The investigation of oxacillinase/metallo-beta-lactamase genes and clonal analysis in carbapenem-resistant *Klebsiella pneumoniae*

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Infections due to carbapenem-resistant *Klebsiella pneumoniae* represent a growing problem nationally. In our study, we aimed to examine carbapenem-resistant *K. pneumoniae* with multiple resistance isolated in the intensive care unit of our hospital. Isolates were investigated for the presence of oxacillinase and metallo-beta-lactamase genes with a view to determining the clonal relationship between the strains intensely over a short period. Strain identification was completed with conventional methods and automated identification kit. OXA-58, OXA-23, OXA-51, OXA-24 and OXA-48 and metallo-beta-lactamase genes IPM, VIM, SPM, SIM, GIM and NDM-1 were investigated with PCR. For clonal relationships of carbapenem-resistant strains, the PFGE experiment was performed. While all of these carbapenem-resistant strains were positive for OXA-48, the resistant genes NDM-1, VIM, KPC, IPM, SPM, SIM, OXA-23, OXA-24, OXA-58 and OXA-51 were not observed. When molecular typing results were investigated, PFGE determined clonal distribution of three pulsotypes. However, it was observed that the strains intensified in a single clone and this was assessed as the outbreak isolate. The results of this study showed the primary enzyme responsible for carbapenem resistance in *K. pneumoniae* strains in our hospital is still OXA-48. To prevent the spread of carbapenem-resistant *K. pneumoniae* isolates, with epidemic potential, national-level monitoring and effective infection control precautions should be enforced.

**Keywords**: *Klebsiella pneumoniae*, carbapenem resistance, oxacillinase, metallo-beta lactamase, clonal analysis.

**INTRODUCTION**

*Klebsiella pneumoniae* is a significant nosocomial pathogen that may cause infections resulting in severe morbidity and mortality especially in intensive care units (ICU) [1]. The infection factor strains generally have multiple resistance and the resulting treatment difficulties are associated with high mortality rates. A variety of studies have reported mortality rates between 30-60% [2]. While treatment of nosocomial infections frequently is carried out with carbapenem group antibiotics, resistance rates have begun to rapidly increase parallel to the use of carbapenem. In fact, during treatment high levels of carbapenem-resistant strains are selected and increased. As a result, determining and monitoring the resistance development against carbapenems carries significant importance from a clinical point of view [3, 4].

The formation of carbapenem-resistance occurs with many different mechanisms. The main mechanism of resistance of *K. pneumoniae* strains to beta-lactam antibiotics including carbapenems is the production of coding beta-lactamase by chromosomes or plasmids. The presence of more than one resistance mechanism reduces the efficacy of antibiotics against the bacteria and
increases the MIC values, causing selection of resistant strains. Accompanying mutations that modify aminoglycosides and enzymes regulating the pump clearance systems, pore defects, AmpC, ESBL and quinolone-resistance are also important for the development of resistance [5]. There are three different classes of beta-lactamase that hydrolyze carbapenems including Ambler class A, class B (metallo-beta lactamas) and class D (oxacillinases) beta-lactamas. Carbapenem-resistance occurs as a result of plasmid-mediated Amp C (pAmpC) beta-lactamas in the bacteria together with reductions in outer membrane permeability linked to pore mutations [6]. Among these, the most commonly seen are KPC in A class, metallo enzyme NDM in B class and in our country undoubtedly OXA-48 from D group [7]. OXA-48 is a serine carbapenemase that can hydrolyze carbapenems commonly observed in hospital-sourced K. pneumoniae isolates. It is identified very frequently in K. pneumoniae strains in our country [8]. Firstly reported from Turkey, OXA-48 was then reported from Central Asia and Europe. As the isolates are generally multi-resistant, treatment choices are very limited [9]. In our study we aimed to examine carbapenem-resistant K. pneumoniae with multiple resistance isolated in the intensive care unit of our hospital intensely over a short period for the presence of oxacillinase and metallo-beta lactamase genes and to determine the clonal relationship between the strains.

# MATERIALS AND METHODS

**Bacterial strains** Our study included 20 carbapenem-resistant strains stored at -20 °C and identified as K. pneumoniae, isolated from clinical samples sent to the Ordu University Medical Faculty Education and Research Hospital Medical Microbiology Laboratory from the intensive care unit between October 2014 and December 2014. Only a single strain from each patient was studied. For control strain the standard Escherichia coli ATCC 25922 strain was used.

**Patients:** Demographic data of patients, length of ICU stay, mortality rates and accompanying diseases were documented. The risk factors for carbapenem-resistance including previous use of carbapenems, advanced age, long duration of stay in the intensive care unit, urinary catheterization, mechanic ventilation, urinary and central venous catheterization, presence of underlying diseases, tracheotomy, immune suppression and surgery before admission to ICU were investigated.

