flora

RESEARCH ARTICLE/KLİNİK ÇALIŞMA

FLORA 2022;27(1):45-54 • doi: 10.5578/flora.20229905

Co-existence of Multiple Resistance Mechanisms in Clinical Isolates of Carbapenem-Resistant *Pseudomonas aeruginosa*

Karbapenem Dirençli Klinik *Pseudomonas aeruginosa* İzolatlarında Çoklu Direnç Mekanizmalarının Birlikte Varlığı

Aylin ÜSKÜDAR-GÜÇLÜ(İD), Hasan Cenk MİRZA(İD), Sezin ÜNLÜ(İD)

Department of Medical Microbiology, Başkent University Faculty of Medicine, Ankara, Turkey

Cite this article as: *Üsküdar-Güçlü A, Mirza HC, Ünlü S. Co-existence of multiple resistance mechanisms in clinical isolates of carbapenem-resistant Pseudomonas aeruginosa. FLORA 2022;27(1):45-54.*

ABSTRACT

Introduction: *Multidrug resistance phenotype of Pseudomonas aeruginosa utilizes several resistant mechanisms to overcome the action of antibiotics. This phenotype is caused by several resistance mechanisms or a combination of thereof. This study aimed to evaluate various resistance mechanisms by phenotypic methods.*

Materials and Methods: *Carbapenem-resistant P. aeruginosa were included in this study. Antimicrobial resistance mechanisms such as efflux pump activity, reduced outer membrane permeability (OMP), various β-lactamase activities, and biofilm formation ability of clinical P. aeruginosa isolates were determined by phenotypic methods.*

Results: *Of the P. aeruginosa isolates, 33.7% (n= 33/98) had a positive efflux pump activity. The co-existence of positive efflux pump activity and Metallo β-lactamase (MBL) production was detected in 30.3% (10/33) of the isolates. In 34.7% of the clinical P. aeruginosa isolates, reduced OMP was detected and 70.6% of them were also biofilm producers. Totally 21.4% (21/98) of P. aeruginosa isolates were evaluated as extended-spectrum beta-lactamase (ESBL) positive. AmpC β-lactamase was detected in 15.3% (n= 15/98) of the clinical P. aeruginosa isolates. MBL activity was detected in 33.7% (n= 33/98) of the clinical P. aeruginosa isolates. Of the MBL-positive isolates, 69.7% were biofilm producers. The co-existence of MBL and reduced OMP was detected in 36.4% (n= 12/33).*

Conclusion: *High resistance of P. aeruginosa was attributed to several resistance mechanisms or a combination of thereof. This infections caused by multidrug-resistant (MDR) P. aeruginosa are difficult to treat due to the co-existence of different resistance mechanisms.*

Key Words: *P. aeruginosa; Biofilm; Antimicrobial resistance mechanisms; Carbapenem resistance*

Received/Geliş Tarihi: 10/09/2021 - Accepted/Kabul Ediliş Tarihi: 24/11/2021

Available Online Date: 24.03.2022

ÖZ

Karbapenem Dirençli Klinik *Pseudomonas aeruginosa* İzolatlarında Çoklu Direnç Mekanizmalarının Birlikte Varlığı

Aylin ÜSKÜDAR-GÜÇLÜ, Hasan Cenk MİRZA, Sezin ÜNLÜ

Başkent Üniversitesi Tıp Fakültesi, Tıbbi Mikrobiyoloji Anabilim Dalı, Ankara, Türkiye

Giriş: *Antibiyotiklerin etkisinden kaçabilmek için Pseudomonas aeruginosa suşları birçok direnç mekanizmasından yararlanır. Çok ilaca dirençli suşlar, farklı direnç mekanizmalarını veya bunların kombinasyonunu kullanır. Bu çalışmada, karbapenem dirençli izolatlarda çeşitli direnç mekanizmalarının fenotipik yöntemlerle değerlendirilmesi amaçlanmıştır.*

Materyal ve Metod: *Karbapenem dirençli P. aeruginosa izolatları bu çalışmaya dahil edilmiştir. Klinik P. aeruginosa izolatlarının efluks pompası aktivitesi, dış membran geçirgenliği, çeşitli β-laktamaz aktiviteleri gibi antimikrobiyal direnç mekanizmaları ve biyofilm oluşturma yetenekleri fenotipik yöntemlerle araştırılmıştır.*

