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Poster Session IV
Molecular detection of bacterial resistance

CARBAPENEMASE- PRODUCING MULTI-DRUG RESISTANT KLEBSIELLA PNEUMONIAE ISOLATES FROM BLOOD CULTURES AND PREVALENCE OF CLASS 1 INTEGRONS

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Objectives: Emergence and dissemination of *Klebsiella pneumoniae,* harboring carbapenemases in various geographic regions represents a significant threat to the management of nosocomial infections. The aim of this study was to examine the clonality of 20 carbapenem-resistant *Klebsiella pneumoniae* clinical isolates from blood cultures, to investigate the prevalence of VIM-1 and KPC-2 genes and to detect class 1 integrons in these isolates. The strains were isolated in our hospital, a tertiary hospital in Athens, Greece, over a two-month period between April to May 2013.

Methods: *Klebsiella pneumoniae* strains were isolated from blood (n=5), urine (n=7), tracheal secretions/sputum (n=7) and cerebrospinal fluid (n=1) clinical samples. Isolates were identified and MICs were determined using automated system Vitec2 (Biomerieux) according to CLSI guidelines. Screening for the presence of a carbapenemase was performed with the Modified Hodge Test. The phenotypic detection of KPC-possessing *K. pneumoniae* isolates was evaluated with boronic acid disks tests. MBL production was screened by E-test MBL.VIM-1 and KPC-2 genes were detected by PCR. The presence of class 1 integrons was verified by PCR with specific primers, designed on the basis of the 5' conserved segment (5'-CS) and the 3'-CS of class 1 integrons. PCR products were cloned and sequenced in order to characterize their content.The clonality of the isolates was examined by PFGE, using the restriction enzyme *XbaI*.

Results: All isolates had MICs ≥16 to meropenem (MER) and imipenem (IMI) and they were additionally resistant to many other antimicrobial agents. Seventeen out of twenty isolates were found positive for the VIM-1 gene, whereas thirteen out of twenty isolates carried the KPC-2 gene. Two isolates were negative for both genes. PFGE results indicated four distinct genotypes, designated A, B, C and D. Six different class 1 integrons of 3100bp, 1913bp, 1616bp, 1242bp, 812bp and 153bp, carrying gene cassettes conferring resistance to different classes of antimicrobials, were detected by PCR in eighteen isolates. Different integrons were detected in each of the four PFGE genotypes.

Conclusions: The fact that four distinct genotypes were involved in the nosocomial spread of the carbapenem resistance, indicates horizontal transfer of the genes involved. The presence of class 1 integrons isolates proves their importance for the dissemination of antibiotic resistance genes. The spread of carbapenemases in *K. pneumoniae* clinical isolates is becoming a clinical concern. Continuous surveillance and control measures are necessary in order to control the spread of these genes.