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Abstract (poster session)

Characterisation of two new variants of 16S rRNA methylase encoding genes, rmtB2 and rmtB3

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Objectives: To characterize two G1405 16S rRNA methylase encoding genes showing 3-4 amino acid changes compared to rmtB from Enterobacteriaceae isolates collected in 2005 and 2006. Methods: Genes encoding RmtB-like were sequenced on both strands. Clinical strains carrying these genes were analyzed. Primers comprising the open reading frame of the rmtB-like genes were used to amplify the entire gene and amplicons were cloned into PCRScript/XL1 Blue E. coli kanR. E. coli DH5alfa was used as a secondary host and transformation plated onto selective media containing 30 mg/L of chloramphenicol. Plasmid preparations of clinical strains were transformed into E. coli DH5alfa by electroporation and selected in media containing 4 mg/L of kanamycin. Susceptibility testing was performed according to CLSI reference broth microdilution methods using extended MIC dilution ranges for amikacin, tobramycin, gentamicin, arbekacin, apramycin, kanamycin, neomycin and streptomycin. Results: rmtB was sequenced in nine strains initially positive by PCR using primers targeting this gene. Six strains carried variants of the rmtB gene: rmtB2 showing three aminoacid changes A41T, I124V and I132V and rmtB3 showing one additional alteration at position 82 (A--V). rmtB2 was detected in 3 isolates from Mexico (2 E. cloacae strains; 2 hospitals) and 1 E. coli from Brazil. rmtB3 was detected among 3 strains from USA (Texas; E. coli) and Mexico (one E. cloacae and one K. pneumoniae). Susceptibility testing demonstrated that isolates carrying rmtB, rmtB2 and rmtB3 had elevated MIC values for amikacin (32-256 mg/L), tobramycin (16-64 mg/L), gentamicin (4-64 mg/L), arbekacin (16-64 mg/L) and kanamycin (64-256 mg/L) when compared to the E. coli host carrying PCRScript plasmid without insert. RmtB-variant produced MIC values for apramycin, neomycin and streptomycin modestly higher (4-8 mg/L) when compared to rmtB (0.25-2 mg/L) expressed in the same genetic background. Plasmids from three of the six clinical strains were transferred to E. coli and MICs were elevated for aminoglycosides (8-256-fold) that are susceptible to G1405 methylation. Conclusions: Two G1405 16S rRNA methylase genes similar to rmtB were detected among several Enterobacteriaceae isolates collected during 2005-2006 from different countries in Latin and North America, suggesting that these variants could be widespread in this geographic region.