

RESEARCH ARTICLE

COMPARISON OF ANTIBIOTIC SUSCEPTIBILITIES OF CARBAPENEM RESISTANT AND CARBAPENEM SUSCEPTIBLE PSEUDOMONAS AERUGINOSA STRAINS AND INVESTIGATING SOME CARBAPENEMASE GENES IN CARBAPENEM RESISTANT STRAINS

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ABSTRACT: Aim: It was aimed to compare the antimicrobial susceptibility of carbapenem resistant (CRPA) and susceptible *P. aeruginosa* (CSPA) strains and to determine the presence of carbapenemase genes in CRPA strains. **Methods:** Fifty CRPA and 251 CSPA were included into the study. Antibiotic susceptibilities were determined using the automated system. The presence of carbapenemase genes (*bla*IMP, *bla*SPM, *bla*AIM, *bla*NDM, *bla*OXA-48, *bla*KPC) in CRPA strains were investigated by multiplex polymerase chain reaction method. **Results:** CRPA isolates were found to be more resistant to amikacin, aztreonam, gentamicin, netilmicin, tobramycin, ciprofloxacin, levofloxacin, cefepime, ceftazidime, piperacillin, piperacillin / tazobactam than CSPA. Amikacin, aztreonam, ceftazidime, ciprofloxacin, colistin, cefepime, gentamicin, levofloxacin, netilmicin, piperacillin, tobramycin, piperacillin/tazobactam MIC values of CRPA strains were found to be higher than MIC values of CSPA strains. The multidrug resistance (MDR) rate was 14,6% and higher in the CRPA group. In the CRPA strains, among *bla*IMP, *bla*VIM, *bla*SPM, *bla*NDM, *bla*KPC, *bla*AIM and *bla*OXA genes, *bla*IMP was found in one strain and *bla*VIM gene in three strains. **Conclusions:** The carbapenem resistance and MDR rate in our study, were found to be lesser than the rates in our country. It was found that CRPA were also more resistant to other antibiotics than CSPA. IMP and VIM type enzymes were found in our study. Together with other studies conducted in our hospital, this study showed that carbapenemases were not common in *P. Aeruginosa* strains isolated in our hospital. Identifying these enzymes epidemiologically is important in preventing the spread of resistance.

KEYWORD: *P. aeruginosa*, antibiotic, resistance, carbapenem, carbapenemase

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INTRODUCTION:

P. aeruginosa causes severe healthcare-associated infections in immunocompromised patients due to high drug resistance and is an opportunistic pathogen associated with ventilator-associated pneumonia [1,2,3]. In addition, due to the frequent occurrence of antibiotic resistance and high drug resistance, it causes difficulties in treatment.⁴

Carbapenems are effective in treating serious infections caused by *P. aeruginosa*. However, increased resistance to carbapenems has been reported worldwide [5,6]. Carbapenem resistant *P. aeruginosa* (CRPA) is the second critical priority bacteria according to the 2017 World Health Organization report [7].

Multidrug-resistant (MDR) *P. aeruginosa* is considered a serious threat according to Centers for Disease Control and Prevention (CDC) report as carbapenem resistance rate is 20% to 30% in this report [8,9].

The reduced membrane permeability, efflux pumps and carbapenemases are the main resistance mechanisms that develop against carbapenems [10]. Carbapenemases are grouped into 4 classes according to Ambler molecular classification. Class A, C and D carbapenemases have serine enzyme, while class B carbapenemases contain zinc enzyme, so it is also called metallo-beta-lactamase (MBL). Class A carbapenemases contain SME (Serratiamarcescens enzyme), NMC (non-metalloenzyme carbapenemase) and IMI (imipenem destructive, hydrolyzing beta-lactamase) enzymes encoded chromosomally. The main enzymes encoded by plasmids are *Klebsiella pneumoniae* carbapenemase (KPC) and Guiana extended spectrum (GES). Enzymes in class B carbapenemases (MBL) are IMP, SIM, SPM, VIM, GIM and NDM-1 (New Delhi MBL-1). Class D carbapenemases are known as OXA-type enzymes because they hydrolyze oxacillin. There are more than 100 enzymes in the OXA class. OXA-51 encoded by chromosomes, OXA-23, OXA-24/OXA40, OXA-48, OXA-58, which are encoded

by plasmids are the most recognized of this class [11]. Carbapenemases are classified as functional as well as molecular classification. Carbapenemases are classified into 2f, 2d and 3 in Karen Bush classification [12].

Comparison of antibiotic susceptibilities of CRPA and carbapenem susceptible *Pseudomonas aeruginosa* (CSPA) strains isolated from clinical samples in the Microbiology Laboratory of the Hatay Mustafa Kemal University (HMKU) Hospital and investigating some carbapenemase genes in CRPA Strains was aimed in this study.

METHODOLOGY:

This study was approved by Clinical Research Ethics Committee of HMKU and was supported by HMKU Scientific Research Projects Coordinator ship with the project number 19.YL.033.

