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SHORT COMMUNICATION

Investigation of metallo-beta-lactamase production in carbapenem-resistant *Pseudomonas aeruginosa* isolated in Kastamonu Training and Research Hospital, Turkey

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ABSTRACT

Aims: The detection of the metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa* isolates is crucial for infection control and public health. The present study aimed to investigate the MBL production in carbapenem-resistant *P. aeruginosa* isolated from various clinical samples in Kastamonu Training and Research Hospital, Turkey.
Methodology and results: Seventy-three carbapenem-resistant *P. aeruginosa* isolates were recovered from different patients between April 2018 and November 2020. Identification of the isolates was performed by conventional methods (culture examination, determination of Gram reaction, and oxidase test) and an automated system (Vitek 2). Antibiotic susceptibility patterns were determined using the Vitek 2 and the results were interpreted based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards. The MBL production was phenotypically investigated using the imipenem-EDTA combined disk test. The presence of beta-lactamase IMP (*bla*$_{IMP}$), beta-lactamase VIM (*bla*$_{VIM}$) and beta-lactamase GIM (*bla*$_{GIM}$) genes were determined using PCR to confirm the MBL production. Seventy-one isolates (97%, n=71/73) were resistant to imipenem, sixty-four isolates (88%, n=64/73) to meropenem and sixty-two isolates (85%, n=62/73) to both imipenem and meropenem. Sixty-five isolates (89%, n=65/73) were defined as multidrug-resistant. The MBL production was detected in 57 isolates (78%, n=57/73) phenotypically. However, the *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{GIM}$ genes were not detected in all the isolates.
Conclusion, significance and impact of study: It was determined that there were no imipenemase (IMP), Verona integron-encoded metallo-beta-lactamase (VIM) and German imipenemase (GIM) type MBLs in carbapenem-resistant *P. aeruginosa* isolated from Kastamonu Training and Research Hospital. MBL production in carbapenem-resistant *P. aeruginosa* strains can be investigated phenotypically. However, confirmation of results with molecular tests is especially significant for epidemiological studies.

Keywords: Carbapenem, metallo-beta-lactamases, PCR, *Pseudomonas aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa is opportunistic pathogen that causes difficult-to-treat infections, especially in inpatients, due to multidrug-resistance (CDC, 2019). Carbapenems (imipenem, meropenem, ertapenem, doripenem, etc.) are beta-lactam antibiotics that are highly effective against bacterial infections. Carbapenems are also considered as one of the last resort antibiotics against infections caused by multidrug-resistant (MDR) pathogens. However, the overuse and misuse of this class of antibiotics have increased the spread of carbapenem resistance bacteria until a worrying level for global public health (Codjoe and Donkor, 2017). Indeed, the carbapenem resistance rate in clinical *P. aeruginosa* isolates has been reported to be >50.0% in Turkey (Castanheira *et al.*, 2014).

Carbapenem resistance can develop due to decrease outer membrane porin (OprD) permeability,

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overexpression of efflux pumps and production of carbapenemase enzymes in *P. aeruginosa* (Elshamy and Aboshanab, 2020). Metallo-beta-lactamases (MBLs) are clinically one of the most significant carbapenemase enzymes. The bacteria producing these enzymes develop resistance to all beta-lactam antibiotics except monobactam (aztreonam). Also, MBLs are not inhibited by beta-lactamase inhibitors such as tazobactam, sulbactam and clavulanic acid (Palzkill, 2013). Their encoding genes are usually carried on plasmids along with other resistance genes. Therefore, the MBL producing bacteria are often resistant to different antibiotic groups as well (Wang and Wang, 2020). There are various MBL enzymes such as imipenemase (IMP), Verona integron-encoded metallo-beta-lactamase (VIM), São Paulo metallo-beta-lactamase (SPM), German imipenemase (GIM), Seoul imipenemase (SIM), Dutch imipenemase (DIM), New Delhi metallo-beta-lactamase (NDM), Florence imipenemase (FIM), etc. defined in *P. aeruginosa* isolates. However, VIM-type has been stated as the most common MBL in *P. aeruginosa* isolates (Abaza *et al.*, 2017). MBLs require zinc ions for their activation. Therefore, they can be inhibited *in vitro* using various metal ion chelators such as ethylene diamine tetraacetic acid (EDTA) and dipicolinic acid (DPA) (Bush *et al.*, 1995). For phenotypic detection of MBL production, tests such as combined disk test, double-disk synergy test (DDST) and G-test have been developed using chelators.

