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Aminoglycoside Resistance:

Updates with a Focus on Acquired 16S Ribosomal RNA Methyltransferases

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INTRODUCTION

The worldwide spread of antibiotic-resistant bacteria, collectively called the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii*, Pseudomonas aeruginosa, and Enterobacter spp) pathogens, has become a major public health concern because of the shortage of effective antimicrobial agents available for treatment.[1] Increasing resistance to β-lactams (penicillins, cephems, monobactams, and carbapenems), fluoroquinolones, and aminoglycosides has become a serious clinical threat because of their heavy use in the treatment of gram-negative infections. Gram-negative bacteria have developed resistance to b-lactams, aminoglycosides, and fluoroquinolones through the production of various β-lactamases, aminoglycoside-modifying enzymes (AMEs)/16S ribosomal RNA (rRNA) methyltransferases (MTases), and substituting key amino acid residues in the QRDRs (quinolone resistance–determining regions) of DNA gyrase (GyrA) and topoisomerase IV (ParC), respectively.[2]

In the 1980s, the use of aminoglycosides became increasingly avoided because of ototoxicity and nephrotoxicity, and was subsequently replaced with β-lactams and fluoroquinolones, which had less toxicity and broader antibacterial spectra. However, with the rapid increase of β-lactam–resistant and fluoroquinolone-resistant bacteria, the clinical usefulness of aminoglycosides has now been revisited, together with an improvement in their safety

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CONFLICT OF INTEREST

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through optimized dosing regimens, as an effective choice in the combined drug therapy against a range of resistant gram-negative bacterial infections.[3]

Streptomycin, produced by *Streptomyces griseus*, was the first clinically introduced aminoglycoside, reported by Jones and colleagues[4] in 1944, followed by neomycin, which was discovered from *Streptomyces fradiae* by Waksman and Lechevalier in 1949.[5] In 1957, Umezawa and colleagues[6] reported kanamycin from *Streptomyces kanamyceticus*, which proved to be effective in treating tuberculosis. Subsequently, gentamicin (1963) and tobramycin (1967) were identified from soil *Actinomycetes*.[7] Kasugamycin, composed of an inositol, an amino sugar, and an amidine carboxylic acid, was also discovered by Umezawa and colleagues,[8] in the 1960s, and this aminoglycoside was used in large amounts in agriculture to treat and prevent rice blast, but not in humans because of its toxicity. Semisynthetic aminoglycosides, such as amikacin (1972, kanamycin A–based), arbekacin (1973, kanamycin B/dibekacin-based), and isepamicin (1977, gentamicin B–based), which have potent activity against both gram-negative and gram-positive bacteria, were further developed.[7]

Clinically available aminoglycosides are structurally classified into 2 major classes: those with a 2-deoxystreptamine (2-DOS) core moiety and those without (eg, streptomycin) (Fig. 1). In addition, aminoglycosides with a 2-DOS core moiety are divided into subgroups, 4,5-disubstituted 2-DOS (neomycin, ribostamycin, paromomycin) and 4,6-disubstituted 2-DOS (kanamycin, gentamicin, tobramycin, amikacin, arbekacin, isepamicin), based on the substituent linkage position (see Fig. 1).

ACTION OF AMINOGLYCOSIDES AND AMINOGLYCOSIDE RESISTANCE MECHANISMS

Aminoglycosides with a 2-DOS core primarily bind to helix 44 of the 16S rRNA comprising bacterial 30S ribosomal subunits. Aminoglycoside binding causes various disruptions in protein synthesis: disturbing transfer RNA (tRNA) translocation, lowering translational fidelity, interfering with the ribosome subunit mobility, disturbing ribosome recycling, and interfering with the formation of intersubunit bridges.[9–14] Aminoglycosides also bind to, and may disturb protein synthesis at, helix 69 of the 23S rRNA in 50S ribosomal subunits.[12,14,15]

Bacteria resist aminoglycosides through a variety of intrinsic and acquired resistance mechanisms.[16] Base mutations within the A site of 16S rRNA, amino acid substitutions in ribosomal proteins, and activated efflux pumps are classic intrinsic aminoglycoside resistance mechanisms in pathogenic bacteria. Production of AMEs, either intrinsic or acquired, is the most common aminoglycoside resistance mechanism. AMEs are divided into 3 groups: acetyltransferase (AAC), phosphotransferase (APH), and adenylyltransferase (AAD or ANT). These AMEs modify NH_3 or OH groups at several positions in aminoglycosides, using cofactors, acetyl–coenzyme A or ATP, thereby deactivating them. The most clinically significant resistance mechanism is acquired 16S rRNA methyltransferase (MTase), because these confer high-level and broad-spectrum aminoglycoside resistance by adding a CH_3 group to specific residues within the A site of 16S rRNA using S-adenosylmethionine (SAM) as the cofactor (Fig. 2). The binding

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affinity of certain aminoglycosides to the CH_3-added 16S rRNA is predicted to be significantly reduced compared with that of the original 16S rRNA, resulting in high-level aminoglycoside resistance.

