



HAL
open science

In vitro activity of apramycin against 16S-RMTase-producing Gram-negative isolates

François Caméléna, Mathilde Liberge, Inès Rezzoug, Manel Merimèche,
Thierry Naas, Béatrice Berçot

► To cite this version:

François Caméléna, Mathilde Liberge, Inès Rezzoug, Manel Merimèche, Thierry Naas, et al.. In vitro activity of apramycin against 16S-RMTase-producing Gram-negative isolates. *Journal of Global Antimicrobial Resistance*, 2023, 33, pp.21-25. 10.1016/j.jgar.2023.02.005 . hal-04088355

HAL Id: hal-04088355

<https://u-paris.hal.science/hal-04088355v1>

Submitted on 4 May 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0
International License



In vitro activity of apramycin against 16S-RMTase-producing Gram-negative isolates

François Caméléna^{a,b,1}, Mathilde Liberge^{a,b,1}, Inès Rezzoug^a, Manel Merimèche^{a,b},
Thierry Naas^{c,d,e}, Béatrice Berçot^{a,b,*}

^a Department of Bacteriology, Saint-Louis-Lariboisière Hospital Group, Assistance Publique-Hôpitaux de Paris, Paris, France

^b Université de Paris Cité, INSERM 1137, IAME, Paris, France

^c Department of Bacteriology-Hygiene, Bicêtre Hospital, Assistance Publique - Hôpitaux de Paris, Le Kremlin-Bicêtre, France

^d Université de Paris-Saclay, INSERM 1184, RESIST Unit, Le Kremlin-Bicêtre, France

^e French National Reference Centre for Antimicrobial Resistance, Le Kremlin-Bicêtre, France

ARTICLE INFO

Article history:

Received 17 November 2022

Revised 10 January 2023

Accepted 2 February 2023

Available online 22 February 2023

Editor by: Stefania Stefani

Keywords:

Aminoglycosides

16S rRNA methyltransferases

ArmA

RmtB

AAC(3)-IV

Gram-negative bacilli

ABSTRACT

Objectives: Apramycin is an aminoglycoside (AG) with a unique structure that is little affected by plasmid-mediated mechanisms of AG resistance, including most AG-modifying enzymes and 16S rRNA methyltransferases (16S-RMTases). We evaluate the activity of apramycin against a collection of 16S-RMTase-producing isolates, including Enterobacterales, non-fermenting bacteria, and carbapenemase producers.

Methods: In total, 164 non-duplicate 16S-RMTase-producing isolates, including 84 Enterobacterales, 53 *Acinetobacter baumannii* and 27 *Pseudomonas aeruginosa* isolates, were included in the study. Whole-genome sequencing (WGS) was performed on all isolates with Illumina technology. The minimum inhibitory concentration (MIC) of apramycin was determined by broth microdilution with customized Sensititre plates (Thermo Fisher Scientific, Dardilly, France).

Results: We found that 95% (156/164) of the 16S-RMTase-producing isolates were susceptible to apramycin, with a MIC₅₀ of 4 mg/L and a MIC₉₀ of 16 mg/L, respectively. Resistance rates were higher in *P. aeruginosa* (11%) than in *A. baumannii* (4%) or Enterobacterales (4%) ($P < 0.0001$ for each comparison). Eight isolates were resistant to apramycin, including one isolate with an MIC >64 mg/L due to the acquisition of the *aac(3)-IV* gene. The genetic environment of the *aac(3)-IV* gene was similar to that in the pAH01–4 plasmid of an *Escherichia coli* isolate from chicken in China.

Conclusion: Resistance to apramycin remains rare in 16S-RMTase-producing isolates. Apramycin may, therefore, be an interesting alternative treatment for infections caused by 16S-RMTase and carbapenemase producers.

© 2023 Published by Elsevier Ltd on behalf of International Society for Antimicrobial Chemotherapy.

This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

1. Introduction

Aminoglycosides (AGs) are broad-spectrum antibiotics used to treat severe infections caused by Gram-positive and Gram-negative bacteria (GNB). The most common mechanism of resistance to AGs involves AG-modifying enzymes (AMEs), which are very frequent in invasive bacteria [1]. However, AME-encoding genes do not confer resistance to all clinically relevant AGs (amikacin, gen-

tamicin, tobramycin, and plazomicin), even in the carbapenemase-producing GNB of the ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species) group [2]. Indeed, plazomicin remains active in most GNB, even carbapenem-resistant Enterobacterales [2].

