Aminoglycosides: An Overview

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Aminoglycosides are natural or semisynthetic antibiotics derived from actinomycetes. They were among the first antibiotics to be introduced for routine clinical use and several examples have been approved for use in humans. They found widespread use as first-line agents in the early days of antimicrobial chemotherapy, but were eventually replaced in the 1980s with cephalosporins, carbapenems, and fluoroquinolones. Aminoglycosides synergize with a variety of other antibacterial classes, which, in combination with the continued increase in the rise of multidrug-resistant bacteria and the potential to improve the safety and efficacy of the class through optimized dosing regimens, has led to a renewed interest in these broad-spectrum and rapidly bactericidal antibacterials.

Aminoglycosides are potent, broad-spectrum antibiotics that act through inhibition of protein synthesis. The class has been a cornerstone of antibacterial chemotherapy since streptomycin (Fig. 1) was first isolated from *Streptomyces griseus* and introduced into clinical use in 1944. Several other members of the class (Fig. 1) were introduced over the intervening years including neomycin (1949, *S. fradiae*), kanamycin (1957, *S. kanamyceticus*), gentamicin (1963, *Micromonospora purpurea*), netilmicin (1967, derived from sisomicin), tobramycin (1967, *S. tenebrarius*), and amikacin (1972, derived from kanamycin). A shift away from systemic use of the class began in the 1980s with the availability of the third-generation cephalosporins, carbapenems, and fluoroquinolones, which were perceived to be less toxic and/or provide broader coverage than the aminoglycosides. However, increasing resistance to these classes of drugs, combined with more extensive knowledge of the basis of aminoglycoside resistance, has led to renewed interest in the legacy aminoglycosides and the development of novel aminoglycosides such as arbekacin and plazomicin (Fig. 4). These latter agents were designed to overcome common aminoglycoside resistance mechanisms thereby maintaining potency against multidrug-resistant (MDR) pathogens.

Additionally, improved dosing schemes have been developed that attempt to reduce aminoglycoside toxicity while maintaining efficacy. Specifically, clinical studies have reported a lower incidence of nephrotoxicity with once-daily dosing (Nicolau et al. 1995). These data
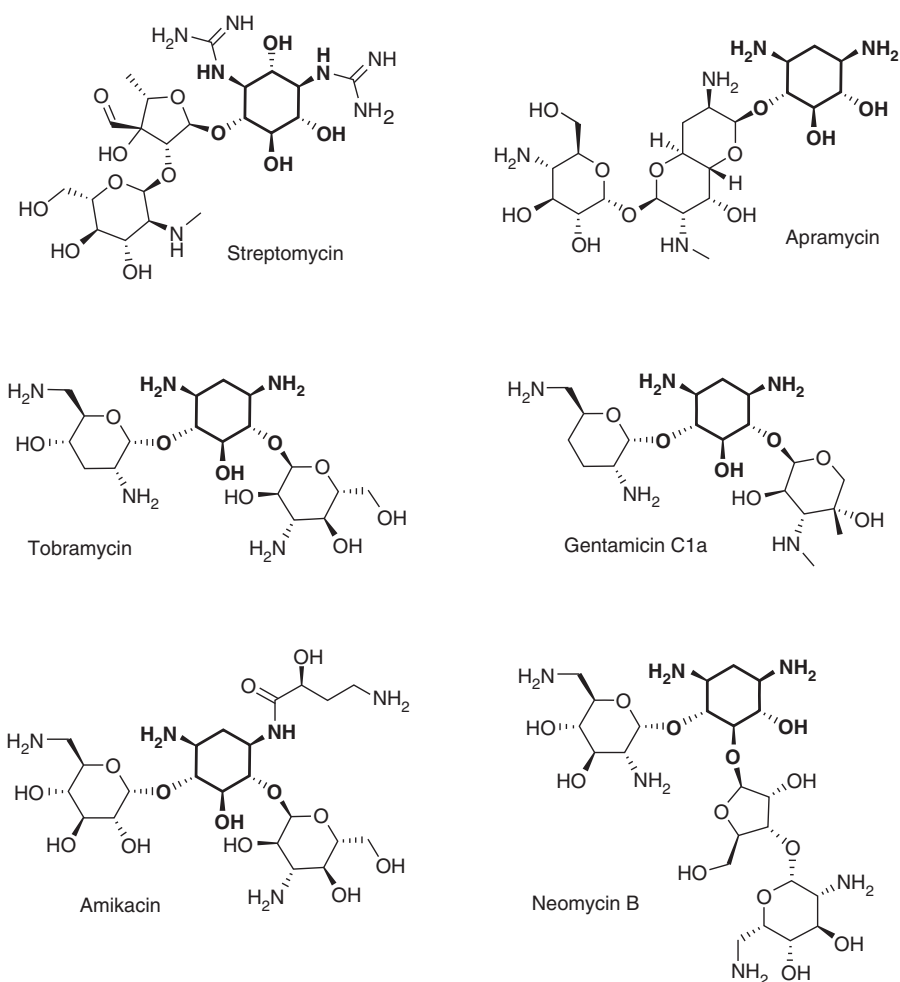

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Figure 1. Structures of representative aminoglycosides, including the atypical aminoglycosides streptomycin and apramycin, 4,6-substituted AGs tobramycin, gentamcin, and amikacin, and the 4,5-substituted AG neomycin. The deoxystreptamine or streptidine rings are in bold.

are consistent with the concept that higher doses given less often should reduce the risk of toxicity while maintaining and possibly enhancing efficacy (Drusano et al. 2007). The advantages of once-daily dosing of aminoglycosides are now widely accepted and, for many infection types, this dosing schedule has become the standard of care (Avent et al. 2011). Additionally, inhaled delivery of aminoglycosides has become an area of renewed interest because it allows for greater local exposure within the lungs with reduced systemic toxicity. Inhaled tobramycin is available in the European Union (EU) and the United States for the treatment of patients with chronic *Pseudomonas aeruginosa* lung infection associated with cystic fibrosis (CF), and inhaled amikacin and arbekacin are in development for potential use in CF and acute respiratory tract infections.

SPECTRUM OF ACTIVITY

Aminoglycosides are active against various Gram-positive and Gram-negative organisms.

