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Resistance Genes and Molecular Epidemiology in Ceftazidime-Avibactam-Resistant *Pseudomonas aeruginosa* Isolates

Seftazidim-Avibaktam Dirençli *Pseudomonas aeruginosa* İzolatlarında Direnç Genleri ve Moleküler Epidemiyoloji

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Abstract

Introduction: Increasing ceftazidime-avibactam (CZA) resistance in *P. aeruginosa* isolates is of serious concern worldwide. Therefore, studies on the underlying resistance mechanisms gain even more importance. The aim of this study was to investigate the presence of beta-lactamase genes reported to be associated with resistance, including metallo-beta-lactamases (MBL), in CZA-resistant *P. aeruginosa* strains and the clonal relationship between isolates.

Materials and Methods: Ceftazidime-avibactam resistant *P. aeruginosa* strains isolated from various clinical specimens between December 2021 and March 2023 were included in this study. MALDI-TOF (VITEK-MS, bioMérieux, France) was used for the identification of strains. Ceftazidime-avibactam susceptibility was determined by disc diffusion method and resistant strains were also studied by gradient diffusion method (MIC strip, Liofilchem, Italy). Antibiotic susceptibility results were evaluated according to the European Committee for Antibiotic Susceptibility Testing criteria. *blaKPC, blaNDM, blaIMP, blaVIM, blaOXA-48, blaOXA-2, blaOXA-10, blaGES, blaPER, blaVEB* genes were determined using polimerase chain reaction, and clonal relationship was investigated by pulsed field gel electrophoresis.

Results: A total of 38 CZA resistant *P. aeruginosa* strains were analyzed. The MIC50 and MIC90 values determined by the gradient diffusion method for CZA were ≥256 mg/l and ≥256 mg/l, respectively; the MIC range was found to be 32-256 mg/l. Among of 38 isolates, 11 (28.9%) isolates including 10 *blaVIM* (26.3%) and one *blaIMP* (2.6%) were positive for the MBL genes. The *blaOXA-10* gene was found to be co-positive in seven of the *blaVIM* positive isolates and in one *blaIMP* positive isolate. In addition, *blaOXA-10* alone was found in nine (23.6%) of the isolates, *blaPER* in two (5.2%) and *blaGES* in one (2.6%). No genes were found in 15 (39.4%) isolates. A total of 15 different genotypes consisting of eight different clusters were identified.

Conclusion: It was determined that MBL production, especially *blaVIM*, was the most common cause of CZA resistance. *blaOXA-10, blaPER* and *blaGES* positivity were also found remarkable. Regarding molecular epidemiology, it was observed that the strains isolated predominantly from ICU patients had different genotypes and exhibited a polyclonal diversity.

Keywords: *Pseudomonas aeruginosa*, ceftazidime-avibactam resistance, metallo-beta-lactamases, PFGE

Öz

Giriş: *P. aeruginosa* izolatlarındaki artan seftazidim-avibaktam (CZA) direnci, dünya genelinde ciddi endişe uyandırmaktadır. Bu yüzden, altta yatan direnç mekanizmaları ile ilgili çalışmalar daha da önem kazanmaktadır. Bu çalışmanın amacı, CZA dirençli *P. aeruginosa* suşlarında metallo-beta-laktamazlar (MBL) dahil, dirençle ilişkili olduğu bildirilmiş beta-laktamaz genlerinin varlığının ve izolatlar arası klonal ilişkinin araştırılmasıdır.

Gereç ve Yöntem: Bu çalışmaya Aralık 2021-Mart 2023 tarihleri arasında, çeşitli klinik örneklerden izole edilmiş, CZA dirençli *P. aeruginosa* suşları dahil edildi. Suşların identifikasyonunda MALDI-TOF (VITEK-MS, bioMérieux, Fransa) kullanıldı. Seftazidim-avibaktam duyarlılığı disk difüzyon yöntemi ile belirlenerek, dirençli bulunan suşlar ayrıca gradiyent difüzyon yöntemi (MIC strip, Liofilchem, İtalya) ile çalışıldı. Antibiyotik duyarlılık

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Öz

sonuçları, Avrupa Antibiyotik Duyarlılık Testi Komitesi kriterlerine göre değerlendirildi. *blaKPC, blaNDM, blaIMP, blaVIM, blaOXA-48, blaOXA-2, blaOXA-10, blaGES, blaPER, blaVEB* genleri polimeraz zincir reaksiyonu ile, izolatlar arası klonal ilişki pulsed field jel elektroforez yöntemi ile belirlendi.

Bulgular: Toplam 38 CZA dirençli *P. aeruginosa* suşu analiz edilmiştir. Seftazidim-avibaktam için gradiyent difüzyon yöntemi ile saptanan MİK50 ve MİK90 değerleri sırasıyla ≥256 mg/l ve ≥256 mg/l; MİK aralığı ise 32-256 mg/l olarak bulunmuştur. Otuz sekiz izolat arasında, 10'u *blaVIM* (%26,3) ve biri *blaIMP* (%2,6) olmak üzere 11 (%28,9) izolat MBL genleri için pozitif bulunmuştur. *blaVIM* pozitif izolatların yedisinde ve *blaIMP* pozitif bir izolatta *blaOXA-10* geni birlikte pozitif saptanmıştır. Ayrıca, izolatların dokuz tanesinde (%23,6) tek başına *blaOXA-10*, ikisinde (%5,2) *blaPER* ve birinde (%2,6) *blaGES* genleri tespit edilmiştir. On beş (%39,4) izolatta ise hiçbir gen bulunmamıştır. Pulsed field jel elektroforezine göre, sekiz farklı kümeden oluşan 15 farklı genotip tespit edilmiştir.