Several studies have documented the worldwide emergence of 16S rRNA methyltransferase (16S-RMTase)-producing isolates [3,4]. These enzymes methylate the 16S ribosomal RNA at the G1405 or A1408 residue (thereby conferring a high level of resistance to all clinically relevant AGs) and are mostly encoded by plasmids [3]. Since their first description in Enterobacterales in the first decade of the 21st century, 11 different acquired 16S-RMTases (ArmA,

* Corresponding author. Department of Bacteriology, Saint-Louis Hospital, Assistance Publique - Hôpitaux de Paris, 1 av. Claude Vellefaux 75010 Paris, France

E-mail address: beatrice.bercot@aphp.fr (B. Berçot).

¹ These authors contributed equally to this work.

RmtA-H, NpmA-B) have been described [3]. ArmA and RmtB, the predominant 16S-RMTase enzymes, have spread worldwide among Enterobacterales and *Acinetobacter baumannii*, whereas the distribution of these genes in *Pseudomonas aeruginosa* is more heterogeneous. The prevalence of these genes varies around the world, with a higher prevalence in Asia. However, recent reports have suggested that isolates producing both 16S-RMTases and carbapenemases are emerging in Europe and Asia, further limiting the options for antibiotic treatment [4–7].

Apramycin is an aminocyclitol aminoglycoside used in veterinary practice for the treatment of colibacillosis, salmonellosis, and enteritis in farm animals [8]. Its structure differs slightly from that of classical AGs, as it has a monosubstituted deoxystreptomine (DOS) moiety compared with the classical AGs (2,6–2-DOS) mentioned above [3]. This unique structure preserves the activity of apramycin against GNB-carrying genes encoding AMEs or 16S-RMTases, with the exception of the *aac(3)-IV* gene, the AAC(2)-encoding gene *apmA*, and genes encoding the rarely found 16S-RMTases methylating the A1408 residue of the 16S rRNA (i.e. *npmA* and *npmB*) [3,9,10]. Apramycin and its derivatives may, therefore, constitute a promising subclass of AGs that could be developed for use in humans. Indeed, EBL-1003 (Juvabis AG, Switzerland), a crystalline free base of apramycin, is currently being developed for use in humans, and a phase I clinical trial has already been launched to assess its potential for use in human medicine (ClinicalTrials.gov NCT04105205).

Few studies have evaluated the in vitro activity of apramycin against Enterobacterales, and even fewer have considered its activity against *P. aeruginosa* and *A. baumannii* isolates [11–13]. Enterobacterales are highly susceptible to apramycin but increasing rates of resistance have been reported in carbapenemase-producing isolates [14]. For *P. aeruginosa* and *A. baumannii*, the minimum inhibitory concentration (MIC) of apramycin seems to be higher, particularly in multidrug-resistant (MDR) isolates [12,13]. There are some data on the susceptibility to apramycin of 16S-RMTase producers, but these studies mainly focused on few bacterial species (Enterobacterales) and on a limited number of strains [13,15]. Although the most common 16S-RMTases do not affect the activity of apramycin, little is known about the presence of apramycin resistance genes in 16S-RMTase producers.

In this study, we evaluated apramycin activity against a collection of 16S-RMTase-producing GNB isolates, including isolates that also produced carbapenemase, by broth microdilution susceptibility testing and whole-genome sequencing (WGS).

2. Materials and methods

2.1. Isolates and 16S-RMTase

We analysed a collection of 164 clinical isolates of GNB expressing a 16S-RMTase cultured from rectal swabs ($n = 69$), urine ($n = 29$), cutaneous samples ($n = 23$), blood ($n = 21$), respiratory samples ($n = 11$), and other samples ($n = 11$). Each clinical isolate (according to the bacterial species) came from a single patient. There were 84 Enterobacterales (49 *Escherichia coli*, 23 *Klebsiella* spp., 5 *Citrobacter freundii*, 2 *Proteus mirabilis*, 3 *Enterobacter cloacae*, and 2 *Providencia stuartii*), 53 *A. baumannii*, and 27 *P. aeruginosa* isolates. The sequences of the 16S-RMTase genes of 134 isolates from the Saint-Louis-Lariboisière-Fernand Widal Hospital Group were obtained by WGS with Illumina technology, as previously described [16]. Briefly, DNA was isolated with automated QIAsymphony extraction (QIAGEN, Crawley, UK) using the DSP DNA Mini Kit (QIAGEN) according to the manufacturer's instructions. The Nextera DNA Sample Preparation Kit (Illumina, San Diego, CA) was used to prepare libraries for sequencing. Samples were sequenced on an Illumina NextSeq or MiSeq sequencer, for 300 cy-

