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Detection of bla_{OXA-48} and clonal relationship in carbapenem resistant *K. pneumoniae* isolates at a tertiary care center in Western Turkey

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ABSTRACT

Background: Klebsiella pneumoniae is an important nosocomial pathogen that can lead to high morbidity and mortality. ESBL and carbapenamase producing strains may cause epidemic situations. The aim of our study was to investigate the molecular epidemiology and clonal relationship between carbapenem resistant *K. pneumoniae* strains isolated from our hospital during a three month period.
Methods: Fourteen carbapenem resistant *K. pneumoniae* strains isolated during April 1st–June 30th 2013 were included. The identification and the antibiotic susceptibility of the strains were studied by Vitek 2 Compact (Biomerieux, France) system. The carbapenemase production of the isolates were investigated by Modified Hodge assay. The bla_{OXA} of the strains was investigated by in house PCR. The clonal relationship between the isolates were studied by pulsed-field gel electrophoresis (PFGE) and automatized repetitive extragenic palindromic PCR (Rep-PCR, DiversiLab sistemi, Biomerieux, France) methods.
Results: All the *K. pneumoniae* isolates were carbapenem resistant; they were all susceptible to gentamycin and colistin. All of them had bla_{OXA-48}. The genotyping analysis revealed that eight isolates were in the same cluster both by Rep-PCR (similarity border ≥95%) and PFGE (Tennover criteriae) analysis. The other isolates did not belong to any other clusters. The strains that are in the same cluster are isolated from the Anesthesiology Intensive Care Unit during a three month period. The cluster ration by both methods was 57%.
Conclusions: All *K. pneumoniae* strains possessed bla_{OXA-48}. The clonal spreading was particularly detected in Anesthesiology Intensive Care Unit. Molecular epidemiological monitorization of nosocomial pathogens may prevent the spread of these multidrug resistant pathogens.

Introduction

Klebsiella pneumoniae is a nosocomial pathogen causing high morbidity and mortality which is frequently isolated from intensive care units with immunosuppressed patients. After the clinical use of extended cephalosporins beginning from the first half of 1980s; the issue of extended spectrum beta-lactamase producing *K. pneumoniae* strains have emerged [1].

Recently infections due to carbapenemase producing *K. pneumoniae* isolates have been isolated and the first strain was detected in Northern Carolina [2].

Carbapenem hydrolysing beta-lactamases belong to Ambler class A,B,D and have been reported worldwide [3]. Carbapenemases are a major trouble due to resistance mechanisms of a wide-range of antimicrobial agents, and infections with carbapenemase-producing *Enterobacteriaceae* are associated with high mortality rates [4–6].

The epidemiological studies on transmission of *K. pneumoniae* isolates leads to differentiation of a single isolate and epidemic strains. The pulsed field gel electrophoresis, plasmid detection, ribotyping and PCR based techniques have replaced serotyping, biotyping, antibiotyping, bacteriophage typing in evaluation of clonal relationship between bacterial isolates. The types of carbapenemases vary among countries, partially depending on the cultural/population exchange relationship between the European countries and the possible reservoirs of each carbapenemase. Carbapenemase producers are mainly identified among *K. pneumoniae*

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Table 1
The bla_{OXA-48} primer sequence and amplification protocol.

Gene	Primer sequence (5′ → 3′)	PCR product (bp)	PCR reactions
blaOXA48	TGTTTTTGGTGGCATCGAT GTAAMRATGCTTGGTTCGC	177	93 °C, 1 min; 55 °C, 1 min; 72 °C, 1 dk (35 cycles), 72 °C, 10 min

and *Escherichia coli*, and still mostly in hospital settings and rarely in the community [7]. The first bla_{OXA-48} producing *K. pneumoniae* strains were isolated in Turkey [8]. Then *Citrobacter* and *E.coli* strains isolated from Lebanon, Belgium, Argentina and US were reported. bla_{OXA-48} is transmitted by plasmids [9].

The aim of our study was to investigate the molecular epidemiology and clonal relationship between carbapenem resistant *K. pneumonaie* strains isolated between April 1st 2013 and June 30th 2013.

Methods

Medicalpark Izmir Hospital is a tertiary care center with three hundred bed. The hospital has 39 bed intensive care unit. The hospital has also 34 bed neonatal intensive care unit, organ transplantation unit and a bone marrow transplantation unit. Fourteen carbapenem resistant *K. pneumoniae* strains isolated from various departments (Anesthesiology Intensive Care Unit, Urology, and Hematology Unit) of Medicalpark Izmir Hospital during April 1st–June 30th 2013. Seven of the strains were isolated from tracheal aspirate specimen, four of blood culture, one from urine and one from sputum specimen. The age of the patients was between 25–86 (mean 55.5). Seven (50%) of the patients were male and seven were female (50%). Ten (71%) patients had central venous catheterization and four of them had no invasive instrumentation. Five (35.7%) of the patients had previous history of hospitalization in an another center. Ten (71%) patients had nasogastric nutrition. Twelve (85.7%) patients had underlying disorders. All the patients had been administered tigecycline, colistin and gentamycin. Four patients were admitted to inpatient services and then dismissed; four of the patients were dismissed after being monitorized at the intensive care unit. Two of them have died. The culture and antibiotic susceptibility were carried out at Medicalpark Izmir Hospital, Department of Medical Microbiology Laboratory and the molecular assays were done at Inonu University School of Medicine, Department of Medical Microbiology. The identification and the antibiotic susceptibility of the strains were studied by Vitek 2 Compact (Biomerieux, France). The carbapenemase production of the isolates were investigated by Modified Hodge assay. The bla_{OXA} of the strains was investigated by in house PCR and the primer design and amplification protocol were shown in Table 1.