Bulgular: *Efluks pompası aktivitesi, P. aeruginosa izolatlarının %33.7'sinde (n= 33/98) saptanmıştır. İzolatların %30.3'ünde (10/33) efluks pompası aktivitesi ve metallo β-laktamaz (MBL) üretiminin birlikteliği tespit edilmiştir. Dış membran geçirgeniğinin azalması klinik P. aeruginosa izolatlarının %34.7'sinde saptanmıştır ve bunların %70.6'sı biyofilm oluşturmaktadır. P. aeruginosa izolatlarının %21.4'ü (n= 21/98) genişlemiş spektrumlu beta laktamaz (GSBL) pozitif olarak saptanmıştır. Klinik P. aeruginosa izolatlarının %15.3'ünde (n= 15/98) AmpC β-laktamaz varlığı saptanmıştır. Klinik P. aeruginosa izolatlarının %33.7'sinde (n= 33/98) MBL aktivitesi saptanmıştır. MBL aktivitesi pozitif izolatların %69.7'si biyofilm oluşturmaktadır. İzolatların %36.4'ünde (n= 12/33) MBL ve azalmış dış membran geçirgenliği birlikteliği saptanmıştır.*

Sonuç: *P. aeruginosa izolatlarındaki karbapenem direncinin, aynı anda çeşitli direnç mekanizmalarının birlikte varlığıyla sağlandığı görülmüştür. Farklı direnç mekanizmalarının aynı anda aktif olmasıyla, çok ilaca dirençli P. aeruginosa'nın neden olduğu infeksiyonların tedavisi güçleşmektedir.*

Anahtar Kelimeler: *P. aeruginosa; Biofilm; Antibiyotik direnç mekanizmaları; Karbapenem direnci*

INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous gram-negative opportunistic pathogen[1,2] that is mostly associated with nosocomial infections[3]. Infections caused by *P. aeruginosa* are associated with significant morbidity and mortality rates[1,3,4]. Eradication of *P. aeruginosa* has become increasingly difficult due to its remarkable capacity of both intrinsic and acquired resistance to antibiotics[1,2,5]. *P. aeruginosa* displays resistance to a variety of antibiotics, including aminoglycosides, quinolones and β-lactams[1,5,6]. Such isolates are mostly referred to as multidrug-resistant (MDR) as described by the European Centre for Disease Prevention and Control (ECDC). According to the European Antimicrobial Resistance Surveillance Network (EARS-Net) report in 2019, the rate of *P. aeruginosa* isolates with combined resistance (resistance to >3 antimicrobial groups, including carbapenems) was 12.1%, and carbapenem resistance was 16.5%[7].

Carbapenems have been used as the last resort of antimicrobials in treating severe infections caused by gram-negative bacteria. However, the clinical use of carbapenem is in danger with the emergence of the high rate of resistance, and carbapenem-resistant *P. aeruginosa* isolates are often resistant to multiple antibiotics[8]. According to the surveys, the rate of carbapenem non-susceptible *P. aeruginosa* strains in Turkey increased from 33.3% in 2011[9] to 44.0% in 2018.

Major antibiotic resistance mechanisms of *P. aeruginosa* are classified into intrinsic, acquired and adaptive resistance. The expression of efflux pumps, reduced outer membrane permeability (OMP), and the production of antibiotic-inactivating enzymes are the intrinsic resistance mechanisms[1]. The acquired resistance of *P. aeruginosa*

can be achieved by either horizontal transfer of resistance genes or mutational changes[10]. The adaptive resistance of *P. aeruginosa* involves the formation of biofilm that limit antibiotic access to the pathogen[11]. Different carbapenem resistance mechanisms have been defined in *P. aeruginosa* so far. These mechanisms include inducible chromosomal AmpC[12], reduced drug uptake due to mutation or lost outer membrane porins, increased drug efflux due to over-expression of efflux pumps, and acquisition of carbapenem-hydrolyzing enzymes[1].

Since antibiotic resistance in *P. aeruginosa* involves the collaboration of restricted uptake through the outer membrane and secondary resistance mechanisms such as efflux and β-lactamase(s), this study aimed to evaluate the resistance mechanisms, including efflux pump activity, outer membrane permeability, various β-lactamase activities, as well as biofilm formation ability of clinical *P. aeruginosa* isolates by phenotypic methods.

MATERIALS and METHODS

This study was approved by Başkent University Institutional Review Board (Project no: KA21/321).

Isolate profile

Previously identified 98 carbapenem resistant clinical *P. aeruginosa* isolates from various clinical samples were included in the study[13].

Detection of Efflux Pump Activity

Ethidium bromide (EtBr) agar cartwheel method was used to detect overexpressed efflux system of tested isolates[14]. Briefly, Trypticase Soy Agar (TSA) (Orlab, Turkey) plates containing EtBr (AppliChem GmbH, Germany) concentrations ranging from 0.0 to 2.5 mg/L were prepared on the same day of the experiment and kept protected from light. Overnight cultures of the bacterial isolates were adjusted to 0.5 McFarland turbidity. Bacterial suspensions were swabbed on the EtBr-TSA plates from the center to the margin of the plate by dividing TSA plates into 6-8 sectors by radial lines that form the cartwheel pattern. The plates were then incubated for 16 hours at 37°C and were examined under a U.V. transilluminator; the minimum concentration of EtBr producing fluorescence of the bacterial mass was recorded, and strains that showed lower fluorescence than the control were defined as positive efflux pump activity (Figure 1A).