cles (150 paired-end reads). All the WGS reads were assembled de novo with SPAdes v3.14. The resistome was evaluated for each isolate with CARD and ResFinder databases. Multilocus sequence typing (MLST) was deduced as described by the protocols of the PubMLST. Sequence data were already available for the other 30 isolates [16].

The 16S-RMTase genes present in the Enterobacterales isolates were *rmtB* in 35 isolates, *armA* in 32, *rmtC* in 11, *rmtF* in 5, and *rmtB* plus *rmtF* in 1 isolate. All the *A. baumannii* isolates producing 16S-RMTases carried *armA*. The 16S-RMTase genes present in *P. aeruginosa* were *rmtF* in 20 isolates, *rmtB* in five, *rmtD* in one, and *armA* in one isolate. The genome of the *E. coli* PT65 isolate, for which the MIC of apramycin was >64 mg/L, was annotated with Prokka and contigs were screened for replicon plasmid content with PlasmidFinder. Further information about isolates is provided in Supplementary Table S1. The genome sequence of isolate PT65 has been deposited in GenBank under BioSample no. SAMN29986909 and BioProject no. PRJNA862645.

2.2. Antimicrobial susceptibility testing

The methods used to determine carbapenems susceptibility were agar disc diffusion (Bio-Rad, Marnes La Coquette, France), MIC gradient strips (E-test, bioMérieux, Marcy l'Etoile, France), or broth microdilution (Sensititre, Thermo Fisher Scientific, Les Ulis, France). The MIC of apramycin was determined by broth microdilution with customized Sensititre plates (Thermo Fisher Scientific). No EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoints are available for apramycin. We therefore used epidemiological breakpoints from the European Food Safety Authority (EFSA) for *E. coli* for Enterobacterales isolates (susceptible ≤ 8 mg/L and resistant > 8 mg/L) and epidemiological cut-off values for *A. baumannii* were applied for non-fermenting bacteria, including *P. aeruginosa* (susceptible ≤ 16 mg/L and resistant > 16 mg/L) [13,15]. *Escherichia coli* strain ATCC 25922 was used as a quality control strain for susceptibility testing. For all 164 isolates, the MICs of gentamicin, amikacin, and tobramycin were determined with MIC gradient strips (E-test, bioMérieux, Marcy l'Etoile, France) to confirm the 16S-RMTase phenotype [i.e. high levels of resistance (MIC ≥ 256 mg/L) to these three AGs].

2.3. Statistical analysis

All statistical analyses were performed with Prism software (Graphpad, San Diego, US). A non-parametric Wilcoxon rank sum was used for comparisons between groups.

3. Results

3.1. Overall apramycin susceptibility

The MIC of apramycin was determined for 164 16S-RMTase-producing isolates: 84 Enterobacterales, 53 *A. baumannii*, and 27 *P. aeruginosa* isolates, including 38, 53, and 4, respectively, producing carbapenemase (Supplementary Table S1). Resistance to carbapenems was observed in 71% (116/164) of the isolates. The distribution of the MIC of apramycin is summarized in Table 1. Overall, the antimicrobial drug susceptibility testing results showed that 95% (156/164) of the 16S-RMTase-producing isolates were susceptible to apramycin, with a MIC₅₀ of 4 mg/L and a MIC₉₀ of 16 mg/L. The MIC of apramycin was significantly higher in non-fermenting bacteria than in Enterobacterales ($P < 0.0001$, Supplementary Fig. S1). For Enterobacterales isolates, the MIC₉₀ was 8 mg/L, with only three isolates having values above the susceptibility breakpoint, including one isolate with an MIC > 64 mg/L (PT65) (Table 1). The MIC of apramycin was similar between isolates that did and

