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ArmA and *RmtB* Were the Predominant 16S RMTase Genes Responsible for Aminoglycoside-resistant Isolates in Korea

Tae Hee Lee,[1,2] **Joo-Hee Hwang**,[3,4] **Woo Kon Lee**,[5] **Min-Kyoung Shin**,[5] **Hye Ryun Woo**,[6] **Kyung Min Chung**,[1,2] **and Chang-Seop Lee**[3,4]

[1]Department of Microbiology and Immunology, Chonbuk National University Medical School, Jeonju, Korea
[2]Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Korea
[3]Department of Internal Medicine, Chonbuk National University Medical School, Jeonju, Korea
[4]Biomedical Research Institute of Chonbuk National University Hospital, Jeonju, Korea
[5]Department of Microbiology, College of Medicine, Gyeongsang National University, Jinju, Korea
[6]Department of New Biology, Daegu Gyeongbuk Institute of Science & Technology, Daegu, Korea

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Address for Correspondence:
Kyung Min Chung, PhD
Department of Microbiology and Immunology, Chonbuk National University Medical School, Jeonju 54896, Republic of Korea.
E-mail: kmin@jbnu.ac.kr

Chang-Seop Lee, MD, PhD
Department of Internal Medicine, Chonbuk National University Medical School, Jeonju, Jeonbuk 54896, Republic of Korea.
E-mail: lcsmd@jbnu.ac.kr

ORCID iDs
Tae Hee Lee
https://orcid.org/0000-0003-3049-8252
Joo-Hee Hwang
https://orcid.org/0000-0002-8616-3411
Woo Kon Lee
https://orcid.org/0000-0003-3913-2265
Min-Kyoung Shin
https://orcid.org/0000-0003-1782-5351

ABSTRACT

Pathogenic gram-negatives that produce 16S ribosomal RNA methyltransferases (16S RMTases) have already been distributed all over the world. To investigate the predominance of aminoglycoside resistance associated with 16S RMTases in Korea, we collected a total of 222 amikacin resistant Gram-negative clinical isolates from patient specimens between 1999 and 2015 from three hospital banks across Korea. *ArmA* and *rmtB* were the predominant 16S RMTase genes responsible for aminoglycoside-resistant isolates circulating in Korean community settings although only one *rmtA*-producing isolate was detected in 2006.

Keywords: Aminoglycoside Resistance; *ArmA*; *RmtB*; Korea

Aminoglycosides are one of the key classes of antimicrobial agents used in the treatment of Gram-negative bacterial infections. These agents bind to the highly conserved A site of the 16S rRNA of the bacterial 30S ribosomal subunits, thereby causing interference with bacterial protein synthesis, which ultimately leads to bacterial death.[1] However, the increase in aminoglycoside-resistant Gram-negative bacteria in recent years is a multi-faceted issue requiring urgent attention. Although several pathways that provide resistance to aminoglycoside antibiotics are known, exogenously acquired 16S ribosomal RNA methyltransferases (16S RMTases) have emerged as a major mechanism of high-level resistance to most clinically important aminoglycosides, including arbekacin, amikacin, tobramycin, and gentamicin in Gram-negative pathogens.[1] In 2003, the first acquired 16S RMTase genes, *armA* and *rmtA*, were identified in *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, respectively.[2] Since then, other plasmid-mediated 16S RMTase genes (*rmtB* through *rmtH*, and *npmA*) have been found in clinical isolates.[2-4] Among them, *armA* and *rmtB* have been found in many species of Gram-negative bacilli in Asia.[5]

In a Korean nationwide surveillance, the amikacin resistance rates of *K. pneumoniae* increased from 8% in 1997 to 13% in 2003.[6,7] The frequency of high-level resistance to amikacin or arbekacin was 9.5% (15/158), 10.3% (13/126), and 17.1% (22/129) for *Enterobacter cloacae*, *Citrobacter*

Hye Ryun Woo
https://orcid.org/0000-0002-0546-052X
Kyung Min Chung
https://orcid.org/0000-0001-6414-9187
Chang-Seop Lee
https://orcid.org/0000-0002-2897-2202

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Disclosure

The authors have no potential conflicts of interest to disclose

Author Contributions

Conceptualization: Chung KM, Lee CS. Data curation: Lee TH, Hwang JH, Chung KM, Lee CS. Investigation: Lee TH, Chung KM, Woo HR. Resources: Lee WK, Shin MK, Lee CS. Writing-original draft: Lee TH, Chung KM, Hwang JH, Lee CS. Writing-review & editing: Chung KM, Lee CS.

freundii and *Serratia marcescens* isolates, respectively.[8] In another study, 24 hospitals and two Community Labs participated in collecting antimicrobial susceptibility data in Korea, and the resistance rates of *K. pneumoniae*, *E. cloacae*, *S. marcescens*, *Acinetobacter* spp., and *P. aeruginosa* to amikacin were high in the hospital laboratories: 15%, 5%, 10%, 48%, and 19%, respectively.[9] Taken together, these results suggest that 16S RMTase-producing bacteria are becoming prevalent in Korea and highlight the need for continued surveillance to investigate the overall trend of aminoglycoside resistance among clinically important pathogens. To provide ongoing insight into aminoglycoside resistance, in this study, we investigated predominant 16S RMTases in Korea from our data and from previously published literatures.