Detection of Reduced Outer Membrane Permeability (OMP)

Evaluation of OMP was performed by determining minimum inhibitory concentration (MIC) values for imipenem (IPM) in the presence and absence of EDTA; a permeabilizer that chelates divalent cations that stabilize molecular interactions in the OM, causing disruption of OMP[15]. The MICs of IPM and IPM+EDTA were determined by broth micro-dilution according to CLSI guidelines[16]. Two-fold serial dilutions of IPM (Oxoid, UK) and IPM+EDTA were prepared in 96-well microtiter plates in a final volume of 100 µL per well. Each well was inoculated with 100 µL of the previously prepared bacterial suspension and incubated at 37°C for 18-20 h. In order to eliminate EDTA effect, it was used at a concentration of 1/4 MIC. A four-fold reduction in the IPM MIC or more in the presence of EDTA indicates OM reduced permeability activity.

Detection of β-lactamases

Extended-spectrum β-lactamase (ESBL) production was detected by the combination disk test (CDT) and the double-disk synergy test (DDST) as described by EUCAST[17]. The inhibition zone around ceftazidime and cefotaxime disks combined with clavulanic acid was compared with the zone around the disks with ceftazidime and cefotaxime alone in CDT method. If diameter of inhibition zone for one of the combination disks was ≥5 mm larger than that of corresponding cephalosporin disk, the test was evaluated as positive. In DDST, disks containing cefotaxime, ceftazidime, and cefepime are applied to plates next to a disk with amoxicillin-clavulanic acid. If the inhibition zones around any of the cephalosporin disks were augmented, or a 'keyhole effect' was observed in the direction of the disk containing clavulanic acid, the test was considered as positive.

Detection of AmpC phenotype was performed by the AmpC Disk Test (ADT)[18]. The AmpC disks were prepared by applying 20 µl of a

1:1 mixture of saline and 100 µl Tris-EDTA to sterile blank disks, allowing the disks to dry. The disks were stored at 2-8°C. The surface of the Mueller-Hinton agar (MHA) plate was inoculated with *E. coli* ATCC 25922 suspension. AmpC disks were rehydrated with 20 µl of saline before use, and several colonies of each test organism were applied to the disk. A cefoxitin (FOX) disk (Oxoid, UK) was placed on the inoculated surface of the MHA. The AmpC disks inoculated with tested bacterial suspension were placed almost touching the FOX disk with the inoculated disks face in contact with the agar surface. After overnight incubation at 37°C, plates were examined for either an indentation or a flattening of the zone of inhibition, indicating enzymatic inactivation of FOX (positive result), or the absence of distortion, indicating no significant inactivation of FOX (negative result).

Detection of AmpC phenotype was also confirmed by Three Dimensional Tests with slight modifications[19]. An overnight culture suspension of the organism *E. coli* ATCC 25922 was adjusted to 0.5 McFarland standard and inoculated on MHA. A 30 µg FOX disc was placed at the center. A linear slit of 3 cm length was cut for each isolate with the help of a pipette tip to form wells. The 20 ul of tested isolates adjusted to 0.5 McFarland were added to each well on MHA. The plates were incubated overnight. The presence of a distorted inhibition zone was interpreted as a positive result for AmpC production.

In order to detect MBL activity of tested isolates, combined disk synergy test (CDST) and double-disk synergy test (DDST) with 0.5 M EDTA were performed. For CDST, two IPM (10 µg) disks were placed 30 mm apart from center to center on the surface of Mueller Hinton agar (MHA) plate, and 10 µl 0.5 M EDTA solution was added to one of them[20]. In CDST, if the zone of inhibition of the IPM-EDTA disk was ≥7 mm more than that of the IPM disk alone, it was considered MBL positive. In the DDST assay, an IPM (10 µg) disk was placed 20 mm apart from a blank disk containing 10 µl of 0.5 M EDTA (750 µg)[3,8]. Enhancement of zone of inhibition between IPM and EDTA disk was considered positive MBL activity[21,22] (Figure 1B).