Bacterial Strains, Identification and Antibiotic susceptibility testing:

The strains were isolated during the period November 2018-January 2020 from the clinical specimens sent to Microbiology Laboratory of HMKU Hospital. Fifty CRPA strains and 251 CSPA strains were included in the study. Identification of the species and antibiotic susceptibilities of these isolates were determined with the Vitek 2 compact system (bioMérieux, Marcy l'Etoile, France) and evaluated according to the European Committee for Antimicrobial Susceptibility Tests (EUCAST) [13]. The isolates which were resistant to at least three of five antibiotic groups (imipenem, cefepime or ceftazidime, piperacillin, aminoglycosides and ofloxacin) were identified as MDR [14].

In the study, among the isolates in the CR group, the colistin sensitivity of three colistin-resistant strains was predetermined using microdilution method, which is a standard method for colistin [13]. Since the strains in the carbapenem susceptible (CS) group were not stored, colistin sensitivity could not be measured by microdilution.

Investigation of Carbapenamase Genes by Multiplex PCR Method:

The DNA of bacteria was extracted with the alkaline lysis method as described by Poirel et al. [15]. The primers for investigating *blaIMP*, *blaVIM*, *blaSPM*, *blaNDM*, *blaKPC*, *blaAIM* and *blaOXA* genes and product lengths were shown in Table 1.

Table 1. The genes studied, and the primers used in this study [15].

Primer	Sequence (5'-3')	Genes	Product length (bp)
IMP-F IMP-R	GGAATAGAGTGGCTTAAAYTCTC GGTTTAAAYAAAACAACCACC	<i>blaIMP</i>	232
VIM-F VIM-R	GATGGTGTTTGGTCGCATA CGAATGCAGCAGACCAG	<i>blaVIM</i>	390
SPM-F SPM-R	AAAATCTGGGTACGCAAACG ACATTATCCGCTGGAACAGG	<i>blaSPM</i>	271
AIM-F AIM-R	CTGAAGGTGTACGGAAACAC GTTCCGCCACCTCGAATTG	<i>blaAIM</i>	322
NDM-F NDM-R	GGTTTGGCGATCTGGTTTTC CGGAATGGCTCATCACGATC	<i>blaNDM</i>	621
OXA-48 OXA-48	GCGTGGTTAAGGATGAACAC CATCAAGTTCAACCCAACCG	<i>blaOXA-48</i>	438
KPC-F KPC-R	CGTCTAGTCTGCTGTCTTG CTTGTCATCCTTGTTAGGCG	<i>blaKPC</i>	798

Multiplex PCR method was performed as previously described by Poirel et al. [15]. Three multiplex reactions were performed; first for detecting *blaIMP*, *blaVIM*, and *blaSPM*, second for detecting *blaNDM* and *blaKPC* and third for detecting *blaAIM* and *blaOXA*.

Statistical Analysis:

Statistical Package for Social Sciences version 23.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Mann-Whitney U test for continuous variables, and chi-square test for nominal variables were used to compare the categorical variables. Statistically significant P-value was considered as 0.05.

RESULTS:

Bacterial Strains, Identification and Antibiotic susceptibility testing:

A total of 301 *P. aeruginosa* strains (50 CR and 251 CS) were included in the study. It was found that 29% of the clinical samples from which *P. aeruginosa* strains were isolated, were sent from intensive care units and 71% of them were from other clinics. Carbapenem resistance rate was found 33.3% in *P. aeruginosa* strains isolated from intensive care units, and 9.8% in *P. aeruginosa* strains isolated from other clinics (P<0,001) (Table 2).

Table 2. Carbapenem susceptibility states of *P. aeruginosa* strains isolated from intensive care units and other clinics

Clinic	Carbapenem Resistant	Carbapenem Susceptible	Total (%)	P
	N (%)	N (%)		
Intensive Care Unit	29 (33,3)	58 (66,7)	7 (28,9)	<0,001
Other Clinics	21 (9,8)	193 (90,2)	14 (71,1)	
Total	50 (16,6)	251 (83,4)	301(100)	

Specimens from which *P. aeruginosa* isolates were isolated, were wound (32.6%), urine (30.6%), sputum (16.9%), tracheal aspirate (10.6%), blood (7.6%), cerebrospinal fluid (CSF) (0.7%), catheter (0.3%), pleural fluid (0.3%) and bronchial lavage (0.3%). No significant relationship was found between carbapenem resistance and the type of sample from which they were isolated (P=0,670).

The antibiotics, to which CSPA strains were most susceptible, were colistin (93.2%), amikacin (86.9%), piperacillin/tazobactam (78.1%), piperacillin (77.7%) and ciprofloxacin (77.7%). The antibiotics to which the strains were most resistant were netilmicin (36.3%), levofloxacin (29.1%), ciprofloxacin (22.3%), aztreonam (17.5%) and ceftazidime (15.5%). The antibiotics to which CRPA strains were most susceptible, were colistin (9.4%), tobramycin (5.6%), gentamicin (5.4%), amikacin (5.4%), netilmicin (3.2%) and ciprofloxacin (3.2%). MIC values of colistin for three strains, which were in the CR group and were resistant to colistin with an automated system, were predetermined by microdilution method. MIC values of colistin were found 4 µg/ml for two strains and 256 µg/ml for one strain, thus all three were found to be resistant to colistin.