Aminoglycosides are particularly potent against members of the Enterobacteriaceae family, including *Escherichia coli*, *Klebsiella pneumoniae* and *K. oxytoca*, *Enterobacter cloacae* and *E. aerogenes*, *Providencia* spp., *Proteus* spp., *Morganella* spp., and *Serratia* spp. (Ristuccia and Cunha 1985; Aggen et al. 2010; Landman et al. 2010). Furthermore, aminoglycosides are active against *Yersinia pestis* (Heine 2015) and *Francisella tularensis* (Ikäheimo et al. 2000), the causative agents of plague and tularemia, respectively. The class also has good activity against *Staphylococcus aureus*, including methicillin-resistant and vancomycin-intermediate and -resistant isolates, *P. aeruginosa* and to a lesser extent *Acinetobacter baumannii* (Ristuccia and Cunha 1985; Karlowsky et al. 2003; Aggen et al. 2010; Landman et al. 2011). Many *Mycobacterium* spp. are also susceptible to aminoglycosides including *Mycobacterium tuberculosis*, *M. fortuitum*, *M. chelonae*, and *M. avium* (Swenson et al. 1985; Ho et al. 1997).

Active electron transport is required for aminoglycoside uptake into cells, so the class inherently lacks activity against anaerobic bacteria (Kislak 1972; Martin et al. 1972; Ramirez and Tolmasky 2010). Aminoglycosides are also inactive against most *Burkholderia* spp. and *Stenotrophomonas* spp. as well as *Streptococcus* spp. and *Enterococcus* spp. (Brogden et al. 1976; Vakulenko and Mobashery 2003; Brooke 2012; Podnecky et al. 2015).

Contemporary large-scale surveillance programs provide an understanding of current aminoglycoside susceptibility among important pathogens associated with common infection types. A recent surveillance study of Gram-negative organisms isolated from patients hospitalized in intensive care units (ICUs) in the United States and the EU found that amikacin and gentamicin showed good activity against key Gram-negative pathogens (Sader et al. 2014). In the United States, 99.5% and 87.9% of *E. coli* isolates were susceptible to amikacin and gentamicin, respectively, according to the Clinical and Laboratory Standards Institute (CLSI) criteria. Likewise, 97.3% and 87.2% of *E. coli* isolates from the EU were susceptible to

amikacin and gentamicin, respectively, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. Among *Klebsiella* spp., 94.8% and 92.7% of U.S. isolates and 90.5% and 83.3% of EU isolates were susceptible to amikacin and gentamicin, respectively. Amikacin was one of the few agents that retained activity against *P. aeruginosa* (97.3% susceptibility in the United States and 84.9% in the EU according to CLSI and EUCAST criteria, respectively). Similarly, aminoglycoside susceptibility rates were high among isolates collected from U.S. medical centers in 2012 (Sader et al. 2015). In this study, CLSI-based susceptibility rates were 99.0%, 88.2%, and 86.3% among *E. coli* and 88.2%, 89.2%, and 82.4% against *K. pneumoniae* for amikacin, gentamicin, and tobramycin, respectively. However, activity was reduced among *K. pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae*, which are often resistant to multiple classes of drugs, for amikacin (42.5% susceptible), gentamicin (50.0% susceptible), and tobramycin (25.0% susceptible). Amikacin was the most active agent tested against *P. aeruginosa* (98% susceptible), followed closely by tobramycin (90% susceptible) and gentamicin (88.0% susceptible). Broad potency was also observed against *S. aureus* (96.0%, 95.0%, and 76.0% for amikacin, gentamicin, and tobramycin, respectively), but these compounds were less active against *A. baumannii* ($\leq$58.0% susceptible).

The broad-spectrum activity of aminoglycosides is enhanced in vitro through synergy with other classes of antimicrobials. This phenomenon, in which the combined effect of two antimicrobial agents is greater than the sum of their individual effects, is particularly well characterized between aminoglycosides and cell-wall-active agents such as β-lactams. In vitro synergy between aminoglycosides and β-lactams has been observed in both Gram-negative and -positive organisms, including wild-type and MDR isolates, using a variety of methodologies (Eliopoulos and Eliopoulos 1988). These in vitro observations have helped to stimulate the use of aminoglycoside-containing combination therapy in the

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treatment of a number of infection types (see below).

MECHANISM OF ACTION

Aminoglycosides inhibit protein synthesis by binding, with high affinity, to the A-site on the 16S ribosomal RNA of the 30S ribosome (Kotra et al. 2000). Although aminoglycoside class members have a different specificity for different regions on the A-site, all alter its conformation. As a result of this interaction, the antibiotic promotes mistranslation by inducing codon misreading on delivery of the aminoacyl transfer RNA. This results in error prone protein synthesis, allowing for incorrect amino acids to assemble into a polypeptide that is subsequently released to cause damage to the cell membrane and elsewhere (Davis et al. 1986; Mingeot-Leclercq et al. 1999; Ramirez and Tolmasky 2010; Wilson 2014). Some aminoglycosides can also impact protein synthesis by blocking elongation or by directly inhibiting initiation (Davis 1987; Kotra et al. 2000; Wilson 2014). The exact mechanism of binding and the subsequent downstream effects varies by chemical structure, but all aminoglycosides are rapidly bactericidal (Davis 1987; Mingeot-Leclercq et al. 1999) and typically produce a prolonged postantibiotic effect (PAE) (Zhanel et al. 1991) The PAE has been shown to be directly related to the length of time that the bacteria take to recover from the inhibition of protein synthesis (Stubbings et al. 2006). It is hypothesized that this is dependent on the eventual disassociation of the antibiotic from its target and exit from the cell.

Aminoglycosides are characterized by a core structure of amino sugars connected via glycosidic linkages to a dibasic aminocyclitol, which is most commonly 2-deoxystreptamine (Mingeot-Leclercq et al. 1999). Aminoglycosides are broadly classified into four subclasses based on the identity of the aminocyclitol moiety: (1) no deoxystreptamine (e.g., streptomycin, which has a streptidine ring); (2) a mono-substituted deoxystreptamine ring (e.g., apramycin); (3) a 4,5-di-substituted deoxystreptamine ring (e.g., neomycin, ribostamycin); or (4) a

4,6-di-substituted deoxystreptamine ring (e.g., gentamicin, amikacin, tobramycin, and plazomicin) (Magnet and Blanchard 2005; Wachino and Arakawa 2012). Examples of each subclass are shown in Figure 1. The core structure is decorated with a variety of amino and hydroxyl substitutions that have a direct influence on the mechanisms of action and susceptibility to various aminoglycoside-modifying enzymes (AMEs) associated with each of the aminoglycosides.