Biofilm Formation Assay

Biofilm formation was assayed in vitro by the method as described by Peeters et al. using a microtiter plate assay[23]. Flat-bottom polystyrene microtiter plates containing 180 µl of Tryptic soy broth (TSB) (Condalab, Spain) were inoculated with 20 µl of bacterial culture adjusted ~1x10^6 CFU/ml. Biofilm formation was quantified by the crystal violet staining method after the incubation period. The supernatant was removed, and the wells were washed thrice with 200 µl phosphate saline buffer (PBS, pH 7.2). By adding 200 µl of 95% methanol into each well for 20 minutes, the biofilms were fixed, and then the supernatant was removed. Then 2% crystal violet solution was added to each well, and 20 min later, the excess dye was removed by washing with PBS three times, and then wells were air-dried. Finally, 200 µl of 95% ethanol was added to wells, followed by incubation for 10 min at room tem-

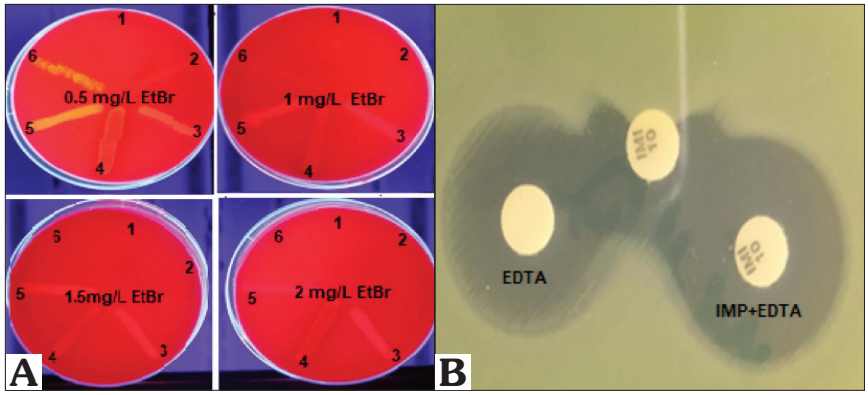

Figure 1. Determination of **(A)** efflux pump activity and **(B)** MBL activities by phenotypic methods. Numbers indicate the tested strains.

Table 1. Percentage of resistance mechanisms and MIC_{50} and MIC_{90} values of meropenem in *P. aeruginosa*

			Meropenem	
Resistance mechanisms	**n**	**%**	**MIC_{50} (µg/ml)**	**MIC_{90} (µg/ml)**
MBL	33	33.6	>32	>32
Efflux Pump Activity	33	33.6	>32	>32
Reduced OMP	34	34.6	>32	>32
ESBL	21	21.4	32	>32
AmpC	15	15.3	>32	>32
Biofilm	75	76.5	>32	>32

perature. The absorbance was measured at 570 nm (BioTek Instruments, ELX 800, USA), and strains were evaluated as negative, weak, intermediate and strong biofilm former[24]. *P. aeruginosa* ATCC 15442 was used as positive control, and *P. aeruginosa* ATCC 27853 as negative control for biofilm formation.

RESULTS

Totally, 98 clinical isolates were included in the study. These isolates were previously confirmed as carbapenem-resistant[13]. Most of them were isolated from trans tracheal aspiration (42.9%), blood culture (20.4%) and wound (10.2%), followed by sterile body side (7.1%), bronchoalveolar lavage (8.2%), sputum (5.1%), and urine (6.1%). Minimum Inhibitory Concentration (MIC) of meropenem varied between 8 to over 32 µg/mL. In 68 isolates (69.4%), MIC was 32 or over 32 µg/mL. MIC was found 16 µg/mL in 20 isolates (20.4%) and 8 µg/mL in 10 isolates (%10.2). 58.8%, 66.7% 69.7% of the isolates having a MIC value of over 32 µg/mL had reduced OMP, efflux pump activity and MBL production, respectively.

Efflux Pump Activity

S. aureus ATCC 29213 was used for efflux pump activity control, and it fluoresced at concentrations of 0.5 mg/L and 1 mg/L of EtBr and did not fluoresce at concentrations higher than 1 mg/L. 33.7% (n= 33/98) of the *P. aeruginosa* isolates showing fluorescence at lower EtBr concentration than the control has a positive efflux pump activity (Table 1). 78.8% (n= 26/33) of them were able to form biofilm. In 42.4% (n= 14/33) of the isolates, positive efflux pump activity was accompanied by reduced OMP. The co-existence of positive efflux pump activity and MBL production was detected in 24.2% (8/33) of the isolates (Table 2).

Reduced Outer Membrane Permeability (OMP)

In 34.7% (n= 34/98) of the isolates, reduced OMP was detected (Table 1) and 70.6% of them were also biofilm producers. Reduced OMP activity and MBL production was detected together in 35.3% (n= 12/34) of the isolates (Table 2).

β-lactamase Activities

ESBL activity was detected in 21 clinical (21.4%) *P. aeruginosa* isolates. AmpC β-lactamase was detected in 15.3% (n= 15/98) of the clinical *P. aeruginosa* isolates. MBL activity was detected in 33.7% (n= 33/98) of the clinical *P. aeruginosa* (Table 1). Out of 33 MBL producer isolates, 12 of them were accompanied by reduced OMP (Table 2). The co-existence of MBL production and reduced OMP activity was accompanied by efflux pump activity in four isolates.