Globally Distributed N7-G1405 16S Ribosomal RNA Methyltransferases ArmA, RmtB, and RmtC

The 16S rRNA MTase gene *armA* was first identified together with *bla*$_{CTX-M3}$ on plasmid pCTX-M3 of *Citrobacter freundii* isolated in 1996, followed by documentation in 2007,[17] and was subsequently found on plasmid pIP1204 of *K pneumoniae* in 2000.[18] The *rmtB* and *rmtC* genes were found on the plasmids of *Serratia marcescens* and *Proteus mirabilis*, respectively, isolated in Japan in the first half of the 2000s.[19,20] Since then, these 3 16S rRNA MTases have been identified globally, primarily found in Enterobacterales, including *Escherichia coli, Klebsiella* spp, *Enterobacter* spp, *S marcescens, Citrobacter* spp, *Proteus* spp, and *Salmonella* spp, isolated from various sources, including humans, livestock, companion animals, and wastewater.[21,22] Regarding glucose-nonfermenting gram-negative bacteria, *armA* has mainly been identified in *A baumannii*, whereas *rmtB*/*rmtC* have rarely been found in that species.[23–26] So far, a few *P aeruginosa* clinical isolates have been reported to carry these 3 16S rRNA MTase genes.[25,27,28] The spread of these MTases has thus far been limited to gram-negative bacteria and has not reached clinically important pathogenic gram-positive bacteria, including *Staphylococcus* spp and *Enterococcus* spp, although the engineered introduction of these MTase genes could confer a high level of aminoglycoside resistance to S aureus, as well as in gram-negative bacteria.[29] One of the clinically concerning issues of these MTase producers is that they tend to also show resistance to β-lactams, fluoroquinolones, polymyxins, and fosfomycin in addition to aminoglycosides through various antimicrobial resistance genetic determinants; for example, β-lactamase genes (*bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{CMY}$, *bla*$_{DHA}$, and *bla*$_{OXA}$), fluoroquinolone-resistance genes (*qnr, aac*(*6′*)*-Ib-cr, qep*, and nucleic mutations in QRDRs of *gyrA*/*parC*), colistin-resistance gene (*mcr*), and the fosfomycin-resistance gene (*fosA*).[21,22,30,31]

ArmA, RmtB, and RmtC 16S rRNA MTases can confer high-level resistance to 4,6-disubstituted 2-DOS (see Fig. 1). The levels of aminoglycoside resistance conferred by these MTases are high (eg, both amikacin and gentamicin have minimum inhibitory concentrations [MICs]   256 μg/mL) compared with those conferred by AMEs.[18–20] These increased MIC values are good indicators of the production of aminoglycoside-resistance 16S rRNA MTases relating to Enterobacterales, *Acinetobacter* spp, and *P aeruginosa*, and can be applied to the initial screening of 16S rRNA MTase producers (discussed later).

ArmA, RmtB, and RmtC 16S rRNA MTases share only modest amino acid identities with each other (up to 30%), but show high three-dimensional structural similarities (Fig. 3).[32,33] These MTases add a CH_3 group to the N7 position of G1405 in 16S rRNA, which causes steric hindrance to the substituent at 3″ position of ring III of 4,6-disubstituted 2-DOS, using SAM as a cofactor.[19,29,33] Moreover, although 16S rRNA MTases recognize the mature 30S ribosomal subunit consisting of 16S rRNA and ribosomal proteins, they do not methylate naked 16S rRNA alone, or mature 70S ribosomes.[29,33]

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N7-G1405 MTases confer resistance to 4,6-disubstituted 2-DOS but not to 4,5-disubstituted 2-DOS or other aminoglycosides (eg, streptomycin and spectinomycin) (Fig. 4). The difference in specificity toward aminoglycosides can be explained based on the binding modes between aminoglycosides and 16S rRNA (see Fig. 4). The N7 position of the G1405 residue is closest to and oriented toward the substituent at 3″ position in the ring III of 4,6-disubstituted 2-DOS. The introduction of a CH_3 residue at the N7 position may lead to a steric clash and/or electrostatic repulsion against the side chain of ring III, leading to reduced binding affinities of aminoglycosides and resulting in increased 4,6-disubstituted 2-DOS resistance. In contrast, ring III and ring IV of 4,5-disubstituted 2-DOS are normally far away from the N7 position of G1405 (see Fig. 4), and the introduction of CH_3 by MTase does not disturb their binding, resulting in almost no change in the MIC values of 4,5-disubstituted 2-DOS (see Fig. 4). Other aminoglycosides, such as streptomycin, that bind to 16S rRNA without interacting with the G1405 position could still bind to 16S rRNA with m^7G1405 and show normal activity.

