Published in final edited form as:
Drug Resist Updat. 2010 December ; 13(6): 151–171. doi:10.1016/j.drup.2010.08.003.

Aminoglycoside Modifying Enzymes

Maria S. Ramirez and **Marcelo E. Tolmasky***
Center for Applied Biotechnology Studies, Department of Biological Science, College of Natural Sciences and Mathematics, California State University Fullerton, Fullerton, California 92834-6850

Abstract

Aminoglycosides have been an essential component of the armamentarium in the treatment of life-threatening infections. Unfortunately, their efficacy has been reduced by the surge and dissemination of resistance. In some cases the levels of resistance reached the point that rendered them virtually useless. Among many known mechanisms of resistance to aminoglycosides, enzymatic modification is the most prevalent in the clinical setting. Aminoglycoside modifying enzymes catalyze the modification at different −OH or $-NH_2$ groups of the 2-deoxystreptamine nucleus or the sugar moieties and can be nucleotidyltranferases, phosphotransferases, or acetyltransferases. The number of aminoglycoside modifying enzymes identified to date as well as the genetic environments where the coding genes are located is impressive and there is virtually no bacteria that is unable to support enzymatic resistance to aminoglycosides. Aside from the development of new aminoglycosides refractory to as many as possible modifying enzymes there are currently two main strategies being pursued to overcome the action of aminoglycoside modifying enzymes. Their successful development would extend the useful life of existing antibiotics that have proven effective in the treatment of infections. These strategies consist of the development of inhibitors of the enzymatic action or of the expression of the modifying enzymes.

Keywords

antibiotic resistance; aminoglycoside; aminoglycoside modifying enzyme; acetyltransferase; nucleotidyltransferase; phosphotransferase; kinase; antisense; RNase P; RNase H; bacterial infection

1. A brief overview of aminoglycoside antibiotics

1.1. General aspects

Aminoglycoside antibiotics are a complex family of compounds characterized for having an aminocyclitol nucleus (streptamine, 2-deoxystreptamine, or streptidine) linked to amino sugars through glycosidic bonds. In addition, other compounds such as spectinomycin, which is an aminocyclitol not linked to amino sugars, or compounds that include the aminocyclitol fortamine are also included in this family (Bryskier, 2005; Veyssier and Bryskier, 2005). Aminoglycosides are primarily used in the treatment of infections caused by gram-negative aerobic bacilli, staphylococci, and other gram-positives (Yao and

*Department of Biological Science College of Natural Sciences and Mathematics California State University Fullerton Fullerton, California 92834-6850 Phone: (714) 278-5263 mtolmasky@fullerton.edu .

Moellering, 2007). However, when used against gram-positives, aminoglycosides are recommended in combination with other antibiotics such as β-lactams or vancomycin with which they exert a synergistic effect probably due to an enhanced uptake (Eliopoulos, 1989; Scaglione et al., 1995; Yao and Moellering, 2007). Due to the nature of the mechanism of uptake of aminoglycosides, which requires respiration, anaerobic bacteria are intrinsically resistant (see below) (Bryan et al., 1979). As it would be expected from a large family of non-identical compounds, different aminoglycosides vary in their activity spectrum. Streptomycin, discovered in 1943, was the first efficient drug against tuberculosis and in 1944 a woman with this disease was cured after treatment with the antibiotic. Currently, streptomycin is still used in combination therapy to treat *Mycobacterium tuberculosis* (Menzies et al., 2009) and other aminoglycosides such as amikacin or kanamycin are used as second line drug in the treatment of resistant *M. tuberculosis* infections (Brossier et al., 2010). Besides *Enterobacteriaceae* and *Pseudomonas aeruginosa*, examples of life-threatening infections that can be treated with aminoglycosides are plague, tularemia, brucellosis, endocarditis caused by enterococci and infections caused by streptococci and enterococci (Yao and Moellering, 2007). Newer non-traditional applications of aminoglycosides include treatment of genetic disorders such as cystic fibrosis, in which about 10% of patients carry a nonsense mutation as opposed to the most common 3-bp deletion that results in the loss of a phenylalanine in the cystic fibrosis transmembrane conductance regulator (Rich et al., 1990), and Duchenne muscular dystrophy, in which 10 - 20% of patients carry a nonsense mutation in the dystrophin gene (Kellermayer, 2006). The property of aminoglycosides to decrease the fidelity of the eukaryotic elongation machinery makes them potential candidates to treat nonsense mutation related genetic disorders such as those mentioned above or others that can benefit from inducing translational readthrough (Hermann, 2007; Kellermayer, 2006; Zingman et al., 2007). Aminoglycosides, mainly gentamicin, have also been used in the treatment of Ménière's disease by intratympanic injection (Dabertrand et al., 2010; Nakashima et al., 2000). Aminoglycoside-based drugs are also inhibitors of reproduction of the HIV virus, showing promise on the treatment of AIDS (reviewed in Houghton et al., 2010)

The most common route of administration of aminoglycosides for systemic infections is parenteral, intramuscular injection or intravenously in cases of severe infections. Oral administration is not possible for these infections due to very low levels of absorption. However, oral administration can be used for decontamination purposes such as to kill bowel flora before intestinal surgery (Vakulenko and Mobashery, 2003; Veyssier and Bryskier, 2005; Yao and Moellering, 2007). Other routes of delivery are sometimes used to increase the concentration of the drug at the site of infection or to limit nephrotoxicity or ototoxicity (Vakulenko and Mobashery, 2003; Veyssier and Bryskier, 2005; Yao and Moellering, 2007). Aminoglycosides exist in a variety of formulations, some experimental, including encapsulation in liposomes or nanoparticles, or aerosolized (Dudley et al., 2008; Kingsley et al., 2006; Pinto-Alphandary et al., 2000). A study has shown that when amikacin-encapsulated liposomes were modified they changed the organ distribution of the antibiotic (Bucke et al., 1998). Aminoglycoside antibiotics are not metabolized, they are excreted as active compounds and they show biphasic elimination with half-lives in the body of 2 – 3 hours (as long as the renal function is normal) and 37 – 100 hours (Veyssier and Bryskier, 2005; Wenk et al., 1979). They are mainly eliminated by glomerular filtration.

Binding to serum proteins, although variable among different aminoglycosides, is low. While no serum binding was demonstrable for gentamicin, tobramycin, or kanamycin, streptomycin was found to be 35% bound in a comparative study (Gordon et al., 1972). Amikacin serum protein binding in patients with spinal cord injury and in able-bodied controls was ~18% (Brunnemann and Segal, 1991). The fraction bound to serum proteins is important because it is only the unbound fraction of a drug that produces a pharmacological effect (Benet and Hoener, 2002; Heinze and Holzgrabe, 2006).

The utilization of aminoglycosides is not free of adverse effects; they have been linked to drug-induced nephrotoxicity and ototoxicity, a problem that limits the doses that can be used. Nephrotoxicity is generally reversible and the most common clinical presentation is nonoliguric acute kidney injury. Other manifestations include a decrease in the glomerular ! ltration rate, enzymuria, aminoaciduria, glycosuria, hypomagnesemia, hypocalcemia, and hypokalemia (Martinez-Salgado et al., 2007; Oliveira et al., 2009). The ototoxicity effects of aminoglycosides include permanent bilaterally severe, high-frequency sensorineural hearing loss and temporary vestibular hypofunction (Guthrie, 2008). The mechanism by which aminoglycosides are ototoxic seems to be related to their ability to sequester and chelate metals forming complexes that are redox active and generate reactive oxygen species, which in turn induce cell damage (Guthrie, 2008). Free radical scavengers as well as iron chelators were shown to attenuate ototoxic effects of aminoglycosides (Nakashima et al., 2000).

1.2. Bacterial uptake

Internalization of aminoglycosides is an important process for their biological activity. Aminoglycosides penetrate the bacterial cell following a three-steps process; a first energy-independent step is followed by two energy-dependent steps (Taber et al., 1987; Tolmasky, 2007a; Vakulenko and Mobashery, 2003; Veyssier and Bryskier, 2005). Two components are needed for accumulation of aminoglycoside molecules inside the cell: ribosomes and the membrane bound respiratory chain. When aminoglycoside molecules are in contact with bacterial cells, the polycationic antibiotic molecules bind to cell's surface anionic compounds such as lipopolysaccharide, phospholipids, and outer membrane proteins in gram-negatives, and teichoich acids and phospholipids in gram-positives. As a result of the binding to anionic sites in the outer membrane, divalent cations that cross-bridge adjacent lipopolysaccharide molecules are displaced resulting in an increase in permeability that leads to the so called "self-promoted uptake" penetration of aminoglycoside molecules to the periplasmic space (Vanhoof et al., 1995). The following process is blocked by inhibitors of electron transport and oxidative phosphorylation (Muir et al., 1984) and is known as "energy-dependent phase I". Although alternatives have been proposed (Nichols and Young, 1985) it is generally accepted that this phase is characterized by the uptake into the cytoplasm of a small number of aminoglycoside molecules in an energy-dependent fashion, and since it needs a functional electron transport system anaerobes tend not to be susceptible to these antibiotics (Bryan and van der Elzen, 1977). The small number of molecules that reach the cytoplasm during energy-dependent phase I induce errors in protein synthesis and the mistranslated membrane proteins cause damage to the integrity of the cytoplasmic membrane when they are inserted triggering the following step known as "energy-dependent phase II". This mechanism is supported by the need for protein synthesis for triggering the energy-dependent phase II (Hurwitz et al., 1981). The aberrant proteins in the damaged membrane facilitate transport of more molecules of antibiotic that increase the level of interference with normal protein synthesis leading to yet more damage in the membrane resulting in an autocatalytic accelerated rate of uptake that ultimately results in death of the cell (Davis, 1988; Nichols, 1989; Taber et al., 1987). The presence of capsule or exopolysaccharide layers seems not to affect diffusion of the aminoglycosides (Nichols et al., 1988).

1.3. Molecular mechanisms of action

Studies on the effect of aminoglycosides on protein synthesis resulted not only in an understanding of the mode of action of these antibiotics but also in contributions to the understanding of the molecular mechanisms of translation fidelity (Davies, 2006; Davis, 1987; Houghton et al.; Magnet and Blanchard, 2005; Majumder et al., 2007; Vakulenko and Mobashery, 2003). It is clear now that pairing of the codon/anticodon nucleotides cannot account for the levels of fidelity observed in selection of the correct aminoacyl-tRNA (Ogle et al., 2001; Ogle et al., 2003). The ribosome plays an active role in stabilization of the cognate tRNA/mRNA association and rejection of near-cognate tRNAs. One of the earliest observations that led to the idea that the ribosome is an active player in the faithful decoding mechanism by modulating tRNA/mRNA interactions was the production of an enzyme that was otherwise absent due to a premature stop codon in specific *Escherichia coli* auxotrophic mutants upon addition of streptomycin (Spotts and Stanier, 1961). These experiments no only helped understanding mechanisms of translation fidelity during protein synthesis but also contributed to the clarification of the misreading-inducing properties of aminoglycosides. It is now well established that the A site is the decoding center of the ribosome, located on the 16S RNA (which together with about 21 proteins composes the 30S subunit of the ribosome). Regions of the 16S RNA establish contact with the cognate codon/anticodon pair and modify their structure resulting in what is known as the closed conformation of the 30S RNA subunit as opposed to the open structure of the empty A site (reviewed in Ogle et al., 2003; Ogle and Ramakrishnan, 2005; Zaher and Green, 2009). Conversely, binding of a near cognate tRNA does not induce the closed state.

The intimate mechanisms by which aminoglycosides interfere with translational fidelity are becoming ever more clear with the dilucidation of crystal structures of complexes between different aminoglycosides and the A site as well as the effects caused by these interactions. Structures of a number of aminoglycosides bound to oligonucleotides containing the decoding A site or the entire subunit have recently been determined by NMR or X-ray crystallography (reviewed in Jana and Deb, 2006; Ogle et al., 2003; Ogle and Ramakrishnan, 2005; Vicens and Westhof, 2003; Zaher and Green, 2009). These studies showed that not all classes of aminoglycosides bind to identical sites of the 16S rRNA but the common effect of their binding is a change of conformation of the A site to one that mimics the closed state induced by interaction between cognate tRNA and mRNA eliminating the proofreading capabilities of the ribosome and thereby promoting mistranslation. With the exception of spectinomycin and kasugamycin, aminoglycosides are bactericidal and their lethality is thought to be due to the secondary effects of inducing mistranslation (Bakker, 1992; Busse et al., 1992; Davis, 1987, 1989; Magnet and Blanchard, 2005; Vakulenko and Mobashery, 2003).

Aminoglycosides such as neomycin and paromomycin have also been shown to inhibit 30S ribosomal subunit assembly although this also could be a secondary effect to protein mistranslation (Mehta and Champney, 2003). Other effects of aminoglycosides include their ability to induce RNA cleavage (Belousoff et al., 2009) or interfere with essential functions such as RNase P, which has been shown to be inhibited by neomycin B due to interference of the antibiotic molecule with the binding of divalent metal ions to the RNA moiety of RNase P (Mikkelsen et al., 1999). These properties could be exploited to develop new aminoglycosides directed to targets other than the ribosome.

Experiments exposing *E. coli* cells to sublethal concentrations of amikacin showed that one of the most susceptible cellular mechanisms is formation of the Z ring, which leads to anomalies in cell division. At these low concentrations of the antibiotic the chromosomes continued replication and were properly located (Possoz et al., 2007).

2. Bacterial resistance to aminoglycoside antibiotics

2.1. Mechanisms of resistance

Aminoglycoside resistance occurs through several mechanisms that can coexist simultaneously in the same cell (Alekshun and Levy, 2007; Houghton et al.; Magnet and Blanchard, 2005; Taber et al., 1987; Tolmasky, 2007a). Described mechanisms include modification of the target by mutation of the 16S rRNA or ribosomal proteins (Galimand et al., 2005; O'Connor et al., 1991); methylation of 16S rRNA, a mechanism found in most aminoglycoside-producing organisms and in clinical strains (Doi and Arakawa, 2007; Galimand et al., 2005); reduced permeability by modification of outer membrane's permeability or diminished inner membrane transport (Hancock, 1981; MacLeod et al., 2000; Over et al., 2001); export outside the cell by active efflux pumps (Aires et al., 1999; Magnet et al., 2001; Rosenberg et al., 2000), one of which has recently been shown to be involved in adaptive resistance (Hocquet et al., 2003); active swarming, a probably non-specific mechanism recently shown in *P. aeruginosa* cells, which exhibited adaptive antibiotic resistance against several antibiotics (Overhage et al., 2008); sequestration of the drug by tight binding to an acetyltransferase of very low activity (Magnet et al., 2003); and enzymatic inactivation of the antibiotic molecule, the most prevalent in the clinical setting and the subject of this review.

3. Aminoglycoside modifying enzymes

Aminoglycoside modifying enzymes catalyze the modification at −OH or $-NH_2$ groups of the 2-deoxystreptamine nucleus or the sugar moieties and can be acetyltransferases (AACs), nucleotidyltranferases (ANTs), or phosphotransferases (APHs) (Fig. 1). The combination of mutagenesis, which leads to continuous generation of new enzyme variants that can utilize an ever growing number of antibiotics as substrates, with the coding genes' ability to transfer at the molecular level as part of integrons, gene cassettes, transposons, or integrative conjugative elements and at the cellular level through conjugation, as part of mobilizable or conjugative plasmids, natural transformation or transduction results in the ability of this resistance mechanism to reach virtually all bacterial types (Tolmasky, 2007b).

The number of aminoglycoside modifying enzymes identified to date as well as the hosts and genetic environments is impressive, therefore the citations and examples described here should be considered representative rather than comprehensive. Furthermore, putative genes coding for aminoglycoside modifying enzymes are being found in complete genome sequences. These genes, for which there is no further information other than the annotation, are not discussed in this review. Summaries including relevant data on aminoglycoside modifying enzymes are shown in Fig. 1 and Tables 1 - 3.

3.1. Aminoglycoside modifying enzymes: nomenclature

There are two main nomenclatures currently in use to identify aminoglycoside modifying enzymes. One of them consists of a three-letter identifier of the activity followed by the site of modification between parenthesis (class), a roman number particular to the resistant profile they confer to the host cells (subclass), and a low case letter that is an individual identifier (Shaw et al., 1993). The parenthesis and the subclass are usually separated by a hyphen but lately some authors have removed it (Oteo et al., 2006). For example, AAC(6′)-Ia represents an *N*-acetyltransferase that catalyzes acetylation at the 6′ position conferring a resistance profile identical to the other AAC(6′)-I enzymes (AAC(6′)-Ib – AAC(6′)-Iaf). In the other nomenclature system the genes are designated *aac*, *aad* and *aph* followed by a capital letter that identifies the site of modification (Novick et al., 1976). Thus, *aacA, aacB, and aacC* identify aminoglycoside 6′-*N*- acetyltransferase, aminoglycoside 2′-*N*-acetyltransferase, and aminoglycoside 3-*N*-acetyltransferase respectively. A number is then added to provide a unique identifier to different genes. Each of the nomenclatures has its own advantages and disadvantages and different authors prefer one to the other but, as it has been suggested before (Tolmasky, 2007a; Vanhoof et al., 1998), it would be convenient to reach consensus and use only one of them to avoid confusion and facilitate following the advances in the field. The confusion is sometimes compounded by different additions or modifications in naming new genes or variants (see below). We suggest that returning to a simpler nomenclature with the support of an internet repository site could facilitate the naming of the genes, avoid duplications, and facilitate further changes when new enzymes with new, and may be unexpected, characteristics are discovered.

3.2. Aminoglycoside modifying enzymes: aminoglycoside N-acetyltransferases (AACs)

AACs belong to the ubiquitous GCN5-related *N*-acetyltransferase (GNAT) superfamily of proteins, which include about 10,000 proteins (Vetting et al., 2005). GNAT enzymes catalyze the acetylation of $-NH_2$ groups in the acceptor molecule using acetyl coenzyme A as donor substrate, in the case of AACs the acceptor is an aminoglycoside antibiotic. The AACs catalyze acetylation at the 1 [AAC(1)], 3 [AAC(3)], 2′ [AAC(2′)], or 6′ [AAC(6′)] positions (Fig. 1 and Table 1). The three dimensional structures of several acetyltransefrases have been resolved (see Table 1), mechanistic and structural aspects of these and other representatives of these enzymes have been thoroughly studied and reviewed (Azucena and Mobashery, 2001; Houghton et al., 2010; Tolmasky, 2007a; Vetting et al., 2005; Wright and Berghuis, 2007).

3.2.1. AAC(1)—To date AAC(1) enzymes have been found in *E. coli* , *Campylobacter* spp., and an actinomycete (Gomez-Luis et al., 1999; Lovering et al., 1987; Sunada et al., 1999). The AAC(1) isolated from *E. coli* catalyzes acetylation of apramycin, butirosin, lividomycin and paromomycin at the 1 position, and catalyzes di-acetylation of ribostamycin and neomycin. The AAC(1) isolated from an actinomycete (strain #8) differed in substrate profile from that one from *E. coli* as apramycin was not acetylated by this enzyme. Furthermore paromomycin was preferentially acetylated at position 1, but 1,2′-di-*N*-acetylparomomycin and 1,6‴-di-*N*-acetylparomomycin were also found as products of the enzymatic reaction (Sunada et al., 1999). These studies also determined that these modifications were not accompanied by a significant reduction of the antibiotic activity. The substrate profile of the AAC(1) isolated from *Campylobacter* spp. was similar to that of the *E. coli* enzyme. This was the only instance in which an AAC(1) was found in clinical isolates. The authors suggested that the gene is located in the chromosome, but these results await confirmation. Although all three enzymes have been named AAC(1), the difference in substrate profile of at least one of them would justify to named them with a subclass number.

3.2.2. AAC(3)—There are nine recognized subclasses of AAC(3) enzymes described to date, all of them in gram-negatives. The subclass AAC(3)-V has been eliminated after confirmation that the only enzyme in this group is identical to AAC(3)-II (Shaw et al., 1993). The subclass AAC(3)-I includes five enzymes that confer resistance to gentamicin, sisomicin, and fortimicin (astromicin) and are present in a large number of *Enterobacteriaceae* and other gram-negative clinical isolates. The X-ray structure of AAC(3)-Ia from *Serratia marcescens* (Javier Teran et al., 1991) complexed to CoA has been determined at 2.3 Å resolution (Wolf et al., 1998), as it is the case with several acetyltransferases this enzyme seems to exist as a dimer under physiological conditions.

All five genes have been found as part of gene cassettes in integrons. The latest gene in this subclass to be reported is *aac(3)-Ie*, which was found in integrons in *Proteus vulgaris*, *P.*

aeruginosa, and within a *Salmonella enterica* subsp. *enterica* genomic island (Gionechetti et al., 2008; Wilson and Hall, 2010).

The subclass AAC(3)-II, which is characterized by resistance to gentamicin, netilmicin, tobramycin, sisomicin, 2′-*N*-ethylnetilmicin, 6′-*N*-ethylnetilmicin and dibekacin (Shaw et al., 1993), includes three enzymes: AAC(3)-IIa and AAC(3)-IIb, which were previously published as AAC(3)-Va and AAC(3)-Vb (see letter and reply van de Klundert and Vliegenthart, 1993), and AAC(3)-IIc. While AAC(3)-IIa has been found in a large variety of genera, AAC(3)-IIb and AAC(3)-IIc have been found in *E. coli, Alcaligenes faecalis* and *S. marcescens* or *E. coli* and *P. aeruginosa* respectively (Dubois et al., 2006; Dubois et al., 2008; Oteo et al., 2006; Shaw et al., 1993). A recent survey of *Enterobacteriaceae* clinical isolates from a Tunisian Hospital showed the presence of undetermined AAC(3)-II enzymes, although the authors suggest the possibility of AAC(3)-IIb, in all genera tested (Dahmen et al., 2010).

There are three enzymes belonging to the subclass AAC(3)-III, all isolated from *P. aeruginosa* isolates. When cloned, the *aac(3)-IIIa* gene was expressed in *P. aeruginosa* but not in *E. coli* (Vliegenthart et al., 1991b). This does not seem to be due to an inactive promoter in *E. coli*. The authors proposed that most probably the mRNA is not completely synthesized or the initiation of translation of the gene is obstructed (Vliegenthart et al., 1991b). There were other early reports of AAC(3)-III enzymes in other genera, e.g., *Klebsiella pneumoniae*, but they seem to be misnamed (for clarification see Vliegenthart et al., 1991b).

The only representative of AAC(3)-IV has been identified in clinical strains of *E. coli* (originally thought to be *Salmonella*) (Brau et al., 1984), *Campylobacter jejuni*, and in environmental *Pseudomonas stutzeri* (Heuer et al., 2002).

Although only AAC(3)-VIa is recognized in the literature within subclass AAC(3)-VI, comparison of the original sequence from *Enterobacter cloacae*, with the more recently isolated genes from *E. coli*, and *S. enterica* show a one amino acid difference (Call et al., 2010; Rather et al., 1993a).

Subclasses AAC(3)-VII, AAC(3)-VIII, AAC(3)-IX, and AAC(3)-X are represented in strains of actinomycetes (Ishikawa et al., 2000; Lopez-Cabrera et al., 1989; Salauze et al., 1991). This latter enzyme was of interest because besides catalyzing acetylation of kanamycin and dibekacin at the 3-amino group it also mediates acetylation the 3″-amino group in arbekacin and amikacin, making this the first AAC detected to have also AAC(3″) activity. Interestingly, while 3″-*N*-acetylamikacin lost most or all antibiotic activity, 3″-*N*-acetylarbekacin was still active (Hotta et al., 1998).

