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REVIEW ARTICLE

Destination of aminoglycoside antibiotics in the 'post-antibiotic era'

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Aminoglycoside antibiotics (AGAs) were developed at the dawn of the antibiotics era and have significantly aided in the treatment of infectious diseases. Aminoglycosides have become one of the four major types of antibiotics in use today and, fortunately, still have an important role in the clinical treatment of severe bacterial infections. In this review, the current usage, modes of action and side effects of AGAs, along with the most common bacterial resistance mechanisms, are outlined. Finally, the recent development situation and possibility of new AGAs in the 'post-antibiotic era' are considered.
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INTRODUCTION

In recent years, antimicrobial resistance (AMR) has emerged as a global health problem. In 2011, the Director-General of the World Health Organization issued an important warning about AMR, with the message 'no action today means no cure tomorrow.'[1] This document sounded a warning bell regarding the imminent arrival of the 'post-antibiotic era,' a time when antibacterial drugs no longer have any effect against pathogenic bacteria.[2,3] The O'Neill Commission also warned that without immediate action, the number of deaths caused by antimicrobial-resistant bacteria will explosively increase worldwide, to approximately 10 million deaths by 2050.[4] With this rising sense of crisis, AMR was recognized as being on par with the global economy and terrorism in terms of importance by the G7 Ise-Shima Summit in 2016. As a result, all of the attending countries agreed to cooperate in working to address the issue of AMR.

In 2013, the United States Centers for Disease Control and Prevention listed several pathogenic bacteria that are considered a serious threat and for which the development of effective antimicrobial drugs is urgently needed.[5] These bacteria include *Clostridium difficile*, carbapenem-resistant Enterobacteriaceae (CRE), drug-resistant *Neisseria gonorrhoeae*, multidrug-resistant (MDR) *Acinetobacter*, drug-resistant *Campylobacter*, extended spectrum β-lactamase-producing Enterobacteriaceae, vancomycin-resistant *Enterococcus*, MDR *Pseudomonas aeruginosa*, drug-resistant non-typhoidal *Salmonella*, drug-resistant *Salmonella* Typhi, drug-resistant *Shigella*, methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Streptococcus pneumoniae* and drug-resistant *Mycobacterium tuberculosis*. Gram-negative bacteria account for more than half of these listed pathogens and the response to MDR Gram-negative bacteria can be used as the framework of future AMR measures. At the time that the list was released, even carbapenems, which are the most powerful antimicrobial agents, showed no effect against CRE, leading the United States of Centers for Disease Control and Prevention to refer to CRE as 'nightmare bacteria.' The threat of CRE have been reported worldwide[6] and the prevalence of new CRE strains that produce the New Delhi metallo-β-lactamase-1 (NDM-1) enzyme has been rapidly increasing in India, Pakistan, Bangladesh, Thailand and China, as well as in several countries in Europe.[7,8] The NDM-1-encoding gene bla_{NDM-1} is contained on a transferable plasmid, meaning that it is easily transferred between bacterial strains/species. As the diffusion of this enzyme is associated with the generation of new drug-resistant bacterial strains, the global dissemination of bla_{NDM-1} is alarming.[9–12] In addition, colistin-resistance gene *mcr-1* has been found in a wide variety of *Escherichia coli* strains in China,[13] leading to the emergence of strains showing resistance to colistin, an antibiotic of last resort. Inevitably, the first case of human infection caused by one of these 'superbugs' was reported in the United States in 2016, causing concern worldwide.[14]

The emergence and spread of MDR bacteria is a global threat to public health and the economy, and urgent countermeasures are required to address the problem of AMR.[15–17] In this review, we outline the current situation of aminoglycoside antibiotics (AGAs) in the viewpoint as the researcher of new antibacterial medicines and discuss the roles and possibilities of aminoglycoside antibiotics in the 'post-antibiotic era.'

AMINOGLYCOSIDE ANTIBIOTICS

Since the discovery of streptomycin[18] by Waksman in 1944, more than 200 kinds of AGAs produced by soil microorganisms (*Streptomyces*, *Micromonospora*, *Streptoalloteichus*, *Bacillus*, etc.) have been discovered. AGAs generally have a broad antimicrobial spectrum and have strong bactericidal activity against most Gram-negative bacteria, including *Acinetobacter*, *Citrobacter*, *Enterobacter*, *E. coli*, *Klebsiella*, *Proteus*, *Providencia*, *Pseudomonas*, *Salmonella*, *Serratia* and *Shigella* species.[19] However, although AGAs show good antimicrobial activity against clinically important Gram-positive bacteria such as staphylococci and

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M. tuberculosis, they are not so effective against streptococci, pneumococci and enterococci. In addition, anaerobic bacteria are generally resistant to AGAs, because transport of the antibiotics into the cell is oxygen dependent.[20–23]

AGAs are chemically stable, have immediate effect against pathogenic bacteria and have a synergistic effect with other antibacterial drugs (for example, β-lactam antibiotics).[19,24] More than 70 years have passed since the first development of AGAs and β-lactam antibiotics have become the mainstay of antimicrobial therapy. However, AGAs remain the backbone of treatment protocols for serious infections caused by Gram-negative bacteria, including antimicrobial-resistant strains.[19,23]

Currently used AGAs

AGAs in the fields of medicine and pharmacology mean basic oligosaccharides, which are glycosides composed of sugars, amino sugars and pseudo sugars such as cyclitols and/or aminocyclitols. They are water-soluble and are characterized by having basic properties.

