ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2008, p. 1204–1205 Vol. 52, No. 3
0066-4804/08/$08.00+0 doi:10.1128/AAC.01320-07

Letters to the Editor

Escherichia coli Isolate Coproducing 16S rRNA Methylase and CTX-M-Type Extended-Spectrum β-Lactamase Isolated from an Outpatient in the United States▽

High-level resistance to aminoglycosides mediated by the production of 16S rRNA methylase among various gram-negative pathogens has been increasingly reported (4). Six 16S rRNA methylase enzymes have been identified: RmtA to RmtD, ArmA, and NpmA (4, 9). We recently described the emergence of ArmA among *Acinetobacter baumannii* strains in North America (3). Here, we report the isolation of an *Escherichia coli* strain coproducing 16S rRNA methylase RmtB and extended-spectrum β-lactamase (ESBL) CTX-M-65 from an ambulatory female with a history of sickle-cell anemia. *E. coli* grew in a urine culture ordered as part of her evaluation for chronic kidney disease. A review of the records indicated that she had been hospitalized briefly 4 months earlier, but the urine culture did not grow *E. coli* during or after that hospitalization. This is the first instance in which RmtB has been shown to be encoded on the same plasmid as an ESBL.

The isolate, *E. coli* ECRB1, was highly resistant to cefotaxime (MIC, 64 μg/ml) and gentamicin, tobramycin, and amikacin (MICs, >256 μg/ml) but was susceptible to trimethoprim-sulfamethoxazole and ciprofloxacin by Etest (AB Biodisk, Solna, Sweden). Transconjugants were obtained by the broth mating method on Luria-Bertani plates containing 50 μg of rifampin/ml and 50 μg of amikacin/ml by using *E. coli* XL1-Blue Rif^r as the recipient. Transformants of *E. coli* DH10B were obtained on Luria-Bertani plates containing 50 μg of amikacin/ml following electroporation with plasmids purified from the parental strain. The MICs of cefotaxime (96 μg/ml) and the three aminoglycosides (≥128 μg/ml) were high for both the transconjugant and transformant strains. The results of PCR analysis of whole-cell lysates for CTX-M genes (6) were positive for both strains. Direct sequencing of the entire structural gene amplified from the transformant confirmed it to encode CTX-M-65, a variant of CTX-M-14. Multiplex PCR analysis of the transconjugant and transformant strains for 16S rRNA methylase genes (4) revealed the presence of *rmtB*. The results of PCR analysis for *qepA*, a quinolone efflux pump gene reported to be carried on the same plasmid as *rmtB* (10), were negative for the transconjugant and transformant strains.

A 2.9-kb BamHI insert containing *rmtB* could be cloned from the transformant into vector pBCSK(−) (Stratagene, La Jolla, CA) with *E. coli* DH10B as the host. This plasmid conferred a high level of resistance to all three aminoglycosides tested (MICs, >256 μg/ml). Full sequencing of this insert revealed a genetic arrangement similar to those reported earlier (5, 10) in that the insert contained bla_{TEM-1} as part of Tn*3* upstream from *rmtB* (Fig. 1). However, the putative transposase gene previously found downstream of *rmtB* was replaced by the 3′ end of another transposase gene comprising IS*26*.

CTX-M-type ESBLs are increasingly associated with community-acquired *E. coli* infections throughout the world, including the United States (2, 8). *rmtB* has been found in strains carrying bla_{CTX-M} but, unlike *armA* (1, 7, 11), on a separate plasmid from bla_{CTX-M}. Our findings indicate, however, that *rmtB* may be captured by the same conjugative plasmid carrying bla_{CTX-M}. The spread of such multidrug resistance plasmids among *E. coli* strains has a potential impact on the empirical management of complicated urinary tract infections that may be treated initially with cephalosporins and aminoglycosides.

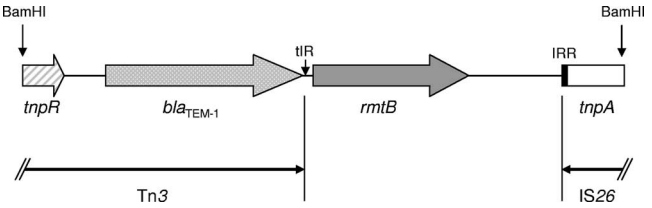

FIG. 1. Genetic support of *rmtB*. *rmtB* is flanked by Tn*3* and IS*26*. *tnpR*, resolvase gene; *tnpA*, transposase gene; tIR, terminal inverted repeat of Tn*3* (5′-CTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCC-3′); IRR, right inverted repeat of IS*26* (5′-GGCACTGTTGCAAA-3′).

Nucleotide sequence accession number. The sequences determined in this work have been deposited in GenBank under accession no. EU213261 and EU213262.

Y.D. is supported by NIH training grant T32 AI007333. D.L.P. has received prior research funding from Pfizer, Elan, Merck, and AstraZeneca and is supported in part by NIH research grant R01 AI070896.

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▽ Published ahead of print on 14 January 2008.