3.2.3. AAC(2′)—These enzymes have been found in gram-negatives and *Mycobacterium*, they mediate modification of several aminoglycosides including gentamicin, tobramycin, dibekacin, kanamycin and netimicin. Only one subclass exists, which includes AAC(2′)-Ia (*Providencia stuartii)*, AAC(2′)-Ib (*Mycobacterium fortuitum* and *Acinetobacter baumannii*), AAC(2′)-Ic (*M. tuberculosis* and *Mycobacterium bovis*), AAC(2′)-Id (*Mycobacterium smegmatis*), and a putative AAC(2′)-Ie identified in the *Mycobacterium leprae* genome (Adams et al., 2008; Ainsa et al., 1997; Hegde et al. 2001; Rather et al., 1993b). A putative AAC(2′) enzyme has been proposed to be part of multidrug resistance in *Stenotrophomonas maltophilia* but it has not been named further (Crossman et al., 2008). Our Blast analysis of the amino acid sequence of this protein against those in GenBank did not show 100% homology with any of the AAC(2′) know enzymes.

3.2.4. AAC(6′)—AAC(6′) enzymes are by far the most common, they are present in gram-negatives as well as gram-positives, the genes have been found in plasmids and chromosomes, and are often part of mobile genetic elements, some of them with unusual structures (Centron and Roy, 2002; Soler Bistue et al., 2008; Tolmasky, 2007a; Tolmasky, 2000). Accordingly, there is a very large volume of information available about them. There are two main subclasses of AAC(6′) enzymes that specify resistance to several aminoglycosides and differ in their activity against amikacin and gentamicin C1. While AAC(6′)-I shows high activity against amikacin and gentamicin C1a and C2 but very low towards gentamicin C1, AAC(6′)-II enzymes actively mediate acetylation of all three forms of gentamicin but not amikacin (Rather et al., 1992; Shaw et al., 1993; Tolmasky, 2007a; Tolmasky et al., 1986; Woloj et al., 1986). A novel enzyme that includes fluoroquinolones as substrates, could be considered a third class because of the change in pattern of substrates but it has been named AAC(6′)-Ib-cr, most probably because it is an evolutionary product of AAC(6′)-Ib by modification of two amino acids, Trp102Arg and Asp179Tyr (Robicsek et al., 2006). Unfortunately, due to the high variability and number of enzymes belonging to this class, the fast pace of research on these enzymes, and the fact that a large number of enzymes have different degrees of similarity in sequence and phenotype, there is a good deal of confusion and lack of consistency in nomenclature and classification of many members. In at least one instance two simultaneously discovered enzymes were named identically (Vanhoof et al., 1998). Enzymes with AAC(6′)-II resistance profiles but with higher identity to AAC(6′)-I enzymes at the amino acid level were named AAC(6′)-I (Casin et al., 2003; Lambert et al., 1994b). Different enzymes have been named identically, for example an acetyltransferase encoded by plasmid pBWH301 was named AAC(6′)-Il (accession number U13880) (Bunny et al., 1995), and the same name was used to name an acetyltransferase from *C. freundii* Cf155 (accession number Z54241) (Hannecart-Pokorni et al., 1997). This latter enzyme was subsequently renamed AAC(6′)-Im (Vanhoof et al., 1998). A search in PubMed shows the title of this paper as “AAC(6′)-Im [corrected]”. However, this enzyme was also called AAC(6′)-Ip by Centrón et al (Centron and Roy, 1998). Another enzyme identified later in *E. coli* and *Entererococcus faecium* was named AAC(6′)-Im (Chow et al., 2001).

The AAC(6′)-I subclass is so highly populated that a double low case letter was necessary to identified them, at the moment the latest published enzyme named as such is the AAC(6′)-Iaf (Kitao et al., 2009). An AAC(6′)-Iai can be found in GenBank but not AAC(6′)-Iag or AAC(6′)-Iah. Variants of AAC(6′)-Ib have been identified with subscripts e.g., AAC(6′)-Ib_3, AAC(6′)-Ib_4, AAC(6′)-Ib_6, and AAC(6′)-Ib_7 and differ at the N-terminus but have similar behavior (Casin et al., 1998). Conversely variant AAC(6′)-Ib_{11}, found in a class 1 integron in *S. Typhimurium*, exhibits a two amino acids difference with AAC(6′)-Ib at positions 118 and 119 that results in an extended resistance spectrum that would merit the definition of a new subclass (Casin et al., 2003). Another variation to the nomenclature used only once is the addition of a prime symbol. The *Pseudomonas fluorescens* BM2687 AAC(6′)-Ib’ is encoded by a gene that has a Ser instead of a Leu residue at position 90, a substitution previously recognized as responsible for changing the resistance profile from subclass I to II (Lambert et al., 1994b; Rather et al., 1992). Besides the addition of a prime symbol, the name of this enzyme is also unusual, although not unique, in that in spite of having a AAC(6′)-II phenotype is called as if belonging to subclass AAC(6′)-I. AAC(6′)-Ib’ also exist as a fusion protein with a nucleotidyltransferase identified as ANT(3″)-Ii/AAC(6′)-IId in a *S. marcescens* integron that includes a group II intron (Centron and Roy, 2002). Considering the total identity between AAC(6′)-IId portion of the *S. marcescens* enzyme and AAC(6′)-Ib’, the name of this latter enzyme should be changed to AAC(6′)-IId. Other modifications to the nomenclature include removal of the roman number that identifies the subclass and the addition of a number, e.g. AAC(6′)-29a, AAC(6′)-29b, AAC(6′)-31, AAC(6′)-32, or AAC(6′)-33 (Gutierrez et al., 2007; Mendes et al., 2007; Poirel et al., 2001; Viedma et al., 2009); or the substitution of the low case letter for a number as in the *S. enterica* AAC(6′)-I30 enzyme (Mulvey et al., 2004). Other recent variations to the nomenclature consist on the addition of whole words or acronyms such as AAC(6′)-Ib-Suzhou (Huang et al., 2008) or AAC(6′)-Isa (Hamano et al., 2004). The monumental number of identified genes together with the *de facto* lack of a unified and agreed nomenclature for AAC(6′) enzymes make it extremely difficult to get a clear nomenclature landscape about these enzymes. The AAC(6′)-Id protein has been mentioned several times in the literature, but the accession number provided (X12618) does not currently correspond to an acetyltransferase and for that reason it has not been included in Table 1.

AAC(6′) enzymes can exist as fusion proteins occupying the N or C terminal region of the composite protein (Zhang et al., 2009). These fused *aac(6′)* genes are usually found within integrons and they can be the result of integrase-mediated recombination events (Centron and Roy, 2002). Interestingly, proteins containing AAC(6′)-I activities have been found fused to APH, ANT, a different AAC, and another AAC(6′)-I activities. AAC(6′)-Ie is located to the amino terminal end of a bifunctional *Enterococcus faecalis* and *Staphylococcus aureus* enzyme with AAC(6′) and APH(2″) activities (Boehr et al., 2004; Ferretti et al., 1986). The *aac(6′)-aph(2″)* gene is usually present in Tn*4001*-like transposons (Culebras and Martinez, 1999). As described above, the AAC(6′)-IId is the carboxy terminal region of the protein fusion that also includes an ANT(3″)-I activity. Fusions of two AAC(6′)-I activities, AAC(6′)-30/AAC(6′)-Ib', or two AAC belonging to different subclasses, AAC(3)-Ib and AAC(6′)-Ib' were found in *P. aeruginosa* integrons (Dubois et al., 2002; Mendes et al., 2004).

Three phylogenetic subgroups have been recognized among AAC(6′)-I and AAC(6′)-II enzymes (Hannecart-Pokorni et al., 1997; Shaw et al., 1993; Shmara et al., 2001) but an alternative theory has been published that proposes that the three groups are less related than thought before and the 6′ acetylating activity has evolved independently at least three times (Salipante and Hall, 2003).

An immunochromatographic method based on the utilization of monoclonal antibodies against the AAC(6′)-Iae has recently been reported (Kitao et al., 2010). This enzyme was selected for these studies because *aac(6′)-Iae* is prevalent in Japan and appears linked to the metallo-!-lactamase gene bla_{IMP} and *ant(3″)-Ia* in the integron In113, making the assay a useful tool to detect multiple drug resistance in *P. aeruginosa* in this country (Kitao et al., 2010). However, 37% of the negative isolates from Japan still showed a multiple drug resistance phenotype and 76% of these negative isolates include *aac(6′)-Ib* and the metallo-!-lactamase gene *bla*IMP-1. At present, the authors of this study are developing an immunochromatography assay targeting AAC(6′)-Ib and metallo-!-lactamase IMP to complement that one targeting AAC(6′)-Iae for a more complete diagnostics tool (Kitao et al., 2010).

AAC(6′)-Ib is probably the most clinically relevant acetyltransferase and is responsible for the resistance to amikacin and other aminoglycosides found in several gram-negatives belonging to the genus *Acinetobacter* and to the *Enterobacteriaceae*, *Pseudomonadaceae*, and *Vibrionaceae* (reviewed in Tolmasky, 2007a; Vakulenko and Mobashery, 2003). It is present in over 70% of AAC(6′)-I-producing gram-negative clinical isolates (Vakulenko and Mobashery, 2003) and, as mentioned above, some of its variants show an extended spectrum including resistance to gentamicin [AAC(6′)-Ib_{11}] (Casin et al., 2003) or reduced susceptibility to quinolones [AAC(6′)-Ib-cr] (Robicsek et al., 2006). Since it was first identified, this latter enzyme has been detected in a large number of geographical regions in numerous genetic environments (Strahilevitz et al., 2009). It is usually found as a gene cassette in different integrons and associated to quinolone resistance genes such as *qnrA1*, *qnrB2*, *qnrB4*, *qnrB6*, *qnrB10*, *qnrS1*, *qnrS2*, and *qepA* or β-lactamase genes such as $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-24}}$, $bla_{\text{DHA-1}}$, $bla_{\text{SHV-12}}$, and $bla_{\text{KPC-2}}$ (Strahilevitz et al., 2009).

The prevalence of AAC(6′)-Ib together with its numerous variants attracted the interest of several research groups that studied them from different points of view. The *aac(6′)-Ib* gene is mostly found as a gene cassette within class 1 integrons or as a defective gene cassette within an unusual structure resembling the variable portion of the integrons but lacking the 5′ and 3′ conserved regions as in Tn*1331* and its derivatives *Tn1331.2*, Tn*1332* or the KQ element (Chamorro et al., 1990; Dery et al., 1997; Poirel et al., 2006; Rice et al., 2008; Sarno et al., 2002; Soler Bistue et al., 2008; Tolmasky et al., 1988; Tolmasky and Crosa, 1987). The structures of Tn*1331* and the modifications occurred for the generation of Tn*1331.2*, Tn*1332*, and the KQ element are shown in Fig. 2. Interestingly, the *aac(6′)-Ib* environment found in these genetic elements has a number of particular characteristics. While in integrons the gene located at the 5′ end of the variable region is preceded by an *attI* recombination site located adjacent to the *intI* gene (Partridge et al., 2000), in these elements there is no *attI* upstream of the *aac(6′)-Ib* gene. Instead, an 8 bp sequence known as *attI1** is found near the beginning of the structural gene at the location where a gene fusion between a bla_{TEM} gene and a precursor of *aac(6′)-Ib* is believed to have occurred, incorporating the first six amino acids of the TEM β-lactamase at the N-terminus of this version of AAC(6′)-Ib (Fig. 2A) (Ramirez et al., 2008; Tolmasky, 1990). These features define an imperfect gene cassette with *attI1** at the 5′ end within the *aac(6′)-Ib* structural gene (Fig. 2A). IntI1 integrase-mediated excision of this imperfect gene cassette could not be detected in cells harboring a recombinant clone with *intI1* under the control of the P_{tac} promoter (Ramirez et al., 2008). Products of evolution of the Tn*1331* transposon by insertion of DNA fragments or duplications have been found and are shown in Fig. 2B. This version of the *aac(6′)-Ib* gene was also found in the chromosome of a *P. mirabilis* isolate as part of a mosaic structure containing several resistance genes (Zong et al., 2009). In this case the upstream region of the gene is derived from Tn*1331* but it shows a different organization, it is preceded by the region located downstream of $bla_{\text{OXA-9}}$ in Tn*1331*. The authors of this report proposed that homologous recombination events between the duplicated regions of Tn*1331* could have led to formation of a circular molecule that could have then been integrated into the chromosome (Fig. 2C) (Zong et al., 2009).

The translation of the *aac(6′)-Ib*$_7$ gene cassette has been studied in some detail. This is one of about 20% of gene cassettes that lack a discernible translation initiation region. Instead, a short open reading frame is located immediately upstream of the structural gene that significantly enhances translation through translational coupling (Hanau-Bercot et al., 2002; Jacquier et al., 2009).

A large number of variants of AAC(6′)-Ib have been found that differ at the N-terminal end, a phenomenon that may be a consequence of the high mobility of the gene. The fact that most of all of these variants are active shows a high flexibility in the structural requirements at this portion of the protein. This property has been proposed to be a contributing factor to the successful distribution and predominance among aminoglycoside resistant *Enterobacteriaceae* (Casin et al., 1998).

The AAC(6′)-Ib protein has been the subject of numerous mutagenesis as well as structural and mechanistic studies (Casin et al., 2003; Chavideh et al., 1999; Kim et al., 2007; Maurice et al., 2008; Panaite and Tolmasky, 1998; Pourreza et al., 2005; Rather et al., 1992; Shmara et al., 2001; Vetting et al., 2004; Vetting et al., 2008). Significant progress in understanding AAC(6′)-Ib and its variants has been achieved recently after the elucidation of the crystal structures of AAC(6′)-Ib and the extended spectrum AAC(6′)-Ib$_{11}$ in conjunction with the construction of a molecular model of AAC(6′)-Ib-cr (Vetting et al., 2008). These studies showed that unlike AAC(6′)-Ii and AAC(6′)-Iy, which are dimers (Draker et al., 2003; Vetting et al., 2004; Wybenga-Groot et al., 1999), AAC(6′)-Ib and AAC(6′)-Ib-cr exist as a monomer while AAC(6′)-Ib_{11} shows monomer/dimer equilibrium (Maurice et al., 2008). Structural features behind the ability of AAC(6′)-Ib to catalyze acetylation of semisynthetic aminoglycosides, as well as the ordered kinetic mechanism could be explained (Maurice et al., 2008). Furthermore, a flexible flap was identified in AAC(6′)-Ib_{11} that might explain its ability to utilize as substrate both amikacin and gentamicin (Maurice et al., 2008). The modeling of AAC(6′)-Ib-cr, which has the substitutions D179Y and W102R with respect to AAC(6′)-Ib, permitted to determine that the Asp179Tyr substitution produces the greatest structural effect that results in an enhanced binding to the antibiotic molecule and the W102R acts by stabilizing the positioning of the Y179 (Robicsek et al., 2006; Strahilevitz et al., 2009). This attractive model explains the effects of each individual substitution. While D179Y is enough to confer a partial resistance phenotype, the effect of W102R is hardly detectable. Another model that emphasizes plasticity in the active site has also been suggested (Maurice et al., 2008). Quick methods for identification and genotyping of *aac(6′)-Ib-cr* have recently been published (Bell et al., 2010; Hidalgo-Grass and Strahilevitz, 2010).

Detailed subcellular localization studies of the AAC(6′)-Ib encoded by Tn*1331* using physical separation methods together with gene fusions to *phoA* in which the signal peptide coding sequence has been removed, and fluorescence microscopy in which the gene was fused to the cyan fluorescent protein demonstrated that the enzyme is evenly distributed within the cytoplasmic compartment of *E. coli* (Dery et al., 2003). Care should be taken when determining the subcellular location of aminoglycoside modifying enzymes. In the past there were contradictory reports indicating that they are located in the periplasmic space or the cytosol (Franklin and Clarke, 2001; Perlin and Lerner, 1981; Tolmasky, 2007a; Vakulenko and Mobashery, 2003; Vliegenthart et al., 1991a). These contradictory findings could be due to the fact that in osmotically shocked *E. coli*, proteins are released through a molecular sieve formed by the damaged cell envelope (Vazquez-Laslop et al., 2001). As a consequence, cytoplasmic proteins small in native size tend to be released after osmotic shock treatment while larger proteins or protein complexes remain inside the cells (Vazquez-Laslop et al., 2001). We confirmed this by extracting the periplasmic proteins by spheroplast formation under different conditions and found that while the controls behaved as expected under all conditions, when we used mild conditions the AAC(6′)-Ib signal was present in the cytosolic extract but when we used harsher conditions a considerable fraction of the total AAC(6′)-Ib was found in the periplasmic extract (Dery et al., 2003; Tolmasky, 2007a).

Two shorter proteins of this class, AAC(6′)-29a and AAC(6′)-29b, have been identified from a multidrug-resistant clinical isolate of *P. aeruginosa* (Magnet et al., 2003; Poirel et al., 2001). The 131-amino acids AAC(‘6)-29b protein was studied in more detail and it was found that it does not mediate resistance by enzymatic modification but rather by tightly binding aminoglycoside molecules, a result that led to the conclusion that the mechanism of aminoglycoside resistance mediated by this protein is by sequestering the drug as a result of tight binding to the molecule (Magnet et al., 2003).

3.3. Aminoglycoside modifying enzymes: aminoglycoside O-nucleotidyltransferases (ANTs)

ANTs mediate inactivation of aminoglycosides by catalyzing the transfer of an AMP group from the donor substrate ATP to and hydroxyl group in the aminoglycoside molecule. There are five classes of ANTs that catalyze adenylylation at the 6 [ANT(6)], 9 [ANT(9)], 4′

[ANT(4′)], 2″ [ANT(2″)], and 3″ [ANT(3″)] positions, of which only ANT(4′) includes two subclasses, I and II (Fig. 1 and Table 2).

3.3.1. ANT(6)—Genes coding for enzymes with related amino acid sequences have been named *ant(6)-Ia*, *ant6*, *ant(6)*, and *aadE*. They all exhibit the same substrate profile (resistance to streptomycin) and therefore belong into the same subclass, but they are not identical. These genes are highly widespread among gram-positive bacteria (Tolmasky, 2007a; Vakulenko and Mobashery, 2003). Two genes called *aadE* with 87% identity at the amino acid level were found in the *E. faecalis* plasmid pRE25 and in *C. jejuni* (Schwarz et al., 2001). Genes coding for ANT enzymes are found in plasmids, transposons, and chromosomes. The *ant(6)* gene is often found in a cluster *ant(6)-sat4-aph(3′)-III* that specifies resistance to aminoglycosides and streptothricin (Cerda et al., 2007). This cluster is part of Tn*5405* and other related transposons, which are distributed among *Staphylococci* and *Enterococci* (Werner et al., 2003) and are located in plasmids and chromosomes. Another gene originally found in *Bacillus subtilis* was named *aadK* (Noguchi et al., 1993) and was subsequently found in other species of *Bacillus* (Vakulenko and Mobashery, 2003). The protein encoded by this gene shows 58% identity and 74% similarity with one encoded by an *aadE* gene (Vakulenko and Mobashery, 2003). A novel *ant(6)* gene, named *ant(6)-Ib*, was recently identified in *Campylobacter fetus* subsp. *fetus* within a transferable pathogenicity island (Abril et al., 2010). This gene is identical to that called *aad(6)* in a contig of an unfinished *Clostridium* genome (accession number NZ_ABDU01000081).

3.3.2. ANT(9)—Two enzymes with the ANT(9) characteristics have been described, ANT(9)-Ia and ANT(9)-Ib, both mediating resistance to spectinomycin. The genes coding for these enzymes were called as *ant(9)-Ia* and *ant(9)-Ib*, but unfortunately they have both also been called *spc* or *aad(9)* facilitating confusion. The amino acid sequences of ANT(9)-Ia and ANT(9)-Ib share 39% identity. ANT(9)-Ia was first described in *S. aureus* and then also in *Enterococcus avium*, *E. faecium*, and *E. faecalis*. In all four bacteria the gene was part of Tn*554* (Mahbub Alam et al., 2005; Murphy, 1985). Our BLAST analysis showed a protein with 100% identity to ANT(9)-Ia present as part of a novel transposon, Tn*6072* (Chen et al., 2010). However, although the gene is correctly named as *spc* it is described as a streptomycin 3′-adenyltransferase. ANT(9)-Ib was found in a plasmid from *E. faecalis* (LeBlanc et al., 1991).

3.3.3. ANT(4′)—ANT(4′)-Ia is found in plasmids of gram-positives such as Staphylococci, Enterococci, and *Bacillus* spp., and the gene has been also named *aadD*, *aadD2*, and *ant(4′, 4″)-I* (Bozdogan et al., 2003; Kobayashi et al., 2001; Muller et al., 1986; Perez-Vazquez et al., 2009). This latter name is due to the fact that this enzyme was found to modify 4′ and 4″ groups, which makes it capable of conferring resistance to dibekacin, an aminoglycoside that lacks a 4′ target (Santanam and Kayser, 1978). Both subclasses, I and II, confer resistance to tobramycin, amikacin, isepamicin, but subclass I also codifies resistance to dibekacin. ANT(4′)-Ia is the only ANT enzyme for which the three dimensional structure has been resolved (Pedersen et al., 1995). An ANT(4′) was also the subject of NMR studies to clarify aspects of the process of the recognition of the substrate (Revuelta et al. 2008). Two ANT(4′)-II enzymes have been described in gram-negative bacilli. These enzymes do not modify dibekacin, and therefore they must be unable to use the position 4″ as target. ANT(4′)-IIa was identified in plasmids of *Pseudomonas* and *Enterobacteriaceae* (Jacoby et al., 1990), and ANT(4′)-IIb was identified more recently in a *P. aeruginosa* transposon (Coyne et al., 2010).

3.3.4. ANT(2″)—This class consists only of ANT(2″)-Ia (Cameron et al., 1986), an enzyme that is widely distributed as a gene cassette in class 1 and 2 integrons (Ramirez et al., 2005;

Vakulenko and Mobashery, 2003) and mediates resistance to gentamicin, tobramycin, dibekacin, sisomicin, and kanamycin. Therefore it is commonly encoded by plasmids and transposons. This enzyme, encoded by a gene more commonly called *aadB*, is present in enterobacteria and non-fermentative gram-negative bacilli.

3.3.5. ANT(3″)—These are the most commonly found ANT enzymes, they specify resistance to spectinomycin and streptomycin, and the coding genes are most commonly named *aadA* (Hollingshead and Vapnek, 1985). At least 22 highly related gene versions are found in GenBank, that are identified as *aadA1* through *aadA24*, but some numbers are missing. The alternative nomenclature for the protein coded for by *aadA1* is ANT(3″)-Ia. Another name used to identify ANT(3″)-Ia is AAD(3″)(9). The *aadA* genes exist as gene cassettes and are part of a large number of integrons, plasmids and transposons. They can be part of unusual gene cassettes and exist as gene fusions as described in the following paragraphs.

In Tn*1331*, the *aadA1* [*ant(3″)-Ia*] gene is present within two unusual gene cassette structures (see Fig. 2A). At the 3′ end of the gene, instead of the usual *attC* site, there is a copy of *attI1**, which may have been formed by an illegitimate recombination event between the *attC* site located 3′ of *aadA1* of an integron and the *attI1* locus located 5′ of $bla_{\text{OXA-9}}$ of another integron in which the $bla_{\text{OXA-9}}$ gene cassette is adjacent to the 5′-conserved sequence (Sarno et al., 2002). The resulting structure defines a gene cassette consisting of *aadA1-attI1** but that lacks the usual *attC* site, and another gene cassette that includes two genes *aadA1-attI1**-$bla_{OXA\text{-}9}$–*attC* (see Fig. 2A) (Ramirez et al., 2008;Sarno et al., 2002;Tolmasky, 1990;Tolmasky and Crosa, 1993). While the *aadA1-attI1** gene cassette is excised by the IntI1 integrase at a very low frequency the gene cassette that includes both genes is fully functional (Ramirez et al., 2008).

The *aadA* genes are also found fused to other resistance enzymes, e.g., in a *P. aeruginosa* class 1 integron *aadA15* is fused 3′ of $bla_{\text{OXA-10}}$ (Yan et al., 2006) and *aadA6* is fused to *aadA10* in another *P. aeruginosa* class 1 integron (Fiett et al., 2006). The *aadA1* and *aadA4* genes were also found disrupted by insertion of IS*26* (Adrian et al., 2000; Han et al., 2008).