MBL positive *P. aeruginosa* can cause serious nosocomial infections, especially in intensive care units (Lucena *et al.*, 2014). Therefore, the detection and monitoring of this resistance mechanism are necessary for infection control and public health. The present study aimed to investigate the MBL production in carbapenem-resistant *P. aeruginosa* isolates isolated from various clinical samples in Kastamonu Training and Research Hospital, Turkey. This is the first report about MBL production in carbapenem-resistant *P. aeruginosa* isolates obtained from Kastamonu Training and Research Hospital, Turkey.

MATERIALS AND METHODS

Bacterial isolates

This study was permitted by the Karabuk University (Turkey), Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (Date: 02.05.2018 and Decision number: 2018-5/4). Seventy-three carbapenem (imipenem and/or meropenem) resistant *P. aeruginosa* were isolated from various clinical samples (respiratory secretions, blood, urine and wound) and sent to Kastamonu Training and Research Hospital Clinical Microbiology Laboratory between April 2018 and November 2020 were included in the study. Isolates belonging to the same patient were excluded from the study. Two isolates were isolated from outpatients and 71 from inpatients. Identification of the isolates was performed using the conventional methods (determination of colony morphology, odor of culture, pyocyanin pigment production, Gram reaction and oxidase test) and Vitek 2 automated system (bioMérieux, Marcy l'Etoile, France). Then, the isolates were stored at –80 °C in nutrient broth (Merck, Darmstadt, Germany) with 20% glycerol (v/v).

Antibiotic susceptibility testing

The antibiotic susceptibility tests were done using the Vitek 2 automated system. The antipseudomonal antibiotics and their concentrations were used as follows: piperacillin/tazobactam (TZP; 2/4, 8/4, 24/4, 32/4, 32/8, 48/8 µg/mL) as penicillin, ceftazidime (CAZ; 0.25, 1, 2, 8, 32 µg/mL) and cefepime (FEP; 0.25, 1, 4, 16, 32 µg/mL) as cephalosporins, imipenem (IPM; 1, 2, 6, 12 µg/mL) and meropenem (MEM; 0.5, 2, 6, 12 µg/mL) as carbapenems, gentamicin (CN; 4, 16, 32 µg/mL) and amikacin (AK; 8, 16, 32 µg/mL) as aminoglycosides and ciprofloxacin (CIP; 0.5, 2, 4 µg/mL) as fluoroquinolone. The results were interpreted based on the breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards. The minimum inhibitory concentration (MIC) values of >16, >8, >8, >4, >8, >4, >16 and >0.5 mg/L were considered to be the resistance breakpoints for TZP, CAZ, FEP, IPM, MEM, CN, AK and CIP, respectively (EUCAST, 2019). Isolates which resistant to at least one agent from three or more antibiotic groups were defined as multidrug resistance (MDR) (Magiorakos *et al.*, 2012).

Phenotypic detection of MBL

The IPM-EDTA combined disk test was performed for the phenotypic detection of the MBL production. Briefly, inoculum of each isolate grown on nutrient agar (Merck) was adjusted to 0.5 McFarland turbidity standards in a densitometer instrument (DEN-1B, Biosan, Riga, Latvia) with the colony suspension method. Then, the prepared suspension was swab in three directions on Mueller Hinton agar (MHA; Merck). Two IPM (10 µg/disk, Oxoid, Hampshire, UK) disks were put on the media. Then, 10 µL of 0.5 M EDTA (pH 8.0; Sigma-Aldrich, St. Louis, MO, USA) was impregnated on one of the disks to obtain the 750 µg concentration and incubate for 18-20 h at 37 °C. The isolate was considered as MBL positive when the zone of inhibition (ZOI) of the IPM-EDTA disk was ≥7 mm than the ZOI of the only IPM disk (Yong *et al.*, 2002). *Klebsiella pneumoniae* NCTC 13440 and *P. aeruginosa* ATCC 27853 were used as positive and negative controls, respectively.