Sporadic N7-G1405 16S Ribosomal RNA Methyltransferases

Contrary to ArmA, RmtB, and RmtC, the spread of RmtA, RmtD, RmtE, RmtF, RmtG, and RmtH largely remain regional. RmtA has been reported in *P aeruginosa* clinical isolates from East Asian countries, Japan, and South Korea.[34–36] Recently, an RmtA-producing *K pneumoniae* was also isolated in Switzerland, the first identification of RmtA in a species other than *P aeruginosa*.[37]

RmtD (RmtD1 to RmtD3) has mainly been found in *P aeruginosa* and Enterobacterales isolated in South America, Argentina, Chile, and Brazil. Notably, Tada and colleagues[38] and Urbanowicz and colleagues[39] reported RmtD3-producing clinical isolates of *P aeruginosa* from Myanmar and Poland, respectively, indicating that RmtD-group MTase may be starting to spread outside South America.

The number of reports for RmtE (RmtE1 to RmtE3) producers are also limited, 3 from the United States (all *E coli*),[40–43] 1 from China (*E coli*),[44] and the last 1, recently, from Myanmar (*P aeruginosa*).[45] *rmtE*-group genes have also been deposited in the GenBank from *A baumannii* and Enterobacter cloacae complex under accession numbers MH572011 and LC511997, respectively.

RmtF is the most prevalent MTase, after ArmA/RmtB/RmtC. The first identification of RmtF was in *K pneumoniae* isolates in La Réunion Island in 2011,[46] followed by Enterobacterales from India,[47–49] United Kingdom,[47,50] Nepal,[51] South Africa,[52] United States,[53] Australia,[54] Egypt,[55] Switzerland,[56] and Ireland,[50] and *P aeruginosa* from Nepal.[57] One of the clinical risks associated with RmtF producers is that they frequently coproduce NDM-group metallo-β-lactamase, which confers carbapenem resistance.

RmtG producers (all *Klebsiella* spp), which often coproduce *Klebsiella pneumoniae* carbapenemase (KPC), have been reported from Chile,[58] United States,[59,60] Brazil,[61,62] India,[48] and Switzerland.[63] The sources of RmtH producers are limited, 1 in *K pneumoniae* from a patient who had been injured in Iraq,[64] and the other also in *K pneumoniae* from a newborn admitted to a hospital in Lebanon.[65] The enzymatic functions of RmtA, RmtD,

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RmtE, RmtF, RmtG, and RmtH are likely the same as those of ArmA, RmtB, and RmtC, in that they methylate the N7 position of G1405.[66]

N1-A1408 16S Ribosomal RNA Methyltransferase

NpmA was first identified in a clinical isolate of *E coli* (sequence type 131) in Japan.[67] This NpmA-producing *E coli* was identified through selection for high-level resistance to apramycin, a veterinary aminoglycoside. NpmA causes a flip of A1408 from h44 in 30S ribosomal subunits (see Fig. 3B)[68] and modifies the N1-A1408 position in 16S rRNA (and N1-G1408 in 16S rRNA[69]). The N1-A1408 position is proximal to ring I of 4,5-disubstituted, 4,6-disubstituted 2-DOS and apramycin (Fig. 5). Methylation at the N1 position of A1408 can confer broader aminoglycoside resistance than that of the N7-G1405 MTases because the spatial position of ring I remains the same regardless of the structures of aminoglycosides, at least for 4,5-disubstituted, 4,6-disubstituted 2-DOS and apramycin (see Fig. 5). Kanazawa and colleagues[70] recently reported that the introduction of a CH_3 group at the N1 position of A1408 prevents the formation of a pseudopair between the ring I of aminoglycosides and the A1408, especially the positively charged N1 atom that electrically prevents the binding of aminoglycosides carrying the NH_3^1 in ring I (eg, amikacin, gentamicin). Nevertheless, they modeled the mode of binding between aminoglycosides with a 6′-OH group in ring I (eg, paromomycin) and m^{1}A1408 and showed that this class of aminoglycoside might still be active against NpmA producers. The extent of MIC increase of paromomycin was, in fact, limited to only 4-fold by NpmA production (see Fig. 5). Therefore, 4,5-disubstituted 2-DOS with the ring I 6′-OH group may be a good starting point for designing the next generation of aminoglycosides that would be active against 16S rRNA MTase producers.[70]

The *E coli npmA* gene was flanked by 2 copies of IS26 elements and located on 115-kb transferable IncF plasmids. Since the first report of *npmA* in 2007, reports of *npmA* are still rare compared with those of N7-G1405 MTase genes, such as *armA*, *rmtB*, and *rmtC*.[71,72] Notably, NpmA2, which has 1 amino acid difference compared with NpmA, was recently identified from *Clostridioides difficile* (discussed later).