The susceptibility of CS and resistant strains to other antibiotics was shown in Table 3. CR strains were found to be more resistant to amikacin, aztreonam, gentamicin, tobramycin, netilmicin, ciprofloxacin, levofloxacin, ceftazidime, cefepime, piperacillin, piperacillin/tazobactam than CS ones (P<0.001). No difference in colistin resistance was found in CS and CR strains (P=1) (Table 3).

Table 3. The susceptibility of carbapenem susceptible and resistant strains to other antibiotics

Antibiotics	Carbapenem Resistant N (%)	Carbapenem Susceptible N (%)	Total N (%)	P
Amikacin	20 (40)	18 (7,2)	38 (12,6)	<0,001
Aztreonam	37 (74)	44 (17,5)	81 (26,9)	<0,001
Gentamicin	23 (46)	17 (6,8)	40 (13,3)	<0,001
Tobramycin	22 (44)	17 (6,8)	39 (12,9)	<0,001
Netilmicin	34 (68)	91 (36,3)	125 (41,5)	<0,001
Ciprofloxacin	34 (68)	56 (22,3)	90 (29,9)	<0,001
Levofloxacin	37 (74)	73 (29,1)	110 (36,5)	<0,001
Ceftazidime	35 (70)	39 (15,5)	74 (24,6)	<0,001
Cefepime	39 (78)	31 (12,4)	70 (23,3)	<0,001
Imipenem	47 (94)	0 (0)	47 (15,6)	<0,001
Meropenem	36 (72)	0 (0)	36 (11,9)	<0,001
Piperacillin	44 (88)	56 (22,3)	100 (33,2)	<0,001
TZP†	41 (82)	55 (21,9)	96 (31,9)	<0,001
Colistin	3 (6)	17 (6,8)	20 (6,6)	1
Total	50 (100)	251 (100)	301 (100)	

†TZP; piperacillin/tazobactam

It was found that CR strains were more resistant to other antibiotics than CS ones (Figure 1).

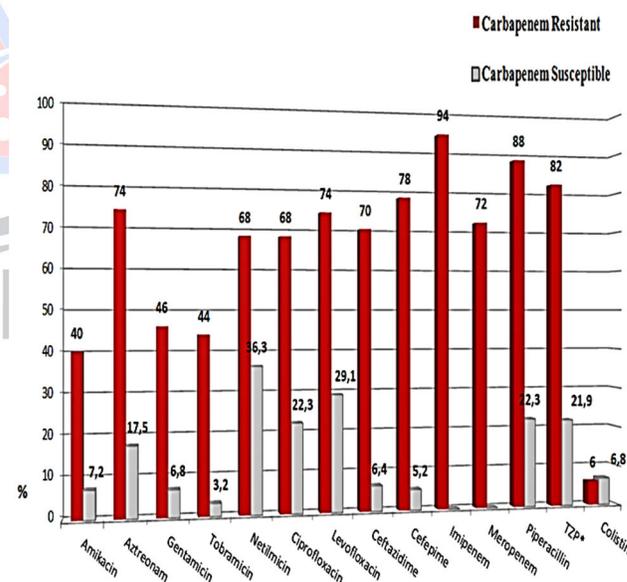


Figure 1. Resistance rates of carbapenem susceptible and resistant strains to other antibiotics

The antibiotics to which all *P. aeruginosa* strains included in the study were most resistant were netilmicin (41.5%), levofloxacin (36.5%),

piperacillin (33.2%), piperacillin/tazobactam (31.9%), ciprofloxacin (29.9%), aztreonam (26.9%), ceftazidime (24.6%) (Figure 2).

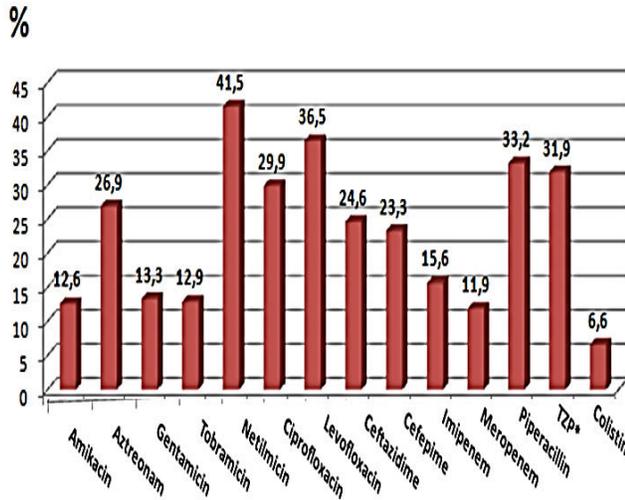


Figure 2. Antibiotic resistance rates of all strains

In CRPA, MIC values of amikacin, gentamicin, tobramycin, netilmicin, aztreonam, ceftazidime, cefepime, ciprofloxacin, levofloxacin, piperacillin, piperacillin/tazobactam were found to be higher than MIC values in CSPA ($p < 0.001$). Colistin MIC values were higher in CRPA than those in CSPA ($p < 0.001$) (Figure 3). Similar to other antibiotic MIC values, aztreonam and differently colistin MIC values were shown in Figure 3.

