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Aminoglycosides versus bacteria – a description of the action, resistance mechanism, and nosocomial battleground

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Abstract

Since 1944, we have come a long way using aminoglycosides as antibiotics. Bacteria also have got them selected with hardier resistance mechanisms. Aminoglycosides are aminocyclitols that kill bacteria by inhibiting protein synthesis as they bind to the 16S rRNA and by disrupting the integrity of bacterial cell membrane. Aminoglycoside resistance mechanisms include: (a) the deactivation of aminoglycosides by *N*-acetylation, adenylylation or *O*-phosphorylation, (b) the reduction of the intracellular concentration of aminoglycosides by changes in outer membrane permeability, decreased inner membrane transport, active efflux, and drug trapping, (c) the alteration of the 30S ribosomal subunit target by mutation, and (d) methylation of the aminoglycoside binding site. There is an alarming increase in resistance outbreaks in hospital setting. Our review explores the molecular understanding of aminoglycoside action and resistance with an aim to minimize the spread of resistance.

Introduction

Prevalence of multidrug resistant strains of several groups of bacteria is becoming a major problem in the whole world both in community acquired infections [1, 2] and hospital settings [3]. Besides other classes of antibiotics, aminoglycoside is an important class of antibiotics used to treat serious infections [4].

These are aminocyclitols that target bacterial ribosomes thereby interfering with protein synthesis. Aminoglycosides are among the most commonly used broad-spectrum antibiotics in the anti-infective armamentarium [5]. They are multifunctional hydrophilic sugars that possess several amino and hydroxyl functionalities [5]. They are particularly active against aerobic, Gram-negative bacteria. Aminoglycoside resistance mechanisms [6] include: (a) the deactivation of aminoglycosides by *N*-acetylation, adenylylation or *O*-phosphorylation, (b) the reduction of the intracellular concentration of aminoglycosides by changes in outer membrane permeability [7], decreased inner membrane transport [8], active efflux [9, 10], and drug trapping [11, 12], (c) the alteration of the 30S ribosomal subunit target by mutation [13], and (d) methylation of the aminoglycoside binding site.

Methylation of A1408 in the N1 position confers resistance to kanamycin, tobramycin, sisomicin, and apramycin but not to gentamicin [14, 15], whereas methylation of G1405 in the N7 position confers resistance to the 4,6-disubstituted deoxystreptamines, including gentamicin [15, 16]. Aminoglycosides are highly potent, broad-spectrum

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antibiotics with many desirable properties for the treatment of life threatening infections [17]. Their history begins in 1944 with streptomycin and was thereafter marked by the successive introduction of a series of milestone compounds (kanamycin, gentamicin, and tobramycin), which definitively established the usefulness of this class of antibiotics for the treatment of Gram-negative bacillary infections. In the 1970s, the semi-synthetic aminoglycosides dibekacin, amikacin, and netilmicin demonstrated the possibility of obtaining compounds which were active against strains that had developed resistance mechanisms towards earlier aminoglycosides as well as displaying distinct toxicological profiles.

As the problem of global antibiotic resistance continues to worsen, aminoglycosides have assumed increasing importance in clinical practice. Their broad antimicrobial spectrum, rapid bactericidal action, and ability to act synergistically with other drugs have made them especially useful in the treatment of serious nosocomial infections. However, as with other drugs, their overuse and misuse lead to the development of resistance in important microbial pathogens.

Genes encoding aminoglycoside-modifying enzymes (AMEs) are often located on plasmids, which permit cell-to-cell dissemination of the aminoglycoside resistance trait. Furthermore, several of these genes are also included in transposons and integrons, which result in their rapid dissemination at molecular level [18]. The crystal structures of aminoglycoside phosphotransferase [APH (3′)], aminoglycoside acetyltransferase [AAC (6′)], and aminoglycoside nucleotidyltransferase [ANT (4′)] are well characterized [19–21]. There is a growing literature on the various aspects of aminoglycoside antibiotics.