The *ant(3″)-Ia* gene is part of numerous transposons, some of them exhaustively studied such as: a) Tn*21* and other related transposons of what is known as the Tn*21* subfamily. These transposons are widely disseminated probably as a result of the association of an integron and a gene conferring resistance to a toxic metal within the same mobile element (Liebert et al., 1999); b) Tn*1331*, already described above; and c) Tn*7*, which includes in its structure a class 2 integron (Hansson et al., 2002).

3.4 Aminoglycoside modifying enzymes: aminoglycoside O-phosphotransferases (APHs)

APHs catalyze the transfer of a phosphate group to the aminoglycoside molecule (Wright and Thompson, 1999). The classes and subclasses are: APH(4)-I, APH(6)-I, APH(9)-I, APH(3′)-I through VII, APH(2″)-I through IV, APH(3″)-I, APH(7″)-I (Fig. 1 and Table 3).

3.4.1. APH(4)—There are two enzymes within the only subclass defined in this group: APH(4)-Ia (Kaster et al., 1983) and APH(4)-Ib (Zalacain et al., 1986), whose genes have also been named *hph* and *hyg*, respectively. These enzymes mediate resistance to hygromycin and are not clinically relevant. These genes have been used in the construction of cloning vehicles for both prokaryotes and eukaryotes (Abhyankar et al., 2009; Gritz and Davies, 1983).

3.4.2. APH(6)—There are 4 enzymes in the only described subclass of APH(6)s, which confer resistance to streptomycin. The *aph(6)-Ia*, also known as *aphD* and *strA*, was originally found in the chromosome of *Streptomyces griseus* (Distler et al., 1987). The *aph(6)-Ib* was also named *sph* and was found in *Streptomyces glaucescens* (Vogtli and Hutter, 1987). The gene coding for APH(6)-Ic is one of three resistance genes present in Tn*5*, a composite transposon found in gram-negatives (Steiniger-White et al., 2004). Although this transposon is not widely distributed, it has been extensively studied and modified as tool for molecular genetics (Steiniger-White et al., 2004). The *aph(6)-Id* gene, also denominated *strB* and *orfI*, was first found in the plasmid RSF1010, a 8,684 bp broad host range multicopy plasmid RSF1010 that can replicate in most gram-negative bacteria and also in gram-positive actinomyces, and is also known as R300B and R1162 (Meyer, 2009). This plasmid was also the first source identified for another APH, *aph(3″)-Ib* (see below), which is contiguous to *aph(6)-Id.* These genes are part of a fragment that includes the genes *repA, repC, sul2, aph(3″)-Ib, and aph(6)-Id* that has been found, complete or in part, within plasmids, integrative conjugative elements, and chromosomal genomic islands (Daly et al., 2005; Gordon et al., 2008). As a consequence of the dissemination of this DNA fragment, the *aph(6)-Id* and *aph(3″)-Ib* genes are found in both gram-positives and gram-negatives.

3.4.3. APH(9)—The *aph(9)-Ia* gene was first found in *Legionella pneumophila* (Suter et al., 1997). BLAST analysis of this nucleotide sequence also showed that there is a gene with 87% homology within the genome of *L. pneumophila* strain Lens that is identified as *aph* (Cazalet et al., 2004). The APH(9)-Ia has been the subject of detailed analysis. The enzyme was overproduced and purified, and it was determined that it does not bind to any tested aminoglycoside other than spectinomycin (Thompson et al., 1998). The Km and kcat values were also determined and the reaction product was purified and characterized by mass spectrometry and ^{1}H, ^{13}C, and ^{31}P NMR (Thompson et al., 1998). Further studies led to determination of the crystal structures of APH(9)-Ia in its apo form, its binary complex with the nucleotide, AMP, and its ternary complex bound with ADP and spectinomycin (Fong et al., 2010). These structures showed that APH(9)-Ia presents similar folding to APH(3′) and APH(2″) enzymes but differs significantly in its substrate binding area and in undergoing a conformation change upon ligand binding (Fong et al., 2010).

The phosphotransferase APH(9)-Ib isolated from *Streptomyces flavopersicus* (*Str. netropsis*) has also been called SpcN and it has no significant homology to that of *L. pneumophila*. A BLAST analysis of this *aph(9)-Ib* gene nucleotide sequence showed 78-79% identity with genes from 3 *Stretomyces spectabilis* strains (Lyutzkanova et al., 1997). Despite of the differences these genes are also called *spcN* in GenBank.

3.4.4. APH(3′)—The APH(3′)-I subclass shows a resistance profile including kanamycin, neomycin, paromomycin, ribostamycin, lividomycin, is composed of three enzymes that are widely distributed mainly among gram-negatives within wide host range plasmids and transposons (Vakulenko and Mobashery, 2003). The *aph(3′)-Ia* gene, also known as *aphA-1*, is part of the well known Tn*903* transposon (Bernardi and Bernardi, 1991) and it is commonly used as marker gene in cloning vehicles. The *aph(3′)-Ib* gene is part of the wide host range conjugative RP4 plasmid (Pansegrau et al., 1987). This gene was originally named *aphA*. The *aph(3′)-Ic* gene, also called *aphA7* and *aphA1-Iab*, is part of plasmids and transposons and its wide distribution includes *Corynebacterium* spp. (Tauch et al., 2000; Vakulenko and Mobashery, 2003). This gene has also been included in cloning vehicles.

The APH(3′)-II subclass includes three isozymes that specify resistance to kanamycin, neomycin, butirosin, paromomycin, and ribostamycin. The APH(3′)-IIa, also known as *aphA-2* is one of the three resistance genes encoded by Tn*5* (Steiniger-White et al., 2004) (see above) and it is used as resistance marker in cloning vectors for both prokaryotes and eukaryotes (Wright and Thompson, 1999). The enzyme coded by this gene has been characterized in detail and its crystal structure in complex with kanamycin has been resolved (Nurizzo et al., 2003; Siregar et al., 1994). The *aph(3′)-IIb* gene was identified in the *P. aeruginosa* chromosome (Winsor et al., 2005) and the third member of this subclass, *aph(3′)-IIc*, was recently defined in *S. maltophilia* but an accession number is not available (Okazaki and Avison, 2007).

The APH(3′)-IIIa is highly disseminated within gram-positives, confers resistance to kanamycin, neomycin, lividomycin, paromomycin, livostamycin, butirosin, amikacin, and isepamicin, and the epidemiological data has been extensively reviewed by Vakulenko and Mobashery (Vakulenko and Mobashery, 2003). Its crystal structure in complex with ADP has been resolved and it shows a close resemblance to kinases from eukaryotes (Hon et al., 1997). An interesting property of this enzyme is that it is competitively inhibited by tobramycin, which one would expect not to be substrate because it lacks a free 3′-hydroxyl group (McKay et al., 1994). However, other aminoglycosides that also lack a free 3′-hydroxyl group like the case of lividomycin can be phosphorylated at the position 5″ (Thompson et al., 1996). This enzyme has also the capability to di-phosphorylate aminoglycosides such as butirosin and neomycin B that have free 3′- and 5″-hydroxyl groups (Hon et al., 1997; Wright and Thompson, 1999). A recent study showed that APH(3′)-IIIa uses only ATP as donor substrate (Shakya and Wright, 2010).

The APH(3′)-IVa coding gene is present in the chromosome of *Bacillus circulans* (Herbert et al., 1983) and those coding for APH(3′)-Va through c are found in the chromosome of actinomycetes (Wright and Thompson, 1999). The resistance profile for this subclass includes neomycin, paromomycin, and ribostamycin. The *aph(3′)-VIa*, also known as *aphA-6*, was described in *A. baumannii* (Martin et al., 1988), and *aph(3′)-VIb* was described in *K. pneumoniae* and *S. marcescens* but an accession number for this gene is not available (Gaynes et al., 1988). The resistance profile specified by this subclass includes kanamycin, neomycin, paromomycin, ribostamycin, butirosin, amikacin, and isepamycin. The *aph(3′)-VIIa*, also known as *aphA-7*, was described in *C. jejuni* and confers resistance to kanamycin and neomycin (Tenover et al., 1989).

3.4.5. APH(2″)—The APH(2″) plays an important role in resistance to gentamicin in gram-positives. There were originally five APH(2″)-I enzymes described in the literature. However, Toth et al. (Toth et al., 2009) recently performed a detailed analysis of the resistance profiles, regiospecificity, and donor substrate preferences of these enzymes and concluded that on the basis of the aminoglycoside recipient substrate profiles presented by APH(2″)-Ib, APH(2″)-Ic, and APH(2″)-Id they should be reclassified as belonging to subclass II. A novel consideration in renaming these enzymes is the inclusion of the donor substrate as a criterion. Contrary to what it was believed at the time of that work, only APH(2!)-Ib among the four APH(2!) enzymes included in Toth et al. study showed a clear preference for ATP as donor of the phosphate group. APH(2!)-Ia and APH(2!)-Ic utilize GTP as the most efficient donor substrate, and APH(2!)-Id shows similar catalytic efficiencies with ATP or GTP (Toth et al., 2009). A contradictory result was obtained with a derivative of APH(2″)-Ib that includes a His_6-tag at the N-terminus, this protein showed a slight selectivity for GTP over ATP but was still able to utilize both NTPs as donor substrates (Shakya and Wright, 2010). Therefore, at least in the case of phosphotransferases of the 2″ class, the subclass nomenclature now considers the recipient as well as the donor substrate profile. In consequence, these authors proposed to change the names of APH(2!)-Ib, -Ic, and -Id to APH(2!)-IIa, -IIIa, and IVa, respectively. The three dimensional structures of these enzymes have been resolved (Smith et al., 2010; Toth et al., 2010a; Toth et al., 2010b). The APH(2″)-Ie has not been included in this analysis and for now it has not been renamed.

The APH(2″)-Ia exists as a fusion to AAC(6′)-Ie, which is located at the N-terminal portion (Ferretti et al., 1986). Cloning both regions as separate genes resulted in active proteins (Ferretti et al., 1986) suggesting that the natural gene arose by gene fusion. However, it is of interest that although the domains do not functionally interact, they are structurally linked in a manner that is important for their stability and conformation and disruption of these interactions results in a negative impact for both activities (Boehr et al., 2004).

The *aph(2″)-Ie* gene was found downstream of a *tnpA* gene in an *Enterococcus casseliflavus* plasmid (Chen et al., 2006).

3.4.6. APH(3″)—The only subclass of APH(3″) enzymes mediates resistance to streptomycin. The APH(3″)-Ia and Ic coding genes were isolated from the chromosomes of *S. griseus* and *M. fortuitum*, respectively (Ramon-Garcia et al., 2006; Trower and Clark, 1990). The *aph(3″)-Ia* gene is also known as *aphE* and *aphD2*. The APH(3″)-Ib coding gene was originally found within the plasmid RSF1010 (Scholz et al., 1989) and then in a large number of plasmids, transposons, integrative conjugative elements, and at least one chromosome (chromosome 1 of *V. cholerae* MJ-1236, accession number CP001485). The gene can also be found named as *strA* (Scholz et al., 1989).

3.4.7. APH(7″)—The APH(7″)-Ia, which mediates resistance to hygromycin, was isolated from *S. hygroscopicus* and the gene has been cloned and engineered to be used in molecular genetic analysis of *Chlamydomonas reinhardtii* (Berthold et al., 2002).

4. Strategies to overcome the effect of aminoglycoside modifying enzymes

The development of new aminoglycosides, which is being pursued using numerous different approaches, is an obvious path to overcome the action of aminoglycoside modifying enzymes. Strategies and perspectives for the generation of novel aminoglycosides or aminoglycoside derivatives such as dimers or conjugates to small molecules have been recently reviewed (see Green et al., 2010; Houghton et al., 2010; Tolmasky, 2007a; Welch et al., 2005). ACHN-490, a novel aminoglycoside named neoglycoside proved to be a promising alternative for treating multiple drug resistance *K. pneumoniae* including those producing KPC !-lactamase (Endimiani et al. 2009). Other approaches such as the utilization of enzymatic inhibitors or strategies to interfere with gene expression could, if successful, reduce or eliminate the need of discarding aminoglycosides due to the broad dissemination of modifying enzymes. All these strategies together have the potential of increasing the armamentarium against the growing threat of multiresistant infections. A summary of the efforts to develop strategies to inhibit the action or biosynthesis of aminoglycoside modifying enzymes follows.

4.1 Inhibitors of aminoglycoside modifying enzymes

Compounds consisting of both substrates covalently linked, known as bisubstrates, are potential tools to inhibit enzymatic reactions that involve the initial formation of a ternary complex through ordered or random binding of the substrates. An aminoglycoside-CoA bisubstrate was first shown to inhibit the activity of AAC(3)-I *in vitro* but not *in vivo*, probably due to the inability of the compound to penetrate the cell wall (Williams and Northrop, 1979). Further research led to synthesis of other bisubstrates of smaller size by using truncated aminoglycosides or CoA. One of the compounds, showed a synergistic effect with kanamycin on the growth of *E. faecium* harboring AAC(6′)-Ii, an enzyme that catalyzes acetylation through an ordered mechanism (Draker et al., 2003; Gao et al., 2005; Gao et al., 2006). Subsequent kinetic and structural studies using AAC(6′)-Iy, which binds the substrates on a random manner, as a target found that the bisubstrates analyzed bind to this enzyme with much lower affinity (Magalhaes et al., 2008). Aminoglycoside–CoA bisubstrates containing sulfonamide, sulfoxide, or sulfone groups were recently synthesized. Only the sulfone- and sulfoxide-containing bisubstrates showed inhibition of AAC(6)-Ii at nanomolar concentrations (Gao et al., 2008).

In another study, cationic antimicrobial peptides were tested as inhibitors of APH(3!)-IIIa, AAC(6!)-Ii, and AAC(6!)-APH(2!). The results showed that the bovine peptide indolicidin and analogs have an inhibitory effect against both aminoglycoside phosphotransferases and aminoglycoside acetyltransferases, albeit by different mechanisms (Boehr et al., 2003). These peptides were the first example of broad-spectrum inhibitors of aminoglycoside resistance enzymes. However, although the research shows enormous potential for therapeutic purposes none of the peptides showed inhibitory effect *in vivo* (Boehr et al., 2003).

Two non-carbohydrate diamine derivatives with inhibitory activity were isolated from a library of compounds. One of these compounds, N-cyclohexyl-N-(3-dimethylamino-propyl)-propane-1,3-diamine, was active against ANT(2″), and the other, N-[2-(3,4-dimethoxyphenyl)-ethyl]-N’-(3-dimethylamino-propyl)-propane-1,3-diamine was active against APH(3′) and ANT(2″) (Welch et al., 2005).

In the case of APHs it has been shown that it is possible to take advantage of the structural relation found between these enzymes and eukaryotic protein kinases. Burk et al. recently reviewed possible strategies to inhibit aminoglycoside phosphotransferases (Burk and Berghuis, 2002). Known inhibitors of eukaryotic protein kinases were tested to determine if they had also activity against two aminoglycoside phosphotransferases, APH(3!)-IIIa and the fusion protein AAC(6!)-APH(2″). The results showed that several of the tested compounds were inhibitors of these enzymes. Compounds belonging to the isoquinolinesulfonamide group were the most active in these experiments (Daigle et al., 1997). Compounds that act as inhibitors can target the antibiotic binding region, the ATP-binding site, or the bridged nature of the active site, which binds both the aminoglycoside and the donor nucleotide. Compounds that target the aminoglycoside binding site would have the potential of showing a broader spectrum by being able to bind the pocket of more than one kind of aminoglycoside modifying enzyme.

Liu et al. synthesized bisubstrate compounds consisting of adenosine tethered covalently to neamine using methylene groups as linkers. Compounds including linkers of 5 – 8 carbons in length acted as competitive inhibitors of APH(3′)-Ia and APH(3′)-IIIa (Liu et al., 2000).

A compound that exhibited a modest level of inhibition of AAC(6′)-Ib has been constructed using non-aminoglycoside-like fragments (Lombes et al., 2008). This could be a first step towards generating a strong inhibitor of this clinically important enzyme.

An interesting approach to beat the activity of modifying enzymes without inhibiting their activity is that proposed by Haddad et al. in which an aminoglycoside is chemically unstable after phosphorylation and spontaneously sheds the phosphate self-regenerating the antibiotic (Haddad et al., 1999). The authors prepared an analog of kanamycin A, whose hydrated variant undergoes spontaneous, non-enzymatic elimination of the phosphate donated by ATP via APH(3′) catalysis (Haddad et al., 1999).

4.2 Inhibition of expression of aminoglycoside modifying enzymes

Inhibition of gene expression by antisense oligonucleotides or oligonucleotide analogs can be achieved by a variety of strategies. A number of them have been explored in bacteria with therapeutic purposes, mainly to target essential genes and inhibit growth. A detailed description of these attempts can be found in recent reviews (Hebert et al., 2008; Lundblad and Altman, 2010; Rasmussen et al., 2007; Woodford and Wareham, 2009). In a few instances the targets of antisense inhibition of gene expression were genes specifying antibiotic resistance rather than essential or virulence genes. Antisense oligonucleotides targeting regulatory regions of the multiple antibiotic resistance operon (*marORAB*) in *E. coli* increased susceptibility to multiple antibiotics and nuclease-resistant phosphorothioate oligonucleotides enhanced the killing effect of nor! oxacin after introduction into competent cells by chemical transformation or electroporation (White et al., 1997). Whether inhibition of resistance occurred by the action of RNase H or steric hindrance has not been determined.

A recombinant clone containing an engineered gene consisting of a *vanH* promoter driving expression of a *vanA* antisense inhibited vancomycin resistance in *E. faecalis* by a dual mechanism. The phosphorylated VanR, a transcriptional activator, is sequestered by the native *vanH* promoter present in the recombinant plasmid reducing expression of the *vanHAX* genes. In addition expression of a *vanA* RNA antisense placed under the control the recombinant plasmid's *vanH* promoter prevents translation of any *vanA* mRNA that is transcribed by forming a duplex followed by degradation (Torres Viera et al., 2001).

Phosphorothioate deoxyribozymes have been used to target genes coding for *mecR1* or *blaR1* in methicillin resistant *S. aureus* and restore susceptibility to methicillin or oxacillin, respectively, after they were delivered inside the cells by electroporation (Hou et al., 2007a; Hou et al., 2007b). Subsequently liposome-encapsulated antisense compounds targeting the *mecA* gene were delivered into untreated *S. aureus.* The compounds induced a reduction of the MICs of commonly used antibiotics for methicillin resistant *S. aureus* clinical isolates and improved the survival rate when administered together with oxacillin to infected mice (Meng et al., 2009).

To inhibit resistance to aminoglycosides mediated by the *aac(6′)-Ib* gene present in Tn*1331* a series of oligodeoxynucleotides targeting mRNA regions identified by RNase H mapping in combination with computer generated secondary structures were synthesized and tested. At least three oligodeoxynucleotides were identified that induced *in vitro* degradation of mRNA, inhibit *in vitro* synthesis of the enzyme, and upon delivery by electroporation significantly reduced the number of cells surviving after exposure to amikacin (Sarno et al., 2003). Although it has not been determined, the most probable mechanism of inhibition *in vivo* is through mRNA degradation by RNase H. However, steric hindrance is a possibility that cannot be discarded at this time. Another approach to reduce or silence expression of *aac(6′)-Ib* consisted of the design of external guide sequences, short antisense RNA molecules that elicit RNase P-mediated degradation of the mRNA, encoded by recombinant plasmids (Guerrier-Takada et al., 1997). This approach had been already used to reverse resistance to ampicillin and chloramphenicol (Guerrier-Takada et al., 1997). Recombinant clones coding for the selected sequences under an inducible promoter were introduced into *E. coli* harboring *aac(6′)-Ib*, and the transformant strains were tested to determine their resistance to amikacin. Two external guide sequences that showed strong binding to the mRNA *in vitro* induced inhibition of expression of the resistance phenotype in cells harboring the *aac(6′)-Ib* gene (Soler Bistue et al., 2007). Although these results were an indication that the use of external guide sequences could be a viable strategy to preserve the efficacy of aminoglycosides, as it is the case with other antisense approaches there are several problems that must be addressed. A crucial one is to find nuclease resistant oligonucleotide analogs that still induce inhibition of gene expression, in this case the analog must behave as RNA with respect to eliciting RNase P degradation of the target mRNA while being impervious to RNases. A survey of a variety of oligoribonucleotide analogs including phosphorothioate oligodeoxynucleotides, 2!-*O*-methyl oligoribonucleotides, phosphorodiamidate morpholino oligomers, or locked nucleic acids (LNA)/DNA co-oligomers showed that selected LNA/DNA co-oligomers elicited RNase P-mediated cleavage of mRNA *in vitro*. Analyses of isosequential LNA/DNA co-oligomers with different numbers and locations of LNA substitutions suggested that different configurations must be tested to identify an oligomer that promotes high enough levels of RNase P cleavage, it is specific, and it is resistant to the action of nucleases. As a results of these assays a configuration of LNA/DNA residues with the desired properties was found for the particular case of inhibition of expression of *aac(6′)-Ib*. Administration of 50 nM of an LNA/DNA co-oligomer to the hyperpermeable *E. coli* AS19 harboring *aac(6′)-Ib* inhibited growth in the presence of amikacin suggesting that the oligoribonucleotide analog induced RNase P-mediated inhibition of expression of the gene (Soler Bistue et al., 2009).

5. Concluding remarks

Inactivation by enzymatic modification is the most prevalent mechanism of resistance to aminoglycoside antibiotics in the clinical setting. The raise and dissemination of aminoglycoside modifying enzymes has reduced the efficacy of these antibiotics and in some cases rendered them virtually unusable. There are three kinds of aminoglycoside modifying enzymes, nucleotidyltranferases, phosphotransferases, or acetyltransferases, which catalyze the modification at different −OH or $-NH_2$ groups in the antibiotic molecule. The large number and ability of the genes coding for these enzymes to evolve, as well as the numerous mobile elements where they are located, results in a high adaptability by these enzymes to utilize new antibiotics as substrates and to efficiently disseminate among bacteria. As a consequence virtually all bacteria of medical interest can support enzymatic resistance to aminoglycosides. Two nomenclature schemes have been proposed in the past, but the dizzying rate of discovery of new genes together with the appearance of enzymes with new characteristics superseded the criteria defined. We suggest that members of the community should engage in a debate to come up with a consensus new nomenclature. We suggest that returning to a simpler nomenclature with the support of an internet repository site could facilitate the naming of the genes, avoid duplications, and facilitate further changes when new enzymes with new, and may be unexpected, characteristics are discovered. The fight to keep aminoglycosides as useful tools in the armamentarium against bacterial infectious diseases includes the development of new aminoglycosides that must be refractory to as many as possible modifying enzymes, the development of inhibitors of aminoglycoside modifying enzymes, and inhibitors of their expression by the action of antisense oligonucleotide analogs.

Acknowledgments

The authors’ work cited in this review article was funded by Public Health Service grant 2R15AI047115 (to M.E.T.) from the National Institutes of Health. M.S.R. is a research career member of C.O.N.I.C.E.T.

