was performed by primer walking analysis on purified DNA of p013.1IncR. The *qnrB19* gene was bracketed by two identical IS*26* insertion sequences (Fig. 1A). Further downstream of the *qnrB19* gene, an *aphA1* gene was identified, followed by a partial IS*26* sequence, which was interrupted by transposable element Tn*5393* harboring a *strB* gene. Two regions, one comprising *qnrB19*, IRR2, and a IS*26* sequence, and a second region comprising *aphA1* and the partial sequence of IS*26* (Fig. 1A and B), both showed one point mutation difference compared to the sequences published previously (6). Both full-length IS*26* elements bracketing the *qnrB19* gene are identical to each other (Fig. 1A) and to the one previously described downstream of *aphA1* (Fig. 1B) (6). IS*Ecp1C*, located with *qnrB19* on Tn*2012* as described by Cattoir et al. (2) (Fig. 1C), was not observed in the present study.

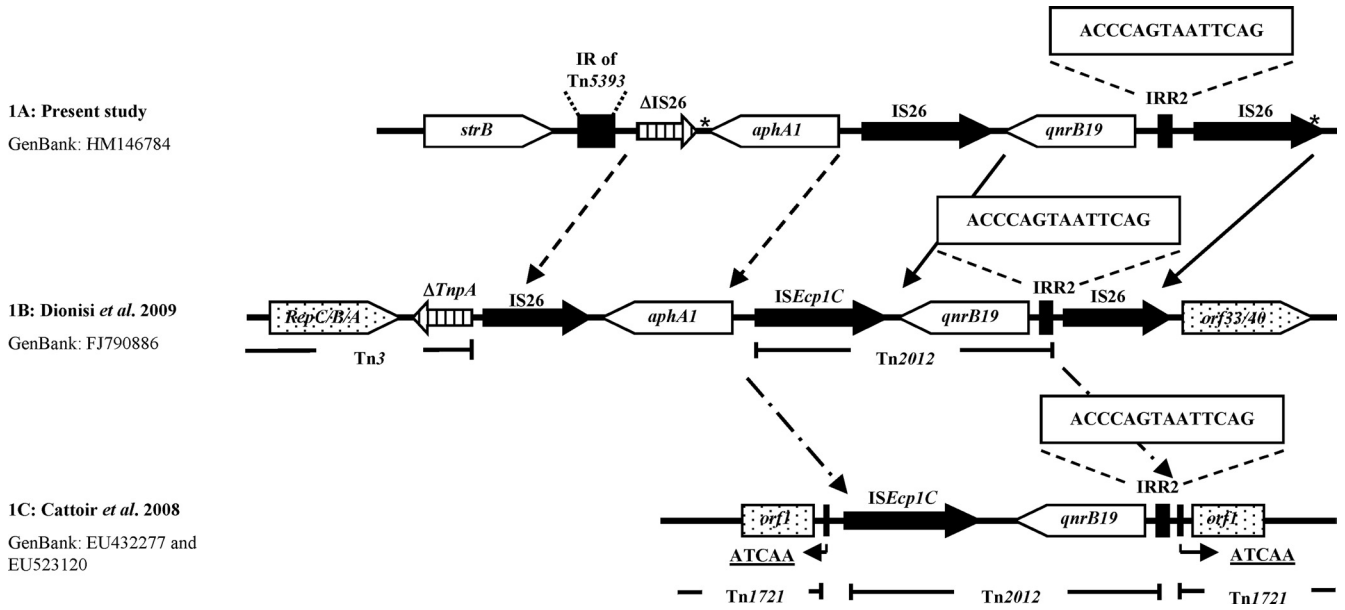

FIG. 1. Genetic environment of the *qnrB19* gene. Schematic overview of sequencing results (A) compared with the *qnrB19* gene with flanking regions found by Dionisi et al. (6) (B) and Cattoir et al. (2) (C). Black arrows indicate insertion sequences or repeat sequences. White arrows indicates antimicrobial resistance genes. Striped arrows indicate partial sequences. Broken-line arrows indicate genes other than antimicrobial resistance genes. An asterisk indicates that the sequence harbors a point mutation compared to the sequence with GenBank accession number FJ790886. (The figure is not drawn to scale.) IR, inverted repeat.

The presence of antimicrobial resistance genes other than *qnr* on p013.1IncR was analyzed by microarray technology using AMR-ve array tubes (Identibac, Addlestone, United Kingdom). The resistance genes *dfr12*, *sul1*, *strB* and integrase gene *intI1* were found in both strains 013.1 and 013.1-T, indicating that these genes all reside on p013.1IncR. The *tetB* gene was observed only in donor strain 013.1.

This is the first report of a *qnrB19* gene found in *E. coli* isolated from veal calves and the first *qnrB19* found on an IncR-type plasmid. The association with different transposable units indicates a high potential for spreading.

Nucleotide sequence accession number. The sequence presented in this study was published in GenBank under accession number HM146784.

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