

Prevalence and molecular characteristics of 16S rRNA methylase gene *rmtB* in amikacin resistant *Escherichia coli* isolated from South Korea

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Abstract: The production of *rmtB*-encoded 16S rRNA methylases has emerged as a novel mechanism promoting high-level resistance toward aminoglycosides in Gram-negative bacteria. Between 2015 and 2017, 636 distinct commensal *Escherichia* (*E.*) *coli* isolates were collected from different farms in South Korea to determine the prevalence and molecular characteristics of *rmtB*. The positive rates of *rmtB* between all the isolates and amikacin-resistant isolates were 1.1 and 100%, respectively. High-level aminoglycoside resistance could be transferred by conjugation from *rmtB*-positive donors to higher amikacin-resistance efficacies. This is the first report of 16S rRNA methylase-encoding genes in *E. coli* isolated from food-producing animals in Korea.

Keywords: *Escherichia coli*, 16S RMTase gene, amikacin resistance, transferability

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The mechanism of the clinically significant aminoglycosides has long been known to be associated with their ability to bind to the bacterial 30S ribosomal subunit and interfere with bacterial protein translation [1]. Serious infections caused by Gram-negative bacteria can often be effectively treated with a combination of aminoglycoside- β -lactam agents [2]. However, the clinical significance and effectiveness of aminoglycosides have been challenged by different bacterial resistance mechanisms. Among the globally disseminated aminoglycoside resistance mechanisms, acquired 16S ribosomal RNA methyltransferases (16S RMTases) in Gram-negative bacteria are the most worrisome due to their ability to compromise the activity of all aminoglycosides [3]. 16S RMTases confer resistance to aminoglycosides by methylating a nucleotide in the aminoglycoside attachment region located in the A-site of the 16S rRNA using S-adenyosyl-L-methionine [2].

The aminoglycoside binding site of the specific nucleotide residues, either the N-7 position of nucleotide G1405 or the N-1 position of nucleotide A1408, in 16S rRNA can be enzymatically modified by various 16S rRNA methyltransferases [4,5]. There are ten acquired N7-G1405 16S RMTase-encoding genes (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtD2*, *rmtE*, *rmtF*, *rmtG*, and *rmtH*), although only one acquired N1-A1408 16S RMTase gene (*npmA*) exclusively confers resistance to aminoglycosides. The first encoded 16S RMTase was discovered in Japan in 1997 on a plasmid harboring *rmtA* in an AGA-resistant *Pseudomonas aeruginosa* strain [6]. Later, in the early 2000s, significant progress in the field of genetic analytical techniques led to discovery of another member of the 16S rRNA methylase gene family, *armA*, in *Klebsiella pneumoniae* isolated from a hospitalized patient in France [7]. Recently, O'Hara et al. [8] identified *rmtH* from a clinical *K. pneumoniae* strain in a wounded male USA soldier returning from the Iraq war.

Despite exhibiting a low clinical prevalence, the clinical significance of 16S RMTases is increasing globally, posing considerable potential health risks due to 16S RMTases being able to confer resistance to many clinically important aminoglycosides, including amikacin. The predominant 16S RMTases are currently encoded by *rmtB* and *armA*, which have spread worldwide to resistant

bacterial strains isolated from humans and livestock [9,10]. In South Korea, 16S RMTase genes has been reported from human clinical *E. coli* isolates, with the most commonly encountered 16S RMTase genes responsible for aminoglycoside-resistance being *armA* and *rmtB* [11].

However, the prevalence of high-level aminoglycoside resistance mediated by 16S rRNA methylases among *E. coli* strains isolated from food-producing animals has not been investigated. In this study, we describe the identification of *rmtB* in a commensal *E. coli* strain recovered from healthy animals. Thus, the aim of this study was to investigate the prevalence of *rmtB* among *E. coli* isolates collected in South Korea and to gain insights into the molecular characterization of these *rmtB*-positive strains.

We screened 636 commensal *E. coli* isolates recovered from fecal samples obtained from clinically healthy animals (341 from cattle, 265 from swine, and 30 from chickens) during a nationwide surveillance study on antimicrobial susceptibility conducted between 2014 and 2017. Fecal samples were freshly collected from cattle, swine, and chickens, and *E. coli* isolates were obtained by culturing on MacConkey and Eosin methylene blue agar.