Table 1 shows representative AGAs currently used in medical, animal husbandry and agricultural fields in G7 countries. Naturally occurring products for medical use include streptomycin, neomycin,[25] kanamycin (kanamycin A),[26] paromomycin,[27,28] spectinomycin,[29] gentamicin,[30] tobramycin[31,32] and ribostamycin.[33] In the 1970s, significant progress was made in the development of effective aminoglycoside derivatives aimed at overcoming the problems of drug resistance and toxicity.[34–36] As a result, semi-synthetic products such as dibekacin,[37] amikacin,[38] arbekacin[39] and isepamicin[40,41] were launched onto the market.

Streptomycin, kanamycin and amikacin are used in the treatment of tuberculosis, and neomycin, kanamycin, gentamicin, tobramycin, ribostamycin, dibekacin, amikacin and isepamicin are used for antimicrobial therapy in a general clinical setting. Spectinomycin, paromomycin and arbekacin are used as specialized therapeutic agents in the treatment of gonorrhea, intestinal amebiasis and MRSA, respectively. Dihydrostreptomycin, used for the treatment of bovine mastitis, was initially produced synthetically by reduction of streptomycin,[42,43] but was later found to be produced naturally by *Streptomyces humidus*.[44] Apramycin[45] is used for the treatment of bacterial diarrhea in livestock. Many of these naturally produced clinical antibiotics are now also being used in veterinary medicine. Kasugamycin[46] and validamycin[47,48] were developed as agrichemicals for rice farming and their use has significantly increased worldwide with the expansion of rice production in recent years. The structures of these AGAs are shown in Figure 1.

Umezawa *et al.* significantly contributed to the development of AGAs. After the discovery and commercialization of kanamycin, he succeeded in developing the practical applications of the therapeutic antimicrobial agents kanamycin B (bekanamycin)[26] and dibekacin, as well as the anti-MRSA drug arbekacin and the pesticide kasugamycin.

The AGAs listed in Figure 1 can be roughly classified into two types based on their chemical structures: those that contain 2-deoxystreptamine (2-DOS) (kanamycin, tobramycin, dibekacin, gentamicin, amikacin, arbekacin, isepamicin, paromomycin, neomycin, ribostamycin and apramycin) and those that do not (streptomycin, dihydrostreptomycin, spectinomycin, kasugamycin and validamycin). There are a much greater number of 2-DOS-containing AGAs (2-DOS-AGAs) and these drugs can be classified further into a 4,5-disubstituted type (paromomycin, neomycin and ribostamycin), a 4,6-disubstituted type (kanamycin, tobramycin, dibekacin, gentamicin, amikacin, arbekacin and isepamicin) and an 'other' type (apramycin) based on differences in the site of sugar attachment.

Based on their antibacterial spectra, the clinically useful AGAs are classified into a group of compounds characterized by anti-tuberculosis activity (including streptomycin, kanamycin and amikacin), a group of compounds with antimicrobial activity against Gram-negative bacteria, except *P. aeruginosa* (including ribostamycin, paromomycin and neomycin) and a highly useful group of compounds with antimicrobial activity against a wide range of bacterial species, including *P. aeruginosa* (including gentamicin, tobramycin, dibekacin, amikacin, arbekacin and isepamicin). Finally, spectinomycin is in a group of its own, as it is only indicated for the use of treatment of penicillinase-producing *N. gonorrhoeae*.

Modes of action

Although the modes of action of AGAs are complicated, the major mechanism involved in their bactericidal activity is their ability to bind to the decoding region of the 16S ribosomal RNA (rRNA) component of the 30S subunit of bacterial ribosomes[49–52] and interfere with various aspects of protein synthesis.[21]

When 2-DOS-AGAs bind to the A-site in the decoding region, the rRNA undergoes a conformational change resulting in a state similar to the 'decoding state,' leading to the misreading of mRNA.[53–58] Peptide chain elongation does not occur due to the failure of the mechanism that ensures translation accuracy, resulting in the synthesis of defective proteins.[36,59] The aberrant proteins inserted and accumulated in the cell membrane lead to altered permeability and the resulting increase in intracellular AGA concentrations is thought to have an important role in the bactericidal effect and the post antibiotic effect of AGAs.[19,24,60] Furthermore, it is thought that 2-DOS-AGAs inhibit translocation by immobilizing peptidyl-tRNA at the A-site of the ribosome, consequently inhibiting protein synthesis.[56,61–63] Some 2-DOS-AGAs (kanamycin, neomycin B and gentamycin) also bind to the 23S rRNA component of the 50S subunit.[64,65] This binding to the allosteric site affects the mobility of ribosomal subunits, which interferes with translation and ribosome recycling.[57,66–68] In addition, the oxidative stress[21,69] caused by superoxide and formation of hydroxyl radicals, and a detrimental effect on the integrity of the cell wall and outer membrane damage[70,71] have been reported.

