

Prevalence of aminoglycoside resistance genes among contemporary Gram-negative resistant isolates collected worldwide

M. Castanheira¹, S.E. Costello¹, R.N. Jones¹, R.E. Mendes¹

¹JMI Laboratories, North Liberty, USA

Objectives: To evaluate the prevalence of aminoglycoside resistance genes, including aminoglycoside modifying enzymes (AMEs) and 16S rRNA methylases (rRNAm_{et}) among Gram-negative isolates displaying distinct R patterns (CLSI criteria) for amikacin (AMK) and/or tobramycin (TOB) and/or gentamicin (GEN).

Methods: 200 Gram-negative (49 *A. baumannii* [ACB], 52 *P. aeruginosa* [PSA] and 99 Enterobacteriaceae [12 species]) randomly selected based on the resistance patterns for aminoglycosides tested by broth microdilution method were evaluated. Isolates were screened by PCR for genes encoding AMEs *aac(3)-I(a-de)*, *aac(3)-IIa*, *aph(3)-Via* (*aphA6[a-j]*), *aac(6)-Ib*, *ant(2'')-Ia*, *ant(3'')-Ia* and *aac(6)-I1* in five reactions. Another 3 reactions targeting rRNAm_{et} *rmt(A-H)*, *armA* and *npmA* were performed for isolates resistant to all aminoglycosides. Amplicons were sequenced and compared to available sequences.

Results: A total of 163 isolates carried 1 or more AME-encoding genes, including 91 Enterobacteriaceae (91.9%), 39 ACB (79.6%) and 33 PSA (63.5%). Variants of *aac(6)-Ib* were the most prevalent (72 isolates; 36.0% overall), and included 29 occurrences *aac(6)-Ib-cr* also encoding quinolone-resistance and one isolate carrying both types. Variations in *aac(6)-Ib* were detected among 20 isolates and L119S (17 alone and with other substitutions in 2 isolates) was the most common. Isolates carrying these genes were 8 ACB, 9 PSA and the remaining 55 isolates among 9 Enterobacteriaceae species (20 *K. pneumoniae* [KPN] and 10 *E. coli*). Nineteen isolates carried *aac(3)-I* variants and 13 harboured *aac(3)-Ia* (11 ACB and 2 *Serratia* spp. mostly resistant to GEN ± AMK, but two resistant to all). Other *aac(3)-I* types were detected only in PSA (resistant to GEN or all). Variants of *aphA6* were observed among 35 isolates all resistant to AMK ± TOB and/or GEN. The most common variants were *aphA6c* (ACB only), *aphA6a* (5 ACB) *aphA6e* detected among 10, 6 and 6 isolates, respectively. Among other AME genes detected were *aacA8* (3 isolates), *aac(3)-Ild* (21), *aac(3)-Ile* (24), *aac(3)-Iva* (1), *ant(3'')-Ia* (51), *aac(6)-I1* (13, all PSA 12 PSA + 1 PVS displaying R to TOB, GEN+TOB or GEM+TOB+AMK). rRNAm_{et} were detected among 21 isolates among 42 isolates displaying R to all aminoglycosides: *armA* was observed among 14 isolates, *rmtB* among 5 Enterobacteriaceae isolates and *rmtF* was noted in two isolates (PSA and KPN). A total of 65 isolates carried 2 AME genes (6 + rRNAm_{et}), 19 carried 3 AME genes (3 + rRNAm_{et}) and 1 isolate carried 4 AME genes.

Conclusions: Aminoglycoside resistance genes are genetically and structurally divergent and were observed among most Enterobacteriaceae tested and various ACB and PSA isolates. These genes are usually carried by genetic elements harbouring other R markers that facilitate their selection and dissemination; however, a scarce number of studies that broadly evaluate these genes have been performed and many issues regarding nomenclature of these genes should be further addressed.