The clonal relationship between the isolates were studied by pulsed-field gel electrophoresis (PFGE) and automatized repetitive extragenic palindromic PCR (Rep-PCR, DiversiLab sistemi, Biomerieux, France) methods. PFGE method was carried out by the protocol of Durmaz et al. [10]. The results were analysed by GelCompar II software system (version 6.0; Applied Maths, Sint-Martens-Latem, Belgium).

Results

All the *K.pneumonaie* isolates were carbapenem resistant; they were all susceptible to gentamycin and colistin. All of them had bla_{OXA-48}. The genotyping analysis revealed that eight isolates were in the same cluster both by Rep-PCR (similarity border ≥95%) and PFGE (Tennover criteriae) analysis. The other isolates did not belong to any other clusters. The strains that are in the same cluster are isolated from the Anesthesiology Intensive Care Unit during a three month period. The cluster ration by both methods was 57% (Fig. 1).

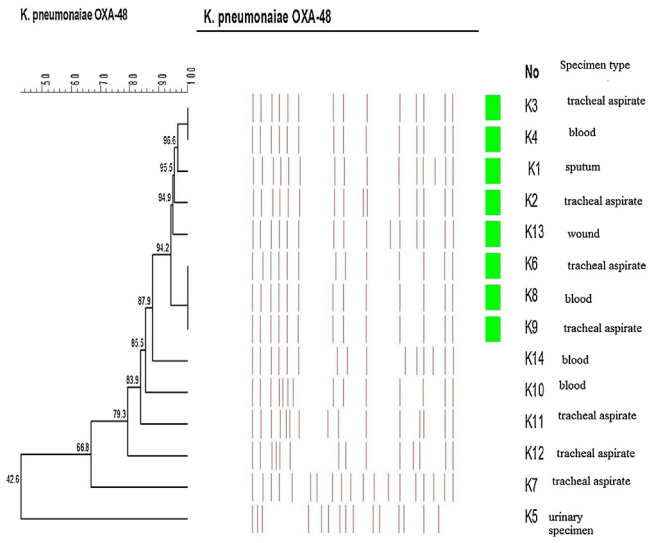

Fig. 1. Similarity analysis of band evaluations, Dice coefficient and UPGMA (unweighted pair wise grouping mathematical averaging) method (position tolerance 1%).

Discussion

Multidrug resistant pathogens have become a global problem recently [11]. *K. pneumoniae* is an important nosocomial pathogen that can cause high morbidity and mortality. After the ESBL producing *K. pneumoniae* strains; an emerging issue of carbapenem resistant *K. pneumoniae* isolates have come up and caused problems in treatment modalities [1,22,11]. Recently the epidemiological studies carried out on *K. pneumoniae* strains consist of plasmid analysis, ribotyping, polymerase chain reaction (PCR) based typing methods and "pulsed-field" gel electrophoresis (PFGE).

Nazik et al. reported various bla_{OXA-48} clones in carbapenem resistant *E. coli* and *Citrobacter koseri* isolates that are spreading carbapenem resistance in different regions of Istanbul in Turkey [12]. Another study reported a single clone spreading from a patient with burn and then that clone was detected in France as well as in Morocco. The authors have emphasized on the international transfer of patients [13]. In our study a single clone causing an epidemic at various departments of the same hospital was detected.

Çiftci et al. studied 23S rRNA, OXA 48 and KPC genes by three different primer sets in two carbapenem resistant *K. pneumoniae* isolates and they showed that both isolates had bla_{OXA-48} gene. KPC was not detected. The authors think that carbapenem resistance is limited among *K. pneumoniae* strains; but the issue of carbapenem resistant *K. pneumoniae* is a growing problem all around the world. Our data showed the monitorization of resistance level and the mechanisms of *K. pneumoniae* strains [14].

Azap et al. reported the molecular epidemiology of eight clones out of 16 carbapenem resistant *K. pneumoniae* strains. No dominant clone is detected and horizontal transmission was reported. The existence of ESBL and porin loss has led to more resistant isolates [15].

In our study all the carbapenem resistant *K. pneumoniae* strains possessed bla_{OXA-48}. Besides a clonal spread was detected in Anesthesiology Intensive Care Unit. Infection control committee meetings were held frequently. Infection control measures are strictly followed after this period. Hand washing and isolation precautions were taken particularly in the intensive care unit. Besides education on hand washing of the intensive care unit staff and high

level disinfection of the surfaces frequently were applied. Proper use of contact precautions were emphasized once again and monitored by the infection control nurses.

The Microbiology Laboratory also informed the units promptly about patients with isolated carbapenem resistant *K. pneumoniae* strains. Rectal cultures were also taken from patients with previous history of hospitalization and monitorized for possible colonization before admission. The rate of the isolated carbapenem resistant *K. pneumoniae* strains have declined.

The bla_{OXA-48} in *K. pneumoniae* was first detected in 2001 in our country. Then the reports followed thereafter. Molecular epidemiological monitorization of nosocomial pathogens may prevent the spread of these multidrug resistant pathogens and help the appropiate precautions being taken. We think that this data will help us to control multidrug resistant *K. peumoniae* strains at our recently opened center.

Acknowledgements

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