Streptomycin has a different binding site to other AGAs in proximity of the decoding center of 16S rRNA. Its binding decreases the translation fidelity by interfering with initial tRNA selection and proofreading.[23,49] In addition, it has been reported that streptomycin disrupts the 70S initiation complex[72] and inhibits the termination reaction of protein synthesis.[73] Spectinomycin also targets a different region of the 16S rRNA from the binding site of other 2-DOS-AGAs.[23] Binding of spectinomycin blocks the attachment of elongation factor G and prevents the translocation of peptidyl-tRNA from the ribosomal A-site, thereby interfering with bacterial protein synthesis.[19,24,74–76]

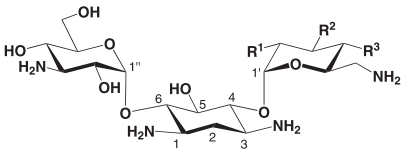
As a cyclitol unit, spectinomycin and kasugamycin contain an actinamine and a D-*chiro*-inositol instead of 2-DOS, respectively. As these two antibiotics do not cause codon misreading such as other AGAs, it can be deduced that the active center of the AGAs that is responsible for the codon misreading is the 2-DOS portion, which is an important pharmacophore required for anchoring drugs to the target RNA.[23,77]

The mechanisms of action of kasugamycin and validamycin differ from those of clinical AGAs. Kasugamycin inhibits the initiation of translation by interfering with the interaction between mRNA and the 30S subunit.[78] In contrast, the antibacterial effects of validamycin are

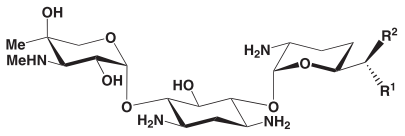
Table 1 Representative aminoglycoside antibiotics currently used in medical, animal husbandry and agricultural fields in G7 countries

Antibiotic	*Origin*		*Discovered*	*Marketed*	*Target organism*	*Adaptive disease*	*Discoverer*	*Producing microorganism*	*Reference*
	Nature	*Semi-synthetic*							
Human medicines									
Streptomycin	○		1944	1946	Acid-fast bacteria	Tuberculosis	Waksman *et al.*	*Streptomyces griseus*	18
Neomycin	○		1949	1950	G+	Bacterial infection	Waksman *et al.*	*Streptomyces fradiae*	25
Kanamycin	○		1957	1958	G+, G–, Acid-fast bacteria	Bacterial infection, Tuberculosis	Umezawa *et al.*	*Streptomyces kanamyceticus*	26
Paromomycin	○		1959	1959	Intestinal protozoa	Intestinal amebiasis	Haskell *et al.*	*Streptomyces rimosus*	27,28
Spectinomycin	○		1961	1967	*N. gonorrhoeae*	Gonocide	Mason *et al.*	*Streptomyces spectabilis*	29
Gentamicin	○		1963	1967	G+, G–	Bacterial infection	Weinstein *et al.*	*Micromonospora purpureochromogenes*	30
Tobramycin	○		1967	1968	G–	Bacterial infection	Stark *et al.*	*Streptomyces tenebrarius*	31,32
Ribostamycin	○		1970	1975	G+, G–	Bacterial infection	Shomura *et al.*	*Streptomyces ribosidificus*	33
Dibekacin		○	1971	1975	G+, G–	Bacterial infection	Umezawa *et al.*	–	37
Amikacin		○	1972	1977	G+, G–, Acid-fast bacteria	Bacterial infection, Tuberculosis	Kawaguchi *et al.*	–	38
Arbekacin		○	1973	1990	MRSA	MRSA infection	Kondo and Umezawa *et al.*	–	39
Isepamicin		○	1975	1988	G–	Bacterial infection	Wright *et al.*	–	40,41
Veterinary and herbal medicines									
Dihydrotreptomycin	○	○	1946	1963	G+, G–	Bacterial infection	Bartz *et al.*	*Streptomyces griseus*	42–44
Apramycin	○		1976	1985	*E. coli*	Bacterial diarrhea	O'Connor *et al.*	*Streptoalloteichus hindustanus*	45
Kasugamycin	○		1965	1970	*Magnaporthe oryzae*	Rice blast	Umezawa *et al.*	*Streptomyces kasugaensis*	46
Validamycin A	○		1970	1972	*Rizoctinia sorani*	Rice sheath blight	Iwase *et al.*	*Streptomyces hygroscopicus*	47,48

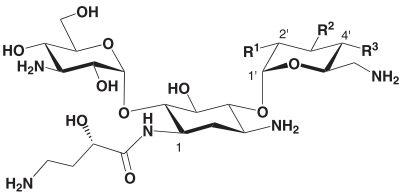
Abbreviations: G+, Gram-positive bacteria; G–, Gram-negative bacteria.



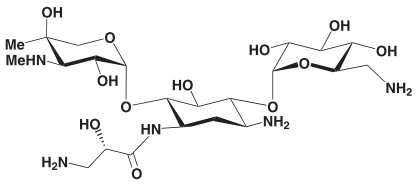
	R^1	R^2	R^3
Kanamycin A	OH	OH	OH
Tobramycin	NH_2	H	OH
Dibekacin	NH_2	H	H



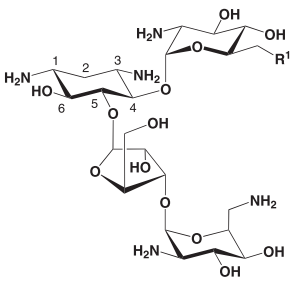
	R^1	R^2
Gentamicin C_1	NHMe	Me
Gentamicin C_{1a}	NH_2	H
Gentamicin C_2	NH_2	Me



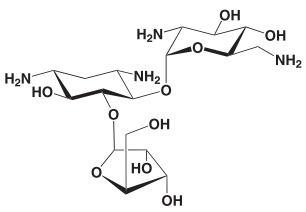
	R^1	R^2	R^3
Amikacin	OH	OH	OH
Arbekacin	NH_2	H	H