Detection of *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{GIM}$ metallo-beta-lactamases genes

The presence of *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{GIM}$ genes in all isolates were investigated by polymerase chain reaction (PCR). The specific primers used are listed in Table 1. Total DNA extraction was done from the isolates using the boiling method. Briefly, 1.2 mL of 0.5 McFarland turbidity standards of the isolates were transferred into

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Table 1: Primers and oligonucleotides sequences used in PCR.

Primer	Oligonucleotide sequences (5' → 3')	Annealing temperature	Product size	Reference
Imp-F	GAATAGAGTGGCTTAAYTCTC	51 °C	188 bp	Ellington *et al.* (2007)
Imp-R	CCAAACYACTASGTTATCT			
Vim-F	GATGGTGTTTGGTCGCATA	52 °C	390 bp	
Vim-R	CGAATGCGCAGCACCAG			
Gim-F	TCGACACACCTTGGTCTGAA		477 bp	
Gim-R	AACTTCCAACTTTGCCATGC			

Table 2: Identification and isolation sources of isolates (n=73).

Isolation source	Colony type	Gram reaction	Oxidase	Vitek 2 identification
Respiratory secterions (n=51)	Rough	Gram-negative basil	Positive	*Pseudomonas aeruginosa*
Blood (n=9)				
Urine (n=7)				
Wound (n=6)				

the sterile microcentrifuge tubes and centrifuged at 13,000× *g* for 5 min. The pellets were suspended in 200 µL Tris-EDTA buffer and heated at 100 °C for 10 min in a dry heating block (BioSan Thermo-Shaker TS-100C). Then, the suspension was placed at room temperature for 3 min and centrifuged at 13,000× *g* for 1 min. The supernatants were transferred to new sterile tubes and used as the DNA template for PCR. Each PCR reaction contained 2 µL DNA template, 0.4 µL 10 pmol/µL of each primer (forward and reverse), 4 µL 5× FIREPol® Master Mix (Solis BioDyne, Tartu, Estonia) and 13.2 µL ultra-pure water. PCR reactions were performed in a thermal cycler (Techne TC-512, Techne Co., Staffs, UK) as follows: initial denaturation at 94 °C for 5 min; 35 cycles of 30 sec at 95 °C, 40 sec at a specific annealing temperature (at 51 °C for bla_{IMP}, at 52 °C for bla_{VIM} and bla_{GIM}) and 50 sec at 72 °C; and a final extension of 5 min at 72 °C (Ellington *et al.*, 2007). In PCR reactions, *P. aeruginosa* ATCC 27853 was used as negative control, while *K. pneumoniae* NCTC 13440, clinical *P. aeruginosa* strain and *Acinetobacter baumannii* strain were served as positive control for bla_{VIM}, bla_{IMP} and bla_{GIM} genes, respectively. All the clinilcal strains have been previously confirmed by DNA sequence analysis contain these resistance genes. The DNA extraction from control bacteria was performed using the boiling method as in the isolates. The PCR products were electrophoresed on a 1% agarose gelstained with RedSafe™ Nucleic Acid Staining Solution (20,000×) (iNtRON Biotechnology, Inc. Seoul, Korea). The results were evaluated in the presence of DNA size marker (GeneRuler 1 kb, Thermo Scientific, CA, USA), visualized under UV transilluminator.

Statistical analysis

The data were analyzed for normality using the Shapiro-Wilks test. Then, the data were analyzed using the Kruskal-Wallis test with IBM-SPSS version 23 for Windows (IBM Inc., Armonk, NY, USA) and $p<0.05$ was recognized as statistically significant.

RESULTS

In the present study, a total of 73 carbapenem-resistant *Pseudomonas aeruginosa* have been isolated from respiratory secretions (n=51/73), blood (n=9/73), urine (n=7/73) and wound (n=6/73) samples. The identification of carbapenem-resistant *P. aeruginosa* has been determined and confirmed by conventional methods and Vitek 2 (Table 2).

Seventy-one (97%) of 73 carbapenem-resistant isolates were found to be resistant to imipenem, 64 (88%) to meropenem and 62 (85%) to both imipenem and meropenem using the automated Vitek 2 system. Resistance rates of the isolates to ciprofloxacin, piperacillin/tazobactam, cefepime, gentamicin, ceftazidime and amikacin were 90% (n=66/73), 84% (n=61/73), 70% (n=51/73), 67% (n=49/73), 64% (n=47/73) and 14% (n=10/73), respectively (Figure 1).