Table 1
Minimum inhibitory concentration (MIC) distributions for apramycin for the 164 non-duplicate 16S-RMTase-producing isolates

Isolates	No. of isolates	MIC ₅₀	MIC ₉₀	Min.	Max.	Resistance ^a n (%)	MIC (mg/L) of apramycin							
							1	2	4	8	16	32	64	>64
All	164	4	16	1	>64	8 (4.9)	3	25	57	33	39	4	2	1
Enterobacterales ^b	84	4	8	1	>64	3 (3.6)	3	23	45	10	1*		1*	1*
ArmA	32	4	8	1	>64	2 (6.3)	2	10	14	4			1*	1*
RmtB	35	4	8	1	8	1 (2.9)	1	2	25	6	1*			
RmtC	11	2	4	2	4	0 (0)		6	5					
RmtF	5	2	4	2	4	0 (0)		4	1					
RmtB + RmtF	1	-	-	2	2	0 (0)		1						
Non-fermenting bacteria	80	16	16	2	64	5 (6.3)	2	12	23	38	4*	1*		
<i>Acinetobacter baumannii</i>	53	8	16	2	64	2 (3.8)	1	12	21	17	1*	1*		
ArmA	53	8	16	2	64	2 (3.8)	1	12	21	17	1*	1*		
<i>Pseudomonas aeruginosa</i>	27	16	32	2	32	3 (11.1)	1	2	21	3*				
RmtB	5	16	32	2	32	1 (20)	1		3	1*				
RmtF	20	16	32	8	32	2 (10)			1	17	2*			
RmtD	1	-	-	-	-	0 (0)				1				
ArmA	1	-	-	-	-	0 (0)				1				

^a The interpretation criteria for apramycin resistance were as follows: MIC >8 mg/L for Enterobacterales and >16 mg/L for *A. baumannii* and *P. aeruginosa*.

^b Enterobacterales were 49 *Escherichia coli*, 23 *Klebsiella* Spp., 5 *Citrobacter freundii*, 2 *Proteus mirabilis*, 3 *Enterobacter cloacae*, and 2 *Providencia stuartii*.

* Isolates resistant to apramycin.

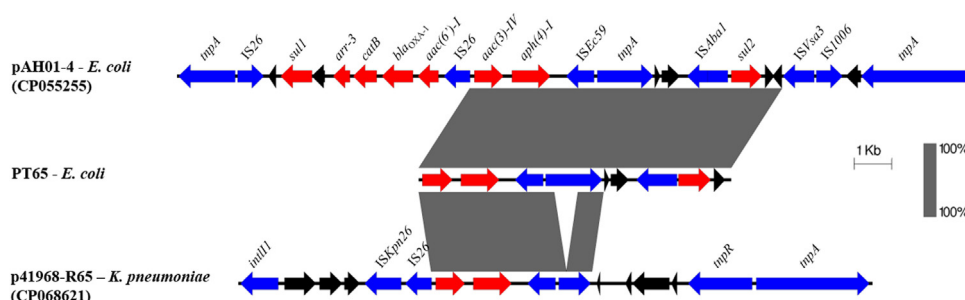


Fig. 1. Genetic structures surrounding the *aac(3)-IV* gene in the *E. coli* PT65 isolate and comparison with plasmids pAH01-4 and p41968-R65. Arrows indicate ORFs and their orientations. The resistance genes are shown in red, mobile genetic elements are shown in blue, and other ORFs are shown in black. Gray shading indicates homologous regions and their degree of sequence identity. Graphical representations were generated with Easyfig v 2.2.2.

did not produce ArmA ($P = 0.47$) (Supplementary Fig. S2, Supplementary Table S2). Apramycin susceptibility rates varied between species, with values of 96% for *E. coli*, 100% for *Klebsiella* Spp., and 92% for other species (including *C. freundii*, *E. cloacae*, *P. mirabilis*, and *P. stuartii*). In non-fermenting bacteria, the mean MIC₉₀ was 16 mg/L, and five isolates were considered resistant (two *A. baumannii* and three *P. aeruginosa* isolates). MIC values were significantly higher in *P. aeruginosa* than in *A. baumannii* ($P < 0.0001$) with MIC₉₀ values of 32 mg/L and 16 mg/L, respectively (Supplementary Fig. S1).