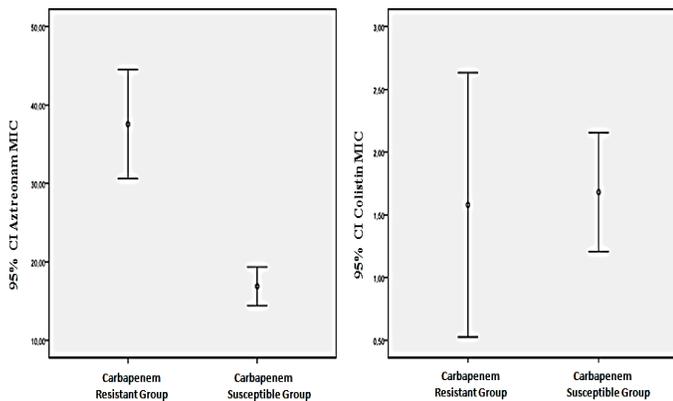


Figure 3. MIC values of aztreonam and colistin in CRPA and CSPA

The rate of MDR was found to be higher in isolates isolated from intensive care units ($P=0.001$) (Table 4).

Table 4. MDR status of strains according to the clinics

Clinic	MDR*		Not MDR*		Total		P
	N	%	N	%	N	%	
Intensive Care Units	22	25,3	65	74,3	87	100	0,001
Other Clinics	22	10,3	192	89,7	214	100	
Total	44	14,6	257	85,4	301	100	

*MDR; Multidrug resistant

Eleven (4.4%) CSPA and 33 (66%) CRPA strains were detected as MDR. MDR rate was found to be higher in CRPA strains ($P < 0.001$) (Table 5).

Table 5. MDR status of strains according to carbapenem susceptibility

Groups	MDR*		Not MDR*		Total		P
	N	%	N	%	N	%	
Carbapenem Susceptible Group	11	4,4	240	95,6	251	100	<0,001
Carbapenem Resistant Group	33	66	17	34	50	100	
Total	44	14,6	257	85,4	301	100	

*MDR; Multidrug resistant

Investigation of Carbapenemase Genes by Multiplex PCR Method:

Among the CR strains, none of the strains included in this study was found to have *blaSPM*, *blaNDM*, *blaKPC*, *blaAIM* and *blaOXA* genes. *blaIMP* was determined in one strain (2%) and *blaVIM* in three strains (6%) (Figure 4).

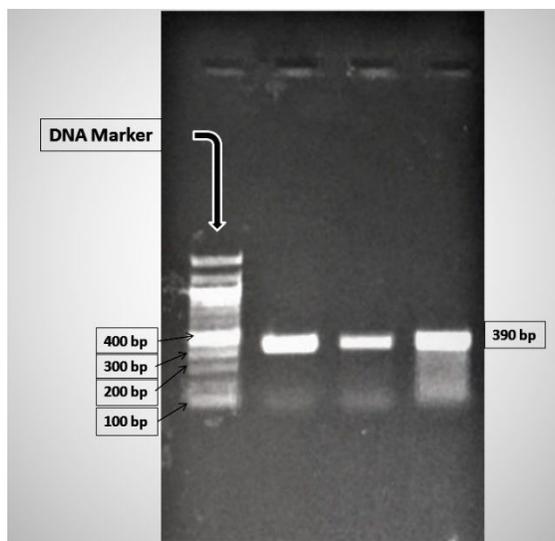


Figure 4. Image of three *blaVIM* positive strains after electrophoresis

A strain containing the *blaIMP* gene was determined to be MDR and resistant to aztreonam, ceftazidime, cefepime, ciprofloxacin, levofloxacin, imipenem, meropenem, netilmicin, piperacillin and piperacillin/tazobactam. One of the three strains containing the *blaVIM* gene was found to be resistant to aztreonam, ceftazidime, ciprofloxacin, levofloxacin, amikacin, gentamicin and tobramycin. Two of these three strains were resistant to cefepime, meropenem and netilmicin, and three were resistant to imipenempiperacillin and piperacillin/ tazobactam.

DISCUSSION:

P.aeruginosa causes serious healthcare-associated infections with high morbidity and mortality rates. Due to multidrug resistance, *P.aeruginosa* is in the serious threats category of the Centers for Disease Control and Prevention (CDC) [8].

In a study we conducted in 2016, 772 *P.aeruginosa* strains isolated in 5 years in our hospital were examined, it was reported that 23.1% of them were isolated from intensive care units [16]. In another study conducted by Pasa et al., again in our hospital, it was reported that 30% of *P.aeruginosa* strains

were isolated from intensive care units [17]. In this study, also, 28.9% of the strains were isolated from intensive care units but this ratio was less than those isolated from other clinics. In this study, as in the study in 2016, *P.aeruginosa* strains were isolated from other services other than intensive care units in our hospital unlike other publications.

In this study, in accordance with the study conducted in our hospital in 2016, we observed that the strains were mostly isolated from wounds, urine, sputum, tracheal aspirate and blood, respectively [16]. It was seen exactly the same sample distribution from which isolates were isolated in the five-year period between 2008-2012 and those isolated in this study between 2018-2020.