Aminoglycoside entry into bacterial cells is comprised of three distinct stages, the first of which increases permeability of the bacterial membrane, whereas the second and third are energy-dependent. The first stage involves electrostatic binding of the polycationic aminoglycoside to the negatively charged components of the bacterial membrane, such as the phospholipids and teichoic acids of Gram-positive organisms and the phospholipids and lipopolysaccharide (LPS) of Gram-negative organisms, followed by displacement of magnesium ions (Davis 1987; Taber et al. 1987; Ramirez and Tolmasky 2010). These cations are responsible for cross bridging and stabilization of the lipid components of the bacterial membrane and their removal leads to disruption of the outer membrane, enhanced permeability, and initiation of aminoglycoside uptake (Hancock et al. 1981, 1991; Hancock 1984; Ramirez and Tolmasky 2010). This phenomenon facilitates entry into the cytoplasm via a slow, energy-dependent, electron-transport-mediated process (Kislak 1972; Martin et al. 1972; Davis 1987; Taber et al. 1987; Ramirez and Tolmasky 2010). Inhibition of protein synthesis and mistranslation of proteins occurs once aminoglycoside molecules access the cytoplasm. These mistranslated proteins insert into and cause damage to the cytoplasmic membrane itself and facilitate subsequent aminoglycoside entry (Nichols and Young 1985; Davis et al. 1986). This then leads to rapid uptake of additional aminoglycoside molecules into the cytoplasm, increased inhibition of protein synthesis, mistranslation, and accelerated cell death (Davis et al. 1986; Davis 1987; Taber et al. 1987; Ramirez and Tolmasky 2010).

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MECHANISMS OF AMINOGLYCOSIDE RESISTANCE

Aminoglycoside resistance takes many different forms including enzymatic modification, target site modification via an enzyme or chromosomal mutation, and efflux. Each of these mechanisms has varying effects on different members of the class and often multiple mechanisms are involved in any given resistant isolate. Resistance to aminoglycosides via target site mutations has not been observed because nearly all prokaryotes, with the exception of *Mycobacterium* spp. (Bercovier et al. 1986) and *Borrelia* spp. (Schwartz et al. 1992), encode multiple copies of rRNA. Although contemporary large-scale surveillance programs provide an understanding of phenotypic aminoglycoside resistance among important pathogens, these studies have generally not focused on the epidemiology of specific resistance mechanisms (Jones et al. 2014; Sader et al. 2015).

Enzymatic Drug Modification

AMEs are often found on plasmids containing multiple resistance elements, including other AMEs or β-lactamases. The mobility of these enzymes might be tied to their origins, which has been hypothesized to be via horizontal gene transfer from the actinomycetes responsible for the natural production of aminoglycosides (Shaw et al. 1993; Ramirez and Tolmasky 2010). More than 100 AMEs have been described and

are broadly categorized into three groups based on their ability to acetylate, phosphorylate, or adenylate amino or hydroxyl groups found at various positions around the aminoglycoside core scaffold (Ramirez and Tolmasky 2010). These modifications decrease the binding affinity of the drug for its target and lead to a loss in antibacterial potency (Llano-Sotelo et al. 2002). These three families of AMEs include aminoglycoside *N*-acetyltransferases (abbreviated AACs), aminoglycoside *O*-nucleotidyltransferases (ANTs), and aminoglycoside *O*-phosphotransferases (APHs). The classes are further divided into subtypes according to the position on the aminoglycoside that the enzyme modifies followed by a Roman numeral and, in some cases, a letter when multiple enzymes exist that modify the same position (Shaw et al. 1993; Ramirez and Tolmasky 2010). The major sites of aminoglycoside modification for the most common AMEs are shown for kanamycin A in Figure 2.
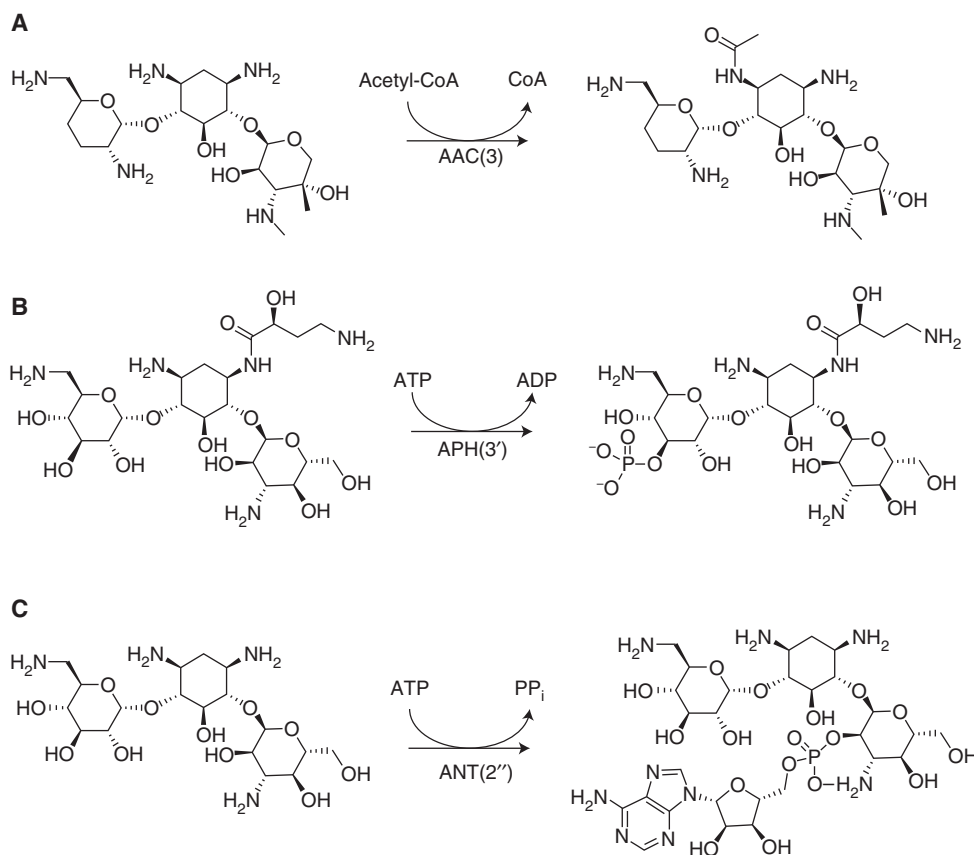
Aminoglycoside Acetyltransferases