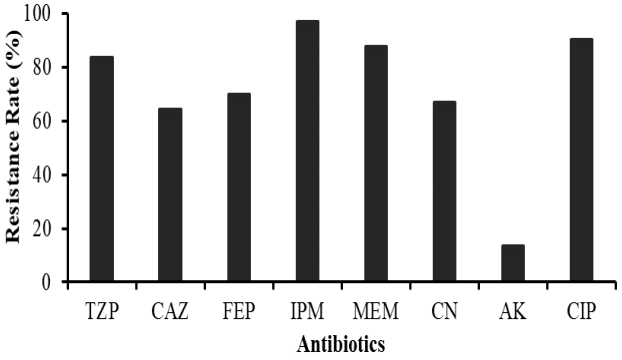

Figure 1: The resistance rate (%) of carbapenem-resistant *P. aeruginosa* isolates to antipseudomonal antibiotics. TZP: Piperacillin/tazobactam, CAZ: Ceftazidime, FEP: Cefepime, IPM: Imipenem, MEM: Meropenem, CN: Gentamicin, AK: Amikacin, CIP: Ciprofloxacine.

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There was no significant difference in antibiotic resistance among clinical samples (respiratory secretions, blood, urine and wound) (p>0.05) (Table 3). Sixty-five isolates (89%) were identified as MDR. Among the MDR isolates (n=65), 62 were resistant to fluoroquinolone (CIP), 60 to penicillin (TZP), 55 to cephalosporins (ceftazidime and/or cefepime) and 49 to aminoglycosides (gentamicin and/or amikacin) (Table 3).

The MBL production was determined in 57 (78%) out of 73 isolates phenotypically and the representive images are shown in Figure 2. However, the *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{GIM}$ genes were not detected in all the isolates. The gel image of the positive and negative controls after PCR is shown in Figure 3.

DISCUSSION

Carbapenems have been used as antibiotics that give confidence to the medical world in combating infection diseases caused by resistant bacteria. However, today the increase in carbapenem resistance is reaching alarming levels (CDC, 2019). The MBL producing *P. aeruginosa* affects the prognosis of the patients is negative, prolongs the hospital stay of patients and increases the mortality (Lucena *et al.*, 2014). For this reason, it is crucial to detect this resistance mechanism early and to take the necessary precautions.

In the current study, the MBL production was detected in 78% of carbapenem-resistant *P. aeruginosa* isolates using the IPM-EDTA combined disk test. The MBL production in carbapenem-resistant *P. aeruginosa* isolates has been reported as 31% (Cesur *et al.*, 2012), 32% (Beşli *et al.*, 2018) and 94% (Aksoy and Tuğrul, 2020) using IPM-EDTA combined disk test in Turkey. The fact that MBL production has been detected in a broad range phenotypically in Turkey may be due to the differences in the antibiotic use policies of the hospitals. As a matter of fact, a high rate of MBL production for Turkey was detected phenotypically in Kastamonu Training and Research Hospital.

In this study, MBL production by carbapenem-resistant *P. aeruginosa* using IPM-EDTA combined disk test. It was reported that the determination of the MBL production using only phenotypic test without the molecular confirmation is incomplete due to the low specificity of the phenotypic tests (EUCAST, 2017). The presence of *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{GIM}$ genes, which were reported to be common in MBL producing *P. aeruginosa* isolates based on the literature, was researched in the presented study. The frequency of *bla*$_{GIM}$, *bla*$_{IMP}$ and *bla*$_{VIM}$ genes have been reported as 3%, 4% and 7%, respectively, in carbapenem-resistant *P. aeruginosa* isolates in Turkey in recent years (Beşli *et al.*, 2018; Vural *et al.*, 2020). However, these genes were not detected in any of the carbapenem-resistant *P. aeruginosa* isolated from our hospital in this study. Although the *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{GIM}$ genes were not found, the phenotypic detection of MBL production at a high rate of 78% might suggest the presence of different MBLs. However, some studies stated that false-positive results could be obtained