P.aeruginosa is difficult to treat due to its natural and acquired antibiotic resistance [18]. Aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, penicillins, monobactams, fosfomicin and polymyxins are the antimicrobial agents most commonly used to treat *P.aeruginosa* infections [19]. Carbapenems are effective in severe *P.aeruginosa* infections. Unfortunately, resistance to carbapenems is increasing worldwide [5,6]. In 2012, 12.5% of the strains reported to the European Center for Disease Prevention and Control (ECDC) were CR, this ratio had exceeded 20% by 2016 [20,21]. In our study, the antibiotics to which *P.aeruginosa* strains were most susceptible were colistin (93.2%), amikacin (86.9%), piperacillin/tazobactam (78.1%), piperacillin (77.7%), ciprofloxacin (77.7%). Sensitivity rates to other antibiotics were determined as 7.3% to tobramycin, 7.1% to levofloxacin, 8.6% to gentamicin, 87.6% to cefepime, 6.4% to netilmicin 79.7% to ceftazidime, 4.8% to aztreonam.

In our hospital between 2007-2009; we found the highest resistance to mezlocillin (50%) in 50 *P.aeruginosa* strains isolated from lower respiratory tract specimens [22]. Researchers found resistance rates to norfloxacin, ciprofloxacin and meropenem as 48%, 46%, 40% respectively. In this study, when the antibiotic resistance of all isolated *P.aeruginosa* strains was examined, the antibiotics to which the

strains were most resistant were piperacillin/tazobactam (53%), ceftazidime (36%), cefepime (34%), gentamicin (27%), amikacin (26%), imipenem (26%) and meropenem (25%). In our study, the antibiotics most resistant to all *P.aeruginosa* strains included in the study were netilmicin (41.5%), levofloxacin (36.5%), piperacillin (33.2%), piperacillin/tazobactam (31.9%), ciprofloxacin (29.9%), aztreonam (26.9%), ceftazidime (24.6%) respectively. There is a change in the course of resistant antibiotics over the years. It is thought that the reason for this may be the change of antibiotics frequently used in clinics. And when comparing the rates of resistance to the same antibiotics in the two studies, the rates of resistance appear to be reduced. In our study in 2016; we found the MDR rate as 11.9%^[16]. In this study, the rate of MDR was found to be 14.6%, and it is observed that the rates did not change much in eight years.

In our study, it was found that CR strains were more resistant to antibiotics except colistin. It was not found statistically significant because of the small number of colistin resistant strains. In addition, it is known that the gold standard method for determining the sensitivity of colistin is the microdilution method and that colistin sensitivities are not reliable in the results of the automated system. In our study, three colistin resistant isolates were found in the CR group and 17 in the CS group with the automated system. Only three colistin resistant isolates in the CR group were studied again by microdilution and all were found to be resistant to colistin.

In their study Rizek et al. isolated a total of 129 CRPA isolated in a hospital in Brazil^[23]. They found that all isolates, except one, were susceptible to colistin.

A total of 1971 *P.aeruginosa* were isolated from various clinical isolates in 32 medical centers in a study conducted in the USA between 2011-2012^[24]. Researchers found that 15.7% of *P.aeruginosa* isolates were MDR, and 8.9% of them were XDR.

In our study; CRPA strains had the highest resistance to piperacillin (88%) and the lowest resistance to amikacin (40%). In addition, resistance to piperacillin/tazobactam 82%, cefepime 78%, aztreonam 74%, levofloxacin 74%, ceftazidime 70%, ciprofloxacin 68%, netilmicin 68%, gentamicin 46% and tobramycin 44% were detected.

Telling et al. investigated 92 *P.aeruginosa* strains from clinical samples collected from five Estonian hospitals^[25]. Among them, 43 isolates were CR, 11 isolates were MDR and 38 strains were both CR and MDR. In addition, they found the highest resistance rate against imipenem (59.8%) and the lowest resistance rate against amikacin (7.6%) in these isolates.

ECDC reported that multidrug resistance in *P.aeruginosa* was lower in Northern Europe and higher in southern and eastern regions in 2018. Less than 5% rates were observed in the Scandinavian countries, Ireland, Luxembourg, Malta, the Netherlands and the United Kingdom; excess of 50% rates were reported in Belarus, Montenegro and Serbia^[26]. According to the geographical distribution of CRPA, most of which were collected between 2009 and 2011, carbapenem resistance rate in Turkey is considered to be 40-49%^[27].

According to the WHO report in 2018; in blood and CSF *P.aeruginosa* isolates in Turkey imipenem and meropenem resistance rate is stated as 38%, and piperacillin/tazobactam, ciprofloxacin/levofloxacin, cefepime, gentamicin/tobramycin, ceftazidime and amikacin resistance rates were stated as 34%, 33%, 28%, 27%, 19% and 12% respectively^[28]. MDR strains rate was reported as 28% of 1451 isolates.