Biofilm Formation Assay

Out of the 98 clinical *P. aeruginosa* isolates, 75 (76.5%) formed biofilm, of which 17 (22.6%) were strong, 19 were intermediate (25.3%), and 39 (52%) were weak biofilm producers. Biofilm formation in relation to other resistance mechanisms was also evaluated. Among the biofilm-forming isolates, 29.3% showed MBL activity, 35.6% had efflux pump activity, 23.3% had ESBL activity, 31.5% had reduced OMP, and 19.2% had AmpC activity (Figure 2).

Table 2. Co-existence of resistance mechanisms in *P. aeruginosa* isolates

Resistance mechanisms		n	%
MBL (n= 33)	Only MBL	4	12.1
	Efflux	8	24.2
	Reduced OMP	12	36.4
	ESBL	3	9.1
	AmpC	6	18.2
	Biofilm	23	69.7
Efflux Pump Activity (n= 33)	Only Efflux	3	9.1
	MBL	8	24.2
	Reduced OMP	14	42.4
	ESBL	10	30.3
	AmpC	5	15.2
	Biofilm	26	78.8
Reduced OMP (n= 34)	Only reduced OMP	4	11.8
	MBL	12	35.2
	Efflux	14	41.1
	ESBL	10	29.4
	AmpC	4	11.8
	Biofilm	24	70.6

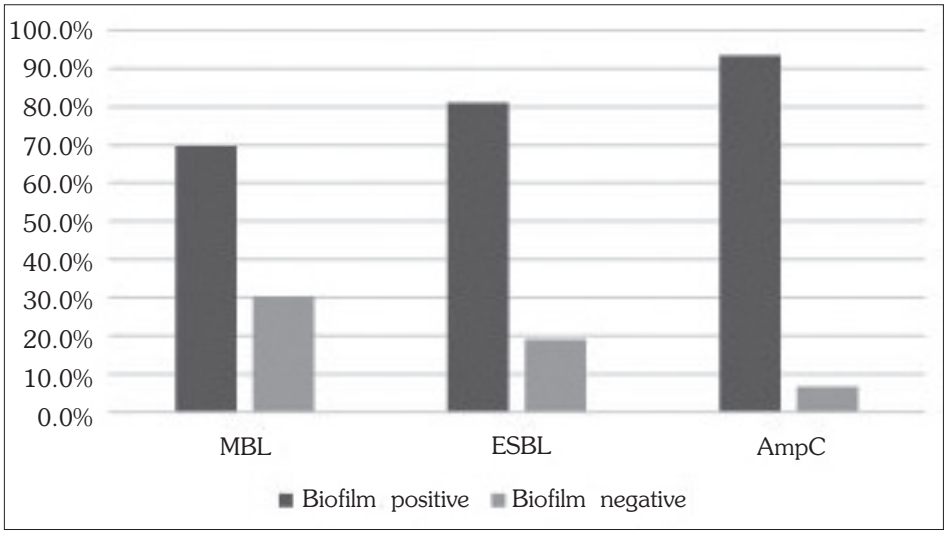

Figure 2. Activities of MBL, ESBL and AmpC among biofilm positive and negative *P. aeruginosa* isolates.

DISCUSSION

Carbapenems are used as the last resort of antibiotics against infections caused by multi-resistant *P. aeruginosa*[25] since they have exceptional stability even in response to extended-spectrum and AmpC β-lactamases. However, the collaboration of reduced outer membrane permeability, and secondary resistance mechanisms such as efflux pumps activity and β-lactamase(s) resulted in[1] significantly high antibiotic resistance in *P. aeruginosa*.

Overproduction of efflux pump activity is one of the most critical resistance mechanisms against carbapenems in *P. aeruginosa*[26]. A previous study has demonstrated that overexpression of efflux pump activity was detected in 56.1% of the *P. aeruginosa* isolates and significantly correlated with carbapenem resistance[27]. Similar result has been obtained from the study conducted in Turkey with 951 *P. aeruginosa* isolates, and it has been reported that 47.6% of the isolates have overproduction of efflux pump activity[28]. In the current study, 33.7% of (n= 33/98) the clinical isolates were found to have efflux pump activity and other resistance mechanisms were found to accompany efflux pump activity. For instance, 42.4% (n= 14/33) of the isolates showing efflux pump activity harbored a second resistance mechanism; reduced OMP (Table 2). In addition to these two resistance mechanisms, MBL activity was detected as a third resistance mechanism in 12.1% of (n= 4/33) the isolates showing efflux pump activity. The co-existence of efflux pump activity, MBL and reduced OMP was found in 4 isolates.

