INVESTIGATION OF KPC GENES BY PCR AND EVALUATION OF CARBAPENEM INACTIVATION METHOD IN CARBAPENEM RESISTANT PSEUDOMONAS AERUGINOSA ISOLATES

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ABSTRACT

Introduction: *Pseudomonas aeruginosa is one of the important human pathogen and causes serious infections. Carbapenems are drug of choice in infections that caused by P. aeruginosa but carbapenem resistance is an emerging problem. In this study we aimed to to investigate the presence of KPC gene, one of the carbapenemase enzymes that cause carbapenem resistance, and carbapenem inactivation method (CIM) of study isolates, in P. aeruginosa isolates which were included in the study.*

Materials and Methods: *DNA extraction of carbapenem resistant isolates was performed by boiling method. After DNA extraction, optimization was performed using the original primers. After optimization, KPC gene was investigated by polymerase chain reaction (PCR) method. In addition, carbapenemase production of isolates was determined by the CIM a phenotypic test.*

Results: *The isolates were identified mostly from the tracheal aspirate cultures (34.5%). The greatest proportion of P. aeruginosa specimens were isolated from internal medicine (36%). KPC gene was not detected in none of the P. aeruginosa isolates by PCR method. According to the results of CIM, 22 were detected positive and 178 were negative.*

Conclusions: *KPC is one of the carbapenamases, but we did not detect in our study and it is not prevelant in P. aeruginosa isolates. It is needed to determine the presence and distribution of carbapenemase genes in healthcare facilities to determine effective infection control measures.*

Keywords: *Carbapenem Resistance, pseudomonas aeruginosa, KPC gene, CIM, class A.*

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Introduction

P. aeruginosa is a gram negative, non-fermentative, oxidase and catalase positive bacillus which causes nosocomial infections with high morbidity and mortality. It is an opportunistic pathogen that can cause diseases especially in immuncomprimised patients. P. aeruginosa is the second most common gram-negative bacterium with nosocomial infection and it is the most common cause of pneumonia in the intensive care unit (ICU)[1,2].

Acquired resistance rates developed by bacteria against antibiotics are increasing all over the world. P. aeruginosa, which is naturally resistant to many antibiotics, also has the ability to develop resistance that has been gained at high levels. For this reason, it has caused severe infections whose treatment is difficult and which are life-threatening[3]. P. aeruginosa strains, have developed multiple resistance against antibiotics, which have become a major problem throughout the world. The incidence of infections with the carbapenem group antibiotic resistant P. aeruginosa strains has increased significantly in recent years[4]. According to the studies, carbapenem resistance for P. aeruginosa can be caused by OprD loss, MexAB-OprM active outflow pumping system, permeability mutations and production of the excessive amounts of chromosomal AmpC and beta-lactamase[5-6]. While resistance rates and enzymes that can be responsible vary according to each country and center, rates are increasing over the years[7]. Very high carbapenem resistance is not reported in

our country when the data collected from abroad and Turkey is compared. Among the carbapenemases encountered in Pseudomonas types, there are serine β-lactamases such as KPC (Klebsiella pneumoniae carbapenemases) and GES in class A according to the molecular classification of Ambler, and OXA-198 in class D[8-9]. A large majority of carbapenemases identified to date in Pseudomonas species form metallo-β-lactamases (MBL) in class B such as VIM, IMP, SPM, GIM, AIM, DIM and NDM[10-11].

Various phenotypic methods are used in the detection of carbapenem resistance. One of these methods is the carbapenem inactivation method (CIM). The working principle of this test is the phenotypic demonstration of inactivation of the carbapenem disc, which is left to incubate with the strain whose carbapenem production will be checked, by enzyme of bacteria[12].

In our study, the presence of KPC gene, which is responsible for the development of resistance to carbapenem antibiotics in P. aeruginosa isolates and the efficacy of carbapenem inactivation method (CIM), a phenotypic test for study isolates, were investigated.

Material and methods

Carbapenem resistant P. aeruginosa isolates isolated from various clinical samples sent between April 2015 and May 2016 to the Medical Microbiology Laboratory of Ondokuz Mayıs University Medical Faculty Hospital were included in the study. Identification of isolates was done using conventional methods and Vitek-MS (Biomeirux, France) automated system. The antibiotic susceptibility was tested with the Vitek 2 Compact (Biomeirux, France) automatization system. P. aeruginosa isolates were stored at -20 °C until the molecular study. The existence of KPC gene was studied by polymerase chain reaction (PCR) using specific primers[13].

DNA extraction were made by the method of boiling. After DNA extraction, optimization was performed using specific KPC primer pairs (KPC-F TGTCACTGTATCGCCGTC, KPC-R CTCAGTGCTCTACAGAAAACC)[13].