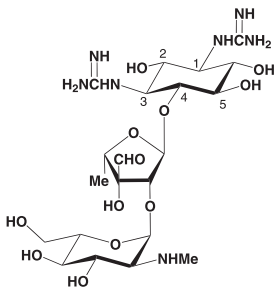
Isepamicin



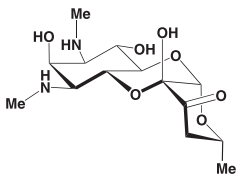
	R^1
Paromomycin	OH
Neomycin B	NH_2



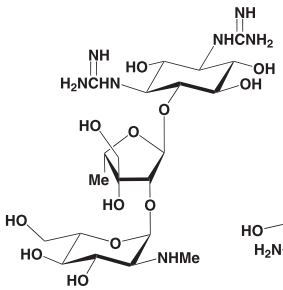
Ribostamycin



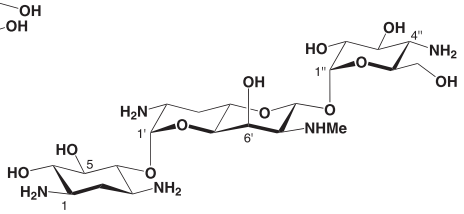
Streptomycin



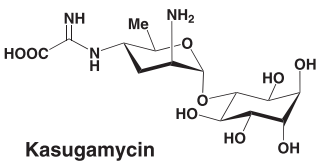
Spectinomycin



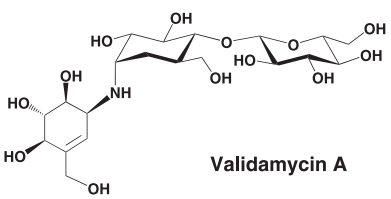
Dihydrostreptomycin



Apramycin



Kasugamycin



Validamycin A

Figure 1 Structures of the aminoglycoside antibiotics listed in Table 1.

attributed to its ability to inhibit trehalase, thereby depleting a major cellular energy source.[79]

Side effects and dose

It is known that nephrotoxicity and ototoxicity are caused by administration of AGAs. Although the damage inflicted by AGAs on the kidney is usually reversible,[80,81] the damage to the inner ear is permanent.[82,83] As the damage caused by AGAs is thought to occur in a concentration-dependent manner,[84] therapeutic drug monitoring is commonly used to predict the effects of the antibiotics and identify any side effects.[85] During the recent development of novel AGAs targeting severe bacterial infections, particular attention is paid to the potential nephrotoxicity of the drugs.

AGAs are not absorbed from the gastrointestinal tract and are generally administered parenterally or topically. After parenteral administration, AGAs are almost entirely excreted in the urine by glomerular filtration without being metabolized within 48 h of administration and some of the drug is reabsorbed via the proximal renal tubular epithelial cells.[86] Endocytic receptor megalin,[87,88] which is highly expressed on the luminal side of proximal tubular epithelial cells, has an affinity for AGAs.[89] Renal cellular necrosis is ultimately caused by AGAs that are taken up via megalin and accumulated in the kidney cells.[90] The degree of damage to the kidneys correlates with the amount of accumulated AGAs.[91]

AGAs exert concentration-dependent antibacterial activity, and effective bactericidal action can be expected by increasing the ratio between the peak concentration (C_{max}) and the minimum inhibitory concentration.[92,93] In addition, AGAs have a post-antibiotic effect,[19] meaning that the bactericidal effect persists even if the blood concentration of the drug decreases below the minimum inhibitory concentration.[94] The duration of this post-antibiotic effect is reported to be extended as C_{max} increases.[95]

In recent years, a method for reducing toxicity has been proposed based on pharmacokinetic/pharmacodynamic studies.[96] A once-daily dosing regimen is recommended, in which a sufficient increase in C_{max} through high-dose administration and an uptake suppression of AGAs into the kidneys under a low trough concentration are expected.[97–103]

Resistance mechanisms

Several mechanisms of bacterial resistance to AGAs have been reported. These include the following: (1) inactivation by aminoglycoside-modifying enzymes; (2) modification of the active site by 16S rRNA methyltransferases; (3) target modification by mutation[75,104] (for example, ribosomal protein mutation[105–110] and point mutation of rRNA[83,111–115]); (4) change of uptake;[23,57,116] and (5) enhancement of efflux systems.[21,24,57,117–119] The most clinically significant resistance mechanisms are inactivation by modifying enzymes, which are generally acquired via the uptake of a transferable plasmid from another resistant strain and modification of the active site by 16S rRNA methyltransferases.

Enzymatic modification of AGAs is mediated by aminoglycoside acetyltransferases, phosphotransferases or nucleotidyltransferases, which are commonly found in both Gram-positive and Gram-negative bacteria.[19,21,24,55,120] These enzymes modify a specific amino group or hydroxyl group in the AGAs. The modifying enzymes and sites of modification for kanamycin and streptomycin are shown in Figure 2.

The first study on the inactivation of kanamycin by modifying enzymes was carried out by Professor Hamao Umezawa and it established a foundation for the subsequent elucidation of the many bacterial AGA resistance mechanisms.[121–124] Based on the study of aminoglycoside-modifying enzymes, the antibacterial agent dibekacin, which is effective against resistant bacteria, was developed by removing the hydroxyl groups, which are subject to enzymatic modification, at the C-3′ and C-4′ positions of kanamycin B. Further study on dibekacin led to the development of arbekacin, an effective medicine for infections caused by MRSA, and which is stable in the presence of most aminoglycoside-inactivating enzymes produced by Gram-positive and Gram-negative bacteria (Figure 3).