MIC testing was conducted according to the recommendations of the Clinical Laboratory Standards Institute (CLSI) (2015), and the resulting MIC values were interpreted using CLSI standards. An inoculum of 10^5 CFU/mL was incubated in Mueller-Hinton broth (MHB) in the presence of two-fold serial dilutions of antimicrobials, with the MIC defined as the lowest concentration of antibiotic that completely inhibited visible growth. The following antimicrobial agents were tested in the described range of concentrations (mg/L): amikacin (0.25-512), streptomycin (0.125-128), gentamicin (0.125-128), kanamycin(0.25-256), neomycin (0.125-256), tobramycin (0.125-256), tetracycline (0.25-512), ampicillin (0.125-256), minocycline (0.25-512), and cephalothin (0.125-128).

PCR amplification to investigate the 16S RMTase-encoding genes (*rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE*, *armA*, and *npmA*) along with other resistance mechanisms was carried on

strains displaying phenotypic resistance for amikacin by employing primer pairs reported in previous studies [4,12,13].

Conjugation experiments were performed to determine the transferability of 16S rRNA methylase genes between the donor and recipient strains using the broth mating technique. The transconjugants were selected for and confirmed by PCR. The MICs for recipient, transconjugant, and donor strains were determined using the broth microdilution method with procedures and interpretation guidelines of the CLSI [14].

High-level amikacin resistance (MIC \geq 512 μ g/mL) was observed in 7 (1.1%) of the 636 assayed *E. coli* isolates. Of the investigated 16S rRNA methylase genes, only *rmtB* was detected in all 7 isolates, whereas all the isolates tested negative for the *armA*, *rmtA*, *rmtC*, *rmtD*, *rmtE*, and *npmA* genes (Table 1). The conjugation assay results confirmed the successful transfer of amikacin resistance to 3 of the 7 *rmtB*-positive isolates by conjugation to the azide-resistant *E. coli* strain J53. The presence of the *rmtB* gene was confirmed by PCR in 3 of the transconjugants, which showed the same resistance profiles for amikacin (MIC \geq 512 μ g/mL) as the donor isolates (Table 2). The three transconjugants were selected with amikacin at a conjugation frequency of 1.73×10^{-2} to 5.1×10^{-3} (number of transconjugants divided by the number of donor cells) (Table 3).

In addition to impermeability and multidrug-active efflux systems, the primary mechanism of aminoglycoside resistance involves modification of the target region by enzymes that are collectively known as aminoglycoside modifying enzymes (AMEs) [3]. We previously reported that commensal *E. coli* isolates from food-producing animals in Korea have a tremendous ability to harbor and transfer AME genes [15]. More recently, 16S rRNA methylases have become an important public health threat due to their ability to confer high-level and broad-spectrum resistance to most clinically relevant aminoglycosides [9]. To the best of our knowledge, we here report for the first time a molecular epidemiological based investigation of 16S rRNAmethylase gene, *rmtB*, carrying *E. coli* strains in Korea. All 7 *rmtB*-carrying isolates

Table 1. MICs, phylogroups and antimicrobial resistance gene profiles for amikacin-resistant *Escherichia coli* isolates of animal origin from South Korea collected between 2015 and 2017

Isolates	Year of isolation	Phylogenetic group	Origin	Amikacin MIC	16S rRNA methyltransferase gene	Other resistance genes
16S-242	2016	D	Pig	\geq 512 μ g/mL	<i>rmtB</i>	<i>TEM</i> , <i>aph3'-1a</i>
DCA4324	2017	D	Pig	\geq 512 μ g/mL	<i>rmtB</i>	<i>tetA</i> , <i>sul1</i> , <i>sul2</i> , <i>TEM</i> , <i>floR</i> , <i>StrAB</i>
DCA4358	2017	A	Pig	\geq 512 μ g/mL	<i>rmtB</i>	<i>tetA</i> , <i>tetB</i> , <i>sul1</i> , <i>sul2</i> , <i>TEM</i> , <i>cat</i> , <i>StrAB</i> , <i>aadB</i> , <i>aph3'-1a</i>
DCA4403	2017	A	Pig	\geq 512 μ g/mL	<i>rmtB</i>	<i>tetA</i> , <i>sul2</i> , <i>TEM</i> , <i>aph3'-1a</i>
EC504	2015	A	Pig	\geq 512 μ g/mL	<i>rmtB</i>	<i>tetB</i> , <i>sul1</i> , <i>sul2</i> , <i>sul3</i> , <i>TEM</i> , <i>cat</i> , <i>StrAB</i> , <i>aph3'-1a</i>
EC716	2015	A	Pig	\geq 512 μ g/mL	<i>rmtB</i>	<i>tetA</i> , <i>sul1</i> , <i>sul2</i> , <i>sul3</i> , <i>TEM</i> , <i>floR</i> , <i>StrAB</i> , <i>aph3'-1a</i>
EC766	2015	A	Pig	\geq 512 μ g/mL	<i>rmtB</i>	<i>tetB</i> , <i>sul1</i> , <i>sul2</i> , <i>TEM</i> , <i>cat</i> , <i>StrAB</i> , <i>aadB</i> , <i>aph3'-1a</i>

