

1 **Complete Sequence of Conjugative IncA/C Plasmid Encoding CMY-2 β -Lactamase and**
2 **RmtE 16S Ribosomal RNA Methyltransferase**

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14 Running title: *rmtE*-carrying IncA/C plasmid

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21 RmtE is a rare 16S-RMTase which was first reported in an aminoglycoside-resistant *Escherichia*
22 *coli* strain of calf origin (1). Subsequently, we reported the first human case of infection caused
23 by RmtE-producing *E. coli* (2). The gene *rmtE* is carried on a self-conjugative plasmid
24 pYDC637 in the latter strain. The present work aimed to elucidate the genetic context of *rmtE*.
25 The sequencing approach has been described previously (3). In brief, the plasmid was extracted
26 from an *E. coli* TOP10 transformant carrying pYDC637 and sequenced on a PacBio RS II
27 sequencing instrument (Pacific Biosciences, Menlo Park, CA). Assembly was also conducted as
28 previously described using the HGAP pipeline (Pacific Biosciences) (3). The plasmid sequence
29 reported in this work appears under accession number KP056256.

30 pYDC637 is an IncA/C plasmid 199,469-bp in size with a G+C content of 52.1%. It
31 harbors 241 predicted open reading frames (ORFs) and is composed of a 144-kb core region and
32 one distinct acquired region spanning 55 kb (Figure 1). The core region includes genes
33 responsible for plasmid replication, horizontal transfer, and stability and maintenance functions,
34 and defines the plasmid backbone (4). This arrangement is shared with *bla*_{CMY-2}-carrying IncA/C
35 plasmids that have been identified in *Aeromonas salmonicida* and *E. coli* from different
36 countries, such as pSN254b, pAR060302, pUMNK88 (GenBank accession nos. KJ909290,
37 FJ621588, and HQ023862, respectively). The core region in pYDC637 encodes the IncA/C
38 replication initiation protein gene *repA* and genes involved in the conjugative transfer of
39 plasmids (*traIDLEKBVACWUN* and *traFHG*). In addition to the *tra* genes, various antimicrobial
40 resistance genes are identified in this region, including *floR* (florfenicol resistance gene), *tet(A)*
41 (tetracycline resistance), *tet(R)*, *strA,B* (streptomycin resistance), *sul2* (sulfonamide resistance)
42 and *bla*_{CMY-2} (cephalosporin resistance), which are highly conserved among *bla*_{CMY-2}-carrying
43 IncA/C plasmids (4).

44 Downstream of the core region, pYDC637 has a distinct acquired region that harbors a
45 variety of antimicrobial resistance genes in two class 1 integrons. The first integron, bounded by
46 two transposase genes found in *A. salmonicida*, carries *aadA1bx* encoding streptomycin
47 resistance as the only gene cassette. Between *aadA1bx* and *qacEΔ1-sul1*, a unit bracketed by two
48 identical copies of a putative *pol* gene is located. Within this unit, *rmtE* is bound by an ISCR20-
49 like element and an IS1294-like insertion sequence. These IS91-like transposable elements are
50 often identified in association with antimicrobial resistance genes including 16S-RMTase genes.
51 For example, *rmtF* and *rmtD1/rmtD2* are flanked by ISCR5-like and ISCR14 elements,
52 respectively (5-7). This unique arrangement suggests that they likely played a role in the initial
53 mobilization of *rmtE*, whose origin remains unknown. It is also possible that the *pol* duplication
54 was created in this process given the putative transposition mechanism. The second integron
55 contains *dfrA17* (trimethoprim resistance) and *aadA5* (streptomycin resistance) as gene cassettes.
56 Thus, the structure of pYDC637 is characterized by incorporation of *rmtE* into a class 1 integron
57 into a *bla*_{CMY-2}-carrying IncA/C plasmid. RmtE remains a rare 16S-RMTase at this point, having
58 been identified only in one animal and one human *E. coli* strains. However, co-production of
59 RmtE and CMY-2 along with other various resistance elements from a broad host range, self-
60 conjugative plasmid suggests its potential for future spread.

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73 **References**

- 74 1. **Davis, M. A., K. N. Baker, L. H. Orfe, D. H. Shah, T. E. Besser, and D. R. Call.** 2010.
75 Discovery of a gene conferring multiple-aminoglycoside resistance in *Escherichia coli*.
76 *Antimicrob Agents Chemother* **54**:2666-2669.
- 77 2. **Lee, C. S., F. Hu, J. I. Rivera, and Y. Doi.** 2014. *Escherichia coli* sequence type 354
78 coproducing CMY-2 cephalosporinase and RmtE 16S rRNA methyltransferase.
79 *Antimicrob Agents Chemother* **58**:4246-4247.
- 80 3. **Li, J. J., C. S. Lee, J. F. Sheng, and Y. Doi.** 2014. Complete sequence of a conjugative
81 IncN plasmid harboring *bla*_{KPC-2}, *bla*_{SHV-12}, and *qnrSI* from an *Escherichia coli* sequence
82 type 648 strain. *Antimicrob Agents Chemother* **58**:6974-6977.
- 83 4. **Call, D. R., R. S. Singer, D. Meng, S. L. Broschat, L. H. Orfe, J. M. Anderson, D. R.**
84 **Herndon, L. S. Kappmeyer, J. B. Daniels, and T. E. Besser.** 2010. *bla*_{CMY-2}-positive
85 IncA/C plasmids from *Escherichia coli* and *Salmonella enterica* are a distinct component
86 of a larger lineage of plasmids. *Antimicrob Agents Chemother* **54**:590-596.
- 87 5. **Toleman, M. A., P. M. Bennett, and T. R. Walsh.** 2006. ISCR elements: novel gene-
88 capturing systems of the 21st century? *Microbiol Mol Biol Rev* **70**:296-316.

- 89 6. **Galimand, M., P. Courvalin, and T. Lambert.** 2012. RmtF, a new member of the
90 aminoglycoside resistance 16S rRNA N7 G1405 methyltransferase family. Antimicrob
91 Agents Chemother **56**:3960-3962.
- 92 7. **Tijet, N., P. Andres, C. Chung, C. Lucero, W. H.-A. Group, D. E. Low, M. Galas, A.**
93 **Corso, A. Petroni, and R. G. Melano.** 2011. *rmtD2*, a new allele of a 16S rRNA
94 methylase gene, has been present in Enterobacteriaceae isolates from Argentina for more
95 than a decade. Antimicrob Agents Chemother **55**:904-909.

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99 **Figure legend**

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