Screening of 16S rRNA Methylases (HLR to Aminoglycosides)

Unlike aminoglycoside-modifying enzymes that vary in their substrate profile, the acquired 16S rRNA methylases confer high level resistance (HLR) to almost all clinically important aminoglycosides.
They have been identified in several nosocomial pathogens, including P, aeruginosa, Serratia marcescens, E. coli, P. mirabilis, K. pneumoniae and Acinetobacter spp., Enterobacter cloacae, Citrobacter freundii (8,9,11).
These enzymes (RmtA, RmtB, RmtC, ArmA) are capable of conferring very high levels of resistance (MIC > 512 µg/ml) against amikacin, gentamicin, isepamicin, netilmicn and tobramycin, while apramycin, neomycin and streptomycin are not affected. The responsible genes armA, rmtA, rmtB, rmtC and rmtD are located in self-tramsmissible plasmids (7).

Screening method

A high-level amikacin resistance (MIC > 512 µg/ml) corresponding to no-zone of inhibition around Amikacin 40 µg Neo-Sensitabs may be used as a marker for screening the 16S rRNA methylase producing strains.
The diffusion test is performed on MH-agar using a 0.5 McF inoculum and incubation at 35-37 °C overnight.
Strains of Enterobacteriaceae and non-fermenters (*P. aeruginosa* and *Acinetobacter* spp.) showing no-zone of inhibition around Amikacin 40 µg Neo-Sensitabs should be suspected of possessing 16S rRNA methylases.

a) Enterobacteriaceae — NpmA-enzyme

16S rRNA methylase **positive** strains will show:

Amikacin:	No zone of inhibition	Resistant
Gentamicin:	No zone of inhibition	Resistant
Netilmicin:	No zone of inhibition	Resistant
Tobramycin:	No zone of inhibition	Resistant
Neomycin 120 µg:	Zone of inhibition ≤ 20 mm or no zone	Resistant
Apramycin100 µg:	Zone of inhibition ≥ 20 mm (6) (S)	Resistant

b) Non-fermenters

16S rRNA methylase **positive** strains will show:

Amikacin:	No zone of inhibition
Gentamicin:	No zone of inhibition
Netilmicin:	No zone of inhibition
Tobramycin:	No zone of inhibition
Neomycin 120 µg:	No zone or small zone
Streptomycin 100 µg:	Small zone in most cases

Galimand et al (5) found in 12 clinical isolates of Enterobacteriaceae the armA gene associated with ESBL beta-lactamase CTXM-3 (cefotaxime zone < ceftazidime zone) on a conjugative plasmid.

Bogaerts et al (9) investigated the presence of 16 SrRNA methylase mediated high level resistance to aminoglycosides in clinical isolates of Enterobacteriaceae from 2 University Hospitals in Belgium. They screened for HLR to gentamicin, tobramycin and amikacin resistance and deleted by PCR, armA genes in 18 *K. pneumoniae*, *E. coli*, *E. aerogenes*, *E. cloacae*, and *C. amalonaticus*, whereas rmtB was detected in a single *E. coli* isolate. These strains were susceptible to Apramycin and Neomycin Neo-Sensitabs (except 2 strains). All 16 SrRNA methylase positive strains produced ESBL's predominantly type CTX-M3 (13).

The concomitant presence of 16S rRNA methylase genes (armA or rmtB) and beta lactamase CTX-M among amikacin-resistant ESBL-producing K. pneumoniae isolates are widely spread in Taiwan (12).

The emergence of 16S rRNA methylases in Enterobactericaceae and non-fermenters (*P. aeruginosa*, *Acinetobacter* spp.) in strains that already are ESBL positive, may result in the spread of multidrug-resistant isolates producing both ESBLs and 16S rRNA methylases becoming an important clinical problem.

Wachino et al (10) describes a new plasmid-mediated 16S rRNA methyltransferase NpmA isolated from E.coli and providing total aminoglycoside resistance (including apramycin, neomycin and streptomycin).

References:
1) Galimand M et al: Plasmid-mediated high-level resistance to aminoglycosides in Enterobacteriaceae due to 16S rRNA methylation. Antimicr. Ag. Chemother., **47**, 2565-2571, 2003.
2) Yohei Doi et al: Plasmid-mediated 16S rRNA methylase in *Serratia marcescens* conferring HLR to aminoglycosides. Antimicr. Ag. Chemother., **48**, 491-6, 2004.
3) Jing-Jou Yan et al: Plasmid-mediated 16S rRNA methylases conferring HL aminoglycoside resistance in *E. coli* and *K. pneumoniae* isolates from 2 Taiwanese hospitals. J. Antimicr. Chemother., **54**, 1007-1012, 2004.
4) Kumkazu Yamane et al: Global spread of multiple aminoglycoside resistance genes. Emerg. Infect. Dis, **11**, 951-953, 2005.
5) Galimand M. et al: Worldwide disseminated armA aminoglycoside resistance methylase gene is borne by composite transposon Tn1548. Antimicr. Ag. Chemother., **49**, 2949-53, 2005.
6) González-Zorn B. et al: Genetic basis of dessemination of armA. J.Antimicr. Chemother., **56**, 583-5, 2005.
7) Wachino Jun-idri et al: Novel plasmid mediated 165 rRNA methylase RmtC found in a P. mirabilis isolate demonstrating extraordinary high-level resistance against various aminoglycosides. Antimicr. Ag. Chemother., **50**, 178-184, 2006.
8) Lee S. et al: Dissemination of 16S rRNA methylase in AmpC producing E. cloacae, C. freundii and S. marcescens in Korea. Clin Microbiol. Infect., **12**, Suppl. 4, P1274, 2006.
9) Bogaerts P. et al: Emergence of ArmA and RmtB aminoglycoside resistance rRNA methylases in Belgium., JAC, 59, 459-464, 2007**.**
10) Wackino Jun-idri et al: Antimicr. Ag. Chemother 51, 4401-09, 2007.
11) Fritsche T. R. et al: Detection of methyltransferases conferring high-level resistance to aminoglycosides in Enterobacteriaceae from Europe, North America and Latin America. Antimicr. Agents Chemother **52**, 1843-1845, 2008.
12) Ling Ma. et al:Widespread dissemination of aminoglycoside resistance genes armA and rmtB in K.pneumoniae isolates in Taiwan, producing CTX-M type ESBL-s. Antimicr. Ag. Chemother **53**, 104-111, 2009.
13) Kim S. et al: 16S rRNA methylase genes in amikacin resistant ESBL producing E.coli and K.pneumoniae isolates at University Hospital in Korea. Abstract, Presentation C2-701, 49th ICAAC, 2009.