Henceforth, we decided the present review covers recent literature dwelling upon the molecular understanding of aminoglycoside action and resistance. Finally there is an outline of strategies to control the spread of resistance.

Chemical structure

The aminoglycosides have a backbone structure consisting of an aminocyclitol ring saturated with amine and hydroxyl substitutions. In the majority of clinically useful aminoglycosides, this aminocyclitol moiety is streptamine or 2-deoxystreptamine (Figure 1). Streptomycin, possessing a streptidine molecule, is the only exception. The aminocyclitol nucleus is connected through glycosidic linkages to various amino sugars (aminoglycosides) [22]. The aminoglycosides can be conveniently divided into three structural types based on the position of their glycosidic linkages. These structural types include the 4,6-disubstituted 2-deoxystreptamines containing most of the clinically useful aminoglycosides such as gentamicin, tobramycin, amikacin, and netilmicin, the 4,5-disubstituted 2-deoxystreptamines (neomycin and paromomycin), and others (streptomycin and spectinomycin).

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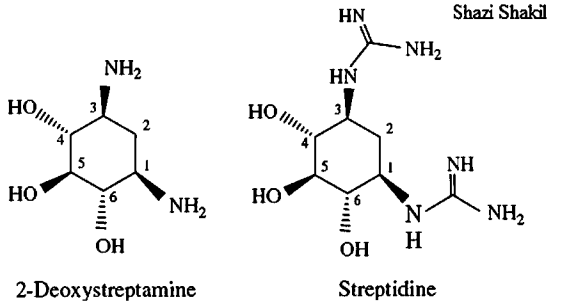

2-Deoxystreptamine Streptidine

Figure 1. Backbone structures of the aminoglycosides.

The aminoglycoside structure is important in understanding their chemical properties. These are basic, strongly polar compounds that are positively charged (cationic). They are highly soluble in water, relatively insoluble in lipids, and have enhanced antimicrobial activity in alkaline rather than acidic environments. As a result, aminoglycosides are minimally absorbed from the gut and penetrate the blood–brain barrier poorly. The cationic nature of the aminoglycosides contributes to their antimicrobial activity. Because of their positive charge, they are able to bind negatively charged lipopolysaccharide of the bacterial cell wall and a variety of intracellular and cell membrane anionic molecules such as DNA, RNA, and phospholipids. Unfortunately, their positive charge at physiological pH also contributes to their toxicities, e.g., nephrotoxicity, ototoxicity, and neuromuscular blockade.

Mechanism underlying antimicrobial action

Being polycationic species, they naturally show binding affinity for negatively charged residues in outer membrane of Gram-negative bacteria and in

the RNA. They kill bacteria by inhibiting protein synthesis as they bind to the 16S rRNA and by disrupting the integrity of bacterial cell membrane. It's worth mentioning that this very uptake process is *self-promoted.* The drug induces disruption of Mg^{2+} bridges between adjacent lipopolysaccharides.

Binding

First, aminoglycosides bind electrostatically to negatively charged residues in the outer membrane of Gram-negative bacteria in a passive, non-energy-dependent process [23]. After diffusing through outer membrane porin channels, they move into the periplasmic space. Now the transport across the cytoplasmic membrane requires metabolic energy from the electron transport system in an oxygen-dependent process. This very phase is known as energy-dependent phase-I (EDP-I). Hence aminoglycosides are less active in anaerobic environment. EDP-I is also inhibited by reduced pH, hyperosmolarity, and divalent cations. In the cytosol, aminoglycosides bind to the 30S subunit of ribosomes through an energy-dependent process (EDP-II) [24].