There are no enzymes that simultaneously modify and inactivate all types of AGAs. However, recent clinical isolates have a tendency to acquire resistance against a wide variety of AGAs by obtaining genes coding for a plurality of modifying inactivation enzymes on a transferable plasmid.

In addition to acquisition of genes encoding aminoglycoside-modifying enzymes, the emergence of highly drug-resistant bacteria that produce enzymes that modify the active site of the 16S rRNA has become prominent in recent years.[125,126] These are enzymes that methylate the specific bases in the 16S rRNA present in the 30S ribosomal subunit that are involved in bacterial protein synthesis. If methylation occurs, AGAs cannot bind to the active site in the 16S rRNA and are therefore ineffective. Resistance caused by these plasmid-mediated 16S rRNA methyltransferases is worrisome, because the plasmids are highly mobile and the enzymes have a wide substrate

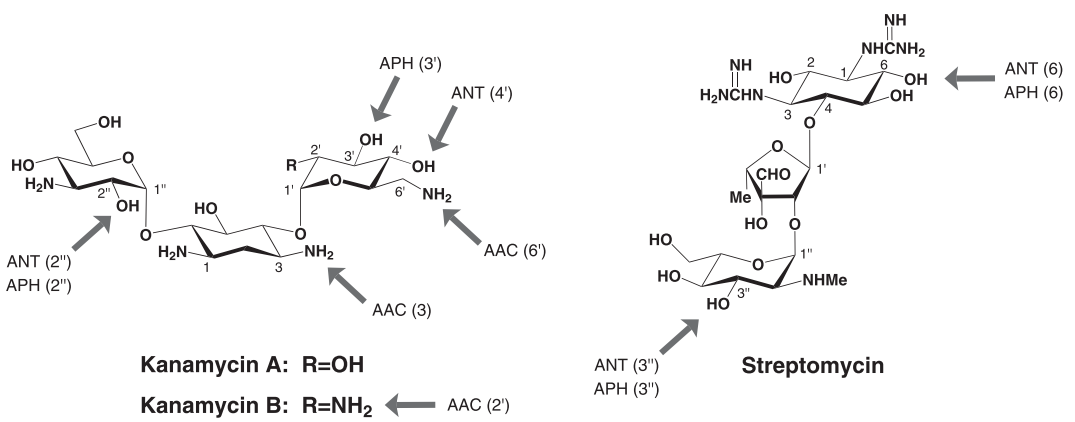


Figure 2 Sites of enzymatic modification of kanamycins and streptomycin. AAC, aminoglycoside acetyltransferase; ANT, aminoglycoside nucleotidyltransferase; APH, aminoglycoside phosphotransferase. A Full color version of this figure is available at *Journal of Antibiotics* online.

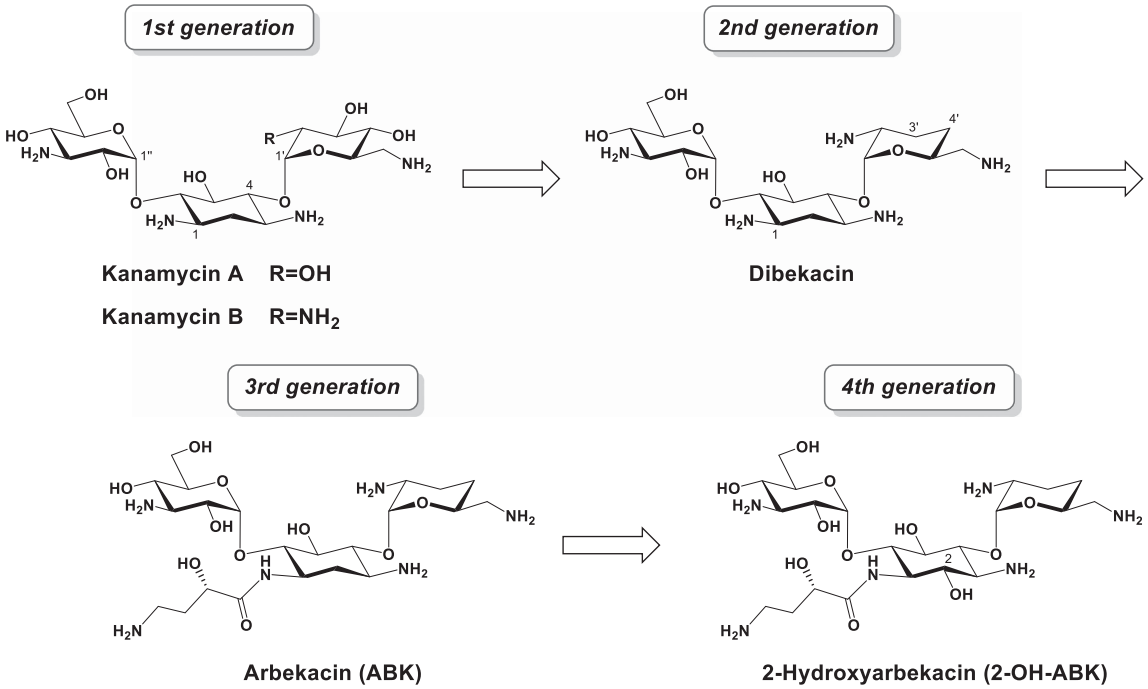


Figure 3 Evolution of kanamycin and the structures of representative compounds. A Full color version of this figure is available at *Journal of Antibiotics* online.