References

Abhyankar MM, Hochreiter AE, Connell SK, Gilchrist CA, Mann BJ, Petri WA Jr. Development of the Gateway system for cloning and expressing genes in *Entamoeba histolytica*. Parasitol Int 2009;58:95–97. [PubMed: 18822389]

Abril C, Brodard I, Perreten V. Two novel antibiotic resistance genes, *tet(44)* and *ant(6)-Ib*, are located within a transferable pathogenicity island in *Campylobacter fetus* subsp. *fetus*. Antimicrob Agents Chemother 2010;54:3052–3055. [PubMed: 20479200]

Adams MD, Goglin K, Molyneaux N, Hujer KM, Lavender H, Jamison JJ, MacDonald IJ, Martin KM, Russo T, Campagnari AA, Hujer AM, Bonomo RA, Gill SR. Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. J Bacteriol 2008;190:8053–8064. [PubMed: 18931120]

Adrian PV, Thomson CJ, Klugman KP, Amyes SG. New gene cassettes for trimethoprim resistance, *dfr13*, and Streptomycin-spectinomycin resistance, *aadA4*, inserted on a class 1 integron. Antimicrob Agents Chemother 2000;44:355–361. [PubMed: 10639362]

Ahmed AM, Nakagawa T, Arakawa E, Ramamurthy T, Shinoda S, Shimamoto T. New aminoglycoside acetyltransferase gene, *aac(3)-Id*, in a class 1 integron from a multiresistant strain of *Vibrio fluvialis* isolated from an infant aged 6 months. J Antimicrob Chemother 2004;53:947–951. [PubMed: 15117923]

Ainsa JA, Perez E, Pelicic V, Berthet FX, Gicquel B, Martin C. Aminoglycoside 2′-N-acetyltransferase genes are universally present in mycobacteria: characterization of the *aac(2′)-Ic* gene from *Mycobacterium tuberculosis* and the *aac(2′)-Id* gene from *Mycobacterium smegmatis*. Mol Microbiol 1997;24:431–441. [PubMed: 9159528]

Aires JR, Kohler T, Nikaido H, Plesiat P. Involvement of an active efflux system in the natural resistance of *Pseudomonas aeruginosa* to aminoglycosides. Antimicrob Agents Chemother 1999;43:2624–2628. [PubMed: 10543738]

Ajiboye RM, Solberg OD, Lee BM, Raphael E, Debroy C, Riley LW. Global spread of mobile antimicrobial drug resistance determinants in human and animal *Escherichia coli* and *Salmonella* strains causing community-acquired infections. Clin Infect Dis 2009;49:365–371. [PubMed: 19538087]

Alekshun MN, Levy SB. Molecular mechanisms of antibacterial multidrug resistance. Cell 2007;128:1037–1050. [PubMed: 17382878]

Allmansberger R, Brau B, Piepersberg W. Genes for gentamicin-(3)-N-acetyl-transferases III and IV. II. Nucleotide sequences of three AAC(3)-III genes and evolutionary aspects. Mol Gen Genet 1985;198:514–520. [PubMed: 3892230]

Azucena E, Mobashery S. Aminoglycoside-modifying enzymes: mechanisms of catalytic processes and inhibition. Drug Resist Updat 2001;4:106–117. [PubMed: 11512519]

Bakker EP. Aminoglycoside and aminocyclitol antibiotics: hygromycin B is an atypical bactericidal compound that exerts effects on cells of *Escherichia coli* characteristics for bacteriostatic aminocyclitols. J Gen Microbiol 1992;138:563–569. [PubMed: 1375624]

Beck E, Ludwig G, Auerswald EA, Reiss B, Schaller H. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn*5*. Gene 1982;19:327–336. [PubMed: 6295884]

Bell JM, Turnidge JD, Andersson P. *aac(6′)-Ib-cr* genotyping by simultaneous high-resolution melting analyses of an unlabeled probe and full-length amplicon. Antimicrob Agents Chemother 2010;54:1378–1380. [PubMed: 20008777]

Belousoff MJ, Graham B, Spiccia L, Tor Y. Cleavage of RNA oligonucleotides by aminoglycosides. Org Biomol Chem 2009;7:30–33. [PubMed: 19081939]

Benet LZ, Hoener BA. Changes in plasma protein binding have little clinical relevance. Clin Pharmacol Ther 2002;71:115–121. [PubMed: 11907485]

Bernardi F, Bernardi A. Inter- and intramolecular transposition of Tn*903*. Mol Gen Genet 1991;227:22–27. [PubMed: 1646385]

Berthold P, Schmitt R, Mages W. An engineered *Streptomyces hygroscopicus aph 7″* gene mediates dominant resistance against hygromycin B in *Chlamydomonas reinhardtii*. Protist 2002;153:401–412. [PubMed: 12627869]

Boehr DD, Daigle DM, Wright GD. Domain-domain interactions in the aminoglycoside antibiotic resistance enzyme AAC(6′)-APH(2″). Biochemistry 2004;43:9846–9855. [PubMed: 15274639]

Boehr DD, Draker KA, Koteva K, Bains M, Hancock RE, Wright GD. Broad-spectrum peptide inhibitors of aminoglycoside antibiotic resistance enzymes. Chem Biol 2003;10:189–196. [PubMed: 12618191]

Bozdogan B, Galopin S, Gerbaud G, Courvalin P, Leclercq R. Chromosomal *aadD2* encodes an aminoglycoside nucleotidyltransferase in *Bacillus clausii*. Antimicrob Agents Chemother 2003;47:1343–1346. [PubMed: 12654668]

Brau B, Pilz U, Piepersberg W. Genes for gentamicin-(3)-*N*-acetyltransferases III and IV: I. Nucleotide sequence of the AAC(3)-IV gene and possible involvement of an IS*140* element in its expression. Mol Gen Genet 1984;193:179–187. [PubMed: 6318050]

Brossier F, Veziris N, Aubry A, Jarlier V, Sougakoff W. Detection by Genotype MTBDRsl test of complex resistance mechanisms to second-line drugs and ethambutol in multidrug-resistant *Mycobacterium tuberculosis* complex isolates. J Clin Microbiol 2010;48:1683–1689. [PubMed: 20335420]

Brunnemann SR, Segal JL. Amikacin serum protein binding in spinal cord injury. Life Sci 1991;49:PL1–5. [PubMed: 2062169]

Bryan L, van der Elzen H. Effects of membrane-energy mutations and cations on streptomycin and gentamicin accumulation by bacteria: a model for entry of streptomycin and gentamicin in susceptible and resistant bacteria. Antimicrob Agents Chemother 1977;12:163–177. [PubMed: 143238]

Bryan LE, Kowand SK, Van Den Elzen HM. Mechanism of aminoglycoside antibiotic resistance in anaerobic bacteria: *Clostridium perfringens* and *Bacteroides fragilis*. Antimicrob Agents Chemother 1979;15:7–13. [PubMed: 218500]

Bryskier, A. Antibiotics and antibacterial agents: classifications and structure-activity relationships. In: Bryskier, A., editor. Antimicrobial agents. ASM Press; Washington, DC: 2005. p. 13-38.

Bucke WE, Leitzke S, Diederichs JE, Borner K, Hahn H, Ehlers S, Muller RH. Surface-modified amikacin-liposomes: organ distribution and interaction with plasma proteins. J Drug Target 1998;5:99–108. [PubMed: 9588866]

Bunny KL, Hall RM, Stokes HW. New mobile gene cassettes containing an aminoglycoside resistance gene, *aacA7*, and a chloramphenicol resistance gene, *catB3*, in an integron in pBWH301. Antimicrob Agents Chemother 1995;39:686–693. [PubMed: 7793874]

Burk DL, Berghuis AM. Protein kinase inhibitors and antibiotic resistance. Pharmacol Ther 2002;93:283–292. [PubMed: 12191620]

Burk DL, Ghuman N, Wybenga-Groot LE, Berghuis AM. X-ray structure of the AAC(6′)-Ii antibiotic resistance enzyme at 1.8 A resolution; examination of oligomeric arrangements in GNAT superfamily members. Protein Sci 2003;12:426–437. [PubMed: 12592013]

Burk DL, Hon WC, Leung AK, Berghuis AM. Structural analyses of nucleotide binding to an aminoglycoside phosphotransferase. Biochemistry 2001;40:8756–8764. [PubMed: 11467935]

Burk DL, Xiong B, Breitbach C, Berghuis AM. Structures of aminoglycoside acetyltransferase AAC(6′)-Ii in a novel crystal form: structural and normal-mode analyses. Acta Crystallogr D Biol Crystallogr 2005;61:1273–1279. [PubMed: 16131761]

Busse HJ, Wostmann C, Bakker EP. The bactericidal action of streptomycin: membrane permeabilization caused by the insertion of mistranslated proteins into the cytoplasmic membrane of *Escherichia coli* and subsequent caging of the antibiotic inside the cells due to degradation of these proteins. J Gen Microbiol 1992;138:551–561. [PubMed: 1375623]

Call DR, Singer RS, Meng D, Broschat SL, Orfe LH, Anderson JM, Herndon DR, Kappmeyer LS, Daniels JB, Besser TE. *bla*CMY-2-positive IncA/C plasmids from *Escherichia coli* and *Salmonella enterica* are a distinct component of a larger lineage of plasmids. Antimicrob Agents Chemother 2010;54:590–596. [PubMed: 19949054]

Cameron FH, Groot Obbink DJ, Ackerman VP, Hall RM. Nucleotide sequence of the AAD(2″) aminoglycoside adenylyltransferase determinant *aadB*. Evolutionary relationship of this region with those surrounding aadA in R538-1 and *dhfrII* in R388. Nucleic Acids Res 1986;14:8625–8635. [PubMed: 3024112]

Casin I, Bordon F, Bertin P, Coutrot A, Podglajen I, Brasseur R, Collatz E. Aminoglycoside 6′-*N*-acetyltransferase variants of the Ib type with altered substrate profile in clinical isolates of *Enterobacter cloacae* and *Citrobacter freundii*. Antimicrob Agents Chemother 1998;42:209–215. [PubMed: 9527761]

Casin I, Hanau-Bercot B, Podglajen I, Vahaboglu H, Collatz E. *Salmonella enterica* serovar Typhimurium *bla*(PER-1)-carrying plasmid pSTI1 encodes an extended-spectrum aminoglycoside 6′-*N*-acetyltransferase of type Ib. Antimicrob Agents Chemother 2003;47:697–703. [PubMed: 12543680]

Cazalet C, Rusniok C, Bruggemann H, Zidane N, Magnier A, Ma L, Tichit M, Jarraud S, Bouchier C, Vandenesch F, Kunst F, Etienne J, Glaser P, Buchrieser C. Evidence in the *Legionella* *pneumophila* genome for exploitation of host cell functions and high genome plasticity. Nat Genet 2004;36:1165–1173. [PubMed: 15467720]

Centron D, Roy PH. Characterization of the 6′-N-aminoglycoside acetyltransferase gene *aac(6′)-Iq* from the integron of a natural multiresistance plasmid. Antimicrob Agents Chemother 1998;42:1506–1508. [PubMed: 9624504]

Centron D, Roy PH. Presence of a group II intron in a multiresistant *Serratia marcescens* strain that harbors three integrons and a novel gene fusion. Antimicrob Agents Chemother 2002;46:1402–1409. [PubMed: 11959575]

Cerda P, Goni P, Millan L, Rubio C, Gomez-Lus R. Detection of the aminoglycosidestreptothricin resistance gene cluster *ant(6)-sat4-aph(3 ′)-III* in commensal viridans group streptococci. Int Microbiol 2007;10:57–60. [PubMed: 17407061]

Chamorro RM, Actis LA, Crosa JH, Tolmasky ME. Dissemination of plasmid-mediated amikacin resistance among pathogenic *Klebsiella pneumoniae*. Medicina (B Aires) 1990;50:543–547. [PubMed: 1966623]

Chavideh R, Sholly S, Panaite D, Tolmasky ME. Effects of F171 mutations in the 6′-*N*-acetyltransferase type Ib [AAC(6′)-Ib] enzyme on susceptibility to aminoglycosides. Antimicrob Agents Chemother 1999;43:2811–2812. [PubMed: 10543772]

Chen L, Mediavilla JR, Smyth DS, Chavda KD, Ionescu R, Roberts RB, Robinson DA, Kreiswirth BN. Identification of a novel transposon (Tn*6072*) and a truncated SCC*mec* element in methicillin-resistant *Staphylococcus aureus* ST239. Antimicrob Agents Chemother. 2010 in press.

Chen YG, Qu TT, Yu YS, Zhou JY, Li LJ. Insertion sequence IS*Ecp1*-like element connected with a novel *aph(2″)* allele [*aph(2″)-Ie*] conferring high-level gentamicin resistance and a novel streptomycin adenylyltransferase gene in *Enterococcus*. J Med Microbiol 2006;55:1521–1525. [PubMed: 17030911]

Chen YT, Lauderdale TL, Liao TL, Shiau YR, Shu HY, Wu KM, Yan JJ, Su IJ, Tsai SF. Sequencing and comparative genomic analysis of pK29, a 269-kilobase conjugative plasmid encoding CMY-8 and CTX-M-3 β- lactamases in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2007;51:3004–3007. [PubMed: 17526756]

Chen YT, Liao TL, Liu YM, Lauderdale TL, Yan JJ, Tsai SF. Mobilization of *qnrB2* and IS*CR1* in plasmids. Antimicrob Agents Chemother 2009;53:1235–1237. [PubMed: 19075060]

Chow JW, Kak V, You I, Kao SJ, Petrin J, Clewell DB, Lerner SA, Miller GH, Shaw KJ. Aminoglycoside resistance genes *aph(2″)-Ib* and *aac(6′)-Im* detected together in strains of both *Escherichia coli* and *Enterococcus faecium*. Antimicrob Agents Chemother 2001;45:2691–2694. [PubMed: 11557456]

Chow JW, Zervos MJ, Lerner SA, Thal LA, Donabedian SM, Jaworski DD, Tsai S, Shaw KJ, Clewell DB. A novel gentamicin resistance gene in *Enterococcus*. Antimicrob Agents Chemother 1997;41:511–514. [PubMed: 9055984]

Costa Y, Galimand M, Leclercq R, Duval J, Courvalin P. Characterization of the chromosomal *aac(6′)-Ii* gene specific for *Enterococcus faecium*. Antimicrob Agents Chemother 1993;37:1896–1903. [PubMed: 8239603]

Coyne S, Courvalin P, Galimand M. Acquisition of multidrug resistance transposon Tn*6061* and IS*6100*-mediated large chromosomal inversions in *Pseudomonas aeruginosa* clinical isolates. Microbiology 2010;156:1448–1458. [PubMed: 20110294]

Crossman LC, Gould VC, Dow JM, Vernikos GS, Okazaki A, Sebaihia M, Saunders D, Arrowsmith C, Carver T, Peters N, Adlem E, Kerhornou A, Lord A, Murphy L, Seeger K, Squares R, Rutter S, Quail MA, Rajandream MA, Harris D, Churcher C, Bentley SD, Parkhill J, Thomson NR, Avison MB. The complete genome, comparative and functional analysis of *Stenotrophomonas maltophilia* reveals an organism heavily shielded by drug resistance determinants. Genome Biol 2008;9:R74. [PubMed: 18419807]

Culebras E, Martinez JL. Aminoglycoside resistance mediated by the bifunctional enzyme 6′-*N*-aminoglycoside acetyltransferase-2″-*O*-aminoglycoside phosphotransferase. Front Biosci 1999;4:D1–8. [PubMed: 9872730]

Dabertrand F, Mironneau J, Henaff M, Macrez N, Morel JL. Comparison between gentamycin and exon skipping treatments to restore ryanodine receptor subtype 2 functions in mdx mouse duodenum myocytes. Eur J Pharmacol 2010;628:36–41. [PubMed: 19944091]

Dahmen S, Bettaieb D, Mansour W, Boujaafar N, Bouallegue O, Arlet G. Characterization and molecular epidemiology of extended-spectrum β-lactamases in clinical isolates of *Enterobacteriaceae* in a Tunisian university hospital. Microb Drug Resist 2010;16:163–170. [PubMed: 20438347]

Daigle DM, McKay GA, Wright GD. Inhibition of aminoglycoside antibiotic resistance enzymes by protein kinase inhibitors. J Biol Chem 1997;272:24755–24758. [PubMed: 9312069]

Daly M, Villa L, Pezzella C, Fanning S, Carattoli A. Comparison of multidrug resistance gene regions between two geographically unrelated *Salmonella* serotypes. J Antimicrob Chemother 2005;55:558–561. [PubMed: 15722395]

Davies JE. Aminoglycosides: ancient and modern. J Antibiot (Tokyo) 2006;59:529–532. [PubMed: 17136885]

Davis BB. The lethal action of aminoglycosides. J Antimicrob Chemother 1988;22:1–3. [PubMed: 3170388]

Davis BD. Mechanism of bactericidal action of aminoglycosides. Microbiol Rev 1987;51:341–350. [PubMed: 3312985]

Davis BD. Non-specific membrane permeability and aminoglycoside action. J Antimicrob Chemother 1989;24:77–78. [PubMed: 2777730]

Del Campo R, Galan JC, Tenorio C, Ruiz-Garbajosa P, Zarazaga M, Torres C, Baquero F. New *aac(6′)-I* genes in *Enterococcus hirae* and *Enterococcus durans*: effect on β-lactam/aminoglycoside synergy. J Antimicrob Chemother 2005;55:1053–1055. [PubMed: 15849260]

Dery KJ, Chavideh R, Waters V, Chamorro R, Tolmasky LS, Tolmasky ME. Characterization of the replication and mobilization regions of the multiresistance *Klebsiella pneumoniae* plasmid pJHCMW1. Plasmid 1997;38:97–105. [PubMed: 9339467]

Dery KJ, Soballe B, Witherspoon MS, Bui D, Koch R, Sherratt DJ, Tolmasky ME. The aminoglycoside 6′-*N*-acetyltransferase type Ib encoded by Tn*1331* is evenly distributed within the cell's cytoplasm. Antimicrob Agents Chemother 2003;47:2897–2902. [PubMed: 12936992]

Distler J, Ebert A, Mansouri K, Pissowotzki K, Stockmann M, Piepersberg W. Gene cluster for streptomycin biosynthesis in *Streptomyces griseus*: nucleotide sequence of three genes and analysis of transcriptional activity. Nucleic Acids Res 1987;15:8041–8056. [PubMed: 3118332]

Doi Y, Arakawa Y. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. Clin Infect Dis 2007;45:88–94. [PubMed: 17554708]

Doi Y, Wachino J, Yamane K, Shibata N, Yagi T, Shibayama K, Kato H, Arakawa Y. Spread of novel aminoglycoside resistance gene *aac(6′)-Iad* among *Acinetobacter* clinical isolates in Japan. Antimicrob Agents Chemother 2004;48:2075–2080. [PubMed: 15155202]

Doublet B, Weill FX, Fabre L, Chaslus-Dancla E, Cloeckaert A. Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster containing a novel 3′-*N*-aminoglycoside acetyltransferase gene cassette, *aac(3)-Id*, in *Salmonella enterica* serovar *newport*. Antimicrob Agents Chemother 2004;48:3806–3812. [PubMed: 15388438]

Draker KA, Northrop DB, Wright GD. Kinetic mechanism of the GCN5-related chromosomal aminoglycoside acetyltransferase AAC(6′)-Ii from *Enterococcus faecium*: evidence of dimer subunit cooperativity. Biochemistry 2003;42:6565–6574. [PubMed: 12767240]

Dubois V, Arpin C, Coulange L, Andre C, Noury P, Quentin C. TEM-21 extended-spectrum β-lactamase in a clinical isolate of *Alcaligenes faecalis* from a nursing home. J Antimicrob Chemother 2006;57:368–369. [PubMed: 16344284]

Dubois V, Arpin C, Dupart V, Scavelli A, Coulange L, Andre C, Fischer I, Grobost F, Brochet JP, Lagrange I, Dutilh B, Jullin J, Noury P, Larribet G, Quentin C. β-lactam and aminoglycoside resistance rates and mechanisms among *Pseudomonas aeruginosa* in French general practice (community and private healthcare centres). J Antimicrob Chemother 2008;62:316–323. [PubMed: 18467306]

Dubois V, Poirel L, Marie C, Arpin C, Nordmann P, Quentin C. Molecular characterization of a novel class 1 integron containing *bla*(GES-1) and a fused product of *aac3-Ib*/*aac6′-Ib'* gene cassettes in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2002;46:638–645. [PubMed: 11850242]

Dudley MN, Loutit J, Griffith DC. Aerosol antibiotics: considerations in pharmacological and clinical evaluation. Curr Opin Biotechnol 2008;19:637–643. [PubMed: 19036576]

Egorova S, Kaftyreva L, Grimont PA, Weill FX. Prevalence and characterization of extended-spectrum cephalosporin-resistant nontyphoidal *Salmonella* isolates in adults in Saint Petersburg, Russia (2002-2005). Microb Drug Resist 2007;13:102–107. [PubMed: 17650961]

Eliopoulos GM. Synergism and antagonism. Infect Dis Clin North Am 1989;3:399–406. [PubMed: 2671129]

Endimiani A, Hujer KM, Hujer AM, Armstrong ES, Choudhary Y, Aggen JB, Bonomo RA. ACHN-490, a neoglycoside with potent in vitro activity against multidrug-resistant *Klebsiella pneumoniae* isolates. Antimicrob Agents Chemother 2009;53:4504–4507. [PubMed: 19770287]

Faldynova M, Pravcova M, Sisak F, Havlickova H, Kolackova I, Cizek A, Karpiskova R, Rychlik I. Evolution of antibiotic resistance in *Salmonella enterica* serovar *typhimurium* strains isolated in the Czech Republic between 1984 and 2002. Antimicrob Agents Chemother 2003;47:2002–2005. [PubMed: 12760885]

Ferretti JJ, Gilmore KS, Courvalin P. Nucleotide sequence analysis of the gene specifying the bifunctional 6′-aminoglycoside acetyltransferase 2″-aminoglycoside phosphotransferase enzyme in *Streptococcus faecalis* and identification and cloning of gene regions specifying the two activities. J Bacteriol 1986;167:631–638. [PubMed: 3015884]

Fiett J, Baraniak A, Mrowka A, Fleischer M, Drulis-Kawa Z, Naumiuk L, Samet A, Hryniewicz W, Gniadkowski M. Molecular epidemiology of acquired-metallo-β-lactamase-producing bacteria in Poland. Antimicrob Agents Chemother 2006;50:880–886. [PubMed: 16495246]

Fong DH, Berghuis AM. Structural basis of APH(3′)-IIIa-mediated resistance to N1-substituted aminoglycoside antibiotics. Antimicrob Agents Chemother 2009;53:3049–3055. [PubMed: 19433564]

Fong DH, Berghuis AM. Substrate promiscuity of an aminoglycoside antibiotic resistance enzyme via target mimicry. EMBO J 2002;21:2323–2331. [PubMed: 12006485]

Fong DH, Lemke CT, Hwang J, Xiong B, Berghuis AM. Structure of the antibiotic resistance factor spectinomycin phosphotransferase from *Legionella pneumophila*. J Biol Chem 2010;285:9545–9555. [PubMed: 20089863]

Franklin K, Clarke AJ. Overexpression and characterization of the chromosomal aminoglycoside 2′-*N*-acetyltransferase of *Providencia stuartii*. Antimicrob Agents Chemother 2001;45:2238–2244. [PubMed: 11451680]

Galimand M, Sabtcheva S, Courvalin P, Lambert T. Worldwide disseminated *armA* aminoglycoside resistance methylase gene is borne by composite transposon Tn*1548*. Antimicrob Agents Chemother 2005;49:2949–2953. [PubMed: 15980373]

Gao F, Yan X, Baettig OM, Berghuis AM, Auclair K. Regio- and chemoselective 6′-*N*-derivatization of aminoglycosides: bisubstrate inhibitors as probes to study aminoglycoside 6′-*N*-acetyltransferases. Angew Chem Int Ed Engl 2005;44:6859–6862. [PubMed: 16206301]

Gao F, Yan X, Shakya T, Baettig OM, Ait-Mohand-Brunet S, Berghuis AM, Wright GD, Auclair K. Synthesis and structure-activity relationships of truncated bisubstrate inhibitors of aminoglycoside 6′-*N*-acetyltransferases. J Med Chem 2006;49:5273–5281. [PubMed: 16913716]

Gao F, Yan X, Zahr O, Larsen A, Vong K, Auclair K. Synthesis and use of sulfonamide-, sulfoxide-, or sulfone-containing aminoglycoside-CoA bisubstrates as mechanistic probes for aminoglycoside *N*-6′-acetyltransferase. Bioorg Med Chem Lett 2008;18:5518–5522. [PubMed: 18805003]

Gaynes R, Groisman E, Nelson E, Casadaban M, Lerner SA. Isolation, characterization, and cloning of a plasmid-borne gene encoding a phosphotransferase that confers high-level amikacin resistance in enteric bacilli. Antimicrob Agents Chemother 1988;32:1379–1384. [PubMed: 2848443]

Gill SR, Fouts DE, Archer GL, Mongodin EF, Deboy RT, Ravel J, Paulsen IT, Kolonay JF, Brinkac L, Beanan M, Dodson RJ, Daugherty SC, Madupu R, Angiuoli SV, Durkin AS, Haft DH, Vamathevan J, Khouri H, Utterback T, Lee C, Dimitrov G, Jiang L, Qin H, Weidman J, Tran K, Kang K, Hance IR, Nelson KE, Fraser CM. Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. J Bacteriol 2005;187:2426–2438. [PubMed: 15774886]

Gionechetti F, Zucca P, Gombac F, Monti-Bragadin C, Lagatolla C, Tonin E, Edalucci E, Vitali LA, Dolzani L. Characterization of antimicrobial resistance and class 1 integrons in *Enterobacteriaceae* isolated from Mediterranean herring gulls (*Larus cachinnans*). Microb Drug Resist 2008;14:93–99. [PubMed: 18476779]

Gomez-Luis, R.; Vergara, Y.; Lopez, L.; Castillo, J.; Rubio, M. 1-N-Aminoglycoside acetyltransferase [AAC(1)] in clinical isolates of *Campylobacter* spp.. Interscience Conference on Antimicrobial Agents and Chemotherapy; San Francisco, CA. 1999.

