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# Exogenously acquired 16S rRNA methyltransferases found in aminoglycoside-resistant pathogenic Gram-negative bacteria: An update

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ABSTRACT

Exogenously acquired 16S rRNA methyltransferase (16S-RMTase) genes responsible for a very high level of resistance against various aminoglycosides have been widely distributed among *Enterobacteriaceae* and glucose-nonfermentative microbes recovered from human and animal. The 16S-RMTases are classified into two subgroups, N7-G1405 16S-RMTases and N1-A1408 16S-RMTases, based on the mode of modification of 16S rRNA. Both MTases add the methyl group of *S*-adenosyl-L-methionine (SAM) to the specific nucleotides at the A-site of 16S rRNA, which interferes with aminoglycoside binding to the target. The genetic determinants responsible for 16S-RMTase production are often mediated by mobile genetic elements like transposons and further embedded into transferable plasmids or chromosome. This genetic apparatus may thus contribute to the rapid worldwide dissemination of the resistance mechanism among pathogenic microbes. More worrisome is the fact that 16S-RMTase genes are frequently associated with other antimicrobial resistance mechanisms such as NDM-1 metallo-β-lactamase and CTX-M-type ESBLs, and some highly pathogenic microbes including *Salmonella* spp. have already acquired these genes. Thus far, 16S-RMTases have been reported from at least 30 countries or regions. The worldwide dissemination of 16S-RMTases is becoming a serious global concern and this implies the necessity to continue investigations on the trend of 16S-RMTases to restrict their further worldwide dissemination.



## 1. Outline of aminoglycosides and related issues

Aminoglycoside antibiotics such as amikacin and gentamicin, often along with β-lactams, have been used for the treatment of serious infections caused by Gram-negative and Gram-positive pathogenic bacteria in clinical settings. In livestock-farming settings, aminoglycosides play a crucial role not only in the treatment of bacterial infections, but also in growth promotion. Aminoglycosides are classified into several groups as 4,6-disubstituted 2-deoxystreptamine (DOS), 4,5-disubstituted DOS, and monosubstituted DOS, on the basis of the difference in their chemical structures (Fig. 1). Most aminoglycosides bind to the decoding aminoacyl-tRNA recognition site (A-site) of the 16S rRNA that composes bacterial 30S ribosome, and subsequently interfere with bacterial growth through blocking of protein synthesis (Fig. 2A and B) (Magnet and Blanchard, 2005). On the other hand, bacteria have been furnished with various resistance mechanisms to cope with aminoglycosides (Poole, 2005). Like other antimicrobial resistance mechanisms, the modes of aminoglycoside resistance in bacteria are divided into (i) enzymatic modification/inactivation of aminoglycosides, (ii) mutation or modification of aminoglycoside-binding site in target molecule, (iii) decreased permeability of aminoglycosides across bacterial membranes, and (iv) augmented efflux of aminoglycosides from cytosol to outside. Among them, the most prevalent and clinically relevant mechanism of aminoglycoside resistance in both Gram-negative and Gram-positive bacteria is inactivation of the agents by aminoglycoside-modifying enzyme (Ramirez and Tolmasky, 2010). Based on their molecular mechanisms, the aminoglycoside-modifying enzymes are further divided into 3 groups, acetyltransferases, nucleotidyltransferases, and phosphotransferases (Ramirez and Tolmasky, 2010). Pathogenic bacteria have acquired these aminoglycoside-modifying enzymes via transferable plasmids carrying bacteria-specific DNA recombination systems like transposons and integrons (Partridge et al., 2009; Tolmasky and Crosa, 1987).

*Actinomycetes* such as *Streptomyces* spp. and *Micromonospora* spp. are the natural producers of aminoglycosides. These aminoglycoside-producing *actinomycetes* are inherently resistant to aminoglycosides, because they harbor intrinsic 16S rRNA methyltransferase (16S-RMTase) genes, that can confer aminoglycoside resistance to bacteria by modifying specific nucleotide residues in the aminoglycoside binding site of 16S rRNA