Since *P. aeruginosa* has been shown to possess reduced OMP as an intrinsic resistance mechanism, the current study also evaluated reduced OMP activity in our clinical isolates. 34.7% of the isolates (n= 34/98) showed reduced OMP activity, and 70.6% (n= 24/34) of them were biofilm producers. The co-existence of reduced OMP and ESBL production was found in 29.4% (n= 10/34) of the isolates (Table 2), and efflux pump activity accompanied 50.0% (n= 5/10) of the isolates as a third resistance mechanism.

Several mechanisms involved in the carbapenem resistance of *P. aeruginosa* includes ESBLs. They are a group of β-lactamases that inactive β-lactams. Since they are encoded on plasmids, they can be easily transferred between strains[29]. A study conducted in Poland has demonstrated that out of 720 clinical *P. aeruginosa* isolates, 110 (%15) detected as ESBL positive by phenotypic tests[30]. In the current study, ESBL production was detected in 21.4% of the isolates. It is known that ESBLs and AmpC are able to contribute resistance to carbapenems when they are combined with porin mutations[31]. In this study, the co-existence of ESBL and AmpC was detected in 5 isolates, and one of them was combined with reduced OMP.

Literature has suggested that solely AmpC-β-lactamase does not contribute to carbapenem-resistance; however, it could definitely increase MICs of β-lactam antibiotics including carbapenems and usually coexisted with another mechanism specially reduced OMP[32,33]. Chika et al. have demonstrated that 36% of tested *P. aeruginosa* isolates were AmpC β-lactamase producers[34]. In this study, 15.3% (n= 15/98) of the clinical *P. aeruginosa* isolate was found to be AmpC β-lactamase producer, and 26.7% (n= 4/15) of them had reduced OMP.

MBL enzyme production from *P. aeruginosa* isolates is one of the most critical resistance mechanisms against carbapenems. MBL genes can be transferred between strains easily since they are located on the chromosome or plasmid[35]. Antimicrobial treatment of severe infections is complicated due to the spread of MBL genes. Even though a previous study has demonstrated significantly high MBL activities, 94.2% of the isolates have been found to have MBL activity by phenotypic methods; the genotypic method have not detected MBL genes[34]. Differences in sensitivities of phenotypic tests can cause these differences. In this study, 33.7% (n= 33/98) of the studied isolates were MBL producers. This rate showed a correlation with other studies that used phenotypic methods for the detection of MBL production. Abbas et al. have reported that 40% of the studied isolates were MBL-positive[15]. Also, a previous study conducted in Egypt has demonstrated that 27% of *P. aeruginosa* isolates were MBL producers[36]. In the current study, 69.7% of MBL-positive isolates were biofilm producers. The co-existence of MBL and reduced OMP was detected in 36.4% (n= 12/33) of the isolates.

An important part of the pathogenesis of *P. aeruginosa* includes biofilm formation. The efficacy of antimicrobials is reduced in isolates that are able to form biofilm. Biofilm forming isolates become more resistant to immune responses since biofilm provide a barrier against

phagocytosis by host immune cells[37,38]. The significantly high number of *P. aeruginosa* included in this study showed biofilm formation with 76.5%. Several studies demonstrated the relation between biofilm formation and the presence of different types of β-lactamases. Chakraborty et al. have demonstrated that *P. aeruginosa* isolates with MBL activity have a strong biofilm-forming ability[39]. This result is in concordance with the current study that demonstrates that 69.7% (n= 23/33) of the MBL producer isolates formed biofilm. Also, 78.8% (n= 26/33) of the isolates showing efflux pump activity were found to have the ability to form biofilm.

CONCLUSION

In conclusion, we believe that results obtained from the current study are significant since these resistance mechanisms overlap, and infections caused by MDR *P. aeruginosa* are challenging to treat.

ACKNOWLEDGEMENT

We would like to thank Ilgin Ata-Vural for her technical assistance.

ETHICS COMMITTEE APPROVAL

This study was approved by Başkent University Institutional Review Board (Project no: KA21/321).

CONFLICT of INTEREST

No conflicts of interest to be declared concerning the publication of this article.