**Strain definition:** Before studying, stored strains were revived and purified by passage over sheep’s blood agar twice. For phenotypic species differentiation traditional methods and the fully automated identification kit VITEK-2 (bioMérieux, France) were used in accordance with the manufacturer’s instructions.

**Antibiotic susceptibility tests:** All K. pneumoniae strains were tested for susceptibility to imipenem, meropenem, amikacin, netilmicin, tetracycline, tigecycline, trimethoprim/sulfamethoxazole, gentamicin, levofloxacin, cefoperazone/sulbactam and colistin using the disk diffusion method and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Minimal inhibitory concentrations (MIC) of imipenem, meropenem, amikacin, netilmicin, tetracycline, tigecycline, trimethoprim/sulfamethoxazole, gentamicin, levofloxacin, cefoperazone/sulbactam and colistin for carbapenem-resistant strains were found using the E-test method (bioMérieux,
were all from the intensive care unit and were isolated from 9 urine, 7 tracheal aspirate and 4 blood samples. In carbapenem-resistant *K. pneumoniae* isolated patients, risk factors including previous use of carbapenem, advanced age, long duration of stay in the intensive care unit, urinary catheterization, mechanical ventilation, central venous catheterization and underlying diseases were noticed. All patients (100%) had urinary catheterization, 19 (95%) had mechanical ventilation and central venous catheterization. Eighteen patients (90%) had other accompanying chronic disease. Of these the most common were chronic obstructive pulmonary disease (70%), heart disease (60%) and diabetes mellitus (45%). When infection was identified, the mean hospital stay of patients was 47.73 days. The death rate of patients included in the study was 75% (15/20).

When antibiotic susceptibility of carbapenem-resistant *K. pneumoniae* strains was assessed, all isolates were susceptible to amikacin and colistin, while 3 strains were susceptible to trimethoprim/sulfamethoxazole. All strains were also resistant for all other antibiotics.

All of these carbapenem-resistant strains were positive for OXA-48, while none of the NDM-1, France) applied according to the manufacturer’s recommendations and interpreted according to the CLSI guidelines [10].

Research of carbapenem resistance mechanisms with molecular methods: The presence of oxacillinase genes OXA-58 group, OXA-23 group, OXA-51 group, OXA-24 group and OXA-48 group and metallo-beta lactamase genes IPM, VIM, GIM, SPM, SIM and NDM-1 causing carbapenem resistance were researched using the polymerase chain reaction (PCR) on the specific gene series (Table 1) of the gene region coding for these enzymes [11-13].

Research of clonal relationship: To research the clonal relationship of carbapenem-resistant strains the PFGE was performed. The *ObaI* enzyme was used for the DNA fragment at the end of lysis [14]. The bands for clonal relationship were assessed visually according to the Tenover criteria [15].

### RESULTS

The study included 20 carbapenem-resistant *K. pneumoniae* strains belonging to 10 male (50%) and 10 female patients (50%). The average age of patients was 77.1 years. The *K. pneumoniae* strains were all from the intensive care unit and were isolated from 9 urine, 7 tracheal aspirate and 4 blood samples. In carbapenem-resistant *K. pneumoniae* isolated patients, risk factors including previous use of carbapenem, advanced age, long duration of stay in the intensive care unit, urinary catheterization, mechanical ventilation, central venous catheterization and underlying diseases were noticed. All patients (100%) had urinary catheterization, 19 (95%) had mechanical ventilation and central venous catheterization. Eighteen patients (90%) had other accompanying chronic disease. Of these the most common were chronic obstructive pulmonary disease (70%), heart disease (60%) and diabetes mellitus (45%). When infection was identified, the mean hospital stay of patients was 47.73 days. The death rate of patients included in the study was 75% (15/20).

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**Figure 1** - Imaging of clonal distribution of strains with PFGE.
The investigation of oxacillinase/metallo-beta-lactamase genes and clonal analysis

VIM, KPC, IPM, SPM, GIM, SIM, OXA-23, OXA-24, OXA-58, and OXA-51 resistant genes were encountered. When results of molecular typing are examined, clonal distribution with PFGE determined 3 pulsotypes. However, strains were observed to intensify in a single clone and this was assessed as the outbreak isolate (Figure 1).