Fitness Costs by 16S Ribosomal RNA Methyltransferase Production in Bacteria

ArmA/RmtB/RmtC are widespread, whereas reports of NpmA have been limited. To explore the difference, some researchers focused on the relationship between the fitness costs of aminoglycoside resistance 16S rRNA MTases and their distribution. Aminoglycoside-resistance 16S rRNA MTases modify the G1405 or A1408 positions, which are close to endogenously methylated residues, C1402 by RsmI and C1407 by RsmF, in *E coli*. Endogenous methylation at G1405 and A1408 may affect the normal process of housekeeping methylation at C1402 and C1407 positions and reduce optimal ribosomal function. G1405 methylation by ArmA production impeded the methylation of the C1402 position, but not C1407, and resulted in growth impairment.[73] In contrast, RmtC impedes methylation at C1407 but is not associated with fitness cost.[74] Although NpmA interfered with the endogenous C1407 methylation, it does not affect cell fitness.[73] Ishizaki and colleagues[75] recently investigated the fitness cost incurred by NpmA production as well and showed low growth rate and cell survival for engineered *E coli* producing NpmA. Overall,

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aminoglycoside-resistance MTases might affect cell fitness cost, but it remains difficult to attribute the difference in the prevalence of N7-G1405 MTase (ArmA, RmtB, RmtC)/N1-A1408 MTase (NpmA) to the fitness costs their production incurs. NpmA confers a lower level of resistance to amikacin and gentamicin compared with N7-G1405 MTases, making it difficult to detect NpmA producers when using frank amikacin and gentamicin resistance as the screening criteria. Screening with apramycin resistance may facilitate identification of more N1-A1408 MTase producers.

Origin of Acquired 16S Ribosomal RNA Methyltransferase Gene

As described earlier, 9 types of acquired N7-G1405 MTases (ArmA, RmtA-RmtH) and N1-A1408 MTase, have thus far been identified in pathogenic gram-negative bacteria. Aminoglycoside-producing Actinomycetales innately possess aminoglycoside-resistance 16S rRNA MTase genes as a self-defense mechanism[22]; however, their G 1 C contents are high, indicating that they are unlikely the direct origin of 16S rRNA MTases of pathogenic bacteria, which have much lower G 1 C contents. Since the first report of N7-G1405 MTases early in the first decade of the 2000s, likely ancestor proteins have not been identified for any of the N7-G1405 MTases. However, Marsh and colleagues[76] recently reported that the potential origin of acquired N1-A1408 16S rRNA MTase gene *npmA* might be the chromosomally encoded 16S rRNA MTase gene carried by some *C difficile* strains. Near-identical nucleotide sequences (99%–100%) were observed between the acquired *npmA* in *E coli* and the chromosomal gene of *C difficile*. NpmA-producing *C difficile* showed a higher level of resistance to aminoglycosides compared with non–NpmA-producing *C difficile*, suggesting that NpmA is associated with aminoglycoside resistance in *C difficile*. However, aminoglycosides are not used in the treatment of *C difficile* infections, and susceptibility breakpoints for *C difficile* have not been defined either. The genetic environment of *E coli npmA* showed little similarity to those found in *C difficile*, whereas some *C difficile* isolates shared 99% genetic identity with each other within the 3-kb regions surrounding *npmA*. It is noteworthy that not every *C difficile* strain has *npmA* on its chromosome. The *npmA* gene found in some *C difficile* isolates might also have been derived from other bacteria.

Screening Methods for 16S Ribosomal RNA Methyltransferase Producers

N7-G1405 MTase producers show high-level resistance to 4,6-disubstitited 2-DOS, such as arbekacin, amikacin, and gentamicin. The MIC values of these 3 aminoglycosides for N7-G1405 MTase producers are mostly greater than 256 μg/mL, and no growth-inhibitory zone is observed around the disks containing these aminoglycosides by the disk diffusion test. Routine microdilution susceptibility testing performed in clinical microbiology laboratories does not generally include high concentrations of aminoglycosides. Thus, a practical approach for screening of potential N7-G1405 MTase producers would be to identify isolates resistant to both amikacin and gentamicin and subject them to manual susceptibility testing, which includes high concentrations of aminoglycosides. This screening strategy is applicable for Enterobacterales, *Acinetobacter* spp, and *P aeruginosa*. In contrast, it must be borne in mind that some nonfermenting gram-negative bacteria, *Pseudomonas* spp, *Burkholderia* spp, and *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* innately show high levels of aminoglycoside resistance and should not be misidentified as aminoglycoside-resistance 16S rRNA MTase producers.

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Because only N1-A1408 MTase producers have so far been detected, it is difficult to discuss screening methods for N1-A1408 MTase producers. In addition, in contrast with N7-G1405 MTase producers, resistance levels toward amikacin and gentamicin conferred by N1-A1408 MTases are similar to those conferred by AMEs. The most remarkable phenotype of N1-A1408 MTase producer is high-level apramycin resistance. The first *E coli* strain producing NpmA was identified through growth on agar plates containing 500 μg/mL apramycin, whereas almost all other tested clinical isolates could not grow on it (Wachino and colleagues, unpublished data, 2005). The only exception was AAC(3)-IV producers, which could also grow on agar plates containing higher concentration of apramycin.

