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Paper Poster Session
Emergence and worldwide outbreaks of carbapenemase-producing bacteria

First occurrence of Vim-1 producing Escherichia coli clinical isolates in a Greek tertiary hospital: inter-species or clonal spread?

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Background: In May 2015 two carbapenem-resistant *E. coli* strains, designated E1 and E2, were isolated in Evaggelismos hospital, Athens, Greece, from two different patients. In both cases co-infection with carbapenem-resistant strains was involved. E1 was recovered from a urine culture which also grew a *Ps. aeruginosa,* designated Ps1, whereas E2 was recovered from a wound infection along with a *Ps. aeruginosa,* designated Ps2 and a *Providencia stuartii,* designated Pr2. Both patients had a prolonged hospital course complicated with multiple episodes of infection, caused by multi-drug resistant *Klebsiella pneumoniae, Ps. aeruginosa* and *P. stuartii.* The aim of this study was to investigate the mechanisms of resistance, their mode of transmission and the clonal relatedness of the two *E. coli* isolates.

Material/methods: All isolates were speciated and MICs were determined by the VITEK2 (bioMerieux, France) automated system. MBL production was additionally screened by Etest MBL (BioMerieux, France). PCR and sequencing of the amplicons was used for the detection of carbapenemases, extended spectrum beta lactamases, plasmid-mediated 16S rRNA methylases, plasmid-mediated quinolone resistance genes, plasmid-mediated AmpC beta lactamases and class 1 integrons. Plasmid extraction was performed by using an alkaline lysis protocol. The potential for conjugational transfer of carbapenem resistance was examined in mating experiments using *Escherichia coli* K12 (lac^+ Rif^r) as the recipient strain. Pulse field gel electrophoresis (PFGE) using *Xba*I digested DNA in order to detect the clonality of the *E. coli* isolates was performed in a Biorad CHEF-DR III system.

Results: The MICs of imipenem (IMI) and meropenem (MER) of all isolates were ≥16 μg/ml and they were found to produce an MBL activity by the Etest. PCR and sequencing revealed the presence of bla_{VIM-1}, bla_{SHV-5}, bla_{TEM-1}, bla_{OXA-10} and bla_{VEB-1} beta lactamases and the rmtB gene in all isolates. All isolates harbored class 1 integrons of different size and content. PCR mapping and sequencing of the regions flanking *bla* genes on plasmid DNA, showed that the bla_{VIM-1} gene was carried on a class 1 integron lacking the 3'CS region along with three other resistance genes encoding cassettes. Conjugation experiments and plasmid analysis revealed the presence of a common, transferable, VIM-encoding plasmid in all isolates. The same plasmid has been isolated in VIM-1 producing *Kl. pneumoniae, Ps. aeruginosa* and *P. stuartii* isolates of our hospital in the past. Isolates E1 and E2 exhibited the same pattern on PFGE, indicating a single clonal origin.

Conclusions: This is the first case of carbapenem-resistant, VIM-1 producing *E. coli* isolates in our hospital. Long hospitalization and co-infection under the selective pressure from the use of broad-spectrum antibiotics, probably led to the horizontal, inter-species spread of resistance, although clonal dissemination of VIM-1 cannot be excluded due to the identical genetic profiles of the two *E. coli* isolates.