**DISCUSSION**

In spite of all infection control precautions taken to prevent infections developing due to microorganisms resistant to multiple medications and outbreaks formed by these microorganisms, carbapenem-resistant *K. pneumoniae* infections are reported with increasing frequency worldwide [16, 17]. Strains producing carbapenemase are multiply resistant as they generally carry other resistance mechanisms and form a serious source of annoyance due to high mortality rates in infections due to carbapenemase-producing strains [18]. As a result determining and monitoring the developing resistance to carbapenems is very important. Especially in the coming years, two large epidemics are expected due to carbapenem-producing microorganisms and one of these is predicted to be caused by hospital-sourced *K. pneumoniae* strains [19]. As a result early description of carbapenem-producing microorganisms is important to prevent hospital outbreaks.

In our country a multi-center MYSTIC study from 2000-2003 found that 99.3% of Gram negative bacteria were susceptible to meropenem, while 97.6% were susceptible to imipenem [20]. Since 2007 the multi-center HITIT study has reported that 3.2% of *K. pneumoniae* strains are imipenem resistant [21]. In SENTRY study conducted in 2007-2009, data collected from 42 centers in the USA found the carbapenem-resistance of *K. pneumoniae* isolates was 6.1% [22]. Over the years the increase in carbapenem resistance is striking.

Carrer et al. reported a hospital sourced outbreak caused by carbapenem-resistant *K. pneumoniae* producing OXA-48 at a university hospital in Istanbul [23]. Aktas et al. in 162 *K. pneumoniae* strains isolated in a six month period between 2004-2005, identified OXA-48 positivity in strains from two pediatric patients with long duration of hospital stay treated with meropenem [24]. In a study of strains isolated between 2004-2007 by Us et al. 26.9% of *K. pneumoniae* strains were found positive for OXA-48 [25]. Another study identifying carbapenemase species sourced in enterobacteria found that 86% of isolates had OXA-48, 10.5% had NDM-1, and 3.5% had VIM gene isolated. This study found no IMP or KMG genes [26]. Various studies in different countries have isolated OXA-48 producing *K. pneumoniae* strains [27-29]. In our study, in carbapenem-resistant *K. pneumoniae* strains isolated from our hospital, similar to previous studies, OXA-48 positivity was identified in all strains, while positivity for oxacillinase genes OXA-23, OXA-51 and OXA-24 and metallo-beta lactamase genes IPM, VIM, GIM, SPM, SIM and NDM-1 causing other carbapenem-resistance was not found. Additionally these strains were isolated within a short time and from the same intensive care unit, it was considered as an outbreak. On clonal analysis all strains were observed to intensify on a single clone and this was assessed as the outbreak isolate.

Among the most important risk factors for carbapenem-resistant *K. pneumoniae* infections are advanced age, long duration antibiotic use, long stay in hospital or intensive care, central venous catheterization, urinary catheterization, mechanical ventilation, underlying diseases, immune suppression, and previous surgical operations. In a study aimed to identify risk factors and mortality determinants in carbapenem-resistant *K. pneumoniae* infection/colonization Jiao et al. found that glycopeptides, use of cefoperazone/sulbactam and tracheostomy were risk factors for carbapenem-resistant *K. pneumoniae* infection/colonization [30]. They also determined that advanced age was a risk factor for carbapenem-resistant *K. pneumoniae* infection/colonization related to mortality. In a study investigating thirty carbapenem-resistant *K. pneumoniae* strains, all strains belonged to the same genotype producing KPC-2 resistant strains. In our study, our group had high risk due to advanced age, use of carbapenem, long stay in the intensive care unit, urinary catheterization, mechanical ventilation, central venous catheterization and underlying diseases. All strains were found to belong to the same genotype producing OXA-48. The death rate in this patient group with carbapenem-producing strains was identified as 75% (15/20). As a result, identification and moni-
toring of carbapenemases is important for treatment and prognosis. Infection control precautions for carbapenemase producing microorganisms are not fully clear. Some studies have identified rectal colonization and recommended rectal screening [31, 32]. Taking isolation precautions may be appropriate in this group of patients. Comprehensive infection control precautions may prevent the spread of these microorganisms [5, 33-35]. Similar efforts as those shown to control vancomycin-resistant enterococci may limit resistant Klebsiella species (25, 36). A study assessing molecular surveillance and clinical results of carbapenem-resistant E. coli and K. pneumoniae infections emphasized that active surveillance and effective infection control strategies should be applied to control the spread of carbapenem-resistant infections [37]. The results of this study showed the primary enzyme responsible for carbapenem-resistance in K. pneumoniae strains in our hospital is still OXA-48. To prevent the spread of carbapenem-resistant K. pneumoniae isolates, with epidemic potential, national-level monitoring and effective infection control precautions should be enforced.

Declaration of interest: No conflict of interests is declared.

REFERENCES

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