3D complex formation

It is well established that aminoglycosides bind to the 30S subunit of the ribosome, which does play an important role in the high-fidelity translation of the genetic material. Atomic structures for both the large and small subunits of the ribosome and high-resolution crystal structures of the 30S subunit with streptomycin, spectinomycin, paromomycin, and hygromycin B have been solved [25]. Along with NMR data for the ribosomal constituents [26], these processes definitely provide important information about the molecular mechanisms of interaction of aminoglycosides with the bacterial ribosome. The 16S rRNA from *E. coli* is a well-studied molecule as far as its interactions with various aminoglycoside antibiotics are concerned. Treatment of rRNA with an aminoglycoside protects several nucleic acid bases in rRNA from chemical modification, implying that these molecules possess high affinities for certain sites in rRNA. This mode of binding was likened to that of enzyme inhibitors, which usually bind to the active sites of enzymes and interfere with their activities [27].

Different classes of aminoglycoside antibiotics bind to different sites on the rRNA, depending on the structural complementarity between the two. For example, neomycin, paromomycin, gentamicin, and kanamycin are believed to bind to the A site on the 16S rRNA in *E. coli* in a similar fashion and were shown to protect bases A1408 and G1494 in chemical footprinting experiments [27]. Four bases, A1408, A1492, A1493, and G1494, in the rRNA A-site interact with tRNA, although with different affinities. Puglisi and coworkers [26, 28, 29] provided structural evidence on the mode of interactions of paromomycin, a representative aminoglycoside of the neomycin class, with a 27-nucleotide RNA template that was designed to mimic the A-site region of the 16S rRNA in *E. coli* (Figure 2a). The design of the RNA template was based on previous knowledge that paromomycin interacts with the C1407·G1494 base pair, A1408, A1493, and U1495 and that these bases are absolutely necessary for high-affinity binding [29]. Additional structural features, such as the pocket created by the asymmetry in the internal loop region due to the presence of A1492 and the base pairing of C1409·G1491 at the lower stem region, are also important. These structural characteristics collectively create a pocket that is optimal for the binding of paromomycin. One more structural study worth mentioning deals with the binding of tobramycin to an RNA aptamer [30]. The RNA aptamer that was used in this study was a 26-nucleotide stem-loop RNA (Figure 2b). There are four mismatched pairs, U7·G20, G8·U19, G9·A18, and U11·U16, in this RNA aptamer that are part of the zippered hairpin loop. Tobramycin binds in this groove partially encapsulated by the surface of the deep groove and the guanine base of the residue G15. In this complex, ring I of tobramycin sits on the floor of the deep groove. One of the amino groups on ring II of tobramycin interacts with the phosphate backbone in the deep groove, and the other amino group is exposed to the solvent. Ring III is positioned in the center of the deep groove, with hydroxyl groups directed towards the floor of the groove. The conformation of the RNA aptamer described above was suggested to be similar to those of the hairpin loops in tRNA and rRNA [31]. The A-site makes weak contacts with the mRNA and tRNA, implying that this region plays a role in recognition of appropriate tRNA via subtle changes in the

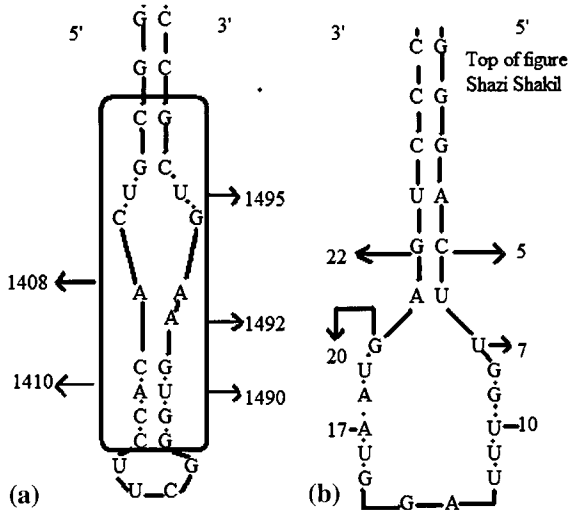

Figure 2. (a) Model of the A-site RNA template used to study the interactions of paromomycin. The boxed portion of the rRNA is the region homologous to the A-site. (b) RNA aptamer template used to study the interactions of tobramycin.

free energy. The binding of aminoglycoside near this site may affect the delicate process of interactions between codon and anticodon [32].