Gordon L, Cloeckaert A, Doublet B, Schwarz S, Bouju-Albert A, Ganiere JP, Le Bris H, Le Fleche-Mateos A, Giraud E. Complete sequence of the *floR*-carrying multiresistance plasmid pAB5S9 from freshwater *Aeromonas bestiarum*. J Antimicrob Chemother 2008;62:65–71. [PubMed: 18413319]

Gordon RC, Regamey C, Kirby WM. Serum protein binding of the aminoglycoside antibiotics. Antimicrob Agents Chemother 1972;2:214–216. [PubMed: 4790560]

Green KD, Chen W, Houghton JL, Fridman M, Garneau-Tsodikova S. Exploring the substrate promiscuity of drug-modifying enzymes for the chemoenzymatic generation of *N*-acylated aminoglycosides. Chembiochem 2010;11:119–126. [PubMed: 19899089]

Gritz L, Davies J. Plasmid-encoded hygromycin B resistance: the sequence of hygromycin B phosphotransferase gene and its expression in *Escherichia coli* and *Saccharomyces cerevisiae*. Gene 1983;25:179–188. [PubMed: 6319235]

Guerrier-Takada C, Salavati R, Altman S. Phenotypic conversion of drug-resistant bacteria to drug sensitivity. Proc Natl Acad Sci U S A 1997;94:8468–8472. [PubMed: 9238000]

Guthrie OW. Aminoglycoside induced ototoxicity. Toxicology 2008;249:91–96. [PubMed: 18514377]

Gutierrez O, Juan C, Cercenado E, Navarro F, Bouza E, Coll P, Perez JL, Oliver A. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa* isolates from Spanish hospitals. Antimicrob Agents Chemother 2007;51:4329–4335. [PubMed: 17938181]

Haddad J, Vakulenko SB, Mobashery S. An antibiotic cloaked by its own resistance enzyme. J. Am. Chem. Soc 1999;121:11922–11923.

Hamano Y, Hoshino Y, Nakamori S, Takagi H. Overexpression and characterization of an aminoglycoside 6′-*N*-acetyltransferase with broad specificity from an epsilon-poly-L-lysine producer, *Streptomyces albulus* IFO14147. J Biochem 2004;136:517–524. [PubMed: 15625322]

Han HL, Jang SJ, Park G, Kook JK, Shin JH, Shin SH, Kim DM, Cheon JS, Moon DS, Park YJ. Identification of an atypical integron carrying an IS*26*-disrupted *aadA1* gene cassette in *Acinetobacter baumannii*. Int J Antimicrob Agents 2008;32:165–169. [PubMed: 18565738]

Hanau-Bercot B, Podglajen I, Casin I, Collatz E. An intrinsic control element for translational initiation in class 1 integrons. Mol Microbiol 2002;44:119–130. [PubMed: 11967073]

Hancock RE. Aminoglycoside uptake and mode of action--with special reference to streptomycin and gentamicin. I. Antagonists and mutants. J Antimicrob Chemother 1981;8:249–276. [PubMed: 6795174]

Hannecart-Pokorni E, Depuydt F, de wit L, van Bossuyt E, Content J, Vanhoof R. Characterization of the 6′-*N*-aminoglycoside acetyltransferase gene *aac(6′)-Im* [corrected] associated with a sulI-type integron. Antimicrob Agents Chemother 1997;41:314–318. [PubMed: 9021185]

Hansson K, Sundstrom L, Pelletier A, Roy PH. IntI2 integron integrase in Tn*7*. J Bacteriol 2002;184:1712–1721. [PubMed: 11872723]

Hebert CG, Valdes JJ, Bentley WE. Beyond silencing-engineering applications of RNA interference and antisense technology for altering cellular phenotype. Curr Opin Biotechnol 2008;19:500–505. [PubMed: 18760358]

Hegde SS, Javid-Majd F, Blanchard JS. Overexpression and mechanistic analysis of chromosomally encoded aminoglycoside 2′-*N*-acetyltransferase (AAC(2′)-Ic) from *Mycobacterium tuberculosis*. J Biol Chem 2001;276:45876–45881. [PubMed: 11590162]

Heinze A, Holzgrabe U. Determination of the extent of protein binding of antibiotics by means of an automated continuous ultrafiltration method. Int J Pharm 2006;311:108–112. [PubMed: 16431044]

Herbert CJ, Giles IG, Akhtar M. The sequence of an antibiotic resistance gene from an antibiotic-producing bacterium. Homologies with transposon genes. FEBS Lett 1983;160:67–71. [PubMed: 6193008]

Hermann T. Aminoglycoside antibiotics: old drugs and new therapeutic approaches. Cell Mol Life Sci 2007;64:1841–1852. [PubMed: 17447006]

Herrero A, Rodicio MR, Echeita MA, Mendoza MC. *Salmonella enterica* serotype Typhimurium carrying hybrid virulence-resistance plasmids (pUO-StVR): a new multidrug-resistant group endemic in Spain. Int J Med Microbiol 2008;298:253–261. [PubMed: 17597002]

Heuer H, Krogerrecklenfort E, Wellington EM, Egan S, Elsas JD, Overbeek L, Collard JM, Guillaume G, Karagouni AD, Nikolakopoulou TL, Smalla K. Gentamicin resistance genes in environmental bacteria: prevalence and transfer. FEMS Microbiol Ecol 2002;42:289–302. [PubMed: 19709289]

Hidalgo-Grass C, Strahilevitz J. High Resolution Melt Curve Analysis for Identification of Single Nucleotide Mutations in the Quinolone Resistance Gene *aac(6′)-Ib-cr*. Antimicrob Agents Chemother 2010;54:3509–3511. [PubMed: 20498320]

Hocquet D, Vogne C, El Garch F, Vejux A, Gotoh N, Lee A, Lomovskaya O, Plesiat P. MexXY-OprM efflux pump is necessary for a adaptive resistance of *Pseudomonas aeruginosa* to aminoglycosides. Antimicrob Agents Chemother 2003;47:1371–1375. [PubMed: 12654672]

Holden MT, Hauser H, Sanders M, Ngo TH, Cherevach I, Cronin A, Goodhead I, Mungall K, Quail MA, Price C, Rabbinowitsch E, Sharp S, Croucher NJ, Chieu TB, Mai NT, Diep TS, Chinh NT, Kehoe M, Leigh JA, Ward PN, Dowson CG, Whatmore AM, Chanter N, Iversen P, Gottschalk M, Slater JD, Smith HE, Spratt BG, Xu J, Ye C, Bentley S, Barrell BG, Schultsz C, Maskell DJ, Parkhill J. Rapid evolution of virulence and drug resistance in the emerging zoonotic pathogen *Streptococcus suis*. PLoS One 2009;4:e6072. [PubMed: 19603075]

Hollingshead S, Vapnek D. Nucleotide sequence analysis of a gene encoding a streptomycin/spectinomycin adenylyltransferase. Plasmid 1985;13:17–30. [PubMed: 2986186]

Hon WC, McKay GA, Thompson PR, Sweet RM, Yang DS, Wright GD, Berghuis AM. Structure of an enzyme required for aminoglycoside antibiotic resistance reveals homology to eukaryotic protein kinases. Cell 1997;89:887–895. [PubMed: 9200607]

Hoshiko S, Nojiri C, Matsunaga K, Katsumata K, Satoh E, Nagaoka K. Nucleotide sequence of the ribostamycin phosphotransferase gene and of its control region in *Streptomyces ribosidificus*. Gene 1988;68:285–296. [PubMed: 2851496]

Hotta K, Sunada A, Ishikawa J, Mizuno S, Ikeda Y, Kondo S. The novel enzymatic 3″-*N*-acetylation of arbekacin by an aminoglycoside 3-*N*- acetyltransferase of *Streptomyces* origin and the resulting activity. J Antibiot (Tokyo) 1998;51:735–742. [PubMed: 9766465]

Hou Z, Meng JR, Niu C, Wang HF, Liu J, Hu BQ, Jia M, Luo XX. Restoration of antibiotic susceptibility in methicillin-resistant *Staphylococcus aureus* by targeting *mecR1* with a phosphorothioate deoxyribozyme. Clin Exp Pharmacol Physiol 2007a;34:1160–1164. [PubMed: 17880371]

Hou Z, Meng JR, Zhao JR, Hu BQ, Liu J, Yan XJ, Jia M, Luo XX. Inhibition of β-lactamase-mediated oxacillin resistance in *Staphylococcus aureus* by a deoxyribozyme. Acta Pharmacol Sin 2007b; 28:1775–1782. [PubMed: 17959028]

Houghton JL, Green KD, Chen W, Garneau-Tsodikova S. The future of aminoglycosides: the end or renaissance? Chembiochem 2010;11:880–902. [PubMed: 20397253]

Huang ZM, Shan H, Mi ZH, Yang HY, Wu L, Chu QJ, Qin L. [Analysis on 168 rRNA methylase genes and aminoglycoside modifying enzymes genes in *Enterobacter cloacae* in China]. Zhonghua Liu Xing Bing Xue Za Zhi 2008;29:369–373. [PubMed: 18843996]

Hurwitz C, Braun CB, Rosano CL. Role of ribosome recycling in uptake of dihydrostreptomycin by sensitive and resistant *Escherichia coli*. Biochim Biophys Acta 1981;652:168–176. [PubMed: 6163463]

Ishikawa J, Sunada A, Oyama R, Hotta K. Identification and characterization of the point mutation which affects the transcription level of the chromosomal 3-*N*-acetyltransferase gene of

Streptomyces griseus SS-1198. Antimicrob Agents Chemother 2000;44:437–440. [PubMed: 10639379]

Jacoby GA, Blaser MJ, Santanam P, Hachler H, Kayser FH, Hare RS, Miller GH. Appearance of amikacin and tobramycin resistance due to 4′-aminoglycoside nucleotidyltransferase [ANT(4′)-II] in gram-negative pathogens. Antimicrob Agents Chemother 1990;34:2381–2386. [PubMed: 1965106]

Jacquier H, Zaoui C, Sanson-le Pors MJ, Mazel D, Bercot B. Translation regulation of integrons gene cassette expression by the *attC* sites. Mol Microbiol 2009;72:1475–1486. [PubMed: 19486293]

Jana S, Deb JK. Molecular understanding of aminoglycoside action and resistance. Appl Microbiol Biotechnol 2006;70:140–150. [PubMed: 16391922]

Teran, F. Javier; Alvarez, M.; Suarez, JE.; Mendoza, MC. Characterization of two aminoglycoside-(3)-*N*-acetyltransferase genes and assay as epidemiological probes. J Antimicrob Chemother 1991;28:333–346. [PubMed: 1960117]

Kao SJ, You I, Clewell DB, Donabedian SM, Zervos MJ, Petrin J, Shaw KJ, Chow JW. Detection of the high-level aminoglycoside resistance gene *aph(2″)-Ib* in *Enterococcus faecium*. Antimicrob Agents Chemother 2000;44:2876–2879. [PubMed: 10991878]

Kaster KR, Burgett SG, Rao RN, Ingolia TD. Analysis of a bacterial hygromycin B resistance gene by transcriptional and translational fusions and by DNA sequencing. Nucleic Acids Res 1983;11:6895–6911. [PubMed: 6314265]

Kehrenberg C, Catry B, Haesebrouck F, de Kruif A, Schwarz S. Novel spectinomycin/streptomycin resistance gene, *aadA14*, from *Pasteurella multocida*. Antimicrob Agents Chemother 2005;49:3046–3049. [PubMed: 15980396]

Kellermayer R. Translational readthrough induction of pathogenic nonsense mutations. Eur J Med Genet 2006;49:445–450. [PubMed: 16740421]

Kim C, Villegas-Estrada A, Hesek D, Mobashery S. Mechanistic characterization of the bifunctional aminoglycoside-modifying enzyme AAC(3)-Ib/AAC(6′)-Ib’ from *Pseudomonas aeruginosa*. Biochemistry 2007;46:5270–5282. [PubMed: 17417880]

Kingsley JD, Dou H, Morehead J, Rabinow B, Gendelman HE, Destache CJ. Nanotechnology: a focus on nanoparticles as a drug delivery system. J Neuroimmune Pharmacol 2006;1:340–350. [PubMed: 18040810]

Kitao T, Miyoshi-Akiyama T, Kirikae T. AAC(6′)-Iaf, a novel aminoglycoside 6′-*N*-acetyltransferase from multidrug-resistant *Pseudomonas aeruginosa* clinical isolates. Antimicrob Agents Chemother 2009;53:2327–2334. [PubMed: 19349516]

Kitao T, Miyoshi-Akiyama T, Shimada K, Tanaka M, Narahara K, Saito N, Kirikae T. Development of an immunochromatographic assay for the rapid detection of AAC(6′)-Iae-producing multidrug-resistant *Pseudomonas aeruginosa*. J Antimicrob Chemother 2010;65:1382–1386. [PubMed: 20478990]

Kobayashi N, Alam M, Nishimoto Y, Urasawa S, Uehara N, Watanabe N. Distribution of aminoglycoside resistance genes in recent clinical isolates of *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus avium*. Epidemiol Infect 2001;126:197–204. [PubMed: 11349969]

Kohl A, Amstutz P, Parizek P, Binz HK, Briand C, Capitani G, Forrer P, Pluckthun A, Grutter MG. Allosteric inhibition of aminoglycoside phosphotransferase by a designed ankyrin repeat protein. Structure 2005;13:1131–1141. [PubMed: 16084385]

Lambert T, Gerbaud G, Courvalin P. Characterization of the chromosomal *aac(6′)-Ij* gene of *Acinetobacter sp.* 13 and the *aac(6′)-Ih* plasmid gene of *Acinetobacter baumannii*. Antimicrob Agents Chemother 1994a;38:1883–1889. [PubMed: 7810994]

Lambert T, Gerbaud G, Galimand M, Courvalin P. Characterization of *Acinetobacter haemolyticus aac(6′)-Ig* gene encoding an aminoglycoside 6′-*N*-acetyltransferase which modifies amikacin. Antimicrob Agents Chemother 1993;37:2093–2100. [PubMed: 8257129]

Lambert T, Ploy MC, Courvalin P. A spontaneous point mutation in the *aac(6′)-Ib’* gene results in altered substrate specificity of aminoglycoside 6′-*N*-acetyltransferase of a *Pseudomonas fluorescens* strain. FEMS Microbiol Lett 1994b;115:297–304. [PubMed: 8138142]

Lambert T, Ploy MC, Denis F, Courvalin P. Characterization of the chromosomal *aac(6′)-Iz* gene of *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother 1999;43:2366–2371. [PubMed: 10508008]

LeBlanc DJ, Lee LN, Inamine JM. Cloning and nucleotide base sequence analysis of a spectinomycin adenyltransferase AAD(9) determinant from *Enterococcus faecalis*. Antimicrob Agents Chemother 1991;35:1804–1810. [PubMed: 1659306]

Lee KY, Hopkins JD, Syvanen M. Direct involvement of IS*26* in an antibiotic resistance operon. J Bacteriol 1990;172:3229–3236. [PubMed: 2160941]

Levings RS, Partridge SR, Lightfoot D, Hall RM, Djordjevic SP. New integron-associated gene cassette encoding a 3-*N*-aminoglycoside acetyltransferase. Antimicrob Agents Chemother 2005;49:1238–1241. [PubMed: 15728939]

Liebert CA, Hall RM, Summers AO. Transposon Tn*21*, flagship of the floating genome. Microbiol Mol Biol Rev 1999;63:507–522. [PubMed: 10477306]

Liu M, Haddad J, Azucena E, Kotra LP, Kirzhner M, Mobashery S. Tethered bisubstrate derivatives as probes for mechanism and as inhibitors of aminoglycoside 3′-phosphotransferases. J Org Chem 2000;65:7422–7431. [PubMed: 11076599]

Llanes C, Neuwirth C, El Garch F, Hocquet D, Plesiat P. Genetic analysis of a multiresistant strain of *Pseudomonas aeruginosa* producing PER-1 β-lactamase. Clin Microbiol Infect 2006;12:270–278. [PubMed: 16451415]

Lombes T, Begis G, Maurice F, Turcaud S, Lecourt T, Dardel F, Micouin L. NMR-guided fragment-based approach for the design of AAC(6′)-Ib ligands. Chembiochem 2008;9:1368–1371. [PubMed: 18464231]

Lopez-Cabrera M, Perez-Gonzalez JA, Heinzel P, Piepersberg W, Jimenez A. Isolation and nucleotide sequencing of an aminocyclitol acetyltransferase gene from *Streptomyces rimosus* forma paromomycinus. J Bacteriol 1989;171:321–328. [PubMed: 2914849]

Lovering AM, White LO, Reeves DS. AAC(1): a new aminoglycoside-acetylating enzyme modifying the Cl aminogroup of apramycin. J Antimicrob Chemother 1987;20:803–813. [PubMed: 3326872]

Lundblad EW, Altman S. Inhibition of gene expression by RNase P. N Biotechnol 2010;27:212–221. [PubMed: 20211282]

Lyutzkanova D, Distler J, Altenbuchner J. A spectinomycin resistance determinant from the spectinomycin producer *Streptomyces flavopersicus*. Microbiology 1997;143(Pt 7):2135–2143. [PubMed: 9245803]

Mabilat C, Lourencao-Vital J, Goussard S, Courvalin P. A new example of physical linkage between Tn*1* and Tn*21*: the antibiotic multiple-resistance region of plasmid pCFF04 encoding extended-spectrum β-lactamase TEM-3. Mol Gen Genet 1992;235:113–121. [PubMed: 1331747]

MacLeod DL, Nelson LE, Shawar RM, Lin BB, Lockwood LG, Dirk JE, Miller GH, Burns JL, Garber RL. Aminoglycoside-resistance mechanisms for cystic fibrosis *Pseudomonas aeruginosa* isolates are unchanged by long-term, intermittent, inhaled tobramycin treatment. J Infect Dis 2000;181:1180–1184. [PubMed: 10720551]

Magalhaes ML, Vetting MW, Gao F, Freiburger L, Auclair K, Blanchard JS. Kinetic and structural analysis of bisubstrate inhibition of the *Salmonella enterica* aminoglycoside 6′-*N*-acetyltransferase. Biochemistry 2008;47:579–584. [PubMed: 18095712]

Magnet S, Blanchard JS. Molecular insights into aminoglycoside action and resistance. Chem Rev 2005;105:477–498. [PubMed: 15700953]

Magnet S, Courvalin P, Lambert T. Activation of the cryptic *aac(6′)-Iy* aminoglycoside resistance gene of *Salmonella* by a chromosomal deletion generating a transcriptional fusion. J Bacteriol 1999;181:6650–6655. [PubMed: 10542165]

Magnet S, Courvalin P, Lambert T. Resistance-nodulation-cell division-type efflux pump involved in aminoglycoside resistance in *Acinetobacter baumannii* strain BM4454. Antimicrob Agents Chemother 2001;45:3375–3380. [PubMed: 11709311]

Magnet S, Smith TA, Zheng R, Nordmann P, Blanchard JS. Aminoglycoside resistance resulting from tight drug binding to an altered aminoglycoside acetyltransferase. Antimicrob Agents Chemother 2003;47:1577–1583. [PubMed: 12709325]

Alam, M. Mahbub; Kobayashi, N.; Ishino, M.; Sumi, A.; Kobayashi, K.; Uehara, N.; Watanabe, N. Detection of a novel *aph(2")* allele (*aph[2"]-Ie*) conferring high-level gentamicin resistance and a spectinomycin resistance gene *ant(9)-Ia* (*aad 9*) in clinical isolates of enterococci. Microb Drug Resist 2005;11:239–247. [PubMed: 16201926]

Majumder, K.; Wei, L.; Annedi, S.; Kotra, LP. Aminoglycoside antibiotics. In: Bonomo, RA.; Tolmasky, ME., editors. Enzyme-mediated resistance to antibiotics: mechanisms, dissemination, and prospects for inhibition. ASM Press; Washington, DC: 2007. p. 7-20.