New Aminoglycoside: Plazomicin

Plazomicin (PLZ), initially known as ACHN-490, is a new, semisynthetic, next-generation aminoglycoside (Fig. 6). PLZ is categorized as one of the essential medicines in the World Health Organization (WHO) model list in 2019.[77] This aminoglycoside was developed by Achaogen Co Ltd in 2009 by adding hydroxylaminobutyric acid to sisomicin at the 1 position and the 2-hydroxyethyl group at the 6′ position. PLZ was approved in 2018 by the Food and Drug Administration (FDA) for the treatment of complicated urinary tract infections and acute pyelonephritis. PLZ was designated to avoid modification by a variety of clinically relevant AMEs, thus its effectiveness is expected to be greater than conventional aminoglycosides for aminoglycoside-resistant pathogens producing AMEs (see Fig. 6).

PLZ has shown high potency in in vitro susceptibility testing against gram-negative bacteria.[78] The susceptibility percentage of *E coli, K pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca, E cloacae* complex, and *S marcescens* to PLZ ranged from 97.6% to 100%, and the MIC_{90} (MIC required to inhibit the growth of 90% of organisms) values of PLZ for these Enterobacteriaceae were 0.5 to 1 μg/mL, which is less than the clinical breakpoint of 2 μg/mL approved by FDA.[78] Overall, PLZ was as potent as or superior to other aminoglycosides, including amikacin, gentamicin, and tobramycin. Compared with these Enterobacteriaceae, PLZ was less active against *P mirabilis* and *Morganella morganii*, with 44.3% and 66.7% susceptibility, respectively, and MIC_{90} values of 4 μg/mL for both, similar or inferior to amikacin, gentamicin, and tobramycin.[78] Compared with Enterobacterales, glucose-nonfermenting gram-negative pathogens, including *P aeruginosa* and *A baumannii*, were less susceptible to PLZ, with MIC_{90} values of 16 and 8 μg/mL, respectively.[78] The activity of PLZ was also significantly lower against *S maltophilia*, with an MIC_{90} value of greater than 64 μg/mL,[78] although this organism also showed natural resistance to other aminoglycosides. It is also noteworthy that PLZ is highly active against ESBL-producing *E coli* and *K pneumoniae*, carbapenemase-producing Enterobacteriaceae, and colistin-resistant Enterobacteriaceae, with susceptibility rates of greater than 90%.[78]

Mechanisms of Plazomicin Resistance

PLZ showed potent activity against clinically relevant AME-producing bacteria, as expected, but a small portion of the tested Enterobacterales strains were highly resistant to PLZ.[79] All these resistant bacteria were reported to be 16S rRNA MTase producers. PLZ has the same ring III structure as sisomicin, whose MIC values are very high for N7-G1405 MTase producers. Thus, it is reasonable that N7-G1405 MTase producers show cross-resistance to

PLZ (MIC>256 μg/mL) (see Fig. 6). N1-A1408 MTase producers also confer PLZ resistance (MIC>256 μg/mL),[80] probably through the 2-hydroxyethyl group at the 6^0 position of ring I interfering with m^1A1408 (see Fig. 6).

Cox and colleagues[80] recently reported the detailed behavior of various AMEs toward PLZ. As expected, production of most AMEs tested, including AAC(3)-Ia, AAC(3)-II, AAC(3)-IV, AAC(6′)-Ib, AAC(6′)-Ib-cr, AAC(6′)-Ie-APH(2″)-Ia, AAC(6′)-Ii, ANT(2″)-Ia, ANT(4′)-Ia, APH(2″)-IIa(-Ib), APH(2″)-IVa(-Id), APH(3′)-IIIa, APH(3″)-Ia, APH(4)-Ia, APH(6)-Ia, and APH(9)-Ia in engineered *E coli* BW25113 strain (originally PLZ MIC 2 μg/mL) conferred no or only slight resistance (2-fold to 4-fold increase in MIC) to PLZ. In contrast, only AAC(2′)-Ia showed a 16-fold increase in PLZ MIC. Cox and colleagues[80] modeled the complex structure of AAC(2′)-Ia and PLZ and confirmed the binding mode between them. AAC(2′)-Ia production is likely one of the causes for PLZ resistance, as observed in organisms such as *Providencia stuartii* that possess chromosomally encoded *aac(2′)*-Ia genes.

SUMMARY

Gram-negative bacteria with high-level aminoglycoside resistance caused by the production of 16S rRNA MTases have spread globally and across a variety of environments since their first identification in the early 2000s, and new variants of the 16S rRNA MTase have since emerged. This development is further complicated by the fact that 16S rRNA MTase producers often carry other clinically relevant resistance genes, including carbapenemase genes (eg, bla_{NDM} and bla_{KPC}) and colistin-resistance genes (*mcr*). The threat of 16S rRNA MTase in the emergence and spread of extensive drug resistance and pandrug resistance among pathogenic gram-negative bacteria therefore should not be underestimated.