The aminoglycoside acetyltransferases or AACs comprise the largest group of AMEs. They are part of the GCN5-related *N*-acetyltransferase (GNAT) superfamily of ∼10,000 described proteins (Ramirez and Tolmasky 2010). These enzymes acetylate amino groups found at various positions on the aminoglycoside scaffold in an acetyl-CoA-dependent reaction (Fig. 3A). There are four main subclasses of this group
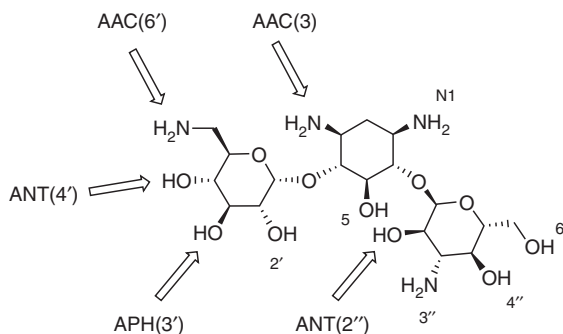
Figure 2. Sites of chemical modification by representative aminoglycoside-modifying enzymes (AMEs) on kanamycin A.

Figure 3. Examples of aminoglycoside modification by AMEs. (*A*) An example of chemical modification of gentamicin catalyzed by the aminoglycoside acetyltransferase AAC(3). (*B*) An example of chemical modification catalyzed by the aminoglycoside phosphotransferase APH(3′) on amikacin. (*C*) Adenylation of the 2″ hydroxyl of kanamycin A catalyzed by the aminoglycoside nucleotidyltransferase ANT(2″).

of enzymes whose nomenclature is derived from the specific amino group that is modified. Specifically, AAC(1) and AAC(3) target the amino groups found at position 1 and 3, respectively, of the 2-deoxystreptamine ring, whereas AAC(2′) and AAC(6′) target amino groups found at the 2′ and 6′ position of the 2,6-dideoxy-2,6-diaminoglucose ring (Mingeot-Leclercq et al. 1999; Wright and Thompson 1999; Azucena and Mobashery 2001; Magnet and Blanchard 2005).

A representative of the AAC family (AAC(6′)-IV) was the first AME found in bacteria to be described in the literature (Okamoto and Suzuki 1965). Since then, >70 members of the AAC family have been described. Fre-

quently observed class members found among Gram-negative bacteria include the AAC(6′)-1 enzyme that leads to amikacin, netilmicin, and tobramycin resistance, AAC(3)-IIa, which is responsible for resistance to gentamicin, tobramycin, and netilmicin, and AAC(3)-I, which modifies gentamicin (Shaw et al. 1993; Castanheira et al. 2015). Less common are the AAC(6′)-APH(2″) hybrid enzyme responsible for high-level aminoglycoside resistance in *Enterococcus faecalis* (Culebras and Martínez 1999), the chromosomally encoded AAC(6′)-Ii enzyme responsible for intrinsic resistance to aminoglycosides among *E. faecium* (Costa et al. 1993) and the chromosomal AAC(2′) enzyme found in *Mycobacterium* spp. and *Provi-*

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dencia stuartii (Rather et al. 1993; Aínsa et al. 1997; Macinga and Rather 1999; Ramirez and Tolmasky 2010). Last, a variant of AAC(6′)-Ib that has acquired the ability to modify fluoroquinolones without significantly altering its activity against aminoglycosides has been identified in clinical isolates of Gram-negative bacteria (Robicsek et al. 2006; Strahilevitz et al. 2009).

Aminoglycoside Phosphotransferases

The second largest group of AMEs is the APHs. This structurally diverse group of enzymes acts like kinases in that they catalyze the ATP-dependent phosphorylation of hydroxyl groups found on aminoglycosides (Fig. 3B). APH enzymes are functionally and structurally similar to the serine–threonine and tyrosine kinases found in eukaryotes (Wright and Thompson 1999). The modifications made by these enzymes lower binding affinity to the target by decreasing the hydrogen bonding potential of aminoglycoside hydroxyl groups with important rRNA residues. Most of the >30 described APH enzymes belong to the APH(3′) subfamily (Kim and Mobashery 2005), although variants that target the 2″ hydroxyl also exist (Ramirez and Tolmasky 2010). These enzymes are found in diverse groups of Gram-negative bacteria, although APH(3′)-IIIa was discovered in *S. aureus* and *Enterococcus* spp. All members of the family lead to kanamycin and neomycin resistance with various members of the family also able to modify a variety of other aminoglycosides, including amikacin and gentamicin B (Shaw et al. 1993).

Aminoglycoside Nucleotidyltransferases

The final group of AMEs is the ANTs. These enzymes act by adding AMP from an ATP donor to hydroxyl groups at the 2″, 3″, 4′, 6, and 9 positions (Fig. 3C). The most clinically relevant members of the class include ANT(2″) and ANT(4′) (Kotra et al. 2000; Magnet and Blanchard 2005), which were first described in *K. pneumoniae* and *S. aureus*, respectively (Benveniste and Davies 1971; Le Goffic et al. 1976).

ANT(2″) broadly effects the activity of 4,6-di-substituted aminoglycosides (Gates 1988), whereas ANT(4′) targets kanamycin A, B, and C, gentamicin A, amikacin, tobramycin, and neomycin B and C. Other members of this class include ANT(3″), ANT(6), and ANT(9), which confer resistance to streptomycin and spectinomycin (Hollingshead and Vapnek 1985; Murphy 1985; Ounissi et al. 1990).

16S rRNA Methylation

Target site modification leading to aminoglycoside resistance occurs via the action of 16S rRNA methyltransferases (RMTs). These enzymes modify specific rRNA nucleotide residues in a manner that blocks aminoglycosides from effectively binding to their target (Beauclerk and Cundliffe 1987; Cundliffe 1989; Wachino and Arakawa 2012). There are two general classes of RMTs that are characterized by the specific nucleotide residues that they modify. These include enzymes that render bacteria resistant to 4,6-di-substituted aminoglycosides via methylation of the N7 position of nucleotide G^{1405} (Thompson et al. 1985; Beauclerk and Cundliffe 1987) and those that affect both 4,6- and 4,5-di-substituted aminoglycosides through methylation of the N1 position of nucleotide A^{1408} (Skeggs et al. 1985; Beauclerk and Cundliffe 1987; Mingeot-Leclercq et al. 1999).