Martin P, Jullien E, Courvalin P. Nucleotide sequence of *Acinetobacter baumannii aphA-6* gene: evolutionary and functional implications of sequence homologies with nucleotide-binding proteins, kinases and other aminoglycoside-modifying enzymes. Mol Microbiol 1988;2:615–625. [PubMed: 2846986]

Martinez-Salgado C, Lopez-Hernandez FJ, Lopez-Novoa JM. Glomerular nephrotoxicity of aminoglycosides. Toxicol Appl Pharmacol 2007;223:86–98. [PubMed: 17602717]

Matsuhashi Y, Murakami T, Nojiri C, Toyama H, Anzai H, Nagaoka K. Mechanisms of aminoglycoside-resistance of *Streptomyces* harboring resistant genes obtained from antibiotic-producers. J Antibiot (Tokyo) 1985;38:279–282. [PubMed: 3838980]

Maurice F, Broutin I, Podglajen I, Benas P, Collatz E, Dardel F. Enzyme structural plasticity and the emergence of broad-spectrum antibiotic resistance. EMBO Rep 2008;9:344–349. [PubMed: 18292754]

Mazodier P, Cossart P, Giraud E, Gasser F. Completion of the nucleotide sequence of the central region of Tn*5* confirms the presence of three resistance genes. Nucleic Acids Res 1985;13:195–205. [PubMed: 3889831]

McKay GA, Thompson PR, Wright GD. Broad spectrum aminoglycoside phosphotransferase type III from Enterococcus: overexpression, purification, and substrate specificity. Biochemistry 1994;33:6936–6944. [PubMed: 8204627]

McKenzie T, Hoshino T, Tanaka T, Sueoka N. The nucleotide sequence of pUB110: some salient features in relation to replication and its regulation. Plasmid 1986;15:93–103. [PubMed: 3010356]

Mehta R, Champney WS. Neomycin and paromomycin inhibit 30S ribosomal subunit assembly in *Staphylococcus aureus*. Curr Microbiol 2003;47:237–243. [PubMed: 14570276]

Mendes R, Toleman M, Ribeiro J, Sader H, Jones R, Walsh T. Integron carrying a novel metallo-b-lactamase gene, *bla*IMP-16, and a fused form of aminoglycoside-resistance gene *aac(6′)-30*/*aac(6′)-Ib*: report from the SENTRY antimicrobial surveillance program. Antimicrob Agents Chemother 2004;48:4693–4702. [PubMed: 15561846]

Mendes RE, Castanheira M, Toleman MA, Sader HS, Jones RN, Walsh TR. Characterization of an integron carrying *bla*IMP-1 and a new aminoglycoside resistance gene, *aac(6′)-31*, and its dissemination among genetically unrelated clinical isolates in a Brazilian hospital. Antimicrob Agents Chemother 2007;51:2611–2614. [PubMed: 17470660]

Meng J, Wang H, Hou Z, Chen T, Fu J, Ma X, He G, Xue X, Jia M, Luo X. Novel anion liposome-encapsulated antisense oligonucleotide restores susceptibility of methicillin-resistant *Staphylococcus aureus* and rescues mice from lethal sepsis by targeting *mecA*. Antimicrob Agents Chemother 2009;53:2871–2878. [PubMed: 19433567]

Menzies D, Benedetti A, Paydar A, Martin I, Royce S, Pai M, Vernon A, Lienhardt C, Burman W. Effect of duration and intermittency of rifampin on tuberculosis treatment outcomes: a systematic review and meta-analysis. PLoS Med 2009;6:e1000146. [PubMed: 19753109]

Meyer R. Replication and conjugative mobilization of broad host-range IncQ plasmids. Plasmid 2009;62:57–70. [PubMed: 19465049]

Michael GB, Cardoso M, Schwarz S. Class 1 integron-associated gene cassettes in *Salmonella enterica* subsp. enterica serovar Agona isolated from pig carcasses in Brazil. J Antimicrob Chemother 2005;55:776–779. [PubMed: 15761062]

Mikkelsen NE, Brannvall M, Virtanen A, Kirsebom LA. Inhibition of RNase P RNA cleavage by aminoglycosides. Proc Natl Acad Sci U S A 1999;96:6155–6160. [PubMed: 10339557]

Mugnier P, Casin I, Bouthors AT, Collatz E. Novel OXA-10-derived extended-spectrum β-lactamases selected *in vivo* or *in vitro*. Antimicrob Agents Chemother 1998a;42:3113–3116. [PubMed: 9835500]

Mugnier P, Podglajen I, Goldstein FW, Collatz E. Carbapenems as inhibitors of OXA-13, a novel, integron-encoded β-lactamase in *Pseudomonas aeruginosa*. Microbiology 1998b;144(Pt 4): 1021–1031. [PubMed: 9579076]

Muir ME, van Heeswyck RS, Wallace BJ. Effect of growth rate on streptomycin accumulation by *Escherichia coli* and *Bacillus megaterium*. J Gen Microbiol 1984;130:2015–2022. [PubMed: 6432955]

Muller RE, Ano T, Imanaka T, Aiba S. Complete nucleotide sequences of *Bacillus plasmids* pUB110dB, pRBH1 and its copy mutants. Mol Gen Genet 1986;202:169–171. [PubMed: 3007933]

Mulvey M, Boyd D, Baker L, Mykytczuk O, Resi E, Asensi M, Rodrigues D, Ng L. Characterization of a *Salmonella enterica* serovar Agona strain harbouring a class 1 integron containing novel OXA-type ß-lactamase ($bla_{\text{OXA-53}}$) and 6′-*N*-aminoglycoside acetyltransferase genes [*aac(6′)-I30*]. J Antimicrob Chemother 2004;54:354–359. [PubMed: 15231768]

Murphy E. Nucleotide sequence of a spectinomycin adenyltransferase AAD(9) determinant from *Staphylococcus aureus* and its relationship to AAD(3″) (9). Mol Gen Genet 1985;200:33–39. [PubMed: 2993813]

Nakashima T, Teranishi M, Hibi T, Kobayashi M, Umemura M. Vestibular and cochlear toxicity of aminoglycosides-a review. Acta Otolaryngol 2000;120:904–911. [PubMed: 11200584]

Nichols WW. The enigma of streptomycin transport. J Antimicrob Chemother 1989;23:673–676. [PubMed: 2759919]

Nichols WW, Dorrington SM, Slack MP, Walmsley HL. Inhibition of tobramycin diffusion by binding to alginate. Antimicrob Agents Chemother 1988;32:518–523. [PubMed: 3132093]

Nichols WW, Young SN. Respiration-dependent uptake of dihydrostreptomycin by *Escherichia coli*. Its irreversible nature and lack of evidence for a uniport process. Biochem J 1985;228:505–512. [PubMed: 2409962]

Nobuta K, Tolmasky ME, Crosa LM, Crosa JH. Sequencing and expression of the 6′-*N*-acetyltransferase gene of transposon Tn*1331* from *Klebsiella pneumoniae*. J Bacteriol 1988;170:3769–3773. [PubMed: 2841303]

Noguchi N, Sasatsu M, Kono M. Genetic mapping in *Bacillus subtilis* 168 of the *aadK* gene which encodes aminoglycoside 6-adenylyltransferase. FEMS Microbiol Lett 1993;114:47–52. [PubMed: 8293959]

Novick RP, Clowes RC, Cohen SN, Curtiss R 3rd, Datta N, Falkow S. Uniform nomenclature for bacterial plasmids: a proposal. Bacteriol Rev 1976;40:168–189. [PubMed: 1267736]

Nurizzo D, Shewry SC, Perlin MH, Brown SA, Dholakia JN, Fuchs RL, Deva T, Baker EN, Smith CA. The crystal structure of aminoglycoside-3′-phosphotransferase-IIa, an enzyme responsible for antibiotic resistance. J Mol Biol 2003;327:491–506. [PubMed: 12628253]

O'Connor M, De Stasio EA, Dahlberg AE. Interaction between 16S ribosomal RNA and ribosomal protein S12: differential effects of paromomycin and streptomycin. Biochimie 1991;73:1493–1500. [PubMed: 1725261]

Ogle JM, Brodersen DE, Clemons WM Jr. Tarry MJ, Carter AP, Ramakrishnan V. Recognition of cognate transfer RNA by the 30S ribosomal subunit. Science 2001;292:897–902. [PubMed: 11340196]

Ogle JM, Carter AP, Ramakrishnan V. Insights into the decoding mechanism from recent ribosome structures. Trends Biochem Sci 2003;28:259–266. [PubMed: 12765838]

Ogle JM, Ramakrishnan V. Structural insights into translational fidelity. Annu Rev Biochem 2005;74:129–177. [PubMed: 15952884]

Ohmiya K, Tanaka T, Noguchi N, O'Hara K, Kono M. Nucleotide sequence of the chromosomal gene coding for the aminoglycoside 6-adenylyltransferase from *Bacillus subtilis* Marburg 168. Gene 1989;78:377–378. [PubMed: 2550327]

Oka A, Sugisaki H, Takanami M. Nucleotide sequence of the kanamycin resistance transposon Tn*903*. J Mol Biol 1981;147:217–226. [PubMed: 6270337]

Okazaki A, Avison MB. Aph(3′)-IIc, an aminoglycoside resistance determinant from *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother 2007;51:359–360. [PubMed: 17088477]

Oliveira JF, Silva CA, Barbieri CD, Oliveira GM, Zanetta DM, Burdmann EA. Prevalence and risk factors for aminoglycoside nephrotoxicity in intensive care units. Antimicrob Agents Chemother 2009;53:2887–2891. [PubMed: 19364846]

Oteo J, Navarro C, Cercenado E, Delgado-Iribarren A, Wilhelmi I, Orden B, Garcia C, Miguelanez S, Perez-Vazquez M, Garcia-Cobos S, Aracil B, Bautista V, Campos J. Spread of *Escherichia coli* strains with high-level cefotaxime and ceftazidime resistance between the community, long-term care facilities, and hospital institutions. J Clin Microbiol 2006;44:2359–2366. [PubMed: 16825350]

Over U, Gur D, Unal S, Miller GH. The changing nature of aminoglycoside resistance mechanisms and prevalence of newly recognized resistance mechanisms in Turkey. Clin Microbiol Infect 2001;7:470–478. [PubMed: 11678929]

Overhage J, Bains M, Brazas MD, Hancock RE. Swarming of *Pseudomonas aeruginosa* is a complex adaptation leading to increased production of virulence factors and antibiotic resistance. J Bacteriol 2008;190:2671–2679. [PubMed: 18245294]

Panaite DM, Tolmasky ME. Characterization of mutants of the 6′-*N*-acetyltransferase encoded by the multiresistance transposon Tn*1331*: effect of Phe171-to-Leu171 and Tyr80-to-Cys80 substitutions. Plasmid 1998;39:123–133. [PubMed: 9514709]

Pansegrau W, Miele L, Lurz R, Lanka E. Nucleotide sequence of the kanamycin resistance determinant of plasmid RP4: homology to other aminoglycoside 3′-phosphotransferases. Plasmid 1987;18:193–204. [PubMed: 2832861]

Parent R, Roy PH. The chloramphenicol acetyltransferase gene of Tn*2424*: a new breed of *cat*. J Bacteriol 1992;174:2891–2897. [PubMed: 1314803]

Parkhill J, Sebaihia M, Preston A, Murphy LD, Thomson N, Harris DE, Holden MT, Churcher CM, Bentley SD, Mungall KL, Cerdeno-Tarraga AM, Temple L, James K, Harris B, Quail MA, Achtman M, Atkin R, Baker S, Basham D, Bason N, Cherevach I, Chillingworth T, Collins M, Cronin A, Davis P, Doggett J, Feltwell T, Goble A, Hamlin N, Hauser H, Holroyd S, Jagels K, Leather S, Moule S, Norberczak H, O'Neil S, Ormond D, Price C, Rabbinowitsch E, Rutter S, Sanders M, Saunders D, Seeger K, Sharp S, Simmonds M, Skelton J, Squares R, Squares S, Stevens K, Unwin L, Whitehead S, Barrell BG, Maskell DJ. Comparative analysis of the genome sequences of *Bordetella pertussis, Bordetella parapertussis* and *Bordetella bronchiseptica*. Nat Genet 2003;35:32–40. [PubMed: 12910271]

Partridge SR, Collis CM, Hall RM. Class 1 integron containing a new gene cassette, *aadA10*, associated with Tn*1404* from R151. Antimicrob Agents Chemother 2002;46:2400–2408. [PubMed: 12121911]

Partridge SR, Recchia GD, Scaramuzzi C, Collis CM, Stokes HW, Hall RM. Definition of the *attI1* site of class 1 integrons. Microbiology 2000;146(Pt 11):2855–2864. [PubMed: 11065364]

Pedersen LC, Benning MM, Holden HM. Structural investigation of the antibiotic and ATP-binding sites in kanamycin nucleotidyltransferase. Biochemistry 1995;34:13305–13311. [PubMed: 7577914]

Perez-Vazquez M, Vindel A, Marcos C, Oteo J, Cuevas O, Trincado P, Bautista V, Grundmann H, Campos J. Spread of invasive Spanish *Staphylococcus aureus* spa-type t067 associated with a high prevalence of the aminoglycoside-modifying enzyme gene *ant(4′)-Ia* and the efflux pump genes *msrA*/*msrB*. J Antimicrob Chemother 2009;63:21–31. [PubMed: 18948410]

Perichon B, Bogaerts P, Lambert T, Frangeul L, Courvalin P, Galimand M. Sequence of conjugative plasmid pIP1206 mediating resistance to aminoglycosides by 16S rRNA methylation and to hydrophilic fluoroquinolones by efflux. Antimicrob Agents Chemother 2008;52:2581–2592. [PubMed: 18458128]

Perlin MH, Lerner SA. Localization of an amikacin 3′-phosphotransferase in *Escherichia coli*. J Bacteriol 1981;147:320–325. [PubMed: 6267007]

Pinto-Alphandary H, Andremont A, Couvreur P. Targeted delivery of antibiotics using liposomes and nanoparticles: research and applications. Int J Antimicrob Agents 2000;13:155–168. [PubMed: 10724019]

Poirel L, Cabanne L, Collet L, Nordmann P. Class II transposon-borne structure harboring metallo-β-lactamase gene *bla*VIM-2 in *Pseudomonas putida*. Antimicrob Agents Chemother 2006;50:2889–2891. [PubMed: 16870796]

Poirel L, Lambert T, Turkoglu S, Ronco E, Gaillard J, Nordmann P. Characterization of Class 1 integrons from *Pseudomonas aeruginosa* that contain the *bla*(VIM-2) carbapenem-hydrolyzing b-lactamase gene and of two novel aminoglycoside resistance gene cassettes. Antimicrob Agents Chemother 2001;45:546–552. [PubMed: 11158753]

Possoz C, Newmark J, Sorto N, Sherratt DJ, Tolmasky ME. Sublethal concentrations of the aminoglycoside amikacin interfere with cell division without affecting chromosome dynamics. Antimicrob Agents Chemother 2007;51:252–256. [PubMed: 17043119]

Pourreza A, Whiterspoon M, Fox J, Newmark J, Bui D, Tolmasky ME. Mutagenesis analysis of a conserved region involved in acetyl coenzyme A binding in the aminoglycoside 6′-N-acetyltransferase type Ib encoded by the plasmid pJHCMW1. Antimicrob Agents Chemother 2005;49:2979–2982. [PubMed: 15980378]

Ramirez MS, Parenteau TR, Centron D, Tolmasky ME. Functional characterization of Tn*1331* gene cassettes. J Antimicrob Chemother 2008;62:669–673. [PubMed: 18632872]

Ramirez MS, Quiroga C, Centron D. Novel rearrangement of a class 2 integron in two non-epidemiologically related isolates of *Acinetobacter baumannii*. Antimicrob Agents Chemother 2005;49:5179–5181. [PubMed: 16304199]

Ramon-Garcia S, Otal I, Martin C, Gomez-Lus R, Ainsa JA. Novel streptomycin resistance gene from *Mycobacterium fortuitum*. Antimicrob Agents Chemother 2006;50:3920–3922. [PubMed: 16954315]

Rasmussen LC, Sperling-Petersen HU, Mortensen KK. Hitting bacteria at the heart of the central dogma: sequence-specific inhibition. Microb Cell Fact 2007;6:24. [PubMed: 17692125]

Rather PN, Mann PA, Mierzwa R, Hare RS, Miller GH, Shaw KJ. Analysis of the *aac(3)-VIa* gene encoding a novel 3-*N*-acetyltransferase. Antimicrob Agents Chemother 1993a;37:2074–2079. [PubMed: 8257126]

Rather PN, Munayyer H, Mann PA, Hare RS, Miller GH, Shaw KJ. Genetic analysis of bacterial acetyltransferases: identification of amino acids determining the specificities of the aminoglycoside 6′-*N*-acetyltransferase Ib and IIa proteins. J Bacteriol 1992;174:3196–3203. [PubMed: 1577689]

Rather PN, Orosz E, Shaw KJ, Hare R, Miller G. Characterization and transcriptional regulation of the 2′-*N*-acetyltransferase gene from *Providencia stuartii*. J Bacteriol 1993b;175:6492–6498. [PubMed: 8407825]

Revilla C, Garcillan-Barcia MP, Fernandez-Lopez R, Thomson NR, Sanders M, Cheung M, Thomas CM, de la Cruz F. Different pathways to acquiring resistance genes illustrated by the recent evolution of IncW plasmids. Antimicrob Agents Chemother 2008;52:1472–1480. [PubMed: 18268088]

Revuelta J, Vacas T, Torrado M, Corzana F, Gonzalez C, Jiménez-Barbero J, Menendez M, Bastida A, Asensio L. NMR-based analysis of aminoglycoside recognition by the resistance enzyme ANT(4!): the pattern of OH/NH3+ substitution determines the preferred antibiotic binding mode and is critical for drug inactivation. J Am Chem Soc 2008;130:5086–5103. [PubMed: 18366171]

Riccio ML, Docquier JD, Dell'Amico E, Luzzaro F, Amicosante G, Rossolini GM. Novel 3-*N*-aminoglycoside acetyltransferase gene, *aac(3)-Ic*, from a *Pseudomonas aeruginosa* integron. Antimicrob Agents Chemother 2003;47:1746–1748. [PubMed: 12709352]

Rice LB, Carias LL, Hutton RA, Rudin SD, Endimiani A, Bonomo RA. The KQ element, a complex genetic region conferring transferable resistance to carbapenems, aminoglycosides, and fluoroquinolones in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2008;52:3427–3429. [PubMed: 18573935]

Rich DP, Anderson MP, Gregory RJ, Cheng SH, Paul S, Jefferson DM, McCann JD, Klinger KW, Smith AE, Welsh MJ. Expression of cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis airway epithelial cells. Nature 1990;347:358–363. [PubMed: 1699126]

Robicsek A, Strahilevitz J, Jacoby GA, Macielag M, Abbanat D, Park CH, Bush K, Hooper DC. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat Med 2006;12:83–88. [PubMed: 16369542]

Rosenberg EY, Ma D, Nikaido H. AcrD of *Escherichia coli* is an aminoglycoside efflux pump. J Bacteriol 2000;182:1754–1756. [PubMed: 10692383]

Rouch DA, Byrne ME, Kong YC, Skurray RA. The *aacA-aphD* gentamicin and kanamycin resistance determinant of Tn*4001* from *Staphylococcus aureus*: expression and nucleotide sequence analysis. J Gen Microbiol 1987;133:3039–3052. [PubMed: 2833561]

Rudant E, Bourlioux P, Courvalin P, Lambert T. Characterization of the *aac(6′)-Ik* gene of *Acinetobacter sp.* 6. FEMS Microbiol Lett 1994;124:49–54. [PubMed: 8001769]

Rudant E, Bouvet P, Courvalin P, Lambert T. Phylogenetic analysis of proteolytic *Acinetobacter* strains based on the sequence of genes encoding aminoglycoside 6′-*N*-acetyltransferases. Syst Appl Microbiol 1999;22:59–67. [PubMed: 10188279]

Sabtcheva S, Galimand M, Gerbaud G, Courvalin P, Lambert T. Aminoglycoside resistance gene *ant(4′)-IIb* of *Pseudomonas aeruginosa* BM4492, a clinical isolate from Bulgaria. Antimicrob Agents Chemother 2003;47:1584–1588. [PubMed: 12709326]

Salauze D, Perez-Gonzalez JA, Piepersberg W, Davies J. Characterisation of aminoglycoside acetyltransferase-encoding genes of neomycin-producing *Micromonospora chalcea* and *Streptomyces fradiae*. Gene 1991;101:143–148. [PubMed: 2060791]

Salipante SJ, Hall BG. Determining the limits of the evolutionary potential of an antibiotic resistance gene. Mol Biol Evol 2003;20:653–659. [PubMed: 12679553]

Sandvang D. Novel streptomycin and spectinomycin resistance gene as a gene cassette within a class 1 integron isolated from *Escherichia coli*. Antimicrob Agents Chemother 1999;43:3036–3038. [PubMed: 10582907]

Santanam P, Kayser FH. Purification and characterization of an aminoglycoside inactivating enzyme from *Staphylococcus epidermidis* FK109 that nucleotidylates the 4′- and 4″-hydroxyl groups of the aminoglycoside antibiotics. J Antibiot (Tokyo) 1978;31:343–351. [PubMed: 659332]

Sarno R, Ha H, Weinsetel N, Tolmasky ME. Inhibition of aminoglycoside 6′-*N*-acetyltransferase type Ib-mediated amikacin resistance by antisense oligodeoxynucleotides. Antimicrob Agents Chemother 2003;47:3296–3304. [PubMed: 14506044]

Sarno R, McGillivary G, Sherratt DJ, Actis LA, Tolmasky ME. Complete nucleotide sequence of *Klebsiella pneumoniae* multiresistance plasmid pJHCMW1. Antimicrob Agents Chemother 2002;46:3422–3427. [PubMed: 12384346]

Scaglione F, Dugnani S, Demartini G, Arcidiacono MM, Cocuzza CE, Fraschini F. Bactericidal kinetics of an *in vitro* infection model of once-daily ceftriaxone plus amikacin against gram-positive and gram-negative bacteria. Chemotherapy 1995;41:239–246. [PubMed: 7555203]

Scholz P, Haring V, Wittmann-Liebold B, Ashman K, Bagdasarian M, Scherzinger E. Complete nucleotide sequence and gene organization of the broad-host-range plasmid RSF1010. Gene 1989;75:271–288. [PubMed: 2653965]

Schwarz FV, Perreten V, Teuber M. Sequence of the 50-kb conjugative multiresistance plasmid pRE25 from *Enterococcus faecalis* RE25. Plasmid 2001;46:170–187. [PubMed: 11735367]

Schwocho LR, Schaffner CP, Miller GH, Hare RS, Shaw KJ. Cloning and characterization of a 3-*N*-aminoglycoside acetyltransferase gene, *aac(3)-Ib*, from *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1995;39:1790–1796. [PubMed: 7486920]

Sekiguchi J, Asagi T, Miyoshi-Akiyama T, Fujino T, Kobayashi I, Morita K, Kikuchi Y, Kuratsuji T, Kirikae T. Multidrug-resistant *Pseudomonas aeruginosa* strain that caused an outbreak in a neurosurgery ward and its *aac(6′)-Iae* gene cassette encoding a novel aminoglycoside acetyltransferase. Antimicrob Agents Chemother 2005;49:3734–3742. [PubMed: 16127047]

Shakya T, Wright GD. Nucleotide selectivity of antibiotic kinases. Antimicrob Agents Chemother 2010;54:1909–1913. [PubMed: 20231391]

Shaw KJ, Cramer CA, Rizzo M, Mierzwa R, Gewain K, Miller GH, Hare RS. Isolation, characterization, and DNA sequence analysis of an AAC(6′)-II gene from *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1989;33:2052–2062. [PubMed: 2515793]

Shaw KJ, Rather PN, Hare RS, Miller GH. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol Rev 1993;57:138–163. [PubMed: 8385262]

Shaw KJ, Rather PN, Sabatelli FJ, Mann P, Munayyer H, Mierzwa R, Petrikkos GL, Hare RS, Miller GH, Bennett P, et al. Characterization of the chromosomal *aac(6′)-Ic* gene from *Serratia marcescens*. Antimicrob Agents Chemother 1992;36:1447–1455. [PubMed: 1354954]

Shmara A, Weinsetel N, Dery KJ, Chavideh R, Tolmasky ME. Systematic analysis of a conserved region of the aminoglycoside 6′-*N*-acetyltransferase type Ib. Antimicrob Agents Chemother 2001;45:3287–3292. [PubMed: 11709299]

Siregar JJ, Lerner SA, Mobashery S. Purification and characterization of aminoglycoside 3′-phosphotransferase type IIa and kinetic comparison with a new mutant enzyme. Antimicrob Agents Chemother 1994;38:641–647. [PubMed: 8031025]

Smith, C.; Toth, M.; Frase, H.; Byrnes, L.; Vakulenko, S. Kinetic and structural characterization of the enterococcal aminoglycoside phosphotransferases from the APH(2″) family. 110th General Meeting of the American Society for Microbiology; San Diego, CA. 2010.