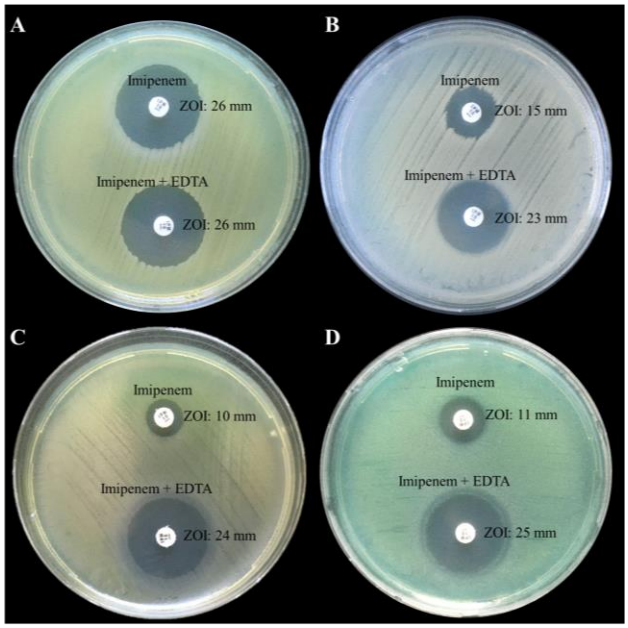

Figure 2: Representive images of the phenotypic detection of the MBL production. A: *P. aeruginosa* ATCC 27853 (negative control), B: *K. pneumoniae* NCTC 13440 (positive control), C: Isolate KPA-8, D: Isolate KPA-59. Imipenem: 10 µg/disk; EDTA: 750 µg/disk; ZOI: Zone of inhibition.

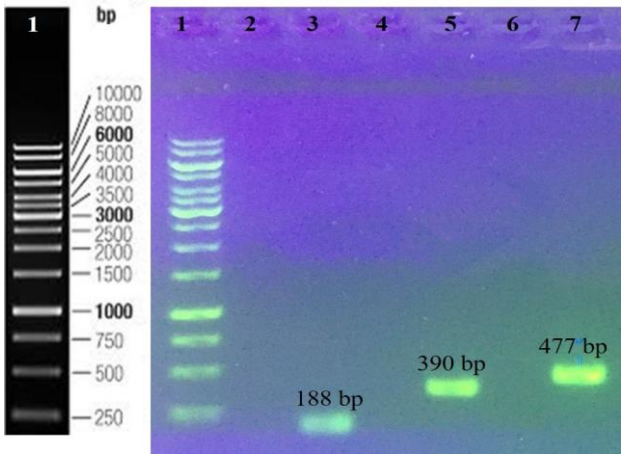

Figure 3: PCR products on agarose gel electrophoresis for MBLs genes. Lane 1: DNA size marker (GeneRuler 1 kb, Thermo Scientific, CA, USA); Lanes 2, 4 and 6: Negative controls for *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{GIM}$ genes, respectively; Lanes 3, 5 and 7: Positve controls for *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{GIM}$ genes, respectively.

in IPM-EDTA combined disk test because EDTA increases the cell wall permeability of bacteria (Aktaş and Kayacan, 2008; Ratkai *et al.*, 2009). A similar situation might have occurred in this study, and thus resulted in high phenotypically MBL production.

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Table 3: Antipseudomonal antibiotics resistance patterns of isolates.