AUTHORSHIP CONTRIBUTIONS

Concept and Design: AÜG

Data Collection or Processing: AÜG, SÜ

Analysis/Interpretation: All of authors

Literature Search: AÜG, HCM

Writing: All of authors

Final Approval: AÜG

REFERENCES

1. *Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnol Adv 2019;37(1):177-92. https://doi.org/10.1016/j.biotechadv.2018.11.013*
2. *Eser O. In: Us AD, Basustaoglu A. (eds). Pseudomonas ve İlişkili Bakteriler. Tıbbi mikrobiyoloji. 7th ed. Ankara: Pelikan Kitabevi; 2013, p.288-95.*
3. *Bassetti M, Vena A, Croxatto A, Righi E, Guery B. How to manage Pseudomonas aeruginosa infections. Drug Context 2018;7:212527. https://doi.org/10.7573/dic.212527*
4. *Barbier F, Andremont A, Wolff M, Bouadma L. Hospital-acquired pneumonia and ventilator-associated pneumonia: recent advances in epidemiology and management. Curr Opin Pulm Med 2013;19:216-28. https://doi.org/10.1097/MCP.0b013e32835f27be*
5. *Lambert PA. Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. J R Soc Med 2002;95(Suppl. 41):22-6.*
6. *Hancock RE, Speert DP. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and impact on treatment. Drug Resist Updat 2000;3:247-55. https://doi.org/10.1054/drup.2000.0152*
7. *European Antimicrobial Resistance Surveillance Network (EARS-Net) Report. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/surveillance-antimicrobial-resistance-Europe-2019.pdf*
8. *Theuretzbacher U. Global antibacterial resistance: The never-ending story. J Glob Antimicrob Resist 2013;1(2):63-9. https://doi.org/10.1016/j.jgar.2013.03.010*
9. *Ulusal Antimikrobiyal Direnç Sürveyans Sistemi Dış Kalite Güvencesi Birinci Yeterlilik Değerlendirmesi Çalışması Raporu. Available from: https://hsgm.saglik.gov.tr/depo/birimler/Mikrobiyoloji_Referans_Laboratuvarlari_ve_Biyolojik_Urunler_DB/uamdss/dkd_raporlar/UAMDSS_2011_yili_DKD_Raporu.pdf?type=file*
10. *Breidenstein EB, de la Fuente Núñez C, Hancock RE. Pseudomonas aeruginosa: all roads lead to resistance. Trends Microbiol 2011;19(8):419-26. https://doi.org/10.1016/j.tim.2011.04.005*
11. *Drenkard E. Antimicrobial resistance of Pseudomonas aeruginosa biofilms. Microbes Infect 2003;5(13):1213-9. https://doi.org/10.1016/j.micinf.2003.08.009*
12. *Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 2009;22(4):582-610. https://doi.org/10.1128/CMR.00040-09*
13. *Mirza HC, Hortaç E, Koçak AA, Demirkaya MH, Yayla B, Güçlü AÜ, Başustaoğlu A. In vitro activity of ceftolozane-tazobactam and ceftazidime-avibactam against clinical isolates of meropenem-non-susceptible Pseudomonas aeruginosa: A two-centre study. J Glob Antimicrob Resist 2020;20:334-8. https://doi.org/10.1016/j.jgar.2019.09.016*
14. *Martins M, McCusker MP, Viveiros M, Couto I, Fanning S, Pagès JM, et al. A simple method for assessment of MDR bacteria for over-expressed efflux pumps. Open Microbiol J 2013;7:72. https://doi.org/10.2174/1874285801307010072*