According to the Central Asian and European Antimicrobial Resistance Surveillance 2019 report; Isolated from blood and CSF in Turkey by *P.aeruginosa* isolates most resistant to imipenem or meropenem (38%), piperacillin/tazobactam (34%), ciprofloxacin or levofloxacin (33%) and cefepime (28%) have been reported against. The MDR rate has also been reported as 25%^[29].

In our study; including 22 (25.3%) *P.aeruginosa* strains isolated from specimens sent from intensive care units and 22 (10.3%) from other services, a total of 44 (14.6%) strains were determined as MDR.11 (4.4%) of CSPA strains and 33 (66%) of CRPA strains were identified as MDR.

According to the Central Asian and European Antimicrobial Resistance Surveillance 2019 report; the rate of MDR *P.aeruginosa* isolates in Turkey was reported to be 25-50%^[28].

In Antibiotic Resistance Threats in USA 2019 report; 37600 cases with MDR *P.aeruginosa* were reported in hospitalized patients in 2014, 37000 in 2015, 36200 in 2016, 32600 in 2017^[8]. In this report, it has been reported that some of the MDR *P.aeruginosa* are resistant to almost all antibiotics, including carbapenems, and 2-3% of the CRPA strains contain genes that enable carbapenemase enzyme secretion^[8].The spread of carbapenemases in Europe started in the second half of the 1990s and was mainly observed in *P.aeruginosa*.Later, a VIM outbreak occurred in *K.pneumoniae* isolates in Greece, followed by a KPC outbreak^[29].

The most important problem-causing carbapenemases are KPC, first reported from the USA in 1996, NDM, which has a high prevalence especially in India and Middle East countries and has been transferred to European countries and OXA-48 which was originated from Turkey. OXA-48-like enzymes have made epidemics in several European countries, and are now spreading rapidly all over the world.Although the most common carbapenemase in our country is OXA-48, different carbapenemases are also reported. These include VIM-5, IMP-1, NDM-1 and most recently KPC-2^[30].

Carbapenemases in *P.aeruginosa* strains reported from Turkey were IMP-1, VIM-2, VIM-5, VIM-38^[31,32,33,34]

In a study conducted by Pasa et al. in our hospital in 2016, MBL was investigated in 100 *P.aeruginosa* strains using the gradient diffusion method and one strain was found positive, and carbapenemases were not genotypically investigated in this study^[17].IMP

and VIM type carbapenemases in *P. aeruginosa* strains have not been studied in our hospital before. However, we investigated IMP1, IMP2, VIM1, VIM2 metallo beta lactamase genes in 150 *Acinetobacter* strains isolated from clinical samples^[35]. We have determined the production of metallo beta lactamase in 44.7% of *Acinetobacter* strains. IMP1, IMP2, VIM1, VIM2 genes were not found in them.

Sekirov et al. investigated the presence of carbapenemase genes (*bla*NDM, *bla*OXA, *bla*KPC, *bla*VIM and *bla*IMP) in 1138 MDR Gram negative isolates between 2010 and 2014 in their study in Canada^[36].It was reported that the carbapenemase gene was detected in 175 (15.4%) of the isolates.

Rizek et al. isolated 217 CRPA and investigated *bla*IMP, *bla*SPM, *bla*VIM, *bla*SIM, *bla*NDM, *bla*KPC, *bla*GES genes in these isolates^[23]. They detected *bla*SPM(32%), *bla*KPC (4.6%), *bla*VIM (3.9%) genes in order of frequency. They found that one strain contains all three genes (*bla*SPM-1, *bla*VIM-2, and *bla*KPC-2).

In the study conducted by Ghamgosha et al. in Iran, it was determined that nine of 191 *P. aeruginosa* isolates produced MBL^[37]. They investigated the presence of *bla*VIM-1, *bla*SPM-1, and *bla*IMP-1 genes in these nine isolates and found that seven isolates had *bla*VIM-1. Rodríguez-Martínez et al. investigated the presence of carbapenemase genes in imipenem or meropenem-resistant or moderately sensitive 22 *P.aeruginosa* isolated in France, and they couldn't find *bla*IMP, *bla*VIM, *bla*SPM and *bla*OXA genes in any of them^[38].Kateete et al. found *bla*IMP-1 36%, *bla*IMP-2 4%, *bla*VIM-1 32%, *bla*SPM 20% and *bla*NDM-1 4% in CRPA isolates^[39]. In a study conducted in Italy, they found 84% *bla*VIM-1 and 16% *bla*VIM-2 in imipenem-resistant 444 *P. aeruginosa* isolates^[40].

CONCLUSION:

In this study, CR strains were found to be more resistant to amikacin, aztreonam, gentamicin, netilmicin, tobramycin, ciprofloxacin, levofloxacin,

cefepime, ceftazidime, piperacillin, piperacillin/tazobactam than CS strains. The imipenem resistance rate was found to be 15.6%, the meropenem resistance rate as 11.9% and the MDR rate as 14.6. and these rates are lower than the rates reported from Turkey in the report published by WHO [28]. It is similar to the results of previous studies with *P.aeruginosa* strains in our hospital. Among the CR strains, one strain was positive for blaIMP gene and three strains for blaVIM gene. Similar to the results of previous studies in our hospital, the rate of carbapenemase enzymes in our hospital was low in this study. Epidemiological identification of these enzymes is important in preventing the spread of resistance. Detection of beta-lactamases and their encoding genes will guide the selection of appropriate antibiotics for treatment of infections.

