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**Poster Session IV**

**Molecular epidemiology of MDR Enterobacteriaceae**

**PREVALENCE OF 16S rRNA METHYLASE GENES IN ENTEROBACTERIAL ISOLATES IN TWO GREEK HOSPITALS IN A THREE YEAR PERIOD**

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**Objectives:** The aim of this study was to investigate the prevalence of 16S rRNA methylase genes in consecutively collected Enterobacteriaceae in Athens, Greece. *RmtB* has been reported previously in 0.2% of *Klebsiella pneumoniae* and 0.4% of *Proteus mirabilis* strains isolated from November 2007 to October 2009 in 'Attikon' University Hospital.

**Methods:** Enterobacteriaceae isolates resistant to both amikacin and gentamicin (n=105) consecutively collected during a three year period (March 2010 - Feb 2013) in our Infectious Diseases Laboratory, as well as 70 MDR (including aminoglycosides) *K. pneumoniae* strains isolated in another tertiary hospital in Athens during 2011, 2012 and 2013, were tested further for MIC determination to amikacin, gentamicin, tobramycin, netilmicin, apramycin and neomycin with the broth dilution technique. Isolates with MICs  $\geq 512$  mg/L to at least the first four aminoglycosides were examined for the presence of 16S rRNA methylase genes (*armA*, *rmtB*, *rmtC*, *rmtA*, *rmtD* and *npmA*) by PCR. Molecular typing was performed by REP-PCR. Carbapenemase production was confirmed by PCR.

**Results:** Thirty one (4.7%) *K. pneumoniae*, fifteen (40.5%) *Providencia stuartii* and three (1.1%) *P. mirabilis* isolates among Enterobacteriaceae consecutively collected were positive for *rmtB* and highly resistant to all clinical used aminoglycosides tested (MICs  $\geq 512$  mg/L). All *rmtB*-bearing *K. pneumoniae* strains were KPC producers with 87.5% of them being also resistant to colistin. All but one *P. stuartii* and one of the three *P. mirabilis* isolates were VIM producers. Among the MDR *K. pneumoniae* isolates of the second hospital, twenty eight (40%) were positive for *rmtB*. Of those isolates twenty were KPC producers, five were VIM producers and three were no carbapenemase producers. The 48.3% of the *rmtB*-positive *K. pneumoniae* isolates belonged to the same clone while two other clones included 22.4% and 13.8% of the isolates, each. All VIM producing *rmtB* - *K. pneumoniae* isolates were clonally unrelated. Three strains belonged to a fourth clone (5.2%) similar to one isolated in our previous study in 2009. All but one *P. stuartii* isolates were clonally related while the three *P. mirabilis* strains although isolated at distant intervals were possibly related.

**Conclusions:** Our data demonstrate an increased prevalence of *rmtB*-positive KPC-producing *K. pneumoniae* and *rmtB*-positive *P. mirabilis* since 2009 as well as a high prevalence of *rmtB* in *P. stuartii*, which have been increasingly isolated in Greek hospitals as a result of the extensive use of antibiotics of last resort such as colistin and tigecycline. The spread of XDR isolates producing both carbapenemases and 16S rRNA methylases raises clinical concern and may become a major therapeutic threat in the future.