The first clinical case of a pathogen with an RMT as a mechanism of aminoglycoside resistance was reported in a *P. aeruginosa* isolate from Japan in 2003 (Yokoyama et al. 2003). This isolate contained a plasmid-encoded RMT, named RmtA for ribosomal methyltransferase A. Subsequently, several additional plasmid-borne RMTs, encoded by the genes *armA*, *rmtB1*, *rmtB2*, *rmtC*, *rmtD*, *rmtD2*, *rmtE*, *rmtF*, *rmtG*, and *rmtH*, have emerged in clinical isolates that show high-level resistance to multiple aminoglycosides (Yokoyama et al. 2003; Doi et al. 2004; Yan et al. 2004; Yamane et al. 2005; Wachino et al. 2006; Wachino and Arakawa 2012). These enzymes modify the G^{1405} nucleotide and, thus, impact the activity of all 4,6-di-substituted aminoglycosides (i.e., amikacin, gentamicin, and tobramycin). In 2007,

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the enzyme NpmA was discovered encoded on a plasmid in an aminoglycoside-resistant *E. coli* clinical isolate, also from Japan (Wachino et al. 2007). This methyltransferase modifies the A^{1408} nucleotide and, thus, impacts 4,6- and 4,5-di-substituted as well as monosubstituted aminoglycosides, conferring pan-aminoglycoside resistance. To date, there has been only one additional report of this enzyme in a clinical isolate (Al Sheikh et al. 2014).

Efflux-Mediated Resistance

Several members of the resistance–nodulation–division (RND) family of efflux systems have been shown to be involved in intrinsic aminoglycoside resistance in various pathogens (Aires et al. 1999; Westbrock-Wadman et al. 1999; Rosenberg et al. 2000; Magnet et al. 2001; Hocquet et al. 2003; Islam et al. 2004). In the opportunistic pathogen *P. aeruginosa*, intrinsic low-level resistance to aminoglycosides, tetracycline and erythromycin, is mediated by the expression of the multiple efflux (Mex) XY-OprM system. MexXY orthologs are found in other species of bacteria. For example, members of the *Burkholderia cenopacia* complex are often intrinsically resistant to aminoglycosides via RND efflux pumps (Buroni et al. 2009). A homologous transporter in *E. coli*, AcrD, participates in efflux of aminoglycosides (Rosenberg et al. 2000) as does the Acinetobacter drug efflux (Ade) ABC efflux system in *A. baumannii* (Magnet et al. 2001) and the major facilitator superfamily (MFS) in *Mycobacteria* spp.

Molecular Epidemiology of Aminoglycoside Resistance Mechanisms

Comprehensive molecular data regarding the current prevalence of specific aminoglycoside resistance mechanisms among common pathogens is sparse. One recent study evaluated the aminoglycoside resistance mechanisms found among 200 Gram-negative bacilli isolates selected at random from an extensive culture collection (Castanheira et al. 2015). Ninety-nine Enterobacteriaceae (91.9% AME-positive), 49 *A. baumannii* (79.6% AME-positive), and 52

P. aeruginosa (63.5% AME positive) with a variety of aminoglycoside resistance profiles were included. A diversity of AME genes were identified with the most prevalent being *aac(6′)-Ib* ($n = 75$), *ant(3′)-Ia* ($n = 51$), and *aac(3)-IIa* ($n = 45$), and with 26 isolates harboring more than one AME gene. In addition, 21 of the isolates were found to carry an RMT including 12 Enterobacteriaceae, seven *A. baumannii*, and two *P. aeruginosa*. Many of these isolates were also resistant to other common antibiotics used to treat Gram-negative infections, highlighting the ability of these resistance mechanisms to spread through conjugation of plasmids and nonreplicative transposons among bacteria (Courvalin 1994; Waters 1999; Dzidic and Bedeković 2003; Feizabadi et al. 2004).

AMEs are commonly found in association with other key resistance elements, such as carbapenemases and extended spectrum β-lactamases (ESBLs). Among 50 carbapenem-resistant *K. pneumoniae* clinical isolates from two U.S. medical centers (80% possessing KPC-2, 10% possessing KPC-3 with all KPC^+ isolates also expressing TEM-1 and SHV-12), 98% of isolates expressed at least one AME (Almaghrabi et al. 2014). Specifically, 98% were positive for *aac(6′)-Ib*, 56% positive for *aph(3′)-Ia*, 38% positive for *aac(3′)-IV*, and 2% positive for *ant(2″)-Ia*. Overall, 40%, 98%, and 16% of the strains were nonsusceptible to gentamicin, tobramycin, and amikacin, respectively. Plazomicin, a novel aminoglycoside in clinical development that was designed to evade AME-based resistance, had MICs that ranged from 0.25 to 1 μg/mL against this set of isolates. AME characterization in 330 aminoglycoside-resistant clinical Enterobacteriaceae isolates from Spain revealed the presence of *aph(3″)-Ib* and *ant(3″)-Ia* genes in 65.4% and 37.5% of the isolates, consistent with 92% phenotypic resistance to streptomycin found in this strain collection (Miró et al. 2013). These isolates were resistant to other aminoglycosides to varying degrees, including gentamicin (18.4%), tobramycin (16.9%), and amikacin (1.5%), indicating the presence of other AMEs; *aph(3′)-Ia* was found in 13.9% of isolates, *aac(3)-IIa* in 12.4%, *aac(6′)-Ib* in 4.2%, *ant(2″)-Ia* in 3.6%, and

aph(3″)-IIa 1.2% (Miró et al. 2013). Underscoring the association of AMEs with resistance elements to other key antibiotic classes, many of these isolates also produced ESBLs and were resistant to the fluoroquinolones.