3.2. Isolates resistant to apramycin

The eight apramycin-resistant isolates consisted of three *P. aeruginosa*, two *A. baumannii*, two *E. coli*, and one *C. freundii* isolate. In previous studies, plasmid-mediated apramycin resistance was attributed principally to the presence of an AME-encoding gene, *aac(3)-IV*, conferring high-level resistance [9]. Here, we found only one *E. coli* isolate (PT65) harbouring the *aac(3)-IV* gene, with an MIC of apramycin >64 mg/L. For the other seven apramycin-resistant isolates (MICs of 16–64 mg/L), we detected no *aac(3)-IV* gene or any other acquired apramycin resistance determinant, including the 16S-RMTase genes *npmA* and *npmB* or the AAC(2) gene *apmA*.

3.3. Characterization of PT65 isolate harbouring *aac(3)-IV* gene

Genomic characterization of the PT65 isolate harbouring the *aac(3)-IV* gene revealed that this *E. coli* isolate belonged to ST156

and carried IncFIB, IncFII, and IncY replicon plasmids. ST156 belongs to phylogroup B1, which contains both human and animal isolates. The *aac(3)-IV* gene was localized in a contig of 8.3 kb that also carried the resistance determinants *aph(4)-I* and *sul*, and multiple mobile genetic elements. The sequence of this contig was 100% identical to sequences from the 146 kb pAH01-4 plasmid (Accession no. CP055255) found in *E. coli* ST602, also belonging to phylogroup B1, which was obtained from a chicken in China. It differed from the recently reported environment of *aac(3)-IV* in the 66 kb p41968-R65 plasmid (Accession no. CP068621), which was obtained from a carbapenem-resistant *K. pneumoniae* ST258 isolate (Fig. 1) [14]. In these genetic environments, the *aac(3)-IV* gene was embedded in a region containing multiples copies of IS6 family elements (IS26, ISEc59, and IS1006).

4. Discussion

We found that apramycin remained active against various 16S-RMTase-producing isolates, including carbapenemase-producing isolates. However, the MIC of apramycin was significantly lower in Enterobacterales than in non-fermenting bacteria, particularly in *P. aeruginosa*. The MIC₅₀s were 4 mg/L for Enterobacterales, 8 mg/L for *A. baumannii*, and 16 mg/L for *P. aeruginosa*, which represent only a 2- to 4-fold increase of apramycin MIC between these bacterial species. Eight isolates (5%, 8/164) were considered resistant to apramycin, mostly non-fermenting bacteria. We identified the *aac(3)-IV* apramycin resistance gene in a single apramycin resistant isolate (PT65), which had the highest MIC (>64 mg/L) observed in

the study. For the seven remaining isolates, genomic characterization failed to identify apramycin resistance determinants previously described. A few isolates with high MICs to apramycin but without the identification of apramycin resistance determinants have been described in other studies [14,15]. We suspect that the resistance mechanisms, such as active efflux and/or reduced outer membrane permeability to AGs, probably contribute to apramycin resistance in these isolates, but further studies are required to address this point.

Our results are consistent with those of previous studies evaluating apramycin on large collections of Enterobacterales and *A. baumannii* isolates, including MDR isolates; however, these previous studies included only a small number of 16S-RMTase producers [13,17]. In our study, the resistance rate in *P. aeruginosa* (11%) was higher than that in other bacteria species (3.6% in Enterobacterales and 3.8% in *A. baumannii*), but we were unable to identify a species-specific resistance determinant. Two studies have reported MIC values for apramycin for MDR *P. aeruginosa*, with susceptibility rates (for a susceptibility breakpoint at ≤ 16 mg/L) ranging from 64% (28/44) to 89% (8/9) of the isolates tested [12,18]. However, no apramycin resistance determinant was identified to explain the resistance phenotype observed in these isolates. Recently, Bolard et al. showed that single amino acid substitutions in elongation factor EF-G1A encoded by *fusA1* gene in *P. aeruginosa*, a key compound of translational machinery, was implicated in AG resistance resulting in an 8- to 16-fold increase in the apramycin MIC [19]. However, no amino acid substitution was detected in the three apramycin-resistant *P. aeruginosa* isolates, hence further studies are needed to elucidate the mechanism of this resistance.