REFERENCES:

- [1] Barbier F, Andremont A, Wolff MveBouadma L. Hospital-acquired pneumonia and ventilator-associated pneumonia: recent advances in epidemiology and management. *Curr Opin Pulm Med* 2013; 19, 3 :216–228.
- [2] Ruhnke M, Arnold RveGastmeier P. Infection control issues in patients with haematological malignancies in the era of multidrug-resistant bacteria. *Lancet Oncol* 2014; 15, 13: 606–619.
- [3] Borgatta B, Lagunes L, Imbiscuso AT, Larrosa MN, Lujan M, Rello J. Infections in intensive care unit adult patients harboring multidrug-resistant *Pseudomonas aeruginosa*: implications for prevention and therapy. *Eur J Clin Microbiol Infect Dis* 2017; 36, 7:1097-1104.
- [4] Brooks GF, Carroll KC, Butel JS, Morse SA veMietzner TA. Bacteriology. In: *Medical Microbiology*. Jawetz, Melnick and Adelbergs, 26th Ed, McGraw-Hill Companies, New York, 2013, s.149-405.
- [5] Suárez C, Peña C, Gavaldà L, Tubau F, Manzur A, Dominguez M.A, Pujol M, Gudiol F, Ariza J Influence of carbapenem resistance on mortality and the dynamics of mortality in *Pseudomonas aeruginosa* bloodstream infection. *Int J Infect Dis* 2010; 14: e73-78.
- [6] Buehrle DJ, Shields RK, Clarke LG, Potoski BA, Clancy CJ and Nguyen MH. Carbapenem-resistant *Pseudomonas aeruginosa* bacteremia: Risk factors for mortality and microbiologic treatment failure. *Antimicrob Agents Chemother* 2016; 61,1: e01243-16.
- [7] Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y, Ouellette M, Outtersson K, Patel J, Cavalieri M, Cox EM, Houchens CR, Grayson ML, Hansen P, Singh N, Theuretzbacher U, Nicola Magrini C, WHO Pathogens Priority List Working Group Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018; 18, 3:318–327.
- [8] Centres for Disease Control and Prevention (US). Antibiotic resistance threats in the United States, 2019. Centres for Disease Control and Prevention, US Department of Health and Human Services.
- [9] Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, Edwards JR, Sievert DM. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011-2014. *Infect Control Hosp Epidemiol* 2016; 37, 11:1288 –1301.
- [10] Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA, Westblade LF. Carbapenemase-Producing Organisms: A Global Scourge. *Clin Infect Dis* 2018; 66, 8 :1290-1297.
- [11] Walther-Rasmussen J. and Hoiby N. OXA-type carbapenemases. *J. Antimicrob Chemother* 2006; 57(3):373-83.
- [12] Nordmann P, Cuzon G. and Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *Lancet Infect Dis* 2009; 9, 4:228-236.
- [13] EUCAST. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 10.0, 2020: 22-27.
- [14] Hassuna NA, Ibrahim Mohamed AH, Abo-Eluoon SM and Hawa R. High prevalence of