No	Isolate Name	Isolation source	TZP[1]	CAZ[2]	FEP[2]	IPM[3]	MEM[3]	CN[4]	AK[4]	CIP[5]
1	KPA-1*	Urine	R	S	R	R	R	R	S	R
2	KPA-2*	Respiratory secterions	S	S	S	R	S	R	S	R
3	KPA-3*	Respiratory secterions	R	R	R	R	R	R	S	R
4	KPA-4*	Respiratory secterions	R	R	R	R	R	R	S	R
5	KPA-5	Respiratory secterions	S	S	S	R	R	S	S	S
6	KPA-6*	Blood	R	S	R	R	R	S	S	R
7	KPA-7*	Respiratory secterions	R	R	R	R	R	R	R	R
8	KPA-8	Respiratory secterions	S	S	S	R	S	S	S	R
9	KPA-9*	Respiratory secterions	R	R	R	R	R	R	S	R
10	KPA-11*	Blood	R	S	S	R	R	S	S	R
11	KPA-12*	Blood	R	R	R	R	R	R	R	R
12	KPA-13	Respiratory secterions	S	S	S	R	S	S	S	S
13	KPA-14*	Respiratory secterions	R	R	S	R	R	S	S	R
14	KPA-15*	Blood	R	R	S	R	R	R	S	R
15	KPA-16*	Respiratory secterions	S	S	S	R	S	R	R	R
16	KPA-17*	Blood	R	R	R	R	R	R	R	R
17	KPA-18*	Respiratory secterions	R	R	R	R	R	R	R	R
18	KPA-19*	Respiratory secterions	R	R	R	R	R	R	R	R
19	KPA-21*	Respiratory secterions	R	R	R	R	R	R	S	R
20	KPA-22*	Respiratory secterions	R	R	R	R	R	R	S	R
21	KPA-23*	Respiratory secterions	R	R	R	R	R	R	S	R
22	KPA-24*	Respiratory secterions	R	R	R	R	R	R	R	R
23	KPA-25	Respiratory secterions	S	S	S	R	R	S	S	R
24	KPA-26	Wound	S	S	S	R	S	S	S	R
25	KPA-28*	Urine	R	R	R	R	R	R	S	R
26	KPA-29*	Respiratory secterions	R	R	R	R	S	R	S	R
27	KPA-30*	Blood	R	S	S	R	R	S	S	R
28	KPA-31*	Respiratory secterions	R	R	R	R	R	R	R	R
29	KPA-33*	Respiratory secterions	R	S	R	R	R	R	S	R
30	KPA-34*	Respiratory secterions	R	S	R	R	R	R	S	R
31	KPA-35*	Respiratory secterions	R	S	S	R	R	S	S	R
32	KPA-36*	Respiratory secterions	R	S	R	R	R	R	S	R
33	KPA-37*	Wound	R	R	R	R	R	R	S	R
34	KPA-38*	Respiratory secterions	S	S	R	R	R	R	S	R
35	KPA-39*	Respiratory secterions	R	R	R	R	R	R	S	R
36	KPA-40*	Respiratory secterions	R	R	R	R	R	R	S	R
37	KPA-41*	Respiratory secterions	R	R	R	R	R	R	S	R
38	KPA-42*	Respiratory secterions	R	R	R	R	R	R	S	R
39	KPA-43*	Urine	R	R	S	R	R	R	S	R
40	KPA-44*	Wound	R	R	R	R	R	S	S	R
41	KPA-45*	Respiratory secterions	R	R	R	R	R	R	S	R
42	KPA-46*	Respiratory secterions	R	R	R	R	R	S	S	R
43	KPA-47*	Respiratory secterions	R	R	R	R	R	R	S	R
44	KPA-48*	Respiratory secterions	R	R	R	R	R	R	R	R
45	KPA-49*	Respiratory secterions	S	S	R	R	R	R	S	R
46	KPA-50*	Respiratory secterions	R	R	R	R	R	R	S	R

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(Continued)

47	KPA-51*	Wound	R	R	R	R	R	R	S	R
48	KPA-52*	Respiratory secterions	R	R	R	R	R	S	S	R
49	KPA-53*	Respiratory secterions	R	R	R	R	R	R	S	S
50	KPA-54*	Wound	R	R	R	R	R	R	S	R
51	KPA-55	Respiratory secterions	S	S	S	R	R	S	S	R
52	KPA-56*	Respiratory secterions	R	R	R	R	R	R	S	R
53	KPA-57*	Respiratory secterions	R	S	R	R	R	R	S	R
54	KPA-58*	Respiratory secterions	R	R	R	R	R	R	S	R
55	KPA-59*	Respiratory secterions	R	R	R	R	R	R	S	R
56	KPA-62*	Respiratory secterions	R	R	R	R	R	S	S	R
57	KPA-63*	Urine	R	R	R	R	R	S	S	R
58	KPA-64*	Urine	R	R	R	R	R	R	S	R
59	KPA-65*	Blood	R	R	R	R	R	S	S	S
60	KPA-67*	Blood	R	S	S	R	R	R	S	R
61	KPA-68*	Respiratory secterions	R	S	S	R	R	S	S	R
62	KPA-69	Respiratory secterions	R	S	S	S	R	S	S	S
63	KPA-70	Respiratory secterions	S	S	S	S	R	S	S	S
64	KPA-71*	Respiratory secterions	R	R	S	R	R	R	S	R
65	KPA-73*	Urine	R	R	R	R	R	S	S	R
66	KPA-74*	Wound	R	R	R	R	R	R	S	S
67	KPA-75*	Urine	R	R	R	R	R	R	S	R
68	KPA-76*	Respiratory secterions	R	R	R	R	R	S	S	R
69	KPA-77*	Respiratory secterions	R	S	S	R	R	R	R	R
70	KPA-78*	Respiratory secterions	S	S	S	R	S	R	S	R
71	KPA-79*	Respiratory secterions	R	S	S	R	S	R	S	R
72	KPA-80*	Blood	R	R	R	R	S	S	S	R
73	KPA-81*	Respiratory secterions	R	R	R	R	R	S	S	R