KPC PCR reaction mixture (50 µl) was prepared by adding 5 μl 10X PCR tamp, 4 μl 25 mM MgCl2, 1 μl dNTP mix (10 mM), 1 μl taq DNA polymerase (5 U/μl), 1 μl KPC-F primer (10 pmol), 1 μl KPC-R primer (10 pmol), 4 μl template DNA, 33 μl purified water. KPC PCR amplification program was determined as 1 cycle of initial denaturation for 5 minutes at 95 °C, 35 cycles of target DNA denaturation for 1 minute at 95 °C, 35 cycles of primer bonding for 1 minute at 60 °C, 35 cycles of primer extension for 1 minute at 72 °C and 1 cycle of the last extension for 10 minutes at 72 °C.

Carbapenem inactivation method (CIM), which is a phenotypic test was applied to study isolates. In this test, the meropenem disc was discarded in sterile distilled water and bacterial suspension mixed in a microcentrifuge tube and left to incubation for two hours.

At the end of two hours, the disc in the microcentrifuge tube was removed and placed in a Mueller hinton agar (MHA) plate where the carbapenem sensitive 0.5 McFarland standard blur E. coli ATCC strain was spread.While it is normally expected that incubation zone is formed around the E. coli strain after 6 or 24 hours incubation, meropenem which is inactivated in the existence of carbapenemase in the tested bacterium can not create an inhibition zone in the E. coli strain. In which case, carbapenemase production is considered positive[14].

Results

In this study, it was determined that tracheal aspirate cultures have the most samples (34.5%) in carbapenem resistant 200 P. aeruginosa isolates according to the distribution of sample species (Table 1). It was stated that, the samples were sent most intensively (36%) from internal medicine service (Table 2). No KPC gene was detected in any of P. aeruginosa isolates (Figure 1). According to CIM result, 22 strains were stated as positive and 178 of them were stated as negative (Figure 2).

Clinic	Number	Percent (%)
Internal medicine	72	36
Neurology Service	21	10.5
Neurosurgery Service	20	10
General Surgery Service	14	7
Pediatric General Service	11	5.5
Intensive Care Service	10	5
Urology Service	8	4
Cardiovascular Surgery Service	7	3.5
Pediatric Emergency Service	6	3

Table 1: Distribution chart of the materials from P. aeruginosa isolated.
**Physical Medicine and Rehabilitation Service, Child Infection, Cardiology Service, Orthopedics and Traumatology Service.*

Antibiotics	R	S	I	Total
Amikacin	31 (15.57%)	161 (80.90%)	7 (3.51%)	199
Gentamicin	49 (24.62%)	150 (75.37%)	-	199
Imipenem	187 (93.5%)	8 (4%)	5 (2.5%)	200
Colistin	3 (1.50%)	196 (98.49%)	-	199
Levofloxacin	87 (44.16%)	86 (43.65%)	24 (12.18%)	197
Meropenem	106 (53%)	15 (7.5%)	79 (39.5%)	200
Piperacillin	123 (% 61.5)	40 (% 20)	37 (18.5)	200
Cefepime	83 (% 41.91)	74 (% 37.37)	41 (% 20.70)	198
Ceftazidime	79 (% 39.69)	82 (% 41.20)	38 (% 19.09)	199
Ciprofloxacin	67 (% 33.83)	115 (% 58.08)	16 (% 8.08)	198

Table 2: Antimicrobial susceptibility results of P. aeruginosa isolates.

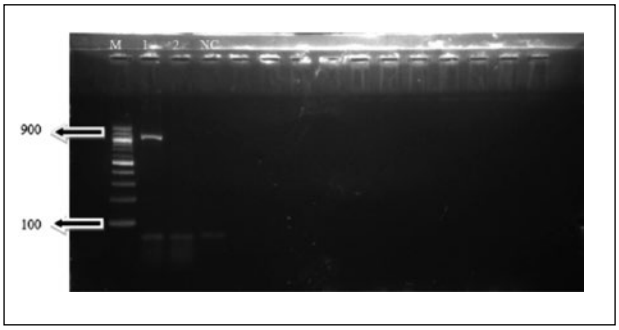

Figure 1: PCR gel image with KPC positive strain (M; marker, 1; KPC positive strain, 2; clinical strain, NC; negative control).

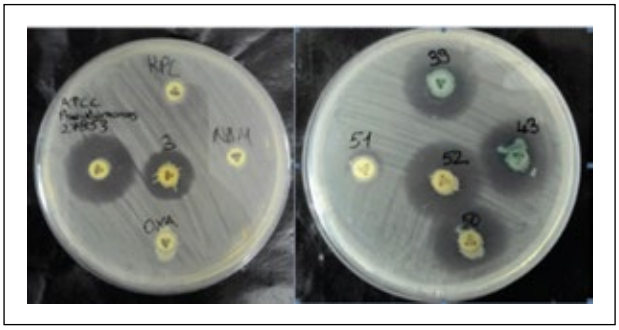

Figure 2: According to carbapenem inactivation method (CIM) evaluation, positive and negative images.