- multidrug resistant *Pseudomonas aeruginosa* recovered from infected burn wounds in children. *Archives of ClinMicrobiol* 2015; 6,4: 1-7.
- [15] Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, Nordmann P. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. *Antimicrob Agents Chemother* 2000;44, 4 :891-897.
- [16] Ozer B, Inci M, Duran N, Kurtgoz S, Alagoz G, Pasa O, Kilic C. Comparison of antibiotic resistance of *Acinetobacter* and *Pseudomonas aeruginosa* strains isolated from intensive care units with other clinics. *Acta Medica Mediterranea* 2016; 32:117-122.
- [17] Pasa O, Ozer B, Duran N, Inci M, Yula E. Beta lactamase enzymes in clinical *Pseudomonas aeruginosa* strains. *West Indian Med J* 2015;16 ;65, 1:40-45.
- [18] Neidig A, Yeung AT, Rosay T, Tettmann B, Stempel N, Rueger M, Lesouhaitier O, Overhage J. TypA is involved in virulence, antimicrobial resistance and biofilm formation in *Pseudomonas aeruginosa*. *BMC Microbiol* 2013;13, 1:77.
- [19] Bassetti M, Vena A, Croxatto A, Righi E and Guery B. How to manage *Pseudomonas aeruginosa* infections. *Drugs in Context*;2018;7.
- [20] Antimicrobial resistance surveillance in Europe 2012 [<http://ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2012>].
- [21] Antimicrobial resistance surveillance in Europe 2016 [<http://ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2016>].
- [22] Ozer B, Duran N, Onlen YandSavas L. Efflux pump genes and antimicrobial resistance of *Pseudomonas aeruginosa* strains isolated from lower respiratory tract infections acquired in an intensive care unit. *The Journal of Antibiotics* 2012;65, 1:9-13.
- [23] Rizek C, Fu L, dos Santos LC, Leite G, Ramos J, Rossi F, Guimaraes T, Levin AS, Costa SF. Characterization of carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates, carrying multiple genes coding for this antibiotic resistance. *Ann ClinMicrobiol Antimicrob* 2014;13, 1:1-5.
- [24] Farrell DJ, Flamm RK, Sader HS and Jones RN. Antimicrobial activity of ceftolozane-tazobactam tested against Enterobacteriaceae and *Pseudomonas aeruginosa* with various resistance patterns isolated in US hospitals (2011-2012). *Antimicrob Agents and Chemother* 2013;57, 12:6305-6310.
- [25] Telling K, Laht M, Brauer A, Remm M, Kisand V, Maimets M, Tenson T, Lutsar I. Multidrug resistant *Pseudomonas aeruginosa* in Estonian hospitals. *BMC Infect Dis* 2018;18, 1:513.
- [26] European Centre for Disease Prevention and Control; Surveillance Atlas of Infectious Diseases [online tool]. Stockholm, 2019 (<https://ecdc.europa.eu/en/antimicrobial-resistance/surveillance-and-disease-data/data-ecdc>, accessed 28 October 2019).
- [27] Hong DJ, Bae IK, Jang IH, Jeong SH, Kang HK, Lee K. Epidemiology and characteristics of metallo-beta-lactamase-producing *Pseudomonas aeruginosa*. *Infect Chemother* 2015;47, 2:81-97.
- [28] World Health Organization. Central Asian and European Surveillance of Antimicrobial Resistance, Annual Report 2019.
- [29] Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen Ø, Seifert H, Woodford N, Nordmann P, European Network on Carbapenemases. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect* 2012; 18, 5:413-431.
- [30] Gulay Z. Molecular Epidemiology of Carbapenemases in Enterobacteriaceae. *Bulletin of Antimicrob Chemother* 2014; 28(suppl 2):73-76.
- [31] Ozgumus OB, Caylan R, Tosun I, Sandalli C, Aydin K, Koksali I. Molecular epidemiology of clinical *Pseudomonas aeruginosa* isolates carrying IMP-1 metallo-beta-lactamase gene in a university hospital in Turkey. *Microbial Drug Resist* 2007;13, 3:191-198.
- [32] Castanheira M, Deshpande LM, Costello A, Davies TA and Jones RN. Epidemiology and carbapenem resistance mechanisms of carbapenem-non-susceptible *Pseudomonas aeruginosa* collected during 2009-11 in 14

- European and Mediterranean countries. J Antimicrob Chemother 2014; 69:1804-1814.
- [33] Walsh TR. The emergence and implications of metallo-beta-lactamases in Gram-negative bacteria. Clin Microbiol Infect 2005;11:2-9.
- [34] Iraz M, Duzgun AO, Cicek AC, Bonnin RA, Ceylan A et al. Characterization of novel VIM carbapenemase, VIM-38, and first detection of GES-5 carbapenem-hydrolyzing beta-lactamases in Pseudomonas aeruginosa in Turkey. Diagn Microbiol Infect Dis 2014; 78, 3 :292-294.
- [35] Ocak M, Özer B, İnci M, Duran N. Antibiotic Resistance and Investigation of IMP-1, IMP-2, VIM-1 and VIM-2 Metallo-β-Lactamases in Acinetobacter Strains Isolated From Clinical Samples. KLIMIK Journal 2015;28, 1:23-27.
- [36] Sekirov I, Croxson MA, Ng C, Azana R, Chang Y, Mataseje L, Boyd D, Mangat C, Mack B, Tadros M, Brodtkin E, Kibsey P, Stefanovic A, Champagne S, Mulvey MR, Hoang LMN. Epidemiologic and genotypic review of carbapenemase-producing organisms in British Columbia, Canada, between 2008 and 2014. J Clin Microbiol 2016;54, 2:317-327.
- [37] Ghamgosha M, Shahrekizahedani S, Kafilzadeh F, Bameri Z, Taheri RA, Farnoosh G. Metallo-beta-lactamase VIM-1, SPM-1, and IMP-1 Genes Among Clinical Pseudomonas aeruginosa Species Isolated in Zahedan, Iran. Jundishapur J Microbiol 2015;8, 4: e17489.
- [38] Rodríguez-Martínez J, Poirel L, Nordmann P. Molecular epidemiology and mechanisms of carbapenem resistance in Pseudomonas aeruginosa. Antimicrob Agents and Chemother 2009; 53, 11:4783-4788.
- [39] Kateete DP, Nakanjako R, Namugenyi J, Erume J, Joloba ML, Najjuka CF. Carbapenem resistant Pseudomonas aeruginosa and Acinetobacter baumannii at Mulago Hospital in Kampala, Uganda (2007-2009). Springerplus 2016;5, 1:1308.
- [40] Lagatolla C, Tonin EA, Monti-Bragadin C, Dolzani L, Gombac F, Bearzi C, Edalucci E, Gionechetti F, Rossolini GM. Endemic carbapenem resistant Pseudomonas aeruginosa with acquired metallo-β-lactamase determinants in European hospital. Emerg Infect Dis 2004;10, 3:535-538.

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