range. 16S rRNA methyltransferases are currently only found in Gram-negative bacteria.[125] Many different methyltransferases have been identified, including ArmA,[127,128] RmtA,[129] RmtB,[130] RmtC,[131] RmtD,[132] RmtE,[133] RmtF,[134] RmtG,[135] RmtH[136] and NpmA,[137] and are classified in two distinct groups based on whether they target the N-7 position of nucleotide G1405 (Liou *et al.*[138]) of the 16S rRNA or the N-1 position of nucleotide A1408 (Beauclerk and Cundliffe,[139] and Dunkle *et al.*[140]) (Figure 4). N7-G1405 16S rRNA methyltransferases mainly confer high-level resistance to the 4,6-disubstituted AGAs, including kanamycin and gentamicin. Methylation of N1-A1408 induces high-level resistance to the 4,6-disubstituted and 4,5-disubstituted AGAs, such as neomycin, and can even confer resistance to apramycin, which has a considerably different molecular structure.[125] Many of these methyltransferases are relatively small (250–290 aa in length)[131,136] and use *S*-adenosyl-L-methionine as a methyl group donor.[57,141] Of particular concern is the finding that experimentally introduced methyltransferase genes can confer aminoglycoside resistance in Gram-positive pathogens.[142]

CRE strains that produce NDM-1-type metallo-β-lactamase often retain their N7-G1405 rRNA methyltransferase-encoding genes.[141,143,144] As these pathogens are highly resistant to a wide range of AGAs, future expansion of epidemic areas is of particular concern.[125]

Recent developments in the design of novel AGAs

At the beginning of this century, many pharmaceutical companies moved away from the development of antibacterial drugs and research into novel antibacterial antibiotics is stagnating. This move was caused in part by the shrinking market for antibiotics in developed countries as well as the fact that bacteria are acquiring antibacterial resistance faster than new drugs can be developed, making it difficult for pharmaceutical companies to recover development costs compared with drugs of other genres. This is compounded by the short administration period of most antibiotics, meaning that they are not profitable in general. However, it is now recognized that AMR is a major threat to global health and the development of novel medicines effective against MDR bacteria is strongly required.

Under such a background, The Infectious Diseases Society of America that raised the sense of crisis, proposed the slogan 'BAD BUGS, NEED DRUGS The 10 x '20 initiative'[145] in 2010. In response to this initiative, research and development activities began with the goal of developing 10 new drugs that are effective against resistant bacteria by 2020 under the cooperation of the government, companies, and academic societies. As a result, multiple new antibacterial drugs, including ceftolozane/tazobactam[146,147] and ceftazidime/avibactam[148,149] for the treatment of MDR Gram-negative bacterial infections, ceftaroline[150] for the treatment of bacterial skin infections and bacterial pneumonia, tedizolid,[151,152] dalbavancin[153,154] and oritavancin[155,156] for the treatment of MRSA, fidaxomicin[157] for the treatment of *C. difficile*-associated diarrhea and bedaquiline[158,159] for the treatment of MDR tuberculosis cases have been developed by the spring of 2017.[160,161] In addition, the antifungal drug isavuconazole[162] was also developed in 2015.

Unfortunately, we are yet to develop drugs effective against Enterobacteriaceaes producing metallo-β-lactamase such as NDM-1, as well as MDR *P. aeruginosa*. However, because these bacteria are still relatively sensitive to AGAs, AGAs are expected to have a significant role as a lead compound in the development of new drugs against these important MDR pathogens.

Recent developments in AGA production can roughly be divided into two categories: (1) novel drugs that improve the effects of existing AGAs by providing a drug delivery system and (2) drugs with a novel