15. *Abbas HA, El Ganiny AM, Kamel HA. Phenotypic and genotypic detection of antibiotic resistance of Pseudomonas aeruginosa isolated from urinary tract infections. Afr Health Sci 2018;18(1):11-21. https://doi.org/10.4314/ahs.v18i1.3*
16. *CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed CLSI supplement M100. Clinical and Laboratory Standards Institute: 2021.*
17. *EUCAST. European Committee on Antimicrobial Susceptibility Testing guidelines for detection of resistance mechanisms and specific resistances for clinical and/or epidemiological importance Version 2.0; 2017.*
18. *Black JA, Smith Moland E, Thomson KS. AmpC disk test for detection of plasmid-mediated AmpC β-Lactamases in Enterobacteriaceae lacking chromosomal AmpC β-Lactamases. J Clin Microbiol 2005;43:3110-3. https://doi.org/10.1128/JCM.43.7.3110-3113.2005*
19. *Maraskolhe DL, Deotale VS, Mendiratta DK, Narang P. Comparision of three laboratory tests for detection of AmpC β Lactamases in Klebsiella species and E. Coli. J Clin Diagn Res 2014;8(6):DC05-DC8. https://doi.org/10.7860/JCDR/2014/8256.4432*
20. *Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-β-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol 2002;40:3798-801. https://doi.org/10.1128/JCM.40.10.3798-3801.2002*
21. *Hemalatha V, Sekar U, Kamat V. Detection of metallo beta-lactamase producing Pseudomonas aeruginosa in hospitalized patients. Indian J Med Res 2005;122:148-52.*
22. *Pandya N, Prajapati S, Mehta S, Kikani K, Joshi P. Evaluation of various methods for detection of metallo-β-lactamase (MBL) production in Gram-negative Bacilli. Int J Biol Med Res 2011;2:775-7*
23. *Peeters E, Nelis HJ, Coenye T. Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. J Microbiol Methods 2008;72:157-65. https://doi.org/10.1016/j.mimet.2007.11.010*
24. *Stepanović S, Vuković D, Hola V, Bonaventura GD, Djukić, S, Ćirković I, Ruzicka F. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. Apmis 2007;115(8):891-9. https://doi.org/10.1111/j.1600-0463.2007.apm_630.x*
25. *Meletis G, Exindari M, Vavatsi N, Sofianou D, Diza E. Mechanisms responsible for the emergence of carbapenem resistance in Pseudomonas aeruginosa. Hippokratia 2012;16(4):303-7.*
26. *Rostami S, Farajzadeh Sheikh A, Shoja S, Farahani A, Tabatabaiefar MA, Jolodar A, et al. Investigating of four main carbapenem-resistance mechanisms in high-level carbapenem resistant Pseudomonas aeruginosa isolated from burn patients. J Chin Med Assoc 2018;81(2):127-32. https://doi.org/10.1016/j.jcma.2017.08.016*
27. *Lee JY, Ko KS. OprD mutations and inactivation, expression of efflux pumps and AmpC, and metallo-β-lactamases in carbapenem-resistant Pseudomonas aeruginosa isolates from South Korea. Int J Antimicrob Agents 2012;40(2):168-72. https://doi.org/10.1016/j.ijantimicag.2012.04.004*
28. *Muderris T, Durmaz R, Ozdem B, Dal T, Unaldı O, Aydogan S, et al. Role of efflux pump and OprD porin expression in carbapenem resistance of Pseudomonas aeruginosa clinical isolates. J Infect Dev Ctries 2018;12(1):1-8. https://doi.org/10.3855/jidc.9486*
29. *Laudy AE, Róg P, Smolińska Król K, Ćmiel M, Słoczyńska A, Patzer J, et al. Prevalence of ESBL-producing Pseudomonas aeruginosa isolates in Warsaw, Poland, detected by various phenotypic and genotypic methods. PLoS One 2017;12(6):e0180121. https://doi.org/10.1371/journal.pone.0180121*
30. *Laudy AE, Róg P, Smolińska Król K, Ćmiel M, Słoczyńska A, Patzer J, et al. Prevalence of ESBL-producing Pseudomonas aeruginosa isolates in Warsaw, Poland, detected by various phenotypic and genotypic methods. PLoS One 2017;12(6):e0180121. https://doi.org/10.1371/journal.pone.0180121*
31. *Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant enterobacteriaceae: the impact and evolution of a global menace. J Infect Dis 2017;215(Suppl. 1):S28-S36. https://doi.org/10.1093/infdis/jiw282*
32. *Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 2009;22(4):582-610. https://doi.org/10.1128/CMR.00040-09*
33. *Mirsalehian A, Kalantar Neyestanaki D, Nourijelyani K, Asadollahi K, Taherikalani M, Emaneini M, et al. Detection of AmpC-β-lactamases producing isolates among carbapenem resistant P. aeruginosa isolated from burn patient. Iran J Microbiol 2014;6(5):306-10.*
34. *Chika E, Charles E, Ifeanyichukwu I, Chigozie U, Carissa D, Michael A. Phenotypic detection of AmpC beta-lactamase among anal Pseudomonasaeruginosa isolates in a Nigerian Abattoir. Arch Clin Microbiol 2016;7.*
35. *Aksoy MD, Tugrul HM. Investigation of metallo-beta-lactamases in carbapenem resistant Pseudomonas aeruginosa strains by phenotypic and genotypic methods. Flora 2020; 25(3):301-7. https://doi.org/10.5578/flora.68921*
36. *Arunagiri K, Sekar B, Sangeetha G, John J. Detection and characterization of metallo-beta-lactamases in Pseudomonas aeruginosa by phenotypic and molecular methods from clinical samples in a tertiary care hospital. West Indian Med J 2012;61(8):778-83.*
37. *Colvin KM, Gordon VD, Murakami K, Borlee BR, Wozniak DJ, Wong GC, Parsek MR. The pel polysaccharide can serve a structural and protective role in the biofilm matrix of Pseudomonas aeruginosa. PLoS Pathog 2011;7:e1001264. https://doi.org/10.1371/journal.ppat.1001264*

38. *Billings N, Millan M, Caldara M, Rusconi R, Tarasova Y, Stocker R, Ribbeck K. The extracellular matrix Component Psl provides fast-acting antibiotic defense in Pseudomonas aeruginosa biofilms. PLoS Pathog 2013;9:e1003526. https://doi.org/10.1371/journal.ppat.1003526*

39. *Chakraborty D, Basu S, Chatterjee P, Dey SK, Das S. Concurrent determination of collagenase and biofilm formation activities in Metallo-beta-lactamase producing Pseudomonas aeruginosa. Int Microbiol Res 2011;2(3):208-12.*

Address for Correspondence/Yazışma Adresi

Dr. Aylin ÜSKÜDAR-GÜÇLÜ

Başkent University Faculty of Medicine, Department of Medical Microbiology, Ankara-Turkey

E-posta: uskudaraylin@gmail.com