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KEY POINTS

- Aminoglycoside-resistant gram-negative pathogens producing 16S ribosomal RNA (rRNA) methyltransferase (MTase) have emerged and spread globally.
- 16S rRNA MTase-producing gram-negative pathogens tend to show a multidrug-resistance profile against β-lactams and fluoroquinolones.
- 16S rRNA MTase producers resist the newly approved aminoglycoside, plazomicin.
- Treatment options are limited for infections caused by multidrug-resistant 16S rRNA MTase producers.

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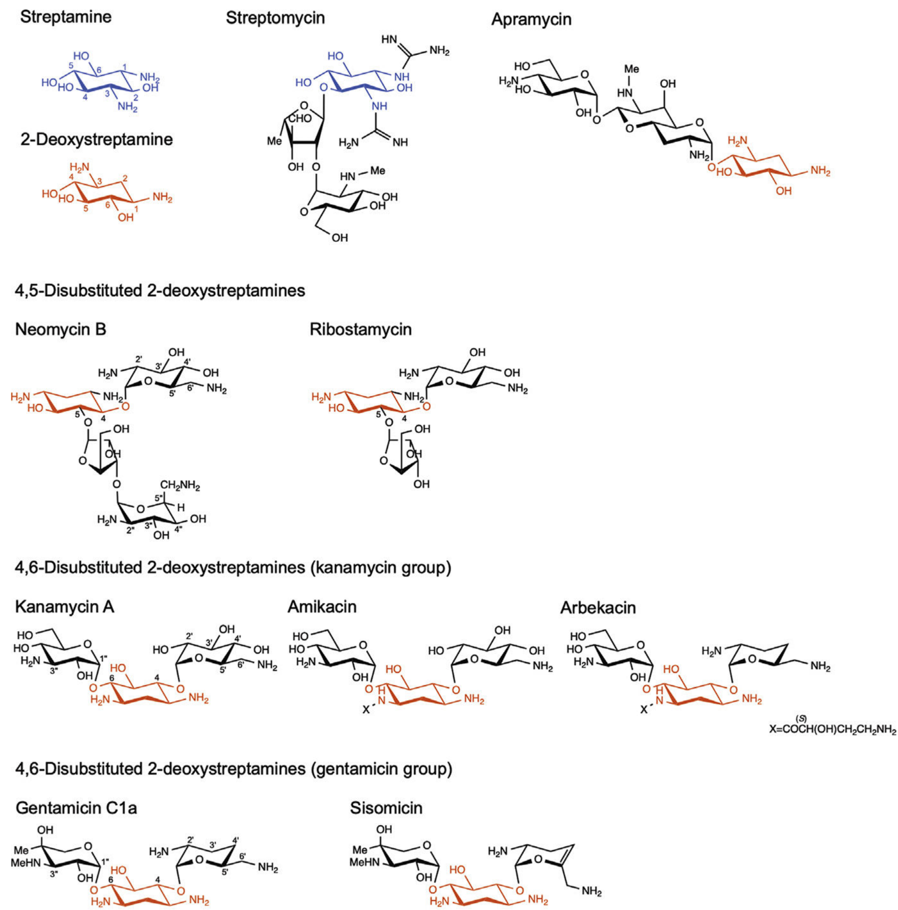

Fig. 1.

Core elements of aminoglycosides and aminoglycoside structures.

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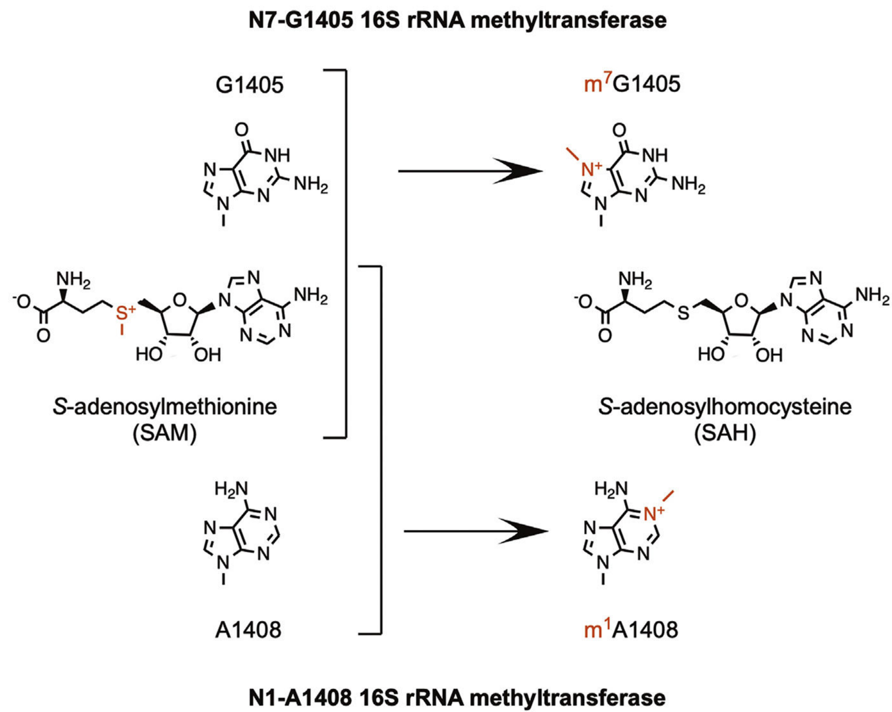

Fig. 2.