Although the overall structure of rRNA is conserved among all species in an evolutionary sense, there are differences that make binding of aminoglycosides more specific by at least a 10-fold higher affinity to the rRNA of prokaryotes than to that of eukaryotes [27, 33, 34]. This is not a large difference in binding affinity and may in part explain the toxic effects of these antibiotics in mammalian systems.

Its noteworthy that several aminoglycoside antibiotics such as neomycin B, tobramycin, and kanamycin A have been dimerized either symmetrically or asymmetrically by using a "tether," and their binding affinities were compared to those of the monomeric parent aminoglycosides [35]. It was suggested that, if there were multiple binding sites on the RNA, the dimerized aminoglycosides should bind with a higher affinity than the parent antibiotic, provided that multiple binding sites are accessible. It was indeed observed that the dimerized aminoglycosides bind to the Tetrahymena ribozyme 20- to 1200-fold better than the parent aminoglycosides. This effect seems to be synergistic with the entropic advantage gained by dimerization [36]. It also indicated that the presence of multiple high affinity binding sites for aminoglycoside antibiotics in an RNA molecule bulge in the RNA sequence is necessary to allow binding of aminoglycosides [37]. By using a specific stem-loop derivative of the RNA aptamer, a series of chemical interference, chemical modification, and mutation studies was performed to understand the structural requirements for binding of tobramycin to the RNA aptamer. This aminoglycoside appeared to interact mainly with the nucleic acid bases in the RNA aptamer but not with the phosphate backbone. The presence of a bulge, however, was proposed to be important for the high affinity binding of tobramycin in a stoichiometric ratio, and it was concluded that a bulge creates a cavity for interactions of the aminoglycoside and the nucleic acid base [38].

Resistance mechanisms

Broadly there are four mechanisms of bacterial resistance to aminoglycosides. Aminoglycoside resistance mechanisms [6] include: (a) the deactivation of aminoglycosides by *N*-acetylation, adenylylation or *O*-phosphorylation, (b) the reduction of the intracellular concentration of aminoglycosides by changes in outer membrane permeability [7], decreased inner membrane transport [8], active efflux [9, 10], and drug trapping [11, 12], (c) the alteration of the 30S ribosomal subunit target by mutation [13], and (d) methylation of the aminoglycoside binding site.

It is noteworthy that more than one mechanism may be at play at the same given time in a bacterium in the case of some classes of drug. First lets discuss about *enzymatic modification* as it is one of the most important mechanisms of aminoglycoside resistance [39–41], resulting in a loss of antibacterial activity due to a diminished affinity for the ribosomal A-site target [42]. The enzymes modifying aminoglycosides are *N*-acetyltransferases (AAC), which use acetyl-coenzyme A as donor and affect amino functions, and *O*-nucleotidyltransferases (ANT) and *O*-phosphotransferases (APH), which both use ATP as donor and affect hydroxyl functions. The functions affected in typical aminoglycosides (kanamycin and gentamicin derivatives) are on positions 3, 29, and 69 for AAC, positions 49 and 20 for ANT, and positions 39 and 20 for APH.

AMEs are often plasmid encoded but are also associated with transposable elements. Horizontal gene transfer of AMEs by conjugation [43–45] is a common phenomenon. The aminoglycoside resistance genes are derived from bacterial genes, which encode enzymes involved in normal cellular metabolism. The selective pressure of aminoglycoside usage causes mutations, which alter the expression of these enzymes, resulting in the ability to modify aminoglycosides. Bacteria can acquire foreign DNA by the mechanisms of transduction, transformation, and conjugation. Two types of genetic elements, self-transferable conjugative plasmids, and transposons facilitate this [46]. Plasmid exchange and dissemination of transposons facilitate the rapid acquisition of a drug resistance phenotype not only within a given species but also among a large variety of bacterial species. In epidemiological surveys, aminoglycoside resistance mechanisms have first been ascertained by examining the susceptibility of the isolates to a panel of clinically used and experimental aminoglycosides with specific susceptibilities to these enzymes (phenotypic characterization) [41]. Such studies quickly led to the recognition of a large diversity of phenotypes with almost every susceptible position in each drug being modified by several distinct enzymes. With the development of molecular biology techniques, a considerably larger number of genes have been characterized so that each phenotype has now been associated with the expression of several distinct proteins with the same aminoglycoside-modifying activity [41]. Large variations in substrate specificity may develop from a few and sometimes a single amino acid change in the protein [47, 48]. Moreover, several genes could derive from one or a few single common ancestors, suggesting a large plasticity in the type of activities a bacterium may express. It is therefore anticipated that bacteria will quickly catch up to or defeat our efforts at making a given aminoglycoside resistant to inactivation by a specific enzyme.

Bacterial *efflux pump* is an energy-dependent (ATP) pump and is now recognized as a major cause of antibiotic resistance. This is particularly true for the multidrug-resistant opportunist pathogens responsible for nosocomial infections. Bacterial species constitutively expressing such transporters are intrinsically resistant to low levels of various antibiotics. However, mutations in the regulatory genes of the pumps or induction of expression in the presence of substrate, can lead to the overexpression of the originally constitutive or pump genes [49, 50]. In the last several years, aminoglycosides were shown to be substrates for a number of multidrug efflux pumps, including members of the five superfamilies of bacterial transporters. The resistance nodulation cell division (RND) transporter superfamily plays an important role in Gram-negative bacteria. The transporters of the RND superfamily use the membrane proton-motive force as energy source. They are localized in the cytoplasmic membrane and in Gram-negative bacteria. They interact with a membrane fusion protein, located in the periplasmic space, and an outer membrane protein to form a continuous tripartite channel able to export substrates directly out of the cell [51, 52]. Several RND proteins were shown to be involved in intrinsic and/or acquired, proton motive force-dependent, aminoglycoside resistance in various Gram-negative pathogens, including *P. aeruginosa, Burkholderia pseudomallei, Acinetobacter baumannii*, and *E. coli* [53].

Another resistance mechanism is *16S rRNA methylation*. Many aminoglycoside producing organisms express rRNA methylases, which are capable of modifying the 16S rRNA molecule at specific drug binding positions [54]. A number of genes encoding such enzymes have been identified from several aminoglycoside producers. The corresponding rRNA methyltransferases form the aminoglycoside resistance family of methyltransferases.

Kanamycin A and B are obtained from *Streptomyces tenjimariensis* and *Streptomyces tenebrarius*, respectively. They catalyze the modification of A1408 at the N1 position and confer high-level resistance to kanamycin, tobramycin, sisomicin, and apramycin, but not gentamicin. Gentamicin A is obtained from the gentamicin producer *Micromonospora purpurea* and kasugamycin is obtained from *S. tenebrarius*. They catalyze the modification of G1405 at the N7 position and conferring high-level resistance only to the 4,6-disubstituted deoxystreptamines including gentamicin [55]. Methylation of these nucleotides presumably abolishes the intermolecular contacts that they make with the drug. The rmtA and rmtB genes were found in clinical isolates of *P. aeruginosa* and *Serratia marcescens*, respectively. These strains were found in Japan, where arbekacin has been used extensively since 1990. They show the high G+C content of the gene (55%). Another 16S rRNA methylase was characterized from *Klebsiella pneumoniae*. The structural gene, armA, was located on plasmid containing several other resistant genes including those conferring resistance to beta-lactams, trimethoprim, sulfonamides, and other aminoglycoside resistance determinants [56].

Epidemiology of resistance to aminoglycosides in the community and hospital settings

High-level aminoglycoside resistance in enterococci is mediated by AMEs, which eliminate the synergic bactericidal effect between cell wall active agents, such as beta-lactams or glycopeptides and virtually all commercially available aminoglycosides, including gentamicin, tobramycin, netilmicin, kanamycin, and amikacin. The most common AMEs in *Enterococcus* spp. are the AAC (6′)-APH (2″), which inactivates gentamicin, kanamycin, tobramycin, netilmicin, and amikacin; APH (3′), which inactivates kanamycin and amikacin; ANT (4′), which inactivates kanamycin, amikacin, and tobramycin; and ANT (6′), which inactivates streptomycin [57]. High-level gentamicin resistance (MIC ≥500 mg/l) is usually mediated by the aac (6′)-Ie-aph (2″)-Ia gene, which encodes the bifunctional enzyme AAC (6′)-APH (2″). In recent years, three new aminoglycoside resistance genes [aph (2″)-Ib, aph (2″)-Ic, and aph (2″)-Id] that also mediate resistance to gentamicin have been detected in enterococci [45]. Zarrilli and coworkers performed a study to investigate the genetic and molecular basis of high-level gentamicin and amikacin resistance in *Enterococcus* species isolated in a university hospital from 1987 to 2003 [45]. Enterococci isolates were typed by pulsed-field gel electrophoresis and for high-level aminoglycoside resistance gene content. Several studies demonstrate that high-level aminoglycoside resistance genes in enterococci are encoded on plasmid or chromosomally located conjugative elements, the most prevalent of which are the conjugative transposons, which mediate the horizontal transfer of resistance determinants [58]. The data reported by Zarrilli et al. [45] showed that high-level resistance to gentamicin, along with the aac (6′)-Ie-aph (2″)-Ia gene, was transferred at a frequency of about 10^{-5} to 10^{-8} per recipient cell by filter mating in 14 of 17 *E. faecalis* and 3 of 4 *E. faecium* different genotypes. However, other studies had shown that high-level gentamicin resistance can be transferred by filter mating at a frequency of about 10^{-2} to 10^{-5} per donor cell [59]. One possible explanation for this discrepancy might be the presence of pheromone responsive plasmids that would increase the frequency of conjugal transfer [59]. In fact, enterococci strains that transfer aminoglycoside resistances at high frequency harbored conjugative gentamicin resistance plasmids [59] while no conjugative plasmids had been detected in parental and transconjugant enterococci analyzed in the study of Zarrilli et al. or in that from others that transfer aminoglycoside resistance at low frequency [60]. However, frequencies of 10^{-5} to 10^{-7} have been reported for plasmids transferring high-level gentamicin resistance in *E. faecium* [58]. Also, the aac (6′)-Ie-aph (2″)-Ia gene, which confers high-level gentamicin resistance, has been identified in very large conjugative plasmids (> 147 kb) [58]. Therefore, the authors could not completely rule out the possibility that low-copy high-molecular weight conjugative plasmids might mediate high-level aminoglycoside resistance in enterococci analyzed in their study.

In conclusion, they had shown that both clonal expansion and the emergence of unique high-level aminoglycoside-resistant strains had contributed to the selection of high-level aminoglycoside resistance in enterococci isolated from the patient populations. The aac (6′)-Ie-aph-(2″)-Ia gene was identified in all high-level gentamicin and

amikacin-resistant *E. faecalis* and *E. faecium* strains and was transferred through *conjugation* by the majority of the strains. Based on these findings, they had postulated that high-level resistance to gentamicin and amikacin among enterococci isolated from patients in their geographical area might also depend on the spread of the aac (6′)-Ie-aph(2″)-Ia gene.

With reference to community setting, a study was performed earlier in Spain [61]. The study showed that despite 4 years of official banning of antibiotic growth promoters in animals, enterococci isolated from food handlers were more resistant than those from healthy volunteers. This suggested the permanence of resistant clones or transferable resistant elements in farms and a possible exchange between food products and humans, or eventually the long-term permanence of certain clones in the food handlers' intestinal tract.

Another noteworthy study performed by Donabedian et al. [62] showed a commonality of gentamicin-resistant determinants and gentamicin-resistant enterococcal isolates among humans, food, and food-producing animals over a broad geographical area. The aac (6′)-Ie-aph (2″)-Ia gene was the most common gene among the gentamicin-resistant isolates evaluated in their study. Since the use of gentamicin in food-producing animals would create selective pressure to increase the emergence and dissemination of gentamicin-resistant enterococci, there was a need to prevent the misuse and overuse of gentamicin in food-producing animals.

Therapeutic significance

Aminoglycosides have versatile clinical utility. Gentamicin, amikacin, and netilmicin are used in meningitis, pneumonia, and sepsis. Streptomycin has applications in tularemia, tuberculosis, and plague. It is an alternative choice for the treatment of brucellosis. Paromomycin is used against amoebic dysentery. Spectinomycin is given to patients of Gonorrhoea. Neomycin finds its applications in burns, wounds, ulcers, and dermatitis. Apart from acting against Gram-negative bacilli, aminoglycosides are also active against staphylococci and certain mycobacteria. These are effective even when the bacterial inoculum is large, and resistance rarely develops during the course of the treatment. These potent antimicrobials are used as prophylactic agents and in the treatment of a variety of clinical situations [63]. Gentamicin is the antibiotic used most often because of its low cost and reliable activity against Gram-negative aerobes [64]. However, local resistance patterns should influence the choice of therapy. In general, gentamicin, tobramycin, and amikacin are used in similar circumstances, often interchangeably. Amikacin is particularly effective when used against bacteria that are resistant to other aminoglycosides because its chemical structure makes it less susceptible to inactivating enzymes. Depending on local patterns of resistance, amikacin may be the preferred agent for serious nosocomial infections caused by Gram-negative bacilli. In clinical practice, they possess many desirable properties, the most important of which may be rapid bactericidal activity against a wide range of pathogens [65]. Streptomycin is the most active aminoglycoside against *Mycobacterium tuberculosis* including many multidrug resistant strains [66]. Merits of aminoglycosides include relatively low cost, rapid bactericidal action, chemical stability, no allergic reaction, broad-spectrum activity, and synergistic action with other antibiotics. Some demerits are nephrotoxicity, ototoxicity, inactivity against anaerobes narrow therapeutic index, and lack of oral absorption. Hence if used cautiously, aminoglycosides have more merits than demerits.

Implications for drug development

The rise of antibiotic resistance is a public health concern that has led to increased interest in studying the ways in which bacteria avoid the effects of antibiotics. Enzymatic inactivation by several families of enzymes has been observed to be the predominant mechanism of resistance to aminoglycoside antibiotics. Reports have become available on the 3D atomic structure of the AMEs, such as kanamycin phosphotransferase and kanamycin nucleotidyl transferase. Relatively little information is known about their exact biochemical mechanism from their 3D structures. Scope is still left to investigate the biochemical mechanisms of resistance and the substrate specificity and catalytic efficiency of these enzymes. The challenge is to determine the 3D structures of three classes of modifying enzymes by X-ray crystallography and to understand the molecular basis for aminoglycoside resistance modification from their 3D structures.

This information could lead to the development of effective and potent inhibitors that will reverse antibiotic resistance. Now time has come to rethink about counter-resistance. There are fewer arguments against the need for new aminoglycosides and strategies to design novel aminoglycoside-modifying enzyme inhibitors to avoid the emergence and dissemination of resistant bacteria. Current research holds out the promise that effective inhibitors of AMEs may eventually restore the usefulness of aminoglycoside antibiotics. With the synthesis of inactivating enzyme-resistant analogs and the introduction of newer, less toxic antimicrobial agents, aminoglycosides continue to serve a useful role in the treatment of serious enterococcal and Gram-negative bacterial infections.

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