Similar to the AMEs, comprehensive data describing the prevalence and molecular epidemiology of RMTs is lacking. A recent literature review described reports of widespread, global dissemination of genes encoding RMTs among isolates from both human and livestock sources (Wachino and Arakawa 2012). Despite the widespread detection of RMTs across many regions, the prevalence appears to vary by region with the highest rates reported in Asia. A SENTRY surveillance study from the Asia-Pacific region (2007–2008) detected genes encoding RMTs in 6.9% (China), 10.5% (India), 1.5% (Hong Kong), 6.1% (Korea), and 5.0% (Taiwan) of Enterobacteriaceae isolates (Bell et al. 2010). Lower rates ($\leq$1.3%) of RMTs among Enterobacteriaceae have been reported from single institution or local studies from European medical centers (Wachino and Arakawa 2012). Similar to the association between AMEs and other key resistance mechanisms, an association between RMTs and specific β-lactamases has been described. Seventy-six percent of the RMT containing isolates described in the SENTRY surveillance study above also possessed a CTX-M ESBL (Bell et al. 2010), and RMTs are frequently found in association with the New Delhi metallo-β-lactamase (NDM) (Berçot et al. 2011; Livermore et al. 2011; Mushtaq et al. 2011; Poirel et al. 2014).

AGENTS IN DEVELOPMENT

Plazomicin is a new aminoglycoside that was specifically engineered to be resistant to the action of the AMEs that are prevalent in key Gram-negative pathogens (Armstrong and Miller 2010). It is synthesized from a sisomicin scaffold that is intrinsically refractory to modification by APH(3′)-III, -VI, and -VII and ANT(4′), which confer amikacin resistance because of an absence of the 3′- and 4′-OH groups. Modification at the N-1 position via addition of a hydroxylaminobutyric acid substituent sterically hinders the action of the AAC(3), ANT(2″), and APH(2″) enzymes, which confer resistance to gentamicin and tobramycin. Finally, addition of a hydroxyethyl substituent at the 6′ position inhibits the action of the AAC(6′) enzymes, which confer resistance to a broad range of agents, including amikacin, tobramycin, and gentamicin (Fig. 4). Importantly, these modifications to sisomicin do not reduce intrinsic potency as has been associated with previous efforts to protect the 6′ position and, as predicted, lead to improved activity against Enterobacteriaceae (MIC$_{90}$ $\leq$2 mg/L) that are resistant to currently available aminoglycosides (Nagabhushan et al. 1982; Aggen et al. 2010). Plazomicin retains vulnerability to modification by AAC(2′)-I, a chromosomal AME found in *P. stuartii* and some mycobacterial species. However, this enzyme is rare, has not been found on a mobile element and has not been shown to have clinical relevance in *Mycobacterium* spp. In addition, plazomicin, like all
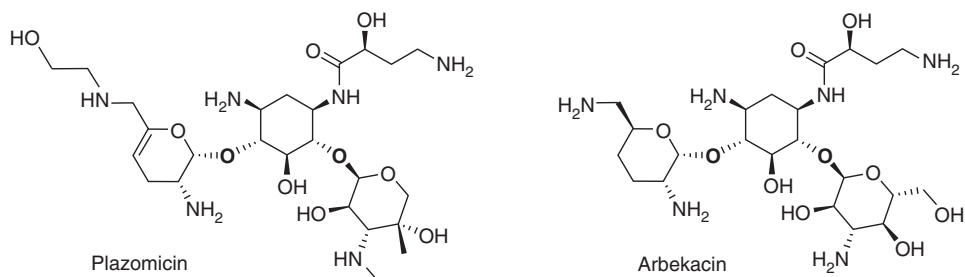
Figure 4. Structures of plazomicin and arbekacin.

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4,6-linked aminoglycosides, is inactive against isolates that produce RMTs. As described above, this mechanism of resistance frequently travels on mobile genetic elements with NDM-positive Enterobacteriaceae, and, thus, plazomicin is not active against many isolates harboring this enzyme (Berçot et al. 2011; Livermore et al. 2011; Mushtaq et al. 2011; Poirel et al. 2014).

Similar to plazomicin, arbekacin, a semisynthetic derivative of dibekacin, was specifically engineered to overcome the action of a subset of clinically important AMEs (Fig. 4). Arbekacin is stable to the action of AMEs commonly found in methicillin-resistant *S. aureus* (MRSA), such as APH, ANT, and AAC and possesses potent activity against this clinically important pathogen (Matsumoto 2014). Although arbekacin has been approved for use in Japan since 1990 for the treatment of sepsis and pneumonia caused by MRSA, this molecule also retains potent activity against key Gram-negative pathogens, including MDR strains, and has more recently gained attention as a potential therapy for infections caused by these organisms. In a recent surveillance study of isolates from hospitalized patients with pneumonia, arbekacin was the most potent aminoglycoside tested against ESBL-producing *E. coli* and was slightly more active than amikacin and tobramycin against ESBL- and KPC-expressing *K. pneumoniae*. Similarly, arbekacin possessed greater activity against *P. aeruginosa* and *Acinetobacter* spp., including MDR isolates, than the other aminoglycosides tested (amikacin, gentamicin, and tobramcin) (Sader et al. 2015). Because of its broad-spectrum in vitro activity against both MDR Gram-positive and Gram-negative pathogens, arbekacin is currently under development as an inhalational agent for the treatment of mechanically ventilated patients with bacterial pneumonia (clinicaltrials.gov/ct2/show/NCT02459158) and is under study at Walter Reed Army Medical Hospital for the treatment of patients with infections caused by MDR organisms that have limited treatment options (clinicaltrials.gov/ct2/show/NCT01659515).

PHARMACOKINETICS AND PHARMACODYNAMICS

Aminoglycosides are poorly absorbed via the gastrointestinal (GI) tract and are, thus, administered via the intravenous or intramuscular route (Ramirez and Tolmasky 2010; Craig 2011). The volume of distribution for members of the aminoglycoside class approaches total body volume, indicating a broad distribution into tissues, including the lung (Simon et al. 1973). This feature has led to extensive use of aminoglycosides as part of combination regimens for the treatment of pneumonia. Aminoglycosides are rapidly cleared through the urinary tract, which also makes these drugs ideal for the treatment of urinary tract infections (Lode et al. 1976; Ramirez and Tolmasky 2010; Craig 2011).