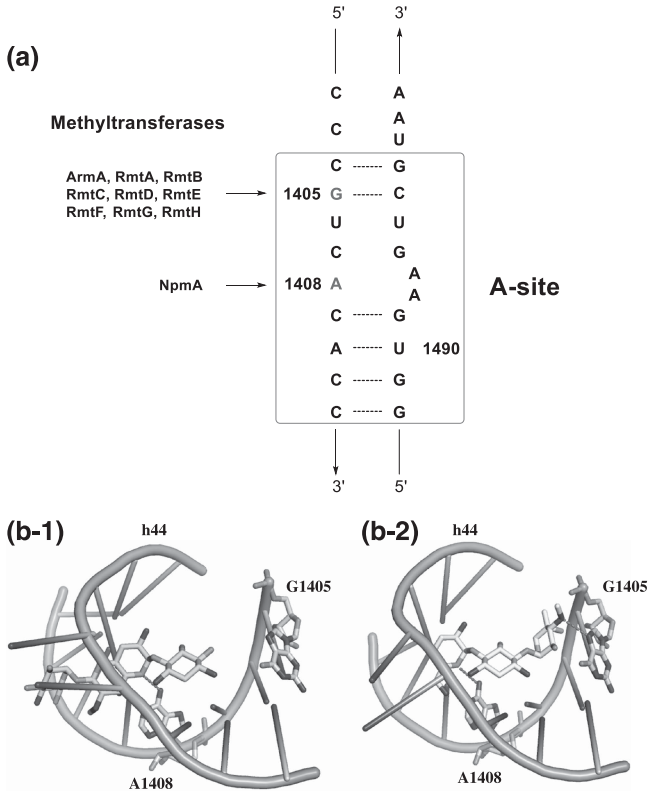


Figure 4 Decoding site in bacterial 16S ribosomal RNA. The ribosomal RNA is numbered according the numbering used in *E. coli* 16S ribosomal RNA. (**a**) Secondary structure[50] of the aminoglycoside-binding site in 16S ribosomal RNA and the methylation positions. The box indicates the sequence corresponding to the highly conserved A-site. (**b**) Interaction of apramycin and gentamicin C_{1a} with the 16S ribosomal RNA in the A-site. (**b-1**) Crystal structure of apramycin with *Thermus thermophilus*; PDB code is 4AQY.[83] (**b-2**) Crystal structure of gentamicin C_{1a} with *E. coli*; PDB code is 2QB9.[66] In all figures, helix 44 of the 30S subunit is represented in the cartoon model, and the A1405 and G1408 residues are further indicated in the stick model. Possible hydrogen bonds are drawn as dashed lines between the antibiotic and the amino group of A1405 or G1408. A Full color version of this figure is available at *Journal of Antibiotics* online.

structure that are effective against MDR bacteria. Examples of developments that fall into these two categories are described below.

Provision of a drug delivery system. **TOBI**[163–165]: Tobramycin is a kanamycin-type AGA and is one component of the nebramycins produced by *Streptomyces tenebrarius*.[31] Tobramycin was first isolated by Eli Lilly and Company in 1967. TOBI is an inhalation solution containing tobramycin as an active ingredient, and it is inhaled into the lungs using a nebulizer or an inhaler device. This medication provides a high topical dose of tobramycin to the lungs of patients with cystic fibrosis, a genetic disorder often characterized by chronic *P. aeruginosa* infection. TOBI significantly improves the lung function in these patients without the adverse side effects observed following parenteral AGA administration. TOBI was developed by the Patho-Genesis Corporation in the United States in 1997, after which the manufacture and sales were handled by Novartis International AG. TOBI is currently approved in more than 40 countries worldwide.

ARIKACE[166–168]: Amikacin[38] is a semi-synthetic AGA produced by introducing a (*S*)-4-amino-2-hydroxybutyric acid into the amino group at the C-1 position of kanamycin and was inspired by the structure of natural antibiotic butirosin.[169,170] Amikacin shows antibacterial activity against Gram-negative bacteria, Gram-positive bacteria (including MRSA) and acid-fast bacteria. In addition, it has the advantage of not being easily inactivated by aminoglycoside-modifying enzymes.

ARIKACE is a form of amikacin enclosed in liposomal nanocapsules and is administered using a nebulizer system. Inhaled liposomal amikacin is expected to penetrate into bacterial biofilms and prolong the release of amikacin in the lungs while minimizing systemic exposure.[171] Phase 3 clinical trials of ARIKACE in patients with non-tuberculous mycobacterial lung infections and phase 2 trials in patients with chronic *P. aeruginosa* in non-cystic fibrosis bronchiectasis are currently being carried out by Imsmed Inc.

BAY41-6551 (Niederman *et al.*[172]): BAY41-6551 is an inhaled amikacin solution being developed by Bayer HealthCare Pharmaceuticals Inc. A global phase 3 clinical trial is being conducted to evaluate the efficacy and safety of adjunctive aerosolized BAY41-6551 in the treatment of intubated and mechanically ventilated patients with Gram-negative pneumonia receiving standard-of-care intravenous antibiotics.

ME1100 (Baba *et al.*[173]) (arbekacin inhalation solution): Arbekacin[39] is a semi-synthetic antibiotic synthesized from antimicrobial drug dibekacin, derived from kanamycin B, by introducing the above-mentioned (*S*)-4-amino-2-hydroxybutyric acid into the amino group at the C-1 position. Arbekacin is a third-generation kanamycin-type drug (Figure 3) and is used clinically for the treatment of pneumonia and sepsis caused by MRSA. ME1100 is a new arbekacin formulation optimized for inhalation. Using a dedicated inhalation device, it is possible to increase the drug concentration in the lower respiratory tract (the main site of infection), resulting in excellent antibacterial effects and a reduction in the systemic side effects.

ME1100 is currently undergoing phase 1b clinical trials in the United States by Meiji Seika Pharma Co., Ltd. It is classified as a Qualified Infectious Disease Product with Fast Track status for the adjunctive treatment of mechanically ventilated patients with bacterial pneumonia caused by MRSA and resistant bacteria such as *Klebsiella pneumoniae*, *P. aeruginosa* and *Acinetobacter*.

AGAs with a novel structure effective against resistant bacteria. **Plazomicin**[174–176]: Plazomicin is a next generation AGA synthetically derived from sisomicin[177,178] by appending an (*S*)-4-amino-2-hydroxybutyric acid to the amino group at the C-1 position and a hydroxyethyl group to the amino group at the C-6′ position (Figure 5). Plazomicin shows excellent antibacterial activity against MRSA, *K. pneumoniae* and Enterobacteriaceae, and it is stable in the presence of various clinically relevant aminoglycoside-modifying enzymes. However, similar to older parenteral AGAs, plazomicin is not effective against NDM-1-producing CRE, resistant bacteria containing a 16S rRNA methyltransferase, and MDR *P. aeruginosa*.[143,179] Plazomicin was designated Fast Track by the United States Food and Drug Administration in 2012, and is currently undergoing phase 3 clinical trials by Achaogen Inc. as a therapeutic agent in patients with complicated urinary tract infections and acute pyelonephritis caused by Enterobacteriaceae, including CRE.