Resistance to apramycin was rare in our collection, with the *aac(3)-IV* gene detected in only one isolate, with an apramycin MIC >64 mg/L. Consistent with our findings, Plattner et al. identified apramycin resistance genes in only 0.7% of the clinical isolate genome sequences in the NCBI National Database of Antibiotic Resistant Organisms, almost exclusively *aac(3)-IV* genes. As a comparison, they identified 16S-RMTase genes in 2.7% of these genomes, suggesting a low level of spread of apramycin resistance genes in bacteria [9].

The genetic environment surrounding *aac(3)-IV* in MLPA65 was identical to sequences from the pAH01–4 plasmid found in *E. coli* ST602. Interestingly, the *aac(3)-IV* gene was flanked by IS26 and ISEc59, which both belonged to IS6 family elements, suggesting possible transposition mediated by these elements [20]. However, none of the IS6 family elements in pAH01–4 contain the 8-bp target site duplications expected for recent transposition, suggesting that this structure may result from homologous recombination.

Apramycin remained active against most of 16S-RMTase-producing isolates, including carbapenemase-coproducing isolates. The efficacy could be better for the treatment of infections due to Enterobacterales, as their apramycin MICs were significantly lower than those of non-fermenting bacteria, particularly in *P. aeruginosa*. Apramycin could be an interesting alternative treatment for infections caused by 16S-RMTase-producing bacteria.

Funding

This work was supported by internal funding

Contributions

FC and BB conceived and designed the study. FC, ML, IR, and MM performed the research. FC, ML, MM, and BB analysed the data. ML and FC wrote the manuscript. All the authors critically revised the manuscript.

Declaration of competing interest

None declared

Acknowledgments

We would like to thank the laboratory staff for carrying out Illumina sequencing of the bacterial isolates. We also wish to thank Julie Sappa of Alex Edelman and Associates for careful reading and English language editing of the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2023.02.005.