The relationship between aminoglycoside pharmacokinetics (PKs) and pharmacodynamics (PDs) has been studied extensively in mice. The PK/PD variable that is most often correlated with efficacy of aminoglycosides is the ratio of area under the concentration–time curve (AUC) to MIC, although peak concentration also appears to play a role. The magnitude of the PK/PD target for aminoglycosides is not as well defined as it is for other antibiotic classes because of, until recently, the lack of development of new aminoglycosides. Available data suggests that significant variations in the PK/PD target exist between species and body site of infection. For example, an AUC/MIC target of 100 was reportedly associated with a 1- to 2-$\log_{10}$ kill in the mouse neutropenic thigh infection model with amikacin and *K. pneumoniae* (Craig 2011), whereas this same group reported better efficacy at the same dose level and with the same strain in the mouse lung infection model (Craig et al. 1991). These results were potentially a result of a longer measured PAE in the lung compared with the thigh (Craig et al. 1991).

AUC/MIC thresholds have been correlated to efficacy in patients in whom extensive PK sampling was also conducted. These case reports and reviews describe a variety of different ways that the AUC/MIC ratio might be used to predict outcomes in patients. Smith et al. (2001)

reviewed 23 patients with intra-abdominal infections ($n = 16$) or lower respiratory infections ($n = 7$) treated with tobramycin infused >30 min to achieve a C_{max} of between 4 and 10 mg/L. A PK/PD model constructed from these patients data showed that improved efficacy was observed when an AUC/MIC ratio of ≥110 was achieved compared with AUC/MIC ratios of <110 (80% clinical cure rate compared with 47%). Other investigators have found similar correlations between exposure and efficacy. Jacobs (2001) describes the need to achieve an AUC/MIC ratio of 25 for less severe infections or in immune competent patients and a ratio of at least 100 in patients with severe infections and/or those that are immune-compromised. Zelenitsky et al. (2003) found significantly improved outcomes in patients with bacteremia treated with gentamicin when AUC/MIC ratios exceeded 70 compared with AUC/MIC ratios <70 (90% cure vs. 45.5%, respectively). However, a much stronger correlation with outcome was noted for C_{max}/MIC with 84% and 90% clinical cure rates for C_{max}/MIC values of 4.8 or 8 compared with 0% clinical cure for C_{max}/MIC < 2.9.

There is substantial evidence that administering larger doses of aminoglycosides less frequently may be associated with improved outcomes compared with providing the same total daily dose over more frequent dosing schedules (Barclay et al. 1999). This dosing approach, known as once daily or extended interval dosing, takes advantage of three features of aminoglycosides—concentration-dependent killing, rapid elimination, and a prolonged PAE. Larger doses are thought to increase target body site concentrations to improve PD while minimizing potential toxicity through allowance for a period of time in which there is little or no drug in circulation. The PAE properties of the class allow for an extended period of killing after the drug is cleared from the body and before the next dose is administered. A number of meta-analyses of results from clinical trials comparing once versus multiple daily administration of aminoglycosides have been published (Barclay et al. 1999). Overall, the results of these studies suggest that once daily dosing is associated with

reduced nephrotoxicity and equal, if not slightly improved, efficacy.

Therapeutic drug management (TDM), the clinical practice of measuring drug concentrations at designated intervals for use in optimizing individualized dosage regimens, has further improved the safety profile of aminoglycosides. Older studies using multiple daily doses of these drugs have shown nephrotoxicity rates of 10% to 20% (Humes et al. 1982; Moore et al. 1984), whereas lower rates of nephrotoxicity ranging from 0% to 14% have been reported with once-daily dosing regimens with dose adjustment guided by the use of TDM (Prins et al. 1993; Nicolau et al. 1995; Murry et al. 1999; Rybak et al. 1999; Buijk et al. 2002). The advantages of once-daily or extended interval dosing of aminoglycosides combined with the use of TDM are now widely accepted and, for many infection types, this dosing approach has become the standard of care (Avent et al. 2011).

CLINICAL USES OF AMINOGLYCOSIDES

The spectrum of activity, rapid bactericidal activity, and favorable chemical and pharmacokinetic properties of aminoglycosides make them a clinically useful class of drugs. Aminoglycosides are used as single agents and in combination with other antibiotics in both empirical and definitive therapy for a broad range of indications (Avent et al. 2011; Jackson et al. 2013).

In patients with serious infections caused by Gram-negative pathogens, the receipt of empiric combination therapy containing at least one antimicrobial agent to which the pathogen is susceptible leads to lower mortality and improved outcomes (Tamma et al. 2012). In addition to helping ensure that the pathogen is adequately covered by at least one active drug, the use of empiric aminoglycoside-β-lactam combination therapy has also been theorized to contribute to improved outcomes by taking advantage of the in vitro synergy observed between these classes and to prevent the emergence of resistance (Pankuch et al. 2010; Le et al. 2011). Although clinical data to support the latter two theoretical benefits of combination therapy are conflicting (Tamma et al. 2012), aminoglyco-

sides are often combined with β-lactams for the empirical treatment of severe sepsis and certain nosocomial infections in patients with a high risk of mortality or when there is concern that the causative pathogen may be resistant to more commonly used agents (American Thoracic Society; Infectious Diseases Society of America 2005; Dellinger et al. 2013). More recently, aminoglycosides have increasingly become important components of therapy for patients infected with MDR pathogens, such as carbapenem-resistant Enterobacteriaceae (CRE), for whom few treatment options remain. In a retrospective cohort study of 50 cases of sepsis caused by carbapenem- and colistin-resistant *K. pneumoniae*, the receipt of gentamicin as part of definitive therapy was associated with lower mortality compared with the receipt of non-gentamicin-containing regimens (Gonzalez-Padilla et al. 2015). The novel aminoglycoside plazomicin (see section on Agents in Development) is currently under phase 3 study for the treatment of serious infections caused by CRE (clinicaltrials.gov/ct2/show/NCT01970371).

Aminoglycosides are also an important component of combination therapy for multidrug-resistant tuberculosis (MDR-TB) and certain non-tuberculous mycobacterial (NTM) infections. Current MDR-TB treatment guidelines recommend inclusion of one of the following agents during the intensive phase of therapy: amikacin, kanamycin, streptomycin, or capreomycin, a cyclic peptide antibiotic that is often considered as an aminoglycoside because of its mechanism of action. Each of these agents possesses potent bactericidal activity against *M. tuberculosis* (Ho et al. 1997) and the choice of agent depends on previous injectable use (if any) and the likelihood of resistance. A meta-analysis including 32 studies with >9000 treatment episodes did not reveal any clear differences in efficacy among the available agents (World Health Organization 2011). Similar to treatment of MDR-TB, combination therapy for patients with fibrocavitary, severe nodular/bronchiectatic or macrolide-resistant lung disease because of the *M. avium* complex generally includes amikacin or streptomycin (Griffith et al. 2007). Among the rapidly growing myco-

bacteria, amikacin is the preferred agent for infections because of *M. fortuitum* or *M. abscessus*, whereas tobramycin is the most active agent against *M. chelonae* (Griffith et al. 2007).