To examine the recent predominance of aminoglycoside resistance associated with 16S RMTases in Korea, a total of 222 potential amikacin resistant Gram-negative clinical isolates from patient specimens were collected between 1999 and 2015 from three hospital banks across Korea and primarily identified by each bank through Vitek Mass Spectrometry: 73 isolates from Chonbuk National University Hospital Culture Collection for Pathogens from 2001 to 2013; 88 isolates from Gyeongsang National University Hospital Culture Collection for Pathogens from 1999 to 2015; 61 isolates from Kyungpook National University Hospital Culture Collection for Pathogens from 2012 to 2015. The specimens were from the following sources: 10 peritoneal fluid, 77 blood, 27 open wound, 6 bile acid, 51 urine, 46 sputum and bronchial washing, 3 ear discharge, and 2 pleural fluid. To further confirm amikacin-resistance and determine the minimum inhibitory concentrations (MICs) of amikacin for the collected isolates, we evaluated the susceptibility to amikacin by using the Etest. Briefly, Etest strips (bioMérieux, Marcy-l'Étoile, France) with amikacin were placed on Mueller-Hinton (MH) agar plates lawn-inoculated with suspension of isolates grown to an optical density of 0.5 McFarland units to determine the MICs of amikacin for each isolate. After incubation, MICs were read directly from the Etest strip described previously.[10] We also screened the amikacin-resistant isolates using polymerase chain reaction (PCR) to detect the known 16S RMTase genes *armA*, *rmtA*, *rmtB*, *rmtB2*, *rmtC*, *rmtD*, *rmtE*, *rmtF*, *rmtG*, and *rmtH*. Briefly, DNA template for PCR were prepared from bacterial isolates cultured in LB broth containing amikacin (10 μg/mL) and previously described oligonucleotides were used to amplify gene fragments from 16S RMTases.[11,12] The PCR products were sequenced, and the sequences were compared with those in the GenBank nucleotide database. A total of 198 (89.2%) isolates were confirmed as amikacin-resistant (> 16 μg/mL) among the 222 collected isolates primarily confirmed as potential aminoglycoside-resistance at the three hospitals in Korea (**Table 1**). Among them, 190 (96.0%) were highly resistant to amikacin (> 256 μg/mL), a resistance phenotype

Table 1. 16S rRNA methyltransferases in amikacin-resistant Gram-negative clinical isolates

Clinical isolates	No. of samples	No. of amikacin resistance (> 16 μg/mL)[a]	No. of high amikacin resistance (> 256 μg/mL)[a]	16S RMTase (No.)
Acinetobacter baumanii	49	45	44	*armA* (40)
Pseudomonas aeruginosa	41	30	28	*armA* (4)
Citrobacter freundii	4	3	3	*armA* (3)
Enterobacter aerogenes	5	3	3	*armA* (3)
E. cloacae	3	3	3	*armA* (3)
Escherichia coli	15	13	11	*armA* (2)
Klebsiella oxytoca	6	5	5	*armA* (5)
K. pneumoniae	84	82	79	*armA* (68)/*rmtB* (1)
Proteus mirabilis	7	6	6	*armA* (5)
Serratia marcescens	8	8	8	*armA* (8)
Total	222	198	190	*armA* (141)/*rmtB* (1)

16S RMTase = 16S ribosomal RNA methyltransferase.
[a]Values in parentheses indicate the concentrations of amikacin using.

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consistent with production of 16S RMTase. By PCR, 142 (74.7%) of the high level-amikacin resistant bacteria were positive for 16S RMTase genes; 141 *armA* and one *rmtB*.

To further investigate whether aminoglycoside resistance was transferable, plasmids from clinical isolates with high-level resistance to amikacin (> 256 μg/mL) and with 16S RMTase genes (*armA* or *rmtB*) were prepared using the alkaline lysis method and were electro-transformed into *Escherichia coli* TOP10 competent cells (Invitrogen, Carlsbad, TX, USA). Transformants were selected on LB agar plates containing 50 μg/mL gentamicin, and gentamicin-resistant transformants were tested for susceptibility to amikacin (256 μg/mL). The plasmids from transformants with both gentamicin and amikacin resistance were isolated, used as template DNAs for the PCR-based detection of 16S RMTases, and analyzed against the GenBank nucleotide database, as described above. Of six *armA* and one *rmtB* from the high-level amikacin-resistant isolates tested, all transferred resistance to amikacin through plasmids.