Sonuç: Çalışmamızda *blaVIM* başta olmak üzere MBL üretiminin CZA direncinin en sık nedeni olduğu saptanmıştır. Bunun dışında *blaOXA-10*, *blaPER* ve *blaGES* pozitifliği de dikkat çekici bulunmuştur. Moleküler epidemiyolojik incelemede, baskın olarak yoğun bakım ünitesi hastalarından izole edilen suşların farklı genotiplere sahip olduğu ve poliklonal bir çeşitlilik sergiledikleri gözlenmiştir.

Anahtar Kelimeler: *Pseudomonas aeruginosa*, seftazidim-avibaktam direnci, metallo-beta-laktamaz, PFGE

Introduction

P. aeruginosa, a gram-negative non-fermentative bacterium, is an important pathogen causing healthcare-associated infections such as bacteremia, pneumonia, and urinary tract infections in the intensive care unit (ICU)[1]. The fact that *P. aeruginosa* has an extraordinary capacity to acquire antimicrobial resistance and the rapid spread of its high-risk clones worldwide has placed it at the top of the list of important pathogens for which new antimicrobials should be developed, as reported by the World Health Organization[2-4]. Ceftazidime-avibactam (CZA), which was developed as an alternative and is also used in our country due to the increasing carbapenem resistance seen in *P. aeruginosa* isolates worldwide, consists of the combination of ceftazidime, a broad-spectrum cephalosporin, and avibactam, a non-beta-lactam beta-lactamase inhibitor. Since ceftolozane-tazobactam, which is reported to be more effective against AmpC-type beta-lactamases frequently seen in *P. aeruginosa* isolates, is not available in our country, CZA can be used as an alternative option if it is found susceptible by in vitro methods. When examined in terms of carbapenemases, while CZA is effective against class A (KPC) and class D (OXA-48) carbapenemases, it is not effective against class B metallo-beta-lactamases (MBL) (NDM, VIM, IMP) due to the absence of serine residues in the active site[5,6]. The *in vitro* activity initially observed for CZA around the world was soon replaced by rapidly progressing resistance reports[7-9]. This situation, which causes serious concern, has accelerated research on CZA resistance mechanisms[1,3,5,10,11]. These studies reported that, in addition to MBL production, *P. aeruginosa* can develop resistance to CZA in different ways. These are stated as enzymatic resistance causing inactivation of antibiotics, chemical modification of the antibiotic target or expression of an alternative target, and changes in cell permeability or expression of efflux pumps[5,6].

In addition to determining the underlying resistance mechanisms in CZA resistant isolates, it is also important to closely monitor the clonal relationship between isolates with molecular epidemiological studies in order to determine the spread within the hospital and take control measures[1,3].

In this study, several beta-lactamase genes reported to be associated with resistance (*blaKPC, blaOXA-48, blaOXA-10, blaOXA-2, blaPER, blaGES, blaVEB*), including MBLs (*blaVIM, blaIMP, blaNDM),* in CZA resistant *P. aeruginosa* isolates and whether the isolates were clonally related to each other were investigated. This study, which is the first to our knowledge on the molecular epidemiology of CZA-resistant *P. aeruginosa* in Turkey, aims to contribute to both local and global literature.

Materials and Methods

Bacterial Isolates

CZA resistant *P. aeruginosa* strains isolated from various clinical samples in the microbiology laboratory of our hospital between December 2021 and March 2023 were included in this study.

Bacterial Identification and Antibiotic Susceptibility Tests

Matrix-mediated laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF) (VITEK-MS, bioMérieux, France) was used to identify the strains, and VITEK 2 automated system (bioMérieux, France) was used to determine antibiotic susceptibility. Ceftazidime-avibactam susceptibility was determined by the standard disk diffusion method using 14 microgram disks (Bioanalyse, Turkey). Isolates with an inhibition zone diameter of ≥17 mm were considered susceptible, and those <17 mm were considered resistant. Strains found to be resistant to CZA were also studied with the gradient diffusion method (MIC strip, Liofilchem, Italy). Those with minimal inhibitory concentration (MIC) values ≤8 mg/l were considered susceptible, and those >8 mg/l were considered resistant.

Colistin susceptibility was studied with the commercial broth microdilution kit MIC-COL (Diagnostics I.n.c., Galanta, Slovakia). Antibiotic susceptibilities of the isolates were determined according to the European Committee on Antibiotic Susceptibility Testing (EUCAST) criteria[12].

Determination of Resistance Genes by Polymerase Chain Reaction (PCR) Method

DNA isolation was performed using the QIAamp DNA midi kit (Qiagen, Hilden, Germany) on the QIAsymphony automated DNA extraction system (Qiