

Aminoglycoside Resistance in Gram-negative Bacilli

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Aminoglycosides are one of the clinically relevant antibiotics. They kill bacteria by binding to bacterial 30S subunit of ribosome. Resistance to aminoglycosides occurs by three different mechanisms: 1. Production of an enzyme that modifies aminoglycosides, 2. Impaired entry of aminoglycoside into the cell by altering the OMP permeability, decreasing inner membrane transport, or active efflux, 3. The receptor protein on the 30S ribosomal subunit may be deleted or

altered as a result of a mutation. By far, enzymatic modification has been the most important mechanism. In this review, the mechanisms of action and resistance, and the prevalence of resistance due to acquisition of enzymes are briefly described. (**Korean J Clin Microbiol 2009;12:57-61**)

Key Words: Aminoglycoside resistance, Gram negative aerobic rods, 30S ribosome subunits

The aminoglycosides/aminocyclitol antibiotics are characterized by two or more amino sugars linked by a glycosidic bond to the aminocyclitol (hexose) ring, which is either streptidine (found in streptomycin) or 2-deoxystreptamine (found in other aminoglycosides) (Fig. 1)[1]. The latter is again classified as 4,5-disubstituted and 4,6-disubstituted. The examples of 4,5-disubstituted are neomycin and paromomycin. Most of other aminoglycosides are 4,6-disubstituted. These are basic, strongly polar compounds that are positively charged (cationic).

Because of their positive charge, they are able to bind negatively charged lipopolysaccharide of the bacterial cell wall and a variety of molecules such as DNA, RNA, and phospholipids.

The primary mechanism of action of aminoglycosides is a decrease in protein synthesis after the drug has bound to the bacterial 30S subunit of the ribosome. Their polycationic nature allows binding to the polyanionic 16S rRNA on the 30S ribosome at the A site (site of codon and anticodon recognition) for tRNA binding. The aminoglycoside-bound bacterial ribosomes then become unavailable for translation of mRNA during protein synthesis, thereby leading to cell death. To reach the intracellular ribosomal binding targets, the initial event is passive diffusion via porin channels across the outer membrane. Drug is then actively transported across the cell membrane into the cytoplasm

by an oxygen-dependent process. Transport may be enhanced by cell wall-active drugs such as penicillin or vancomycin: This enhancement maybe the basis of the synergism of these antibiotics with aminoglycosides[2].

Resistance to aminoglycosides occurs by three different mechanisms: 1. Production of an enzyme that modifies aminoglycosides (aminoglycoside modifying enzymes, AMEs) 2. Impaired entry of aminoglycoside into the cell by altering the OMP permeability, decreasing inner membrane transport, or active efflux 3. The receptor protein on the 30S ribosomal subunit may be deleted or altered as a result of a mutation. By far, enzymatic modification is (has been) the most important mechanism. These enzymes are assigned to three groups: (a) acetyltransferases (acetylation of an aminogroup-AAC), (b) phosphotransferases (phosphorylation of a hydroxyl group-APH) and (c) adenylation (adenylation of a hydroxyl group-AAD or ANT). These AMEs are often plasmid encoded but are also associated with transposable elements. Sites of AME modification of aminoglycosides are identified by a standard numbering system. The first sugar moiety at the 4 position has positions numbered with a single prime (1' to 6'); the second sugar moiety at the 6 position has positions numbered with a double prime (1'' to 6'') (Fig. 2)[1,3].

Aminoglycoside resistance mechanisms can be ascertained by examining the susceptibility of the strains to a panel of aminoglycosides (phenotypic characterization) (Table 1)[4]. However, in case of harboring more than two enzymes, the phenotype will not match any one of them.

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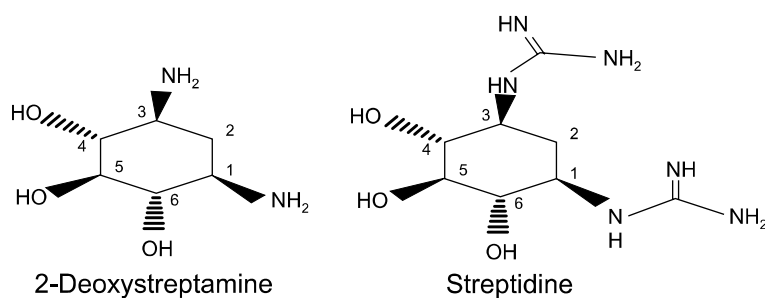


Fig. 1. Backbone structures of the aminoglycosides.

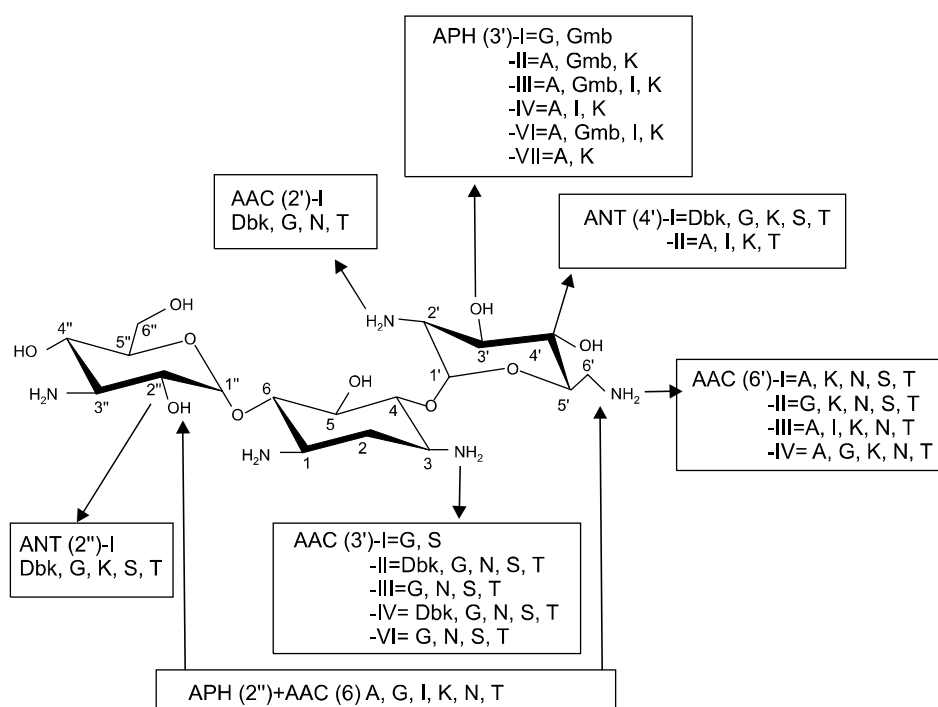


Fig. 2. Aminoglycoside-modifying enzymes and their substrates. A, amikacin; Dbk, dibekacin; G, gentamicin; Gmb, gentamicin B; I, isepamicin; K, kanamycin A; N, netilmicin; S, sisomicin; T, tobramycin.

This enzyme is important because it confers resistance to amikacin, netilmicin, kanamycin, and tobramycin and its gene is located within integrons and transposons[5,6].

In Korean isolates of AmpC-producing *Enterobacteriaceae*, the prevalence of *aac(6')-Ib* was 41.3%, 18.7%, and 6.6%, respectively in *Enterobacter cloacae*, *Citrobacter freundii* and *Serratia marcescens*[6]. Interestingly, of the *aac(6')-Ib* or *aac(6')-Ib-cr* harboring isolates, only about only 25~30% were nonsusceptible to amikacin[6,7] and this finding needs further investigation. Moreover, the fact that AAC(6')-I coexists very frequently with other antibiotic-inactivating enzymes such as β -lactamases, carbapenemases or other aminoglycosidases alarms us[7].

For *P. aeruginosa*, Miller et al.[8] brought together several studies carried out worldwide. Results showed that in Europe, between 1988 and 1993, *aac(6')-II* was the most prevalent (2.1

~70.3%, avr. 32.5%), followed by *ant(2'')-I* (1.7~45.2%, avr. 16.9%), and non-enzymatic resistance (4.3~23.7%, average of 14.0%). In a study conducted with *P. aeruginosa* isolates showing high-level resistance to gentamicin and netilmicin and strains presenting a low-level resistance to all aminoglycosides including apramycin, collected in 2000 from community and private healthcare centers[9], the predominance of the three enzymes (*aac(6')-Ib*, *ant(2'')-Ia*, *aac(3)-IIc*) was observed (36.5%, 21.2%, and 7.7%, respectively), and the non-enzymatic resistance was 34.6%. In addition, the presence of *aac(6')-Ib*, which acetylates kanamycin, tobramycin, netilmicin, amikacin (KTNA), but not gentamicin, was regularly associated with a decreased gentamicin susceptibility. And it was assigned to the amino acid sequence at position 119, a leucine being associated with amikacin resistance and a serine with gentamicin resistance.

In Korean nationwide study, of the 250 isolates of *P. aerugi-*

Table 1. Phenotypic characteristics of isolates producing aminoglycoside modifying enzymes (cited from EUCAST expert rules in antimicrobial susceptibility testing)

Phenotype	Enzyme	St	Sp	K	A	G	Nt	T	Nm
St	APH(3'')	R	S	S	S	S	S	S	S
St Sp	ANT(3'')(9)	R	R	S	S	S	S	S	S
G	AAC(3)-I	S	S	S	S	R	s/r	s/r	S
K	APH(3')-I	S	S	R	S	S	S	S	R
K A	APH(3')-VI	S	S	R	R	S	S	S	R
G T	AAC(3)-VI*	S	S	S	S	R	s/r	R	S
T K A	ANT(4')-II	S	S	R	R	S	S	R	S
G T Nt Nm	AAC(2')-I AAC(3)-IV	S	S	S	S	R	R	R	R
K G T	ANT(2')-I	S	S	R	S	R	S	r	S
K T A Nt	AAC(6')-I	S	S	R	r	S	R	R	S
K T G Nt	AAC(3)-II	S	S	R	S	R	R	R	S
K T G A Nt	Impermeability±different enzymes	R	R	R	R	R	R	R	R

*AAC(3)-IIa.

Abbreviations: S, streptomycin; Sp, spectinomycin; K, kanamycin; A, amikacin; G, gentamicin; Nt, netilmicin; T, tobramycin; Nm, neomycin; APH, aminoglycoside phosphotransferase; ANT, aminoglycoside nucleotidyltransferase; AAC, aminoglycoside acetyltransferase; R, resistance; r, reduced zones but likely to remain susceptible at BSAC breakpoints; S, susceptible.

nosa collected in 2002, the amikacin resistance rate was 22% (55 isolates), and of them, aph(3')-VI, ant(2'')-I, and aac(6')-I were found in 37, 32, and 18 isolates, respectively, alone or in combination[10]. Thirty-six strains harboured two or more types of enzymes, of which a combination of aac(6')-I and ant(2'')-I was the most frequent (24/36 isolates). The association with class 1 integron was confirmed with all of the ant(2'')-I, 6 of 18 aac(6')-I, but none of the aph(3')-VI. The difference in the enzyme distribution might be derived from the difference in selection of bacterial population and aminoglycoside usage.

More recently, 16S rRNA methylases (*RmtA*, *RmtB*, *RmtC*, *RmtD*, *ArmA* and *NpmA*) conferring resistance to almost all aminoglycosides, including arbekacin, has emerged and disseminated via mobile elements. Of these, *armA* gene has been found to be widely disseminated among various species[11], and was found on a large plasmid which carries a type 1 integron that mediates various gene cassettes responsible for multiple antimicrobial resistance[12]. In Korean isolates of *Enterobacteriaceae* collected between 1995 and 1998 and between 2001 and 2006 at a university hospital, *armA* was first appeared in 1997[13]. Among the *E. cloacae*, *C. freundii*, and *S. marcescens* collected in 2003, amikacin resistance rate was 9.5%, 10.3% and 17.1%, respectively. About 84.5% of the amikacin-resistant isolates showed high-level (MIC > 512 µg/mL), and almost all of them

harboured *armA* gene[14]. Among arbekacin or amikacin-non-susceptible *K. pneumoniae* and *A. baumannii*, 53.8% and 53.3% harbored *armA*, respectively, and most *armA*-harboring *A. baumannii* were nonsusceptible to carbapenem[15].

rmtA gene is likely associated with the mercury-resistant transposon Tn5041[16]; the *rmtB* gene was found in the flanking region of *Tn3*-like structure[17]. The *rmtC* was located downstream of an *ISEcpI*-like element containing *tnpA*[18]. *NpmA* is unique in that it gives resistance to apramycin and neomycin, due to methylation of the A1408 position at the A site of 16S rRNA; all other 16S rRNA methylases methylate the G1405 position[19].

Aminoglycosides is also the substrates for a number of multi-drug efflux pumps, including the resistance nodulation cell division (RND) transporter superfamily, which plays an important role in Gram-negative bacteria. Several RND proteins are involved in aminoglycoside resistance in various Gram-negative pathogens, including *P. aeruginosa*, *Burkholderia pseudomallei*, *Acinetobacter baumannii*, and *E. coli*[20].

Despite these various resistance mechanisms, aminoglycosides are still valuable weapons in antimicrobial treatment, particularly in the treatment of serious Gram-negative nosocomial infections[5]. To use the aminoglycosides properly, accurate detection of resistance and efforts to prevent the spread of resistant

isolates are required.

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=국문초록=

그람음성 세균에서의 Aminoglycoside 내성

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박연준

Aminoglycoside는 임상적으로 유용한 항균제 중 하나로, 세균의 리보솜의 30S subunit에 결합함으로써 살균 작용을 보인다. 이 약제에 대한 내성은 세 가지 기전에 의해 일어난다. 첫째는 aminoglycoside를 불활화시키는 효소의 생성이고, 둘째는 세균의 세포외막 투과성의 변화, 능동적 유출에 의해 약제가 세균 세포 내로 이동하지 못하게 되는 기전이며, 셋째는 리보솜 단백질의 변화이다. 현재까지는 효소에 의한 내성이 가장 흔하다. 본 종설에서는 aminoglycoside의 작용 및 내성 기전, aminoglycoside 불활화 효소에 의한 내성의 빈도 등에 대해 간략히 살펴보았다. [대한임상미생물학회지 2009;12: 57-61]

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