We reviewed 419 articles using the keywords "16S rRNA methyltransferase" or "16S methyltransferase" or "Korea" in PubMed for articles published from 1974 to 2017 to study overall trends over time of 16S RMTases from clinical isolates in Korea. A total of 10 papers related to 16S RMTases from clinical isolates were published in Korea from 2006 to 2017 (**Table 2**).[7,8,13-20] Among 331 amikacin resistant gram-negative organism (63 *E. coli*; 149

Table 2. Ten published 16S rRNA methyltransferases articles related to clinical isolates from Korea

Ref. No.	Reported year	Year of sample collection	Sample selection of bacterial strains	Clinical isolates (No.)	16S RMTases (No.)
7	2006[a]	2003/2005	Amikacin resistant isolates	*E. coli* (11)	*armA* (3)
				K. pneumoniae (26)	*armA* (14)/*rmtB* (1)
				C. freundii (4)	*armA* (1)
				E. cloacae (4)	*armA* (2)
				S. marcescens (8)	*armA* (4)
				Acinetobacter spp. (30)	*armA* (14)
				P. aeruginosa (27)	None
8	2006	March–July 2003	High-level resistant to amikacin	*E. cloacae* (15)	*armA* (13)
				C. freundii (13)	*armA* (12)/*rmtB* (1)
				S. marcescens (22)	*armA* (21)
13	2007	2006	ESBL producing isolates	*K. pneumoniae* (44)	*armA* (30)
14	2007[a]	July 2004	Amikacin resistant isolates	*Providencia* spp. (3)	*armA* (1)
15	2008[a]	1995–1998/2001–2006	Amikacin resistant isolates	*E. coli* (52)	*ArmA* (28)/*rmtB* (18)
				K. pneumoniae (123)	*armA* (88)/*rmtB* (32)
				K. spp. (2)	*armA* (2)
				E. cloacae (23)	*armA* (19)
				Enteroabcter spp. (7)	*armA* (6)
				S. marcescens (6)	*armA* (6)
				C. freundii (4)	*armA* (3)/*rmtB* (1)
				M. morganii (1)	*armA* (1)
16	2009[a]	1995–2005	High-level resistant to amikacin	*K. pneumoniae* (40)	*armA* (28)/*rmtB* (9)
				E. coli (25)	*armA* (10)/*rmtB* (9)
				E. cloacae (18)	*armA* (14)
				S. marcescens (6)	*armA* (6)
				C. freundii (3)	*armA* (3)
				Enterobacter sakazakii (1)	*armA* (1)
				Klebsiella oxytoca (1)	*armA* (1)
				M. morganii (1)	*armA* (1)
17	2009[a]	2005–2006	MDR isolates	*P. aeruginosa* (11)	*rmtA* (1)
18	2009	2005	Isolates from urine	*E. coli* (264)	*armA* (4)/*rmtB* (1)
19	2010[a]	2008–2009	MDR isolates	*P. aeruginosa* (100)	*armA* (14)
20	2013	2010	MDR isolates	*A. baumannii* (31)	*armA* (28)

Acinetobacter spp. = *Acinetobacter* species, *Providencia* spp. = *Providencia* species, *K.* spp. = *Klebsiella* species, *Enterobacter* spp. = *Enterobacter* species, ESBL = extended spectrum beta lactamase, MDR = multidrug resistant.
[a]From Kyungpook National University Hospital.

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K. pneumoniae; 2 *K.* spp.; 8 *C. freundii*; 27 *E. cloacae*; 7 *E.* spp.; 14 *S. marcescens*; 30 *Acinetobacter* spp.; 27 *P. aeruginosa*; 3 *Providencia* spp.; 1 *Morganella morganii*), *armA* was 192 and *rmtB* was 52 isolates.[7,14,15] In 145 high-level amikacin resistant isolates, *armA* was 110 and *rmtB* was 19 isolates.[8,16] In the other 5 articles, *armA* was detected 30 of 44 extended spectrum β-lactamase producing *K. pneumonia*, 14 of 111 multi-drug resistant (MDR) *P. aeruginosa*, 28 of 31 MDR *Acinetobacter baumannii*, 4 of 264 isolates from urines.[13,17,19,20] One *rmtB* was from urinary isolate *E. coli*.[18] Interestingly, only one *P. aeruginosa* isolate with *rmtA* was reported in 2006.[17]

Taken together, this study provides recent information regarding the 16S RMTase genes responsible for aminoglycoside-resistant isolates circulating in Korean community settings. *ArmA* and *rmtB* were the predominant 16S RMTase genes, and one RmtA-producing isolate was detected back in 2006 but not since then. Given that *armA* and *rmtB* have been found worldwide and that other 16S RMTase genes (*rmtA, rmtC, rmtD, rmtF, rmtG* and *rmtH*) are regionally spread, further studies with a larger number of clinical isolates are needed to confirm the presence of a variety of 16S RMTases among aminoglycoside-resistant bacteria in Korea.

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