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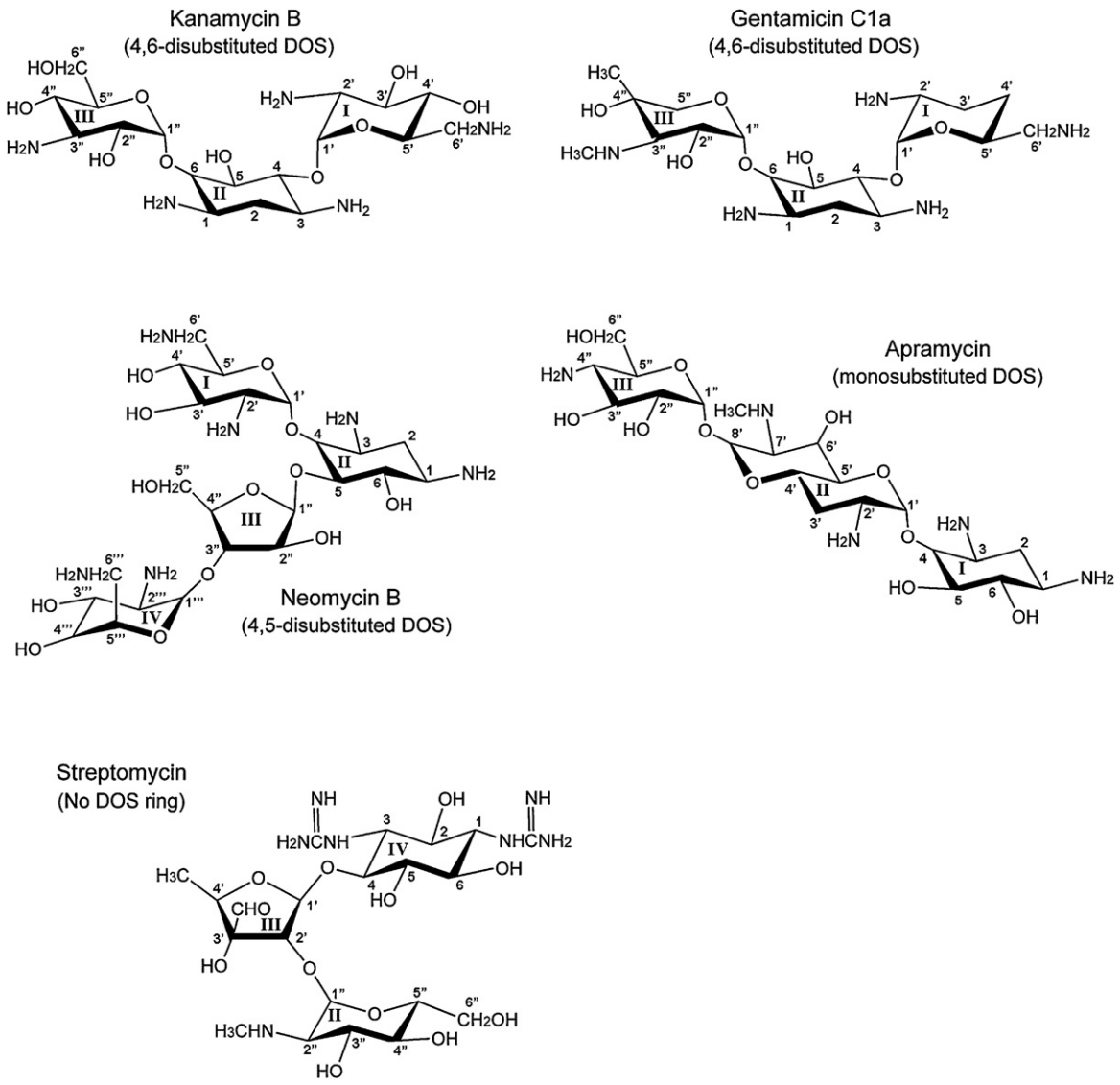


**Fig. 1.** Chemical structures of major aminoglycosides. Many clinically important aminoglycosides usually belong to the group of 4,6-disubstituted 2-deoxystreptamine (DOS). Among the 4.6-disubstituted DOS, kanamycin, amikacin, tobramycin, and arbekacin are classified into kanamycin group, and gentamicin, sisomicin, and isepamicin are the members of gentamicin group.

(Fig. 2B) (Cundliffe, 1989). Although the aminoglycoside resistance mechanisms through 16S rRNA protection are inherent in aminoglycoside-producing *actinomycetes*, this trait has not been found among the pathogenic bacterial species that cause infectious diseases in human or animal.

However, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* clinical isolates that show high-level resistance to clinically useful aminoglycosides through the production of acquired 16S-RMTases were identified in France and Japan, respectively, in 2003 (Galimand et al., 2003; Yokoyama et al., 2003). These 16S-RMTase genes were mostly located on transferable plasmids, and could be easily transferred to other bacterial species. After these reports, the number of 16S-RMTase-producing Gram-negative bacteria with virulence potential isolated from human and livestock have gradually increased (Doi and Arakawa, 2007). In recent years, the global spread of 16S-RMTase producers has been a concern in association with the rapid worldwide dissemination of the members of *Enterobacteriaceae* that produce NDM-1 metallo-β-lactamase (MBL), because these two enzymes are often coproduced (Mushtaq et al., 2011; Poirel et al., 2011b). Now, global dissemination of the pathogenic microorganisms exhibiting a multidrug-resistant nature by coproduction of NDM-1 MBL and 16S-RMTase, is becoming a serious threat to human health. This review describes the genetic and biochemical characteristics of the 16S-RMTases responsible for aminoglycoside resistance, with focus on those found in pathogenic Gram-negative bacteria, their epidemiology, as well as the practical screening method for early identification of 16S-RMTase producers.

## 2. Intrinsic 16S-RMTases of aminoglycoside-producing *actinomycetes*

### 2.1. Aminoglycoside-producing actinomycetes

In the 1980s, a number of aminoglycoside-producing *actinomycetes* like *Streptomyces* and *Micromonospora* species were found to harbor specific 16S-RMTase genes to protect themselves from their own intrinsic aminoglycosides (Fig. 3) (Cundliffe, 1989). Thereby, these aminoglycoside-producing *actinomycetes*