Discussion

P. aeruginosa is responsible for 10% -15% of nosocomial infections[15]. The resistance developed by microorganisms against antibiotics becomes a problem all around the world[16]. Although the rapidly increasing carbapenem resistance of P. aeruginosa strains in recent years is noteworthy, the most effective antibiotics used in the treatment of P. aeruginosa infections are carbapenems, which have the largest spectrum in the beta lactam group antibiotics[17].

In Colombia, except for the Enterobacteriacea family, according to the results of the first report of KPC gene, it has been reported that KPC gene was detected in 3 P. aeruginosa strains[8].

The appearance of KPC-2 gene in different species of Enterobacteriacea and the spread of this gene to P. aeruginosa strains in different countries emphasizes the potential for global expansion. This situation has been associated with Tn4401, a common transposon[18-19]. As a result of bacteraemia occurs after liver transplantation, KPC-2 gene was detected in isolated Enterobacter cloacae and Pseudomonas putida strains[20]. In their study, Akpaka et al. (2009) reported KPC gene in P. aeruginosa isolates in Trinidad and Tobago[21]. De Araujo Jacome et al. (2012) reported P. aeruginosa strains producing KPC-2 for the first time from two patients hospitalized in Brazilian intensive care unit[22]. Lari et al. (2014) first reported KPC gene in Iran as a result of PCR on 241 P. aeruginosa strains isolated from hospitalized burn injuries in a study they performed[23].

SPM-1 gene region was detected in 33 of carbapenem resistant 129 P. aeruginosa isolates isolated from the hospitalized patients between 1998 and 2012, in 4 of them VIM-2 gene region was detected and in 3 of them GES-3 gene region was detected. SPM-1 and KPC-2 association was found in 9 strains and also SPM-1, VIM-2 and KPC-2 association was found in 1 strain[24].

During 8 years of their study, Ramirez et al. (2013) compared the isolates of P. aeruginosa before and after epidemics caused by K. pneumoniae isolates producing KPC and found that KPC gene was not found on the isolates obtained before epidemics, but it was found on 33 of 76 isolates (43%) obtained after the epidemics[25].

Poirel et al. (2011) investigated 11 apart resistance genes (IMP, VIM, NDM, SPM, AIM, DIM, GIM, SIM, KPC, BIC, OXA-48) with multiplex method in their study[26]. They detected VIM-2 resistance gene in P. aeruginosa strains and the IMP-1 resistance gene in K. pneumoniae strains. However, they did not detect KPC gene in P. aeruginosa isolates. Robledo et al. (2010) could not identify KPC genes in P. aeruginosa isolates in a study of them[27]. Resistance genes (PER, GES, KPC, VIM, IMP, OXA) were investigated in 195 ceftazidime-resistant P. aeruginosa strains isolated from the hospitalized patients by real-time PCR method. However, KPC and PER genes were not detected[28].

A new phenotypic test called the carbapenem inactivation method (CIM) was developed to de-

tect carbapenemase activity in gram-negative bacilli within six hours. This method has been found to be highly compatible with the results obtained with PZR to detect genes encoding KPC, NDM, OXA-48, VIM, IMP and OXA-23 carbapenemases. Comparing the results of PZR and CIM determined by genes producing several carbapenemase in Enterobacteriaceae, P. aeruginosa and A. baumannii isolates; CIM has been shown to be a phenotypic screening method that can reliably detect carbapenemase activity. It was observed that, 67 isolates (16.3%) whose carbapenemase gene determined by PCR method of 411 isolates tested in the study were positive and according to CIM result, 65 (97.0%) of 67 isolates were positive. It was seen that KPC gene was not detected in the Pseudomonas isolates used in the study[12].

By the study performed between 2008 and 2014, in which Bayramoglu et al. (2016) obtain various clinical specimens and include Enterobacteriaceae isolates which are resistant for at least one carbapenems (ertapenem, imipenem or meropenem), all isolates included carbapenemase gene in CIM and PCR was found as positive, isolates that did not include carbapenemase gene was found as negative[29]. Sensitivity and specificity of the method were calculated as 100%.

The specificity and sensitivity of CIM in Enterobacteriaceae members was respectively determined as 100%, 100% by Van der Zwaluw et al. (2015) and 100% and 98.8% by Tijet et al. (2016) [12-30]. In these studies it was preferred to perform the evaluation of CIM after one night of incubation. However, in the study performed by Bayramoglu et al. (2016), it was determined that positive results were obtained even after evaluations were carried out after 6 hours of incubation[29].

Our study was not determined clearly because that we just considered the existence of KPC gene to check whether results of our study match up with the results of CIM.

Conclusion

Carbapenem resistance is rapidly increasing in P. aeruginosa isolates as well as in other gram negative bacterias. KPC gene, one of the genes causing this resistance, is not yet widely found in isolates of P. aeruginosa. KPC gene was not also detected in the isolates of our study. In the following periods, it will be helpful to perform studies with more isolates.

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