Table 2. Antimicrobial resistance profile of *rmtB*-harboring *Escherichia coli* transconjugants

Transconjugants	16S rRNA methylase gene	MIC value ($\mu\text{g/mL}$)					MEM
		AMK	CAZ/CLA	CAZ	CTX	CTX/CLA	
TC504	<i>rmtB</i>	≥ 512	0.5	0.5	0.5	0.25	0.06
TC716	<i>rmtB</i>	≥ 512	0.5	0.25	1	1	0.06
TC358	<i>rmtB</i>	≥ 512	0.25	0.5	1	1	0.06
ATCC29212 [#]	-	2	0.25	0.25	1	0.5	0.03

AMK, amikacin; CAZ/CLA, ceftazidime-clavulanic acid; CTX/CLA, cefotaxime-clavulanic acid; MEM, meropenem; ATCC, American Type Culture Collection.

Table 3. Transferability and conjugation frequencies of *rmtB*-harboring *Escherichia coli* isolates from food-producing animals in South Korea

Isolates	Number of CFU/ μL of isolates	Transferability	Number of CFU/ μL of transconjugants	Conjugation efficiency
16S-242	$2.54 \times 10^6/\mu\text{L}$	-	-	-
DCA4324	$2.48 \times 10^6/\mu\text{L}$	-	-	-
DCA4358	$2.54 \times 10^6/\mu\text{L}$	+	$4.4 \times 10^4/\mu\text{L}$	1.73×10^{-2}
DCA4403	$2.5 \times 10^5/\mu\text{L}$	-	-	-
EC504	$2.66 \times 10^6/\mu\text{L}$	+	$1.92 \times 10^5/\mu\text{L}$	7.2×10^{-2}
EC716	$3.35 \times 10^6/\mu\text{L}$	+	$1.7 \times 10^4/\mu\text{L}$	5.1×10^{-3}
EC766	$2.78 \times 10^6/\mu\text{L}$	-	-	-
<i>E. coli</i> J53	$1.28 \times 10^6/\mu\text{L}$	Recipient	Recipient	

were highly resistant to amikacin (MIC value of $\geq 512 \mu\text{g/mL}$), a resistance phenotype consistent with production of a 16S RMTase.

In this study, the overall prevalence (1.1%) of 16S rRNA methylase genes in *E. coli* isolates was similar to that previously reported in a Taiwanese study (0.4%) [16], but lower than was observed in a study from China (5.4%) [17]. Generally, a low relatively low prevalence of acquired 16S RMTases has been reported, with lower prevalence rates of 1% or less having been reported in different regions of the world [18].

Although we investigated all the known 16S RMTase genes, we only detected the *rmtB* gene, which is the most prevalent 16S RMTase gene among *Enterobacteriaceae* isolates [4]. 16S rRNA methylase genes have been linked to other resistance determinants, such as AME genes, *tet* genes, *bla*_{TEM}, *cat*, *sul* genes, and *floR* (Table 1). Our data are consistent with those of other studies, indicating that these resistance determinants are co-harbored on a similar plasmid [17]. Amikacin resistance was transferrable from three of seven *rmtB*-positive strains by conjugation. The MICs of amikacin against transconjugants were uniform ($\geq 512 \mu\text{g/mL}$), suggesting a similar expression of the *rmtB* gene in the transconjugants. PCR analyses also confirmed the presence of *rmtB* in three transconjugants. Similarly, a previous study showed that the *rmtB* gene was readily transferable to a recipient strain since the gene can be both plasmid and chromosomally encoded [19].

In conclusion, the prevalence of 16S rRNA methylases, while still low, is increasing and will continue to be reported

globally. Plasmid-mediated 16S rRNA methylase *rmtB* gene was detected among commensal *E. coli* isolates from food-producing animals in South Korea. These results have a strong impact on the treatment options used in both veterinary and human medicine since 16S rRNA methylases can hamper the effectiveness of most clinically relevant aminoglycoside antibiotics.

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