Mechanisms of methylation of G1405 and A1408 residues in 16S rRNA by aminoglycoside resistance 16S rRNA MTases.

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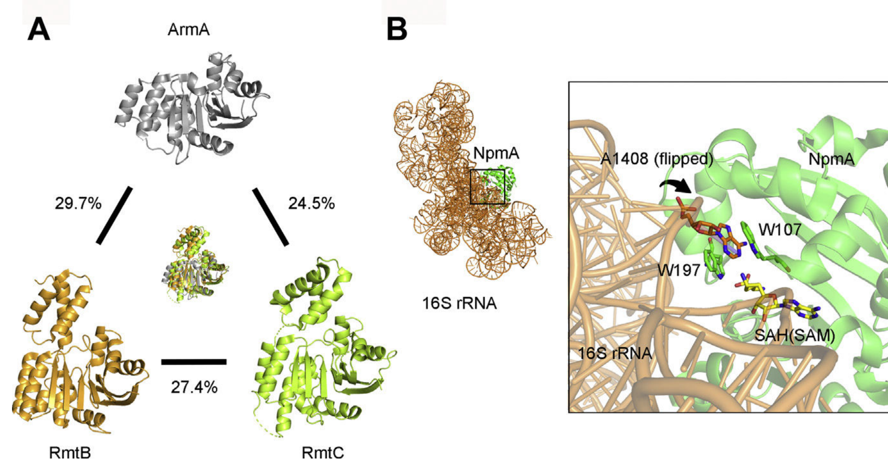

Fig. 3.

(*A*) Three-dimensional structures of ArmA (*gray*), RmtB (*orange*), and RmtC (*light green*). These figures were rendered with Protein Data Bank (PDB) data (PDB identifier [ID], 3FZG, 3FRH, and 6PQB). The percentages indicate amino acid identities. (*B*) Binding mode between 16S rRNA (*orange*) and NpmA (*green*). The S-adenosyl-L-homocysteine (SAH) molecule is shown in yellow sticks and the A1408 residue in orange sticks. The 2 tryptophan residues (W107 and W197) of NpmA are shown in green sticks. This figure was rendered with PDB data (PDB ID, 4OX9).

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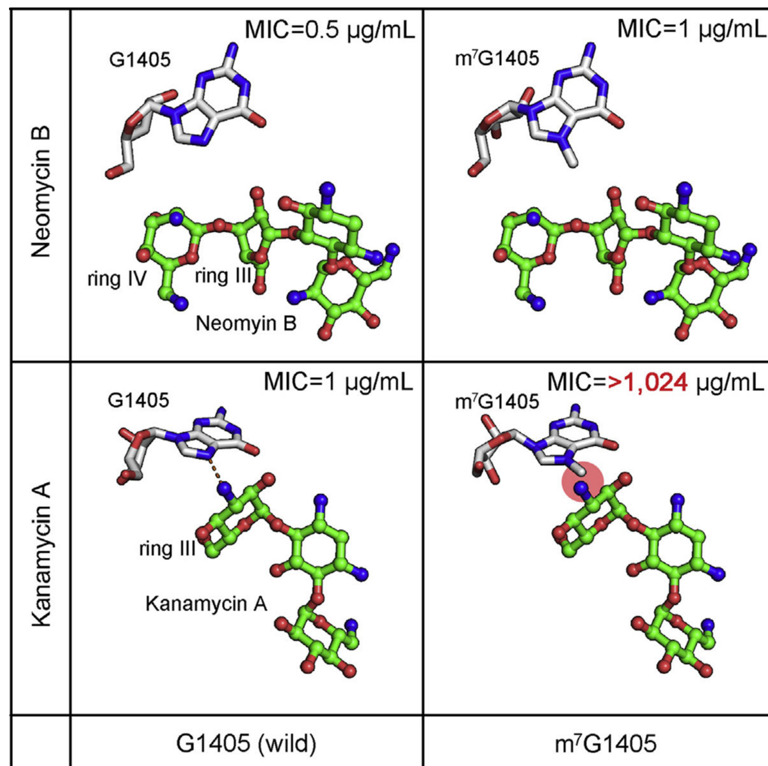

Fig. 4.

Molecular models of binding mode between neomycin B/kanamycin A and wild G1405/ m^7G1405 in 16S rRNA. These figures were rendered based on crystal structures (PDB ID, 2ESI and 2ET4). Basic residues and aminoglycoside molecules are depicted in silver and green sticks, respectively, and the orange dashed lines indicate hydrogen bonds. The red translucent circle indicates the predicted position of the steric clash between the residue and aminoglycoside. MIC values were cited from references.[20] (*From* Wachino J, Yamane K, Shibayama K, et al. Novel plasmid-mediated 16S rRNA methylase, RmtC, found in a *Proteus mirabilis* isolate demonstrating extraordinary high-level resistance against various aminoglycosides. *Antimicrob Agents Chemother* 2006;50(1):178–84 https://doi.org/10.1128/AAC.50.1.178-184.2006; with permission.)