References

- [1] Ramirez MS, Tolmasky ME. Aminoglycoside modifying enzymes. Drug Resist Updat 2010;13:151–71. doi:10.1016/j.drup.2010.08.003.
- [2] Castanheira M, Deshpande LM, Woosley LN, Serio AW, Krause KM, Flamm RK. Activity of plazomicin compared with other aminoglycosides against isolates from European and adjacent countries, including Enterobacteriaceae molecularly characterized for aminoglycoside-modifying enzymes and other resistance mechanisms. J Antimicrob Chemother 2018;73:3346–54. doi:10.1093/jac/dky344.
- [3] Wachino J-I, Doi Y, Arakawa Y. Aminoglycoside resistance: updates with a focus on acquired 16S ribosomal RNA methyltransferases. Infect Dis Clin North Am 2020;34:887–902. doi:10.1016/j.idc.2020.06.002.
- [4] Taylor E, Bal AM, Balakrishnan I, Brown NM, Burns P, Clark M, et al. A prospective surveillance study to determine the prevalence of 16S rRNA methyltransferase-producing Gram-negative bacteria in the UK. J Antimicrob Chemother 2021;76:2428–36. doi:10.1093/jac/dkab186.
- [5] Taylor E, Jauneikaite E, Sriskandan S, Woodford N, Hopkins KL. Detection and characterisation of 16S rRNA methyltransferase-producing *Pseudomonas aeruginosa* from the UK and Republic of Ireland from 2003–2015. Int J Antimicrob Agents 2022;59:106550. doi:10.1016/j.ijantimicag.2022.106550.
- [6] Taylor E, Jauneikaite E, Sriskandan S, Woodford N, Hopkins KL. Novel 16S rRNA methyltransferase RmtE3 in *Acinetobacter baumannii* ST79. J Med Microbiol 2022;71:1–6. doi:10.1099/jmm.0.001531.
- [7] Xia Q, Wang H, Zhang A, Wang T, Zhang Y. Prevalence of 16S rRNA methylase conferring high-level aminoglycoside resistance in *Escherichia coli* in China. Int J Antimicrob Agents 2011;37:387–8. doi:10.1016/j.ijantimicag.2011.01.004.
- [8] Livermore DM, Mushtaq S, Warner M, Zhang J-C, Maharjan S, Doumith M, et al. Activity of aminoglycosides, including ACHN-490, against carbapenem-resistant Enterobacteriaceae isolates. J Antimicrob Chemother 2011;66:48–53. doi:10.1093/jac/dkq408.
- [9] Plattner M, Gysin M, Haldimann K, Becker K, Hobbie SN. Epidemiologic, phenotypic, and structural characterization of minoglycoside-resistance gene *aac(3)-IV*. Int J Mol Sci 2020;21:6133. doi:10.3390/ijms21176133.
- [10] Bordeleau E, Stogios PJ, Evdokimova E, Koteva K, Savchenko A, Wright GD. *ApmA* is a unique aminoglycoside antibiotic acetyltransferase that inactivates apramycin. MBio 2021;12:1–11. doi:10.1128/mBio.02705-20.
- [11] Fernández-Martínez M, Miró E, Ortega A, Bou G, González-López JJ, Oliver A, et al. Molecular identification of aminoglycoside-modifying enzymes in clinical isolates of *Escherichia coli* resistant to amoxicillin/clavulanic acid isolated in Spain. Int J Antimicrob Agents 2015;46:157–63. doi:10.1016/j.ijantimicag.2015.03.008.
- [12] Kang AD, Smith KP, Eliopoulos GM, Berg AH, McCoy C, Kirby JE. In vitro apramycin activity against multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Diagn Microbiol Infect Dis 2017;88:188–91. doi:10.1016/j.diagmicrobio.2017.03.006.
- [13] Juhas M, Widlake E, Teo J, Huseby DL, Tyrrell JM, Polikanov YS, et al. In vitro activity of apramycin against multidrug-, carbapenem- and aminoglycoside-resistant enterobacteriaceae and *Acinetobacter baumannii*. J Antimicrob Chemother 2019;74:944–52. doi:10.1093/jac/dky546.
- [14] Hao M, Schuyler J, Zhang H, Shashkina E, Du H, Fouts DE, et al. Apramycin resistance in epidemic carbapenem-resistant *Klebsiella pneumoniae* ST258 strains. J Antimicrob Chemother 2021;76:2017–23. doi:10.1093/jac/dkab131.
- [15] Roch M, Sierra R, Sands K, Martins WMBS, Schrenzel J, Walsh TR, et al. Vertical and horizontal dissemination of an IncC plasmid harbouring rmtB 16S rRNA methylase gene, conferring resistance to plazomicin, among invasive ST258 and ST16 KPC-producing *Klebsiella pneumoniae*. J Glob Antimicrob Resist 2021;24:183–9. doi:10.1016/j.jgar.2020.12.006.
- [16] Caméléna F, Morel F, Merimèche M, Decousser J-W, Jacquier H, Clermont O, et al. Genomic characterization of 16S rRNA methyltransferase-producing *Escherichia coli* isolates from the Parisian area, France. J Antimicrob Chemother 2020;75:1726–35. doi:10.1093/jac/dkaa105.
- [17] Gysin M, Hon PY, Tan P, Sengduanghachanh A, Simmalavong M, Hinthong P, et al. Apramycin susceptibility of multidrug-resistant Gram-negative blood culture isolates in five countries in Southeast Asia. Int J Antimicrob Agents 2022;60:106659. doi:10.1016/j.ijantimicag.2022.106659.

- [18] Becker K, Cao S, Nilsson A, Erlandsson M, Hotop S-K, Kuka J, et al. Antibacterial activity of apramycin at acidic pH warrants wide therapeutic window in the treatment of complicated urinary tract infections and acute pyelonephritis. *EBioMedicine* 2021;73:103652. doi:[10.1016/j.ebiom.2021.103652](https://doi.org/10.1016/j.ebiom.2021.103652).
- [19] Bolard A, Plésiat P, Jeannot K. Mutations in gene *fusA1* as a novel mechanism of aminoglycoside resistance in clinical strains of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2018;62:1–10. doi:[10.1128/AAC.01835-17](https://doi.org/10.1128/AAC.01835-17).
- [20] Varani A, He S, Siguier P, Ross K, Chandler M. The IS6 family, a clinically important group of insertion sequences including IS26. *Mob DNA* 2021;12:11. doi:[10.1186/s13100-021-00239-x](https://doi.org/10.1186/s13100-021-00239-x).