CARBAPENEMASE- PRODUCING MULTI-DRUG RESISTANT *Klebsiella pneumoniae* ISOLATES FROM BLOOD CULTURES AND PREVALENCE OF CLASS 1 INTEGRONS

E. Kraniotaki[1], E. Perivolioti[1], M. Nepka[1], Z. Psaroudaki[1], A. Argyropoulou[1], K. Fountoulis[1]

[1]Department of Clinical Microbiology, Evaggelismos Hospital, Athens, Greece

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INTRODUCTION AND PURPOSE

Emergence and dissemination of *Klebsiella pneumoniae,* harboring carbapenemases in various geographic regions represents a significant threat to the management of nosocomial infections (1).

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, in April 2013.

METHODS

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 phenotyping 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 (1, 2), using primer pairs:

VIM-F 5'-AGTGGTGAGTATCCGACAG-3' (forward)
VIM-R 5'-ATGAAAGTGCGTGGAGAC-3' (reverse) and
KPC-F 5'-ATGTCACTGTATCGCCGTCT-3' (forward)
KPC-R 5'-TTTTCAGAGCCTTACTGCCC-3' (reverse)

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' conserved segment (3'-CS) of class 1 integrons (3). 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. Eighteen out of twenty isolates were found positive for the VIM-1 gene, whereas seventeen out of twenty isolates carried the KPC-2 gene. PFGE results indicated four distinct genotypes, designated A, B, C and D (Figure 1).

Six different class 1 integrons of 3100bp, 1913bp, 1616bp, 1242bp, 812bp and 153bp, were detected by PCR in eighteen isolates (Figure 2). Different integrons were detected in each of the four PFGE genotypes (Table 1). Sequencing revealed the gene cassette arrays of the six class 1 integrons (Figure 3).

Figure 1. PFGE patterns of clinical isolates. Lanes 1, 2, 4, 13 and 14, genotype A; lanes 3, 5 and 6, genotype B; lanes 7, 8, 9, 11 and 12, genotype C; lane 10, genotype D. Lane M, lambda ladder PFGE marker (Biorad).

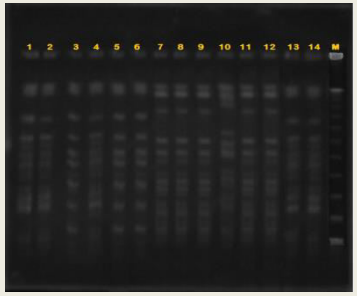


Figure 2. PCR for class 1 integrons. Lanes 2, 7 and 9, clinical isolates carrying a 1913bp integron; lane 4, isolate carrying a 1616bp integron; lane 5, isolate carrying a 1242bp integron; lanes 1 and 6, isolates carrying a 153bp integron; lane 10, isolate carrying a 3100bp, a 1616bp and a 812bp integron and lane 3, isolate carrying a 1913bp and a 153bp integron**.** Lane M, 1kb molecular size marker (Invitrogen).

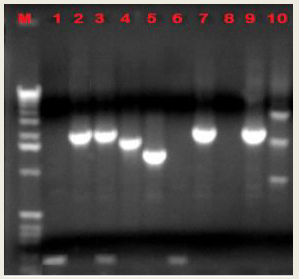
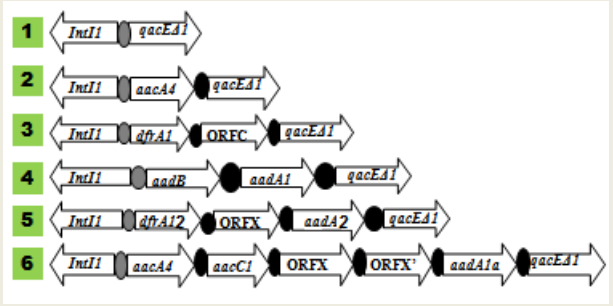


Table 1. Distribution of class 1 integrons in clinical isolates according to PFGE genotypes.

PFGE genotype	Class 1 Integrons
A	800bp/800bp+1616bp+3100bp/ No integrons
B	1242bp
C	1913bp/153bp/1913bp+153bp
D	1616bp

Figure 3. Physical maps of the class 1 integrons of 153bp (1), 812bp (2); 1242bp (3), 1616bp (4), 1913bp (5) and 3100bp (6). Coding sequences are indicated by arrows with the corresponding names; the *attI* and *attC* sites are indicated by grey and black filled circles respectively.



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.

REFERENCES

1. Bradford P, *et al.* Clin Infect Dis 2004; 39(1): 55-60. **2.** Tsakris A, *et al.* J Clin Microbiol 2000; 38(3):1290-2. **3.** Levesque C, *et al.* Antimicrob Agents Chemother 1995; 39(1):185-91.