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# *Escherichia coli* Sequence Type 354 Coproducing CMY-2 Cephalosporinase and RmtE 16S rRNA Methyltransferase

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Production of acquired 16S rRNA methyltransferase (16S RMTase) is an emerging mechanism of high-level aminoglycoside resistance in clinically relevant Gram-negative bacteria. So far, seven plasmid-mediated 16S RMTases have been reported worldwide (1). Of the known 16S RMTases, RmtE has been reported in only one *Escherichia coli* strain, which was identified from a calf in the United States (2). Here, we report the first documented case of human infection by RmtE-producing *E. coli*.

*E. coli* YDC637 was isolated from fluid from a Jackson-Pratt drain in a 54-year-old male patient who had been admitted to a tertiary-care hospital in western Pennsylvania in 2013. He had undergone orthotopic liver transplantation twice for ulcerative colitis and primary sclerosing cholangitis. He had received multiple courses of antimicrobial agents, including piperacillin-tazobactam, ciprofloxacin, metronidazole, ertapenem, vancomycin, linezolid, and caspofungin, for recurrent cholangitis within a month before this presentation. *E. coli* YDC637 was resistant to ceftazidime, ceftriaxone, ciprofloxacin, trimethoprim-sulfamethoxazole, amikacin, gentamicin, and tobramycin and susceptible to cefepime, imipenem, and meropenem. Of note, inhibitory zones were not present around amikacin, gentamicin, and tobramycin disks, which suggested high-level aminoglycoside resistance conferred by the production of 16S RMTase.

Phylogenetic typing and multilocus sequence typing (MLST) were conducted as described previously (3, 4); these processes assigned *E. coli* YDC637 to phylogenetic group D and sequence type 354 (ST354). We then conducted a series of PCR amplification reactions to detect a 16S RMTase gene using positive-control strains possessing *armA* and *rmtA* through *rmtH*. As a result, *rmtE* encoding the 16S RMTase RmtE was identified, which was confirmed by sequencing. The plasmid of *E. coli* YDC637 was then extracted by the alkaline lysis method and used to transform *E. coli* TOP10 competent cells, selected by resistance to 50 μg/ml gentamicin. Consequently, a transformant demonstrating high-level resistance to aminoglycosides and harboring an *rmtE*-carrying plasmid (pYDC637) was obtained. The MICs of key aminoglycosides and β-lactams for *E. coli* YDC637 and *E. coli* TOP10 (pYDC637) are shown in Table 1. Of note, the transformant was resistant to ceftazidime in addition to aminoglycosides, but phenotypic testing for the production of extended-spectrum β-lactamase (ESBL) was negative. This resistance was explained by the presence of $bla_{CMY-2}$, the gene for plasmid-mediated CMY-2 cephalosporinase, which was determined by PCR and sequencing (5). The size of this plasmid carrying *rmtE* and $bla_{CMY-2}$ was estimated to be approximately 195 kb by S1 nuclease pulsed-field gel electrophoresis (PFGE), and its replicon type was determined to be IncA/C (6). In addition to aminoglycosides and expanded-spectrum cephalosporins, this plasmid conferred resistance to tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole.

**TABLE 1** MICs of *E. coli* YDC637, its transformant, and control strains

| Antimicrobial agent | MIC (μg/ml) for: *E. coli* YDC637 | *E. coli* TOP10(pYDC637) | *E. coli* TOP10 |
|---|---|---|---|
| Imipenem | 0.38 | 0.50 | 0.38 |
| Ertapenem | 0.25 | 0.032 | 0.006 |
| Ceftazidime | 24 | 24 | 0.5 |
| Cefotaxime | ≥32 | 8 | 0.094 |
| Piperacillin-tazobactam | 2 | 2 | 1.5 |
| Gentamicin | ≥256 | ≥256 | 0.5 |
| Tobramycin | ≥1,024 | 512 | 0.75 |
| Amikacin | ≥256 | ≥256 | 1.0 |

Phylogenetic group D ST354 *E. coli* has been identified in association with CMY-2 production in humans (7) and a stray dog (8). The incompatibility group of the plasmids was not reported in these studies, but $bla_{CMY-2}$ is known to be most commonly encoded on IncA/C plasmids, followed by IncI1 plasmids (9). One may therefore hypothesize that this gene was acquired by an IncA/C, $bla_{CMY-2}$-carrying plasmid in *E. coli* YDC637.

In summary, we here report a group D ST354 *E. coli* coproducing RmtE and CMY-2 that caused invasive human disease. The IncA/C plasmid carrying *rmtE* and $bla_{CMY-2}$ also conferred resistance to tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole in addition to aminoglycosides and cephalosporins, making it an exceptionally multidrug-resistant plasmid.

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