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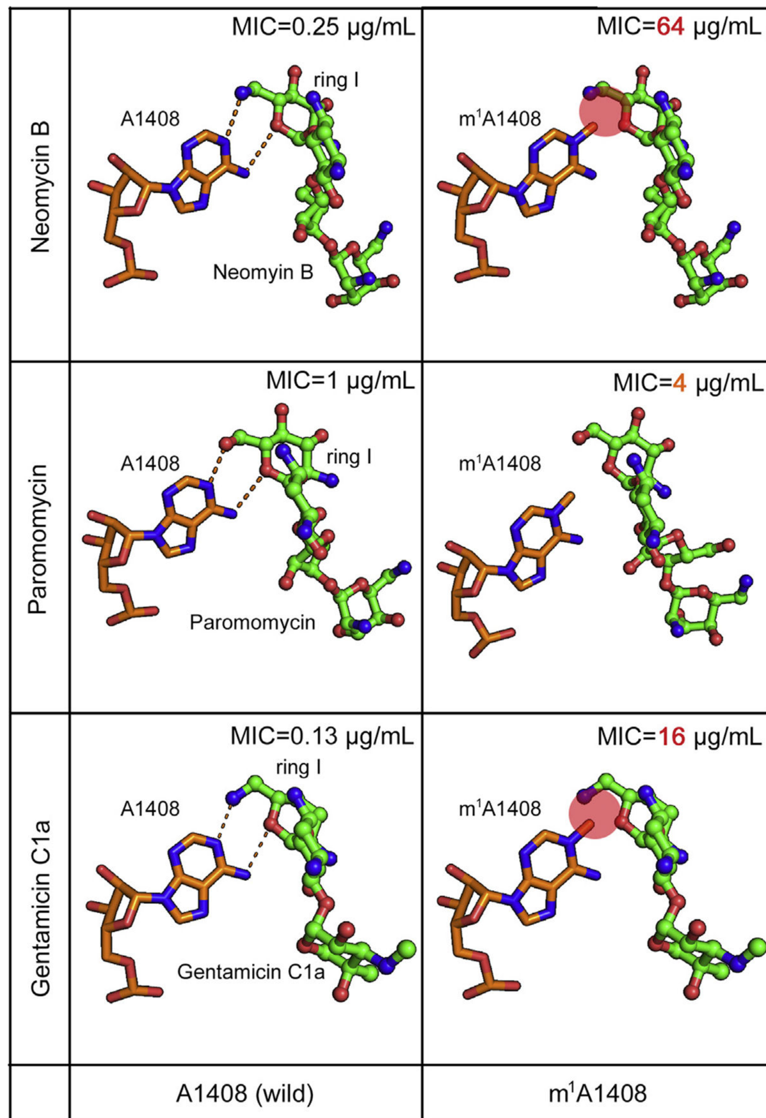

Fig. 5.

Molecular models of binding mode between neomycin B/paromomycin/gentamicin C1a and wild A1408/m^{1}A1408 in 16S rRNA. The figures were rendered based on crystal structures (PDB ID, 2ET4, 5ZEM, 5ZEJ, and 2ET3). Basic residues and aminoglycoside molecules are depicted in orange and green sticks, respectively, and orange dashed lines indicate hydrogen bonds. The red translucent circle indicates the predicted position of the steric clash between the residue and aminoglycoside. MIC values were cited from references.[67] (*From* Wachino J, Shibayama K, Kurokawa H, et al. Novel plasmid-mediated 16S rRNA m1A1408 methyltransferase, NpmA, found in a clinically isolated *Escherichia coli* strain resistant to structurally diverse aminoglycosides. *Antimicrob Agents Chemother* 2007;51(12):4401–9; with permission.)

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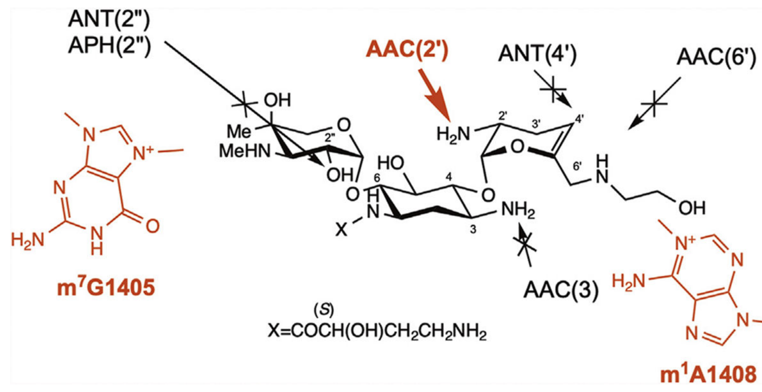

Fig. 6.

Structure of plazomicin and modification targets of AMEs.