Bistue, A.J. Soler; Birshan, D.; Tomaras, AP.; Dandekar, M.; Tran, T.; Newmark, J.; Bui, D.; Gupta, N.; Hernandez, K.; Sarno, R.; Zorreguieta, A.; Actis, LA.; Tolmasky, ME. *Klebsiella pneumoniae* multiresistance plasmid pMET1: similarity with the *Yersinia pestis* plasmid pCRY and integrative conjugative elements. PLoS One 2008;3:e1800. [PubMed: 18350140]

Bistue, A.J. Soler; Ha, H.; Sarno, R.; Don, M.; Zorreguieta, A.; Tolmasky, ME. External guide sequences targeting the *aac(6′)-Ib* mRNA induce inhibition of amikacin resistance. Antimicrob Agents Chemother 2007;51:1918–1925. [PubMed: 17387154]

Bistue, A.J. Soler; Martin, FA.; Vozza, N.; Ha, H.; Joaquin, JC.; Zorreguieta, A.; Tolmasky, ME. Inhibition of *aac(6′)-Ib*-mediated amikacin resistance by nuclease-resistant external guide sequences in bacteria. Proc Natl Acad Sci U S A 2009;106:13230–13235. [PubMed: 19666539]

Spotts CR, Stanier RY. Mechanism of streptomycin action on bacteria: a unitary hypothesis. Nature 1961;192:633–637. [PubMed: 13915908]

Steiniger-White M, Rayment I, Reznikoff WS. Structure/function insights into Tn*5* transposition. Curr Opin Struct Biol 2004;14:50–57. [PubMed: 15102449]

Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, Brinkman FS, Hufnagle WO, Kowalik DJ, Lagrou M, Garber RL, Goltry L, Tolentino E, Westbrock-Wadman S, Yuan Y, Brody LL, Coulter SN, Folger KR, Kas A, Larbig K, Lim R, Smith K, Spencer D, Wong GK, Wu Z, Paulsen IT, Reizer J, Saier MH, Hancock RE, Lory S, Olson MV. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. Nature 2000;406:959–964. [PubMed: 10984043]

Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A. Plasmid-mediated quinolone resistance: a multifaceted threat. Clin Microbiol Rev 2009;22:664–689. [PubMed: 19822894]

Sunada A, Nakajima M, Ikeda Y, Kondo S, Hotta K. Enzymatic 1-*N*-acetylation of paromomycin by an actinomycete strain #8 with multiple aminoglycoside resistance and paromomycin sensitivity. J Antibiot (Tokyo) 1999;52:809–814. [PubMed: 10726929]

Suter TM, Viswanathan VK, Cianciotto NP. Isolation of a gene encoding a novel spectinomycin phosphotransferase from *Legionella pneumophila*. Antimicrob Agents Chemother 1997;41:1385–1388. [PubMed: 9174205]

Taber HW, Mueller JP, Miller PF, Arrow AS. Bacterial uptake of aminoglycoside antibiotics. Microbiol Rev 1987;51:439–457. [PubMed: 3325794]

Tauch A, Gotker S, Puhler A, Kalinowski J, Thierbach G. The 27.8-kb R-plasmid pTET3 from *Corynebacterium glutamicum* encodes the aminoglycoside adenyltransferase gene cassette *aadA9* and the regulated tetracycline efflux system Tet 33 flanked by active copies of the widespread insertion sequence IS*6100*. Plasmid 2002;48:117–129. [PubMed: 12383729]

Tauch A, Krieft S, Kalinowski J, Puhler A. The 51,409-bp R-plasmid pTP10 from the multiresistant clinical isolate *Corynebacterium striatum* M82B is composed of DNA segments initially identified in soil bacteria and in plant, animal, and human pathogens. Mol Gen Genet 2000;263:1–11. [PubMed: 10732668]

Tennstedt T, Szczepanowski R, Braun S, Puhler A, Schluter A. Occurrence of integron-associated resistance gene cassettes located on antibiotic resistance plasmids isolated from a wastewater treatment plant. FEMS Microbiol Ecol 2003;45:239–252. [PubMed: 19719593]

Tenover FC, Filpula D, Phillips KL, Plorde JJ. Cloning and sequencing of a gene encoding an aminoglycoside 6′-*N*-acetyltransferase from an R factor of *Citrobacter diversus*. J Bacteriol 1988;170:471–473. [PubMed: 2826403]

Tenover FC, Gilbert T, O'Hara P. Nucleotide sequence of a novel kanamycin resistance gene, *aphA-7*, from *Campylobacter jejuni* and comparison to other kanamycin phosphotransferase genes. Plasmid 1989;22:52–58. [PubMed: 2550983]

Teran FJ, Suarez JE, Mendoza MC. Cloning, sequencing, and use as a molecular probe of a gene encoding an aminoglycoside 6′-*N*-acetyltransferase of broad substrate profile. Antimicrob Agents Chemother 1991;35:714–719. [PubMed: 2069376]

Thompson CJ, Gray GS. Nucleotide sequence of a streptomycete aminoglycoside phosphotransferase gene and its relationship to phosphotransferases encoded by resistance plasmids. Proc Natl Acad Sci U S A 1983;80:5190–5194. [PubMed: 6310563]

Thompson PR, Hughes DW, Cianciotto NP, Wright GD. Spectinomycin kinase from *Legionella pneumophila*. Characterization of substrate specificity and identification of catalytically important residues. J Biol Chem 1998;273:14788–14795. [PubMed: 9614079]

Thompson PR, Hughes DW, Wright GD. Regiospecificity of aminoglycoside phosphotransferase from Enterococci and Staphylococci (APH(3′)-IIIa). Biochemistry 1996;35:8686–8695. [PubMed: 8679631]

Tolmasky, ME. Aminoglycoside-modifying enzymes: characteristics, localization, and dissemination. In: Bonomo, RA.; Tolmasky, ME., editors. Enzyme-mediated resistance to antibiotics: mechanisms, dissemination, and prospects for inhibition. ASM Press; Washington, DC: 2007a. p. 35-52.

Tolmasky ME. Bacterial resistance to aminoglycosides and β-lactams: the Tn*1331* transposon paradigm. Front Biosci 2000;5:D20–29. [PubMed: 10702385]

Tolmasky, ME. Overview of dissemination mechanisms of genes coding for resistance to antibiotics. In: Bonomo, RA.; Tolmasky, ME., editors. Enzyme-mediated resistance to antibiotics: mechanisms, dissemination, and prospects for inhibition. ASM Press; Washington, DC: 2007b. p. 267-270.

Tolmasky ME. Sequencing and expression of *aadA*, *bla*, and *tnpR* from the multiresistance transposon Tn*1331*. Plasmid 1990;24:218–226. [PubMed: 1963948]

Tolmasky ME, Chamorro RM, Crosa JH, Marini PM. Transposon-mediated amikacin resistance in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 1988;32:1416–1420. [PubMed: 2848445]

Tolmasky ME, Crosa JH. Genetic organization of antibiotic resistance genes (*aac(6′)-Ib*, *aadA*, and *oxa9*) in the multiresistance transposon Tn*1331*. Plasmid 1993;29:31–40. [PubMed: 8382826]

Tolmasky ME, Crosa JH. Tn*1331*, a novel multiresistance transposon encoding resistance to amikacin and ampicillin in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 1987;31:1955–1960. [PubMed: 2830842]

Tolmasky ME, Roberts M, Woloj M, Crosa JH. Molecular cloning of amikacin resistance determinants from a *Klebsiella pneumoniae* plasmid. Antimicrob Agents Chemother 1986;30:315–320. [PubMed: 3021052]

Toriya M, Sakakibara M, Matsushita K, Morohoshi T. Nucleotide sequence of aminoglycoside 6′-*N*-acetyltransferase [AAC(6′)] determinant from *Serratia* sp. 45. Chem Pharm Bull (Tokyo) 1992;40:2473–2477. [PubMed: 1339319]

Viera, C. Torres; Tsiodras, S.; Gold, HS.; Coakley, EP.; Wennersten, C.; Eliopoulos, GM.; Moellering, RC., Jr.; Inouye, RT. Restoration of vancomycin susceptibility in *Enterococcus faecalis* by antiresistance determinant gene transfer. Antimicrob Agents Chemother 2001;45:973–975. [PubMed: 11181395]

Toth M, Chow JW, Mobashery S, Vakulenko SB. Source of phosphate in the enzymic reaction as a point of distinction among aminoglycoside 2″- phosphotransferases. J Biol Chem 2009;284:6690–6696. [PubMed: 19158087]

Toth M, Frase H, Antunes NT, Smith CA, Vakulenko SB. Crystal structure and kinetic mechanism of aminoglycoside phosphotransferase-2″-IVa. Protein Sci. 2010a in press.

Toth M, Vakulenko S, Smith CA. Purification, crystallization and preliminary X-ray analysis of *Enterococcus casseliflavus* aminoglycoside-2″- phosphotransferase-IVa. Acta Crystallogr Sect F Struct Biol Cryst Commun 2010b;66:81–84.

van Nhieu, G. Tran; Collatz, E. Primary structure of an aminoglycoside 6′-*N*-acetyltransferase AAC(6′)-4, fused *in vivo* with the signal peptide of the Tn*3*-encoded β-lactamase. J Bacteriol 1987;169:5708–5714. [PubMed: 2824444]

Trieu-Cuot P, Courvalin P. Nucleotide sequence of the *Streptococcus faecalis* plasmid gene encoding the 3′5″-aminoglycoside phosphotransferase type III. Gene 1983;23:331–341. [PubMed: 6313476]

Trower MK, Clark KG. PCR cloning of a streptomycin phosphotransferase (*aphE*) gene from *Streptomyces griseus* ATCC 12475. Nucleic Acids Res 1990;18:4615. [PubMed: 2167474]

Tsai SF, Zervos MJ, Clewell DB, Donabedian SM, Sahm DF, Chow JW. A new high-level gentamicin resistance gene, *aph(2″)-Id*, in *Enterococcus* spp. Antimicrob Agents Chemother 1998;42:1229–1232. [PubMed: 9593155]

Vakulenko SB, Mobashery S. Versatility of aminoglycosides and prospects for their future. Clin Microbiol Rev 2003;16:430–450. [PubMed: 12857776]

van de Klundert JA, Vliegenthart JS. Nomenclature of aminoglycoside resistance genes: a comment. Antimicrob Agents Chemother 1993;37:927–928. [PubMed: 8494396]

Vanhoof R, Hannecart-Pokorni E, Content J. Nomenclature of genes encoding aminoglycoside-modifying enzymes. Antimicrob Agents Chemother 1998;42:483. [PubMed: 9527817]

Vanhoof R, Sonck P, Hannecart-Pokorni E. The role of lipopolysaccharide anionic binding sites in aminoglycoside uptake in *Stenotrophomonas* (*Xanthomonas*) *maltophilia*. J Antimicrob Chemother 1995;35:167–171. [PubMed: 7768765]

Vazquez-Laslop N, Lee H, Hu R, Neyfakh AA. Molecular sieve mechanism of selective release of cytoplasmic proteins by osmotically shocked *Escherichia coli*. J Bacteriol 2001;183:2399–2404. [PubMed: 11274096]

Vetting MW, Hegde SS, Javid-Majd F, Blanchard JS, Roderick SL. Aminoglycoside 2′-*N*-acetyltransferase from *Mycobacterium tuberculosis* in complex with coenzyme A and aminoglycoside substrates. Nat Struct Biol 2002;9:653–658. [PubMed: 12161746]

Vetting MW, LP S.d.C. Yu M, Hegde SS, Magnet S, Roderick SL, Blanchard JS. Structure and functions of the GNAT superfamily of acetyltransferases. Arch Biochem Biophys 2005;433:212–226. [PubMed: 15581578]

Vetting MW, Magnet S, Nieves E, Roderick SL, Blanchard JS. A bacterial acetyltransferase capable of regioselective *N*-acetylation of antibiotics and histones. Chem Biol 2004;11:565–573. [PubMed: 15123251]

Vetting MW, Park CH, Hegde SS, Jacoby GA, Hooper DC, Blanchard JS. Mechanistic and structural analysis of aminoglycoside *N*-acetyltransferase AAC(6′)-Ib and its bifunctional, fluoroquinolone-active AAC(6′)-Ib-cr variant. Biochemistry 2008;47:9825–9835. [PubMed: 18710261]

Veyssier, P.; Bryskier, A. Aminocyclitol aminoglycosides. In: Bryskier, A., editor. Antimicrobial agents. ASM Press; Washington, DC: 2005. p. 453-469.

Vicens Q, Westhof E. Molecular recognition of aminoglycoside antibiotics by ribosomal RNA and resistance enzymes: an analysis of x-ray crystal structures. Biopolymers 2003;70:42–57. [PubMed: 12925992]

Viedma E, Juan C, Acosta J, Zamorano L, Otero JR, Sanz F, Chaves F, Oliver A. Nosocomial spread of colistin-only-sensitive sequence type 235 *Pseudomonas aeruginosa* isolates producing the extended-spectrum β-lactamases GES-1 and GES-5 in Spain. Antimicrob Agents Chemother 2009;53:4930–4933. [PubMed: 19738007]

Vliegenthart JS, Ketelaar-van Gaalen PA, Eelhart J, van de Klundert JA. Localisation of the aminoglycoside-(3)-*N*-acetyltransferase isoenzyme II in *Escherichia coli*. FEMS Microbiol Lett 1991a;65:101–105. [PubMed: 1874395]

Vliegenthart JS, Ketelaar-van Gaalen PA, van de Klundert JA. Nucleotide sequence of the *aacC3* gene, a gentamicin resistance determinant encoding aminoglycoside-(3)-*N*-acetyltransferase III expressed in *Pseudomonas aeruginosa* but not in *Escherichia coli*. Antimicrob Agents Chemother 1991b;35:892–897. [PubMed: 1649572]

Vogtli M, Hutter R. Characterisation of the hydroxystreptomycin phosphotransferase gene (*sph*) of *Streptomyces glaucescens*: nucleotide sequence and promoter analysis. Mol Gen Genet 1987;208:195–203. [PubMed: 3039305]

Wei Q, Jiang X, Yang Z, Chen N, Chen X, Li G, Lu Y. *dfrA27*, a new integron-associated trimethoprim resistance gene from *Escherichia coli*. J Antimicrob Chemother 2009;63:405–406. [PubMed: 19008256]

Welch KT, Virga KG, Whittemore NA, Ozen C, Wright E, Brown CL, Lee RE, Serpersu EH. Discovery of non-carbohydrate inhibitors of aminoglycoside-modifying enzymes. Bioorg Med Chem 2005;13:6252–6263. [PubMed: 16140014]

Wenk M, Spring P, Vozeh S, Follath F. Multicompartment pharmacokinetics of netilmicin. Eur J Clin Pharmacol 1979;16:331–334. [PubMed: 520400]

Werner G, Hildebrandt B, Witte W. Linkage of *erm(B)* and *aadE-sat4-aphA-3* in multiple-resistant *Enterococcus faecium* isolates of different ecological origins. Microb Drug Resist 2003;9(Suppl 1):S9–16. [PubMed: 14633362]

White DG, Maneewannakul K, von Hofe E, Zillman M, Eisenberg W, Field AK, Levy SB. Inhibition of the multiple antibiotic resistance (*mar*) operon in *Escherichia coli* by antisense DNA analogs. Antimicrob Agents Chemother 1997;41:2699–2704. [PubMed: 9420041]

Williams JW, Northrop DB. Synthesis of a tight-binding, multisubstrate analog inhibitor of gentamicin acetyltransferase I. J Antibiot (Tokyo) 1979;32:1147–1154. [PubMed: 393684]

Wilson NL, Hall RM. Unusual class 1 integron configuration found in *Salmonella* genomic island 2 from *Salmonella enterica* serovar Emek. Antimicrob Agents Chemother 2010;54:513–516. [PubMed: 19884375]

Winsor GL, Lo R, Sui SJ, Ung KS, Huang S, Cheng D, Ching WK, Hancock RE, Brinkman FS. *Pseudomonas aeruginosa* Genome Database and PseudoCAP: facilitating community-based, continually updated, genome annotation. Nucleic Acids Res 2005;33:D338–D343. [PubMed: 15608211]

Wohlleben W, Arnold W, Bissonnette L, Pelletier A, Tanguay A, Roy PH, Gamboa GC, Barry GF, Aubert E, Davies J, et al. On the evolution of Tn*21*-like multiresistance transposons: sequence analysis of the gene (*aacC1*) for gentamicin acetyltransferase-3-I(AAC(3)-I), another member of the Tn*21*-based expression cassette. Mol Gen Genet 1989;217:202–208. [PubMed: 2549372]

Wolf E, Vassilev A, Makino Y, Sali A, Nakatani Y, Burley SK. Crystal structure of a GCN5-related *N*-acetyltransferase: *Serratia marcescens* aminoglycoside 3-N-acetyltransferase. Cell 1998;94:439–449. [PubMed: 9727487]

Woloj M, Tolmasky ME, Roberts MC, Crosa JH. Plasmid-encoded amikacin resistance in multiresistant strains of *Klebsiella pneumoniae* isolated from neonates with meningitis. Antimicrob Agents Chemother 1986;29:315–319. [PubMed: 3521478]

Woodford N, Wareham DW. Tackling antibiotic resistance: a dose of common antisense? J Antimicrob Chemother 2009;63:225–229. [PubMed: 19004840]

Wright, G.; Berghuis, A. Structural aspects of aminoglycoside-modifying enzymes. In: Bonomo, RA.; Tolmasky, ME., editors. Enzyme-mediated resistance to antibiotics: mechanisms, dissemination, and prospects for inhibition. ASM Press; Washington, DC: 2007. p. 21-33.

Wright GD, Thompson PR. Aminoglycoside phosphotransferases: proteins, structure, and mechanism. Front Biosci 1999;4:D9–21. [PubMed: 9872733]

Wybenga-Groot LE, Draker K, Wright GD, Berghuis AM. Crystal structure of an aminoglycoside 6′-*N*-acetyltransferase: defining the GCN5-related N-acetyltransferase superfamily fold. Structure 1999;7:497–507. [PubMed: 10378269]

Yan JJ, Hsueh PR, Lu JJ, Chang FY, Ko WC, Wu JJ. Characterization of acquired β-lactamases and their genetic support in multidrug-resistant *Pseudomonas aeruginosa* isolates in Taiwan: the prevalence of unusual integrons. J Antimicrob Chemother 2006;58:530–536. [PubMed: 16816399]

Yao, J.; Moellering, R. Antibacterial agents. In: Murray, P.; Baron, E.; Jorgensen, J.; Landry, M.; Pfaller, M., editors. Manual of Clinical Microbiology. American Society for Microbiology Press; Washington, DC: 2007. p. 1077-1113.

Young PG, Walanj R, Lakshmi V, Byrnes LJ, Metcalf P, Baker EN, Vakulenko SB, Smith CA. The crystal structures of substrate and nucleotide complexes of *Enterococcus faecium* aminoglycoside-2″-phosphotransferase-IIa [APH(2″)-IIa] provide insights into substrate selectivity in the APH(2″) subfamily. J Bacteriol 2009;191:4133–4143. [PubMed: 19429619]

Zaher HS, Green R. Fidelity at the molecular level: lessons from protein synthesis. Cell 2009;136:746–762. [PubMed: 19239893]

Zalacain M, Gonzalez A, Guerrero MC, Mattaliano RJ, Malpartida F, Jimenez A. Nucleotide sequence of the hygromycin B phosphotransferase gene from *Streptomyces hygroscopicus*. Nucleic Acids Res 1986;14:1565–1581. [PubMed: 3005976]

Zhang W, Fisher JF, Mobashery S. The bifunctional enzymes of antibiotic resistance. Curr Opin Microbiol 2009;12:505–511. [PubMed: 19615931]

Zingman LV, Park S, Olson TM, Alekseev AE, Terzic A. Aminoglycoside-induced translational read-through in disease: overcoming nonsense mutations by pharmacogenetic therapy. Clin Pharmacol Ther 2007;81:99–103. [PubMed: 17186006]

Zong Z, Partridge SR, Iredell JR. A *bla*VEB-1 variant, *bla*VEB-6, associated with repeated elements in a complex genetic structure. Antimicrob Agents Chemother 2009;53:1693–1697. [PubMed: 19139283]

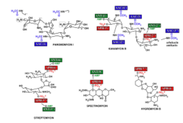

Fig. 1.

Representative aminoglycosides and modification sites by AAC, ANT, and APH enzymes. An example of each kind of modification is shown on one of the substrates. The square and oval on positions 2′ and 6″ in paromomycin I indicate that although this molecule is preferentially acetylated at the position 1, 1,2′-di-*N*-acetylparomomycin and 1,6″-di-*N*-acetylparomomycin are also found as products of the enzymatic reaction (Sunada et al., 1999). AAC(3)-X can catalyze acetylation at the 3″-amino group in arbekacin and amikacin (Hotta et al. 1998).

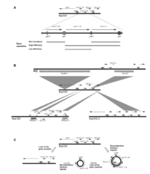

Fig. 2.

A. Genetic map of the Tn*1331* transposon with the region including genes *aac(6′)-Ib*, *aadA1* and bla_{OXA-9} amplified. Circles and ovals represent *attC* and *attI1** loci respectively. For clarity the points of potential crossover reactions are not indicated but they can be found in Ramirez et al. (Ramirez et al., 2008). Regions with a gene cassette structure are indicated below the genetic map by bars of different patterns. Their functionality as determined in recombination assays in the presence of IntI1 expressed from a recombinant clone harboring *intI1* under the control of the P_{tac} promoter is shown. Directly repeated regions are shown as gray boxes on the sequences. B. Genetic maps of Tn*1331*, Tn*1331.2*, Tn*1332*, and the KQ element. Shadowed areas show the fragments inserted within the Tn*1331* sequence that generated the other three genetic elements. C. Model for generation of a circular molecule containing *aac(6′)-Ib* (Zong et al., 2009). The white box indicates the DNA region that is found upstream of the gene in *P. mirabilis* JIE273. GC, gene cassette. Circular molecules are not drawn to scale.

Table 1

Aminoglycoside *N*-acetyltransferases

AACs	Gene names	Genetic location	Accession number	Host	References
AAC(1)				*E. coli, Actinomycete, Campylobacter* spp.	(Gomez-Luis et al., 1999; Lovering et al., 1987; Sunada et al., 1999)
AAC(3)-Ia **C**	*aac(3)-Ia, aacC1*	Plasmid, transposon, integron	X15852, AF550679	*S. marcescens, E. coli, Acinetobacter baumannii, Klebsiella pneumoniae, Klebsiella oxytoca, P. aeruginosa, Salmonella typhimurium, Proteus mirabilis*	(Javier Teran et al., 1991; Wohlleben et al., 1989)
AAC(3)-Ib	*aac(3)-Ib*	Integron	L06157	*P. aeruginosa*	(Schwocho et al., 1995)
AAC(3)-Ic	*aac(3)-Ic*	Integron	AJ511268	*P. aeruginosa*	(Riccio et al., 2003)
AAC(3)-Id	*aac(3)-Id*	Genomic island, integron	AY458224	*S. enterica, P. mirabilis, Vibrio fluvialis*	(Doublet et al., 2004)
AAC(3)-Ie	*aac(3)-Ie, aacCA5*	Integron	AY463797, DQ520937, AY463797	*S. enterica, P. mirabilis, P. aeruginosa*	(Gionechetti et al., 2008; Levings et al., 2005)
AAC(3)-IIa	*aac(3)-IIa, aaC3, aacC5, aacC2, aac(3)-Va*	Plasmid	X13543	*K. pneumoniae, E. cloacae, Actinobacillus pleuropneumoniae, S. typhimurium, Citrobacter freundii*	(Allmansberger et al., 1985)
AAC(3)-IIb	*aac(3)-IIb, aac(3)-Vb*		M97172	*E. coli, A. faecalis, S. marcescens*	(Rather et al., 1992) (Dahmen et al., 2010)
AAC(3)-IIc	*aac(3)-IIc, aacC2*	Plasmid	X54723	*E. coli, P. aeruginosa*	(Dubois et al., 2008)
AAC(3)-IIIa	*aac(3)-IIIa, aacC3*	Chromosome	X55652	*P. aeruginosa*	(Vliegenthart et al., 1991a)
AAC(3)-IIIb	*aac(3)-IIIb*		L06160	*P. aeruginosa*	
AAC(3)-IIIc	*aac(3)-IIIc, ant(2")-Ib*		L06161	*P. aeruginosa*	
AAC(3)-IVa	*aac(3)-IVa*	Plasmid	X01385, AY216678, AJ493432	*E. coli, C. jejuni, P. stutzeri*	(Brau et al., 1984; Heuer et al., 2002)
AAC(3)-VIa	*aac(3)-VIa*	Plasmid	M88012, NC_009140 NC_009838	*E. cloacae, S. enterica, E. coli*	(Rather et al., 1993a) (Call et al., 2010)

