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LETTER TO THE EDITOR

Complete Sequence of Conjugative IncA/C Plasmid Encoding CMY-2 β-Lactamase and RmtE 16S rRNA Methyltransferase

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RmtE is a rare 16S-RMTase which was first reported in an aminoglycoside-resistant *Escherichia coli* strain of calf origin (1). Subsequently, we reported the first human case of infection caused by RmtE-producing *E. coli* (2). The *rmtE* gene is carried on a self-conjugative plasmid (pYDC637) in the latter strain. The present work aimed to elucidate the genetic context of *rmtE*. The sequencing approach has been described previously (3). In brief, the plasmid was extracted from an *E. coli* TOP10 transformant carrying pYDC637 and sequenced on a PacBio RS II sequencing instrument (Pacific Biosciences, Menlo Park, CA). Assembly was also conducted using the HGAP pipeline (Pacific Biosciences) as previously described (3).

pYDC637 is an IncA/C plasmid 199,469 bp in size with a G+C content of 52.1%. It harbors 241 predicted open reading frames (ORFs) and is composed of a 144-kb core region and one distinct acquired region spanning 55 kb (Fig. 1). The core region includes genes responsible for plasmid replication, horizontal transfer, and stability and maintenance functions and defines the plasmid backbone (4). This arrangement is shared with bla_{CMY-2}-carrying IncA/C plasmids that have been identified in *Aeromonas salmonicida* and *E. coli* isolates from different countries, such as pSN254b, pAR060302, and pUMNK88 (GenBank accession no. KJ909290, FJ621588, and HQ023862, respectively). The core region in pYDC637 encodes IncA/C replication initiation protein gene *repA* and genes involved in the conjugative transfer of plasmids (*traIDLEKBVACWUN* and *traFHG*). In addition to the *tra* genes, various antimicrobial resistance genes are identified in this region, including *floR* (florfenicol resistance gene), *tet*(A) (tetracycline resistance), *tet*(R), *strA* and *strB* (*strA*,*B*) (streptomycin resistance), *sul*2 (sulfonamide resistance), and bla_{CMY-2} (cephalosporin resistance), which are highly conserved among bla_{CMY-2}-carrying IncA/C plasmids (4).

Downstream of the core region, pYDC637 has a distinct acquired region that harbors a variety of antimicrobial resistance genes in two class 1 integrons. The first integron, bounded by two transposase genes found in *A. salmonicida*, carries *aadA1bx* (encoding streptomycin resistance) as the only gene cassette. A unit bracketed by two identical copies of a putative *pol* gene is located between *aadA1bx* and *qacEΔ1-sul1*. Within this unit, *rmtE* is bound by an IS*CR20*-like element and an IS*1294*-like insertion sequence. These IS*91*-like transposable elements are often identified in association with antimicrobial resistance genes, including

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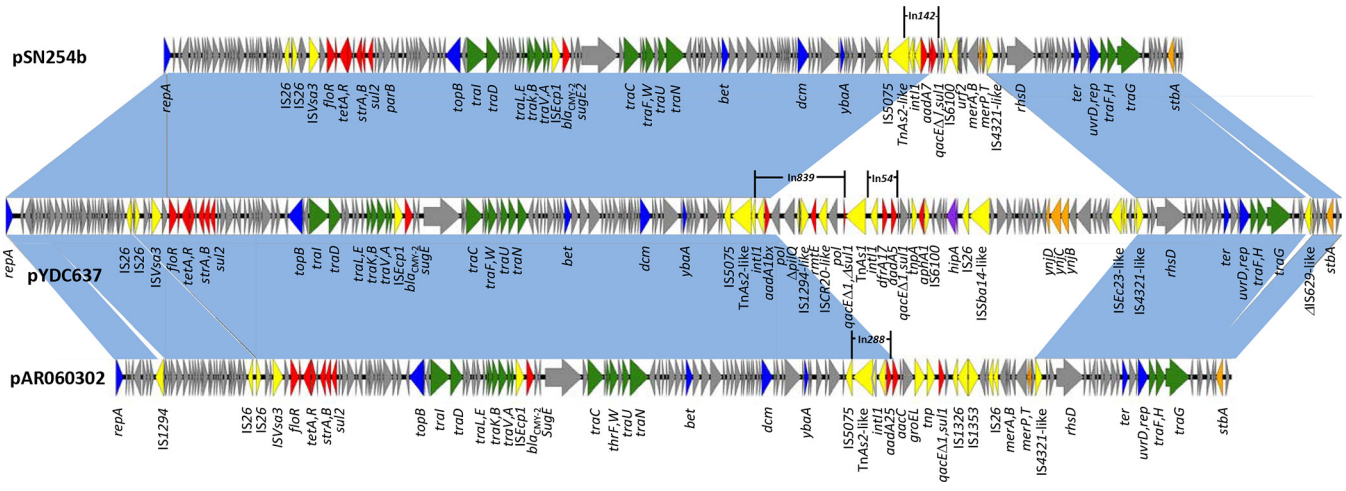

FIG 1 Comparative analysis of IncA/C plasmid pYDC637 (GenBank accession no. KP056256) with two other bla_{CMY-2}-carrying IncA/C plasmids, pSN254b (KJ909290) and pAR060302 (FJ621588). Light-blue shading indicates shared backbone regions with a high degree of homology. ORFs are indicated by arrows and are colored according to their putative functions. Dark-blue arrows indicate replication-associated genes. Green arrows indicate genes that are associated with plasmid conjugal transfers, and brown arrows indicate genes that are involved in plasmid stability. Red arrows indicate antimicrobial resistance genes. Yellow arrows indicate accessory genes of mobile elements. Dark-purple arrows indicate other backbone genes.

16S-RMTase genes. For example, *rmtF* and *rmtD1-rmtD2* are flanked by IS*CR5*-like and IS*CR14* elements, respectively (5–7). This unique arrangement suggests that they likely played a role in the initial mobilization of *rmtE*, whose origin remains unknown. It is also possible that the *pol* duplication was created in this process given the putative transposition mechanism. The second integron contains *dfrA17* (trimethoprim resistance) and *aadA5* (streptomycin resistance) as gene cassettes. Thus, the structure of pYDC637 is characterized by incorporation of *rmtE* in a class 1 integron into a bla_{CMY-2}-carrying IncA/C plasmid. RmtE remains a rare 16S-RMTase at this point, having been identified in only one animal *E. coli* strain and one human *E. coli* strain. However, coproduction of RmtE and CMY-2 along with other various resistance elements from a broad-host-range, self-conjugative plasmid suggests its potential for future spread.

Nucleotide sequence accession number. The plasmid sequence reported in this work appears under accession number KP056256.

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