2-Hydroxyarbekacin (2-OH-ABK)[180–182]: 2-OH-ABK is a semi-synthetic AGA specifically designed to reduce the nephrotoxicity of the anti-MRSA drug arbekacin, which is a fourth-generation kanamycin-type drug (Figure 3). Although many aminoglycoside derivatives have been synthesized in an attempt to improve the antibacterial activity and reduce the toxicity,[34,35] a kanamycin-type derivative modified the C-2 position has not been reported.

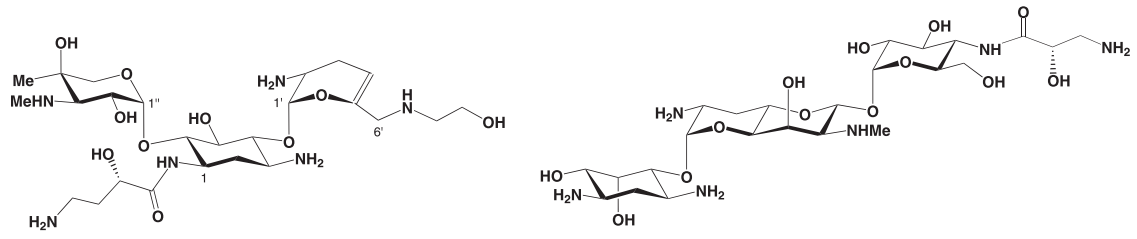


Figure 5 Structures of plazomicin and TS3112.

2-OH-ABK is a derivative in which an equatorial hydroxyl group has been introduced at the C-2 position of arbekacin, resulting in a markedly lower nephrotoxicity than that of arbekacin. In addition, 2-OH-ABK was effective against arbekacin-resistant bacteria and showed better antibacterial activity than arbekacin against MRSA and *P. aeruginosa*. Initially, 2-OH-ABK was synthesized through the condensation of mono-saccharide with pseudo-disaccharide but has recently been efficiently synthesized[183] from the natural product 2-hydroxykanamycin B.[184]

TS3112 (Takahashi *et al.*[185]): Among the AGAs, apramycin contains a unique sugar molecule, aprosamine, in which the aminooctadiose, which has a *trans*-decalin structure, is glycosidically linked to 2-DOS. The inhibition of translocation is thought to be the major mode of action of apramycin.[186,187] Clinical CRE isolates are usually relatively sensitive to apramycin having such a characteristic structure.[143,188] TS3112 is an apramycin derivative developed at the Institute of Microbial Chemistry and it shows excellent activity against 16S rRNA methyltransferase-producing bacteria that are highly resistant to AGAs and against MDR NDM-1-producing Enterobacteriaceae (Figure 5). In addition, because TS3112 is effective against *P. aeruginosa* and *Acinetobacter*, including resistant strains, it is likely to become a key drug in the treatment of infectious diseases caused by MDR Gram-negative bacteria.[189]

Future directions for AGAs

In February 2017, the World Health Organization published the first bacteria catalogue 'World Health Organization priority pathogens list for R&D of new antibiotics' to identify bacterial pathogens that pose the greatest threat to human health.[190] According to this list, carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *P. aeruginosa* and carbapenem-resistant extended spectrum β-lactamase-producing Enterobacteriaceae are included in the most urgent category (Priority 1: critical). These pathogens symbolize the arrival of the "post-antibiotic era", and the list strongly outlines the threat posed by MDR Gram-negative bacteria to global public health.

Although existing AGAs are still mainly effective against members of the family Enterobacteriaceae, including *E. coli* and *K. pneumoniae*, AGAs are inactive against highly resistant bacteria producing 16S rRNA methyltransferases. Many of the emerging CRE strains that express various resistance enzymes, including NDM-1, frequently also contain 16S rRNA methyltransferases along with carbapenemase. Currently, only colistin is effective for treatment of infections caused by these resistant bacteria, although colistin-resistant CRE are already being reported.[191] Therefore, the development of next-generation AGAs, effective against 16S rRNA methyltransferase-producing MDR Gram-negative bacteria and with reduced side effects, is essential for the treatment of serious bacterial infections.

CONCLUSIONS

This review covered the current usage, modes of action, side effects and resistance mechanisms of bacteria against representative AGAs, and summarized recent developments in the discovery of novel aminoglycoside drugs in the face of the 'post-antibiotic era.' In the case of tobramycin, amikacin and arbekacin, novel drug delivery systems are being developed to obtain increased efficacy and reduced systemic side effects by administering antibiotics directly to the infection site. 2-OH-ABK was developed via a challenging synthesis process, but this synthetic success is an example of how the nephrotoxicity of AGAs can be reduced while still maintaining high antibacterial activity. Finally, the unique apramycin derivative TS3112, which is effective against MDR Enterobacteriaceae, *Acinetobacter* and *P. aeruginosa* strains producing 16S rRNA methyltransferases, is an example of the type of next-generation AGAs that could be used in the 'post-antibiotic era.' If we can continue with this recent progress, AGAs could greatly contribute to global public health by aiding in the fight against drug-resistant bacteria.

DEDICATION

This review article is dedicated to Professor Hamao Umezawa with respect and admiration for his achievements in science and medicine.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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