AACs	Gene names	Genetic location	Accession number	Host	References
AAC(3)-VIIa	*aac(3)-VIIa*, *aacC7*	Chromosome	M22999	*Streptomyces rimosus*	(Lopez-Cabrera et al., 1989)
AAC(3)-VIIIa	*aac(3)-VIIIa*, *aacC8*	Chromosome	M55426	*Streptomyces fradiae*	(Salauze et al., 1991)
AAC(3)-IXa	*aac(3)-IXa*, *aacC9*	Chromosome	M55427	*Micromonospora chalcea*	(Salauze et al., 1991)
AAC(3)-X	*aac(3)-Xa*	Chromosome	AB028210	*Streptomyces griseus*	(Ishikawa et al., 2000)
AAC(2′)-Ia	*aac(2′)-Ia*	Chromosome	L06156	*P. stuartii*	(Rather et al., 1993b)
AAC(2′)-Ib	*aac(2′)-Ib*	Chromosome	CP001172	*M. fortuitum*, *A. baumannii*	(Adams et al., 2008; Ainsa et al., 1997)
AAC(2′)-Ic **C**	*aac(2′)-Ic*	Chromosome	CP001658, NC_002945	*M. tuberculosis*, *M. bovis*	(Ainsa et al., 1997)
AAC(2′)-Id	*aac(2′)-Id*	Chromosome	NC_008596	*M. smegmatis*	(Ainsa et al., 1997)
AAC(2′)-Ie	*aac(2′)-Ie*	Chromosome		*M. leprae*	(Ainsa et al., 1997)
Putative AAC(2′)		Chromosome	AM743169	*S. maltophilia*	(Crossman et al., 2008)
AAC(6′)-Ia	*aac(6′)-Ia*, *aacA1*	Plasmid, transposon, integron	M18967, AF047479, M86913	*Citrobacter diversus*, *E. coli*, *K. pneumoniae*, *Shigella sonnei*	(Tenover et al., 1988), (Parent and Roy, 1992)
AAC(6′)-Ib **C**	*aac(6′)-Ib*, *aac(6′)-4* *aacA4*	Plasmid, transposon, integron	M21682, M23634, AF479774	*K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. enterica*, *K. oxytoca*, *S. maltophilia*, *E. cloacae*	(Nobuta et al., 1988; Tran van Nhieu and Collatz, 1987)
AAC(6′)-Ib’	*aac(6′)-Ib’*, *aac(6′)-Ib*$_6$	Integron	L25617, AJ584652, L25666	*P. fluorescens* , *P. aeruginosa*	(Lambert et al., 1994b; Mendes et al., 2004); (Casin et al., 1998)
AAC(6′)-Ic	*aac(6′)-Ic*	Chromosome	M94066	*S. marcescens*	(Shaw et al., 1992)
AAC(6′)-Ie	*aac(6′)-Ie*, *aac(6′)*-bifuncional	Transposon	M18086	*S. aureus*, *Macrococcus caseolyticus*, *E. faecalis*, *Enterococcus faecium*	(Rouch et al., 1987)
AAC(6′)-If	*aac(6′)-If*	Plasmid	X55353	*E. cloacae*	(Teran et al., 1991)
AAC(6′)-Ig	*aac(6′)-Ig*	Chromosome	L09246	*Acinetobacter haemolyticus*	(Lambert et al., 1993)
AAC(6′)-Ih	*aac(6′)-Ih*	Plasmid	L29044	*A. baumannii*	(Lambert et al., 1994a)
AAC(6′)-Ii **C**	*aac(6′)-Ii*	Chromosome	L12710	*Enterococcus* spp.	(Costa et al., 1993; Draker et al., 2003; Wybenga-Groot et al., 1999)
AAC(6′)-Ij	*aac(6′)-Ij*	Chromosome	L29045	*Acinetobacter genomosp. 13*	(Lambert et al., 1994a)

NIH-PA Author Manuscript
NIH-PA Author Manuscript

AACs	Gene names	Genetic location	Accession number	Host	References
AAC(6′)-Ik	*aac(6′)-Ik*	Chromosome	L29510	*Acinetobacter sp.*	(Rudant et al., 1994)
AAC(6′)-Ip	*aac(6′)-Il, aac(6′)-Im, aac(6′)-Ip*	Integron	Z54241	*C. freundii*	(Hannecart-Pokorni et al., 1997)
AAC(6′)-Iq	*aac(6′)-Iq*	Plasmid, integron	AF047556	*K. pneumoniae*	(Centron and Roy, 1998)
AAC(6′)-Im	*aac(6′)-Im*	Plasmid	AF337947	*E. coli, E. faecium*	(Chow et al., 2001)
AAC(6′)-Il	*aac(6′)-Il, aacA7*	Plasmid, integron	U13880	*Enterobacter aerogenes*	(Bunny et al., 1995)
AAC(6′)-Ir	*aac(6′)-Ir*	Chromosome	AF031326	*Acinetobacter genomosp. 14*	(Rudant et al., 1999)
AAC(6′)-Is	*aac(6′)-Is*	Chromosome	AF031327	*Acinetobacter genomosp. 15*	(Rudant et al., 1999)
AAC(6′)-Isa	*aac(6′)-Isa*	Plasmid	AB116646	*Streptomyces albulus*	(Hamano et al., 2004)
AAC(6′)-It	*aac(6′)-It*	Chromosome	AF031328	*A. genomosp. 16*	(Rudant et al., 1999)
AAC(6′)-Iu	*aac(6′)-Iu*	Chromosome	AF031329	*A. genomosp. 17*	(Rudant et al., 1999)
AAC(6′)-Iv	*aac(6′)-Iv*	Chromosome	AF031330	*Acinetobacter sp.*	(Rudant et al., 1999)
AAC(6′)-Iw	*aac(6′)-Iw*	Chromosome	AF031331	*Acinetobacter sp.*	(Rudant et al., 1999)
AAC(6′)-Ix	*aac(6′)-Ix*	Chromosome	AF031332	*Acinetobacter sp.*	(Rudant et al., 1999)
AAC(6′)-Iy **C**	*aac(6′)-Iy*	Chromosome	AF144881	*S. enteritidis, S. enterica*	(Magnet et al., 1999)
AAC(6′)-Iz	*aac(6′)-Iz*	Chromosome	AF140221	*S. maltophilia*	(Lambert et al., 1999)
AAC(6′)-Iaa	*aac(6′)-Iaa*	Chromosome	NC_003197	*S. typhimurium*	(Salipante and Hall, 2003)
AAC(6′)-Iad	*aac(6′)-Iad*	Plasmid	AB119105	*Acinetobacter genomosp. 3*	(Doi et al., 2004)
AAC(6′)-Iae	*aac(6′)-Iae*	Integron	AB104852	*P. aeruginosa, S. enterica*	(Sekiguchi et al., 2005)
AAC(6′)-Iaf	*aac(6′)-Iaf*	Plasmid, integron	AB462903	*P. aeruginosa*	(Kitao et al., 2009)
AAC(6′)-Iai	*aac(6′)-Iai*	Plasmid, integron	EU886977	*P. aeruginosa*	
AAC(6′)-Ib_3	*aac(6′)*-Ib_3, *aac(6′)*-Ib_5	integron	X60321	*P. aeruginosa*	(Mabilat et al., 1992); (Casin et al., 1998)
AAC(6′)-Ib_4	*aac(6′)*-Ib_4		S49888	*Serratia spp.*	(Toriya et al., 1992)
AAC(6′)-Ib_7	*aac(6′)*-Ib_7	Plasmid	Y11946	*E. cloacae, C. freundii*	(Casin et al., 1998),
AAC(6′)-Ib_8	*aac(6′)*-Ib_8	Plasmid	Y11947	*E. cloacae*	(Casin et al., 1998)
AAC(6′)-Ib_9	*aac(6′)*-Ib_9	Integron	AF043381	*P. aeruginosa*	(Mugnier et al., 1998a)

AACs	Gene names	Genetic location	Accession number	Host	References
AAC(6′)-Ib_{10}	*aac(6′)-Ib_{10}*	Integron		*P. aeruginosa*	(Mugnier et al., 1998b)
AAC(6′)-Ib_{11} **C**	*aac(6′)-Ib_{11}*	Integron	AY136758	*S. enterica*	(Casin et al., 2003)
AAC(6′)-29a	*aac(6′)-29a*	Integron	AF263519	*P. aeruginosa*	(Poirel et al., 2001)
AAC(6′)-29b	*aac(6′)-29b*	Integron	AF263520	*P. aeruginosa*	(Poirel et al., 2001)
AAC(6′)-31	*aac(6′)-31*	Integron	AM28348, AM283490	*Pseudomonas putida, A. baumannii, K. pneumoniae*	(Mendes et al., 2007)
AAC(6′)-32	*aac(6′)-32*	Plasmid, integron	EF614235	*P. aeruginosa*	(Gutierrez et al., 2007)
AAC(6′)-33	*aac(6′)-33*	Integron	GQ337064	*P. aeruginosa*	(Viedma et al., 2009)
AAC(6′)-I30	*aac(6′)-I30*	Integron	AY289608	*S. enterica*	(Mulvey et al., 2004)
AAC(6′)-Iid	*aac(6′)-Iid*	Chrosmome	AJ584700	*Enterococcus durans*	(Del Campo et al., 2005)
AAC(6′)-Iih	*aac(6′)-Iih*	Chromosome	AJ584701	*Enterococcus hirae*	(Del Campo et al., 2005)
AAC(6′)-Ib-Suzhou	*aac(6′)-Ib-Suzhou*		EF37562, EU085533	*E. cloacae, K. pneumoniae*	(Huang et al., 2008)
AAC(6′)-Ib-Hangzhou	*aac(6′)-Ib-Hangzhou*		FJ503047	*A. baumannii*	
AAC(6′)-SK	*aac(6′)-sk*	Chromosome	AB164230	*Streptomyces kanamyceticus*	(Matsuhashi et al., 1985)
AAC(6′)-IIa	*aac(6′)-IIa*	Plasmid, integron	M29695	*P. aeruginosa, S. enterica*	(Shaw et al., 1989)
AAC(6′)-IIb	*aac(6′)-Iib*	Integron	L06163	*P. fluorescens*	
AAC(6′)-IIc	*aac(6′)-IIc*	Plasmid, integron	NC_012555	*E. cloacae*	(Chen et al., 2009)
AAC(6′)-Ib-cr	*aac(6′)-Ib-cr*	Plasmid, transposon, integron	DQ303918	*Enterobacteriaceae*	(Robicsek et al., 2006)
AAC(6′)-Ie-APH(2″)-Ia	*aac(6′)-aph (2″)*	Plasmid, transposon	M18086, M13771	*S.aureus, E. faecalis, E. faecium, Staphylococcus. warneri*	(Rouch et al., 1987)
ANT(3″)-Ii-AAC(6′)-IId	*ant(3″)-Ii-aac(6′)-IId, ant(3″)-Ih-aac(6′)-IId*	Integron	AF453998	*S. marcescens*	(Centron and Roy, 2002)
AAC(6′)-30/AAC(6′)-Ib’	*aac(6′)-30/aac(6′)-Ib’*	Integron	AJ584652	*P. aeruginosa*	(Mendes et al., 2004)
AAC(3)-Ib/AAC(6′)-Ib”	*aac(3)-Ib/aac(6′)-Ib”*	Integron	AF355189	*P. aeruginosa*	(Dubois et al., 2002)

Only representative hosts, references and accession numbers are shown.

C, three dimensional structure has been resolved. AAC(3)-Ia pdb id: 1BO4 (Wolf et al., 1998). AAC(2′)-Ic pdb id: 1M44, 1M4D (in complex with CoA and tobramycin), 1M4G (in complex with CoA and ribostamycin), 1M4I (in complex with CoA and kanamycin A) (Vetting et al., 2002). AAC(6′)-Ib pdb id: 1V0C (in complex with kanamycin C and AcetylCoA), 2BUE (in complex with ribostamycin and

CoA), 2VQY (in complex with parmomycin and AcetylCoA (Vetting et al., 2008); 2PRB (in complex with CoA), 2QIR (in complex with CoA and kanamycin) (Maurice et al., 2008). AAC(6′)-Ib11 pdb id: 2PR8 (Maurice et al., 2008). AAC(6′)-Ii pdb id: 2A4N (in complex with CoA) (Burk et al., 2005), 1N71 (in complex with CoA) (Burk et al., 2003), 1B87 (in complex with AcetylCoA) (Wybenga-Groot et al., 1999). AAC(6′)-Iy pdb id: 2VBQ (in complex with bisubstrate analog CoA-S-monomethy-acetylneamine) (Magalhaes et al., 2008), 1S3Z (in complex with CoA and ribostamycin), 1S5K (in complex with CoA and N-terminal His(6)-tag, crystal form 1), 1S60 (in complex with CoA and N-terminal His(6)-tag, crystal form 2) (Vetting et al., 2004).

Table 2

Aminoglycoside *O*-nucleotydyltransferases

ANTs	gene names	Genetic location	Accession number	Host	References
ANT(6)-Ia	*ant(6)-Ia, ant6, aadE*	Plasmid, chromosome	NC_006663, NC_012924, GQ900487	*Staphylococcus epidermidis, E. faecium, Streptococcus suis, S. aureus*	(Gill et al., 2005; Holden et al., 2009)
	ant6	Plasmid	AB247327	*E. faecalis*	
	aadE	Chromosome	NC_013853	*Streptococcus mitis*	
	aadK	Chromosome	M26879	*B. subtilis, Bacillus* spp.	(Noguchi et al., 1993; Ohmiya et al., 1989)
	aadE	Plasmid	AJ489618	*C. jejuni*	
	aad(6)	Plasmid	NC_008445, AY712687	*E. faecalis, Streptococcus oralis*	(Cerda et al., 2007; Schwarz et al., 2001)
ANT(6)-Ib	*ant(6)-Ib*	Transferable pathogenicity island	FN594949, NZ_ABDU0 1000081	*C. fetus subsp. fetus, B. subtilis*	(Abril et al., 2010)
ANT(9)-Ia	*ant(9)-Ia, aad(9), spc*	Plasmid, transposon	X02588, GU235985	*S. aureus, Enterococcus* spp., *Sathylococcus sciuri*	(Murphy, 1985)
ANT(9)-Ib	*ant(9)-Ib, aad(9), spc*	Plasmid	M69221	*E. faecalis*	(LeBlanc et al., 1991)
ANT(4′)-Ia **C**	*ant(4′)-Ia, aadD2, aadD, ant(4′,4″)-I*	Plasmid	U35229, M19465	*S. epidermidis, S. aureus, Enterococcus* spp., *Bacillus* spp.	(McKenzie et al., 1986; Santanam and Kayser, 1978)
ANT(4′)-IIa	*ant(4′)-IIa*	Plasmid	M98270	*P. aeruginosa, Enterobacteriaceae*	(Jacoby et al., 1990)
ANT(4′)-IIb	*ant(4′)-IIb*	Transposon	AY114142	*P. aeruginosa*	(Sabtcheva et al., 2003)
ANT(2″)-Ia	*ant(2″)-Ia, aadB*	Plasmid, integron	X04555	*P. aeruginosa, K. pneumoniae , Morganella morganii, E. coli, S. typhimurium, C. freundii, A. baumannii*	(Cameron et al., 1986)
ANT(3″)-Ia	*ant(3″)-Ia, aadA, aadA1, aad(3″)(9)*	Plasmid, transposon, integron	X02340	*Enterobacteriaceae, A. baumannii, P. aeruginosa, Vibrio cholerae*	(Hollingshead and Vapnek, 1985; Tolmasky, 1990)
	aadA2	Plasmid, integron	NC_010870	*K. peneumoniae, Salmonella* spp., *Corynebacterium glutamicum, C. freundii, Aeromonas* spp.	(Chen et al., 2007)

ANTs	gene names	Genetic location	Accession number	Host	References
	aadA3	Plasmid, transposon, integron	AF047479	*E. coli*	(Parent and Roy, 1992)
	aadA4	Plasmid, chromosome	NC_002928, NC_010558	*Bordetella parapertussis, E. coli*	(Parkhill et al., 2003; Perichon et al., 2008)
	aadA5	Plasmid, transposon, integron	AF137361	*E. coli, K. pneumoniae, Kluyvera georgiana, P. aeruginosa, E. cloacae*	(Sandvang, 1999)
	aadA6	Integron	AM087411	*P. aeruginosa*	(Fiett et al., 2006)
	aadA7	Integron	AB114632	*V. fluvialis, P. aeruginosa, E. coli, V. cholerae, S. enterica*	(Ahmed et al., 2004)
	aadA8	Plasmid, integron	AY139603	*V. cholerae, K. pneumoniae, Bacillus endophyticus*	(Tennstedt et al., 2003)
	aadA9	Plasmid	NC_003227	*C. glutamicum*	(Tauch et al., 2002)
	aadA10	Plasmid, integron	AM087405	*P. aeruginosa, E. coli.*	(Fiett et al., 2006; Partridge et al., 2002)
	aadA11	Integron	AJ567827, AY758206	*E. coli, P. aeruginosa.*	(Llanes et al., 2006)
	aadA12	Integron	FJ381668	*E. coli, Yersinia enterocolitica, S. enterica*	(Ajiboye et al., 2009)
	aadA13	Plasmid, integron	NC_010643	*Pseudomonas rettgeri, P. aeruginosa, Y. enterocolitica, E. coli*	(Revilla et al., 2008)
	aadA14	Plasmid	AJ884726	*Pasteurella multocida*	(Kehrenberg et al., 2005)
	aadA15	Integron	DQ393783	*P. aeruginosa*	(Yan et al., 2006)
	aadA16	Plasmid, integron	EU675686	*E. coli, V. cholerae, K. pneumoniae*	(Wei et al., 2009)
	aadA17	Integron	FJ460181	*Aeromonas media*	
	aadA21	Integron	AY171244	*Salmonella* spp.	(Faldynova et al., 2003)
	aadA22	Plasmid, integron	AM261837	*S. enterica, E. coli*	(Herrero et al., 2008)
	aadA23	Integron	AJ809407	*S. enterica*	(Michael et al., 2005)
	aadA24	Integron	DQ677333	*Salmonella* spp.	(Egorova et al., 2007)
	aadA6/aadA10	Integron	AM087405	*P. aeruginosa*	(Fiett et al., 2006)

Only representative hosts, references and accession numbers are shown.

C, three dimensional structure has been resolved. ANT(4′)-Ia pdb id: 1KNY (Pedersen et al., 1995).

NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 3

Aminoglycoside *O*-phosphotransferases

APHs	gene names	Genetic location	Accesion number	Host	References
APH(4)-Ia	*aph(4)-Ia, hph*	Plasmid	V01499	*E. coli*	(Kaster et al., 1983)
APH(4)-Ib	*aph(4)-Ib, hyg*	Chromosome	X03615	*Streptomyces hygroscopicus*	(Zalacain et al., 1986)
APH(6)-Ia	*aph(6)-Ia, aphD, strA*	Chromosome	Y00459	*S. griseus*	(Distler et al., 1987)
APH(6)-Ib	*aph(6)-Ib, sph*	Chromosome	X05648	*S. glaucescens*	(Vogtli and Hutter, 1987)
APH(6)-Ic	*aph(6)-Ic, str*	Transposon	X01702	*S. enterica, P. aeruginosa, E. coli*	(Mazodier et al., 1985; Steiniger-White et al., 2004)
APH(6)-Id	*aph(6)-Id, strB, orfI*	Plasmid, integrative conjugative element, chromosomal genomic islands	M28829	*K. pneumoniae, Salmonella* spp., *E. coli, Shigella flexneri, Providencia alcalifaciens, Pseudomonas* spp., *V. cholerae, Edwardsiella tarda, Pasteurella multocida, Aeromonas bestiarum*	(Daly et al., 2005; Gordon et al., 2008; Meyer, 2009; Scholz et al., 1989)
APH(9)-Ia **C**	*aph(9)-Ia*	Chromosome	U94857, CR628337	*L. pneumophila*	(Suter et al., 1997)
APH(9)-Ib	*aph(9)-Ib, spcN*	Chromosome	U70376	*S. flavopersicus*	(Lyutzkanova et al., 1997)
APH(3′)-Ia	*aph(3′)-Ia, aphA-1*	Transposon	V00359	*E. coli, S. enterica*	(Oka et al., 1981)
APH(3′)-Ib	*aph(3′)-Ib, aphA-like*	Plasmid	M20305	*E. coli*	(Pansegrau et al., 1987)
APH(3′)-Ic	*aph(3′)-Ic, apha1-1AB, apha7*	Plasmid, transposon, genomic island	M37910	*K. pneumoniae, A. baumannii, S. marcescens, Corynebacterium* spp., *Photobacterium* spp., *Citrobacter* spp.	(Lee et al., 1990; Tauch et al., 2000)
APH(3′)-IIa **C**	*aph(3′)-Iia, aphA-2*	Transposon	V00618	*E. coli*	(Beck et al., 1982)
APH(3′)-IIb	*aph(3′)-IIb*	Chromosome	NC_002516	*P. aeruginosa*	(Stover et al., 2000)
APH(3′)-IIc	*aph(3′)-IIc*	Chromosome		*S. maltophilia*	(Okazaki and Avison, 2007)
APH(3′)-IIIa **C**	*aph(3′)-IIIa*	Plasmid	V01547	*S. aureus, Enterococcus* spp.	(Trieu-Cuot and Courvalin, 1983)
APH(3′)-IVa	*aph(3′)-Iva, aphA4*	Chromosome	X01986	*B. circulans*	(Herbert et al., 1983)
APH(3′)-Va	*aph(3′)-Va, aphA-5a*	Chromosome	K00432	*Streptomyces fradiae*	(Thompson and Gray, 1983)

APHs	gene names	Genetic location	Accesion number	Host	References
APH(3′)-Vb	*aph(3′)-Vb, aphA-5b, rph*	Chromosome	M22126	*Streptomyces ribosidificus*	(Hoshiko et al., 1988)
APH(3′)-Vc	*aph(3′)-Vc, aphA-5c*	Chromosome	S81599	*M. chalcea*	(Salauze et al., 1991)
APH(3′)-VIa	*aph(3′)-Via, aphA-6*	Plasmid	X07753	*A. baumannii*	(Martin et al., 1988)
APH(3′)-VIb	*aph(3′)-VIb*	Plasmid		*K. pneumoniae, S. marcescens*	(Gaynes et al., 1988)
APH(3′)-VIIa	*aph(3′)-VIIa, aphA-7*	Plasmid	M29953	*C. jejuni*	(Tenover et al., 1989)
APH(2″)-Ia	*aph(2″)-Ia, aph(2″)-bifunctional*	Plasmid	AP003367	*S. aureus, Clostridium difficile, Streptococcus mitis, E. faecium*	(Ferretti et al., 1986)
APH(2″)-IIa **C**	*aph(2″)-Iia, aph(2′)-Ib*	Chromosome	AF207840, AF337947	*E. faecium, E. coli*	(Kao et al., 2000)
APH(2″)-IIIa **C**	*aph(2″)-IIIa, aph(2′)-Ic*	Plasmid	U51479	*Enterococcus gallinarum*	(Chow et al., 1997)
APH(2″)-IVa **C**	*aph(2″)-Iva, aph(2″)-Id*	Chromosome	AF016483	*E. casseliflavus*	(Tsai et al., 1998)
APH(2″)-Ie	*aph(2″)-Ie*	Plasmid, transposon	AY939911	*E. faecium , E. casseliflavus*	(Chen et al., 2006)
APH(3″)-Ia	*aph(3″)-Ia, aphE, aphD2*	Chromosome	X53527	*S. griseus*	(Trower and Clark, 1990)
APH(3″)-Ib	*aph(3″)-Ib, strA, orfH*	Plasmid, transposon, integrative conjugative elements, chromosome	M28829	*Enterobacteriaceae, Pseudomonas* spp.	(Scholz et al., 1989)
APH(3″)-Ic	*aph(3″)-Ic*	Chromosome	DQ336355	*M. fortuitum*	(Ramon-Garcia et al., 2006)
APH(7″)-Ia	*aph(7″)-Ia, aph7*!	Chromosome		*S. hygroscopicus*	(Berthold et al., 2002)

Only representative hosts, references and accession numbers are shown.

C, three dimensional structure has been resolved. APH(9)-Ia pdb id: 3I0O (in complex with ADP and Spectinomcyin), 3I0Q (in complex with AMP), 3I1A (Fong et al., 2010). APH(3′)-IIa pdb id: 1ND4 (Nurizzo et al., 2003). APH(3′)-IIIa pdb id: 1J7I, 1J7L (in complex with ADP), 1J7U (in comlex with APPNP) (Burk et al., 2001), 1L8T (in complex with ADP and kanamycin A) (Fong and Berghuis, 2002) 3H8P (in complex with AMPPNP and butirosin A) (Fong and Berghuis, 2009), 2BKK (in complex with the inhibitor AR_3A) (Kohl et al., 2005). APH(2″)-IIa pdb id: 3HAV (in complex with ATP and streptomycin), 3HAM (in complex with gentamicin) (Young et al., 2009).