Aminoglycosides remain the preferred therapy for certain zoonotic infections such as plague and tularemia. Although streptomycin has traditionally been the agent of choice for these infection types, gentamicin is now widely used because of the broader availability of this agent as well as data suggesting similar efficacy to streptomycin (Mwengee et al. 2006; Snowden and Stovall 2011). Aminoglycosides are bactericidal against these organisms and the use of bacteriostatic agents, such as doxycycline or chloramphenicol has led to treatment failures (Dennis et al. 2001; Snowden and Stovall 2011).

Inhaled tobramycin therapy in CF patients with chronic lung infection caused by *P. aeruginosa* has been shown to improve respiratory function, decrease hospitalizations, and reduce systemic antibiotic use (Ramsey et al. 1999), and has contributed to a significant increase in survival for these patients (Sawicki et al. 2012). Inhaled aminoglycosides, alone or as part of combination therapy, are currently under evaluation as adjunctive agents for treatment of additional respiratory infection types including chronic lung infections associated with non-CF bronchiectasis and refractory NTM infections of the lung, and for the prevention and/or treatment of ventilator associated infections, tracheobronchitis (VAT), and pneumonia (VAP). Regarding the latter indication, although definitive data from large randomized trials is not available, a number of small studies focused on the prevention or treatment of VAP have provided encouraging results. A meta-analysis of eight comparative trials of prophylactic aerosolized antibiotics (four of which used inhaled tobramycin or gentamicin) found that ICU-acquired pneumonia was less common in the group of patients that received antibiotic prophylaxis compared with those who received no prophylaxis (Falagas et al. 2006). Similarly, a meta-analysis of five comparative trials of adjunctive aerosolized antibiotics in the treatment of VAP, each of which evaluated an inhaled aminoglycoside versus placebo or no therapy, re-

vealed that administration of aerosolized antibiotics was associated with better treatment success compared with control in both the intent-to-treat and clinically evaluable populations (Ioannidou et al. 2007). The relative inefficiency of drug delivery through the ventilator circuit is a significant challenge with the use of aerosolized antibiotics for the treatment of ventilator-associated infections. To overcome this issue, BAY41-6551, an investigational drug–device combination of amikacin specifically formulated for inhalation, has been developed. This drug–device combination is currently under phase 3 study as adjunctive therapy in intubated and mechanically ventilated patients with Gram-negative pneumonia (clinicaltrials .gov/ct2/show/NCT01799993 and clinicaltrials .gov/ct2/show/results/NCT00805168).

Because of their poor oral bioavailability, aminoglycosides are a key element of oropharyngeal or gut decolonization/decontamination regimens, including those targeting MDR pathogens. The purpose of these decontamination regimens is to eradicate potential pathogens from the oropharynx and digestive tract of patients at risk for nosocomial or postoperative infections. Selective digestive decontamination (SDD) consists of the oropharyngeal and gastric administration of non-absorbable antibiotics lacking anaerobic activity (often a polymyxin, an aminoglycoside, and amphotericin) along with a short course of systemic antibiotic therapy, whereas selective oropharyngeal decontamination (SOD) consists of application of non-absorbable antibiotics to the oropharynx alone. More than 50 randomized studies and 10 meta-analyses of SDD/SOD have been published. Overall, these data suggest that SDD/SOD are associated with improved survival in ICU patients (Price et al. 2014) and SDD is associated with a reduction in the rate of postoperative infection, including anastomotic leakage, in patients undergoing elective GI surgery (Abis et al. 2013; Roos et al. 2013). Despite these successes in the use of SOD and SDD to improve patient outcomes in the setting of low levels of antibiotic resistance, controversy remains regarding their effectiveness in the setting of high levels of antibiotic resistance as well as their im-

pact on antibiotic resistance. No relationship between the use of SDD or SOD and the development of antimicrobial resistance has been shown in individual studies or meta-analyses in the setting of low antibiotic resistance (Daneman et al. 2013; Plantinga and Bonten 2015) and an international multicenter study of the effects of SDD and SOD on ICU-level antibiotic resistance in countries with higher levels of resistance is currently ongoing (clinicaltrials.gov/ct2/show/NCT02208154).

The ability of paromomycin to bind to eukaryotic ribosomes has led to the use of this agent in the treatment of protozoal infections. Like other aminoglycosides, oral paromomycin is poorly absorbed and may be used for the treatment of noninvasive amebiasis, cryptosporidiosis, trichomoniasis, and giardiasis in patients in whom other agents are contraindicated (Stover et al. 2012). More recently, this agent has been used to treat both cutaneous and visceral leishmaniasis. Topical paromomycin yielded a significantly higher cure rate compared with control therapy in patients with cutaneous leishmaniasis caused by *Leishmania major* (Ben Salah et al. 2013). Intramuscular paromomycin monotherapy was noninferior to standard amphotericin B therapy in a randomized control trial in patients with visceral disease in India (Sundar et al. 2007) and short-course combination regimens containing this agent were also noninferior to standard therapy with fewer adverse events (Sundar et al. 2011).

CONCLUSIONS

The aminoglycosides are a critical component of the current antibacterial arsenal. Their broad spectrum of activity, rapid bactericidal action, and favorable chemical and pharmacokinetic properties make them a clinically useful class of drugs across numerous infection types, including certain protozoal infections. The use of aminoglycosides waned as a result of the emergence of other classes of broad-spectrum agents with improved safety profiles, but the emergence of MDR pathogens has led to renewed interest in this class of drugs. Improved understanding of the drivers of toxicity and

efficacy has led to the implementation of optimized dosing regimens that improve safety while maintaining efficacy. Increased understanding of key aminoglycoside resistance mechanisms combined with innovative medicinal chemistry approaches have led to the synthesis and development of novel agents specifically designed to evade resistance while maintaining potency against fully susceptible isolates. Given the dearth of new agents in the antibiotic pipeline and the ever-increasing specter of resistance, further optimization of the aminoglycoside scaffold to generate new agents with superior potency against MDR pathogens as well as an improved safety profile is warranted.

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