KPA: Kastamonu *Pseudomonas aeruginosa*, *: Multidrug-resistant (MDR), TZP: Piperacillin/tazobactam, CAZ: Ceftazidime, FEP: Cefepime, IPM: Imipenem, MEM: Meropenem, CN: Gentamicin, AK: Amikacin, CIP: Ciprofloxacine, [1]: Penicillin, [2]: Cephalosporin, [3]: Carbapenem, [4]: Aminoglycoside, [5]: Fluoroquinolone, R: Resistant, S: Susceptible. There was no significant difference (p>0.05) in antibiotic resistance among clinical samples.

In other similar studies, it was stated that carbapenem resistance might resulted from decreased OprD permeability, overexpression of efflux pumps and the production of other carbapenemases (Petrova *et al.*, 2017; Aksoy and Tuğrul, 2020). Many studies have shown that the decrease in OprD permeability plays a significant role in the development of carbapenem-resistance in *P. aeruginosa* isolates (Fehlberg *et al.*, 2012; Castanheira *et al.*, 2014; Li *et al.*, 2020). OprD is the main porin used by carbapenems for diffussion into *P. aeruginosa*. The decrease or loss of OprD, a porin protein that allows carbapenems to pass into the periplasmic region, causes carbapenems unable to pass through the outer membrane of bacteria cells (Papp-Wallace *et al.*, 2011). One of the reasons for carbapenem resistance in our isolates might be the decrease in OprD permeability. Besides, the isolates were also found to be more susceptible to other beta-lactam antibiotics as ceftazidime, cefepime and piperacillin/tazobactam than carbapenems. The MBL producing isolates are expected to be resistant to all beta-lactam antibiotics except aztreonam (Palzkill, 2013). If the isolates in this study had produced MBL, they would have been expected to be at least as resistant to other beta-lactam antibiotics as carbapenems. These results support that the carbapenem resistance in our isolates may be due to a mechanism other than MBL.

It was determined that the resistance rates of the isolates to antipseudomonal antibiotics other than amikacin were between 64% to 90%. The lowest resistance rate was found against amikacin (14%). Previous studies showed that carbapenem-resistant *P. aeruginosa* isolates had a low resistance rate against amikacin (Beşli *et al.*, 2018; Vural *et al.*, 2020). The low resistance of amikacin might be due to its rare use in our hospital. Thus, amikacin can be used as an alternative drug in carbapenem-resistant *P. aeruginosa* infections in Kastamonu Training and Research Hospital. On the other hand, it was determined that the isolates were most resistant to ciprofloxacin (90%) among other antibiotics. Ciprofloxacin resistance was not reported at high rates in carbapenem-resistant *P. aeruginosa* isolates in the

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previous studies (Beşli *et al.*, 2018; Vural *et al.*, 2020; Wang and Wang, 2020). Nevertheless, the high resistance determined against ciprofloxacin in our study might be due to the widespread use of this drug in Kastamonu Training and Research Hospital.

CONCLUSION

As a result, there were no IMP, VIM and GIM-type MBLs among carbapenem-resistant *P. aeruginosa* isolates in Kastamonu Training and Research Hospital. MBL production in carbapenem-resistant *P. aeruginosa* strains can be investigated phenotypically. However, confirmation of results with molecular tests is especially significant for epidemiological studies. The carbapenem resistance in these isolates might be caused by different resistance mechanisms such as decreased OprD permeability. It is recommended to investigate the other carbapenem resistance mechanisms for these isolates in further studies.

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CONFLICT OF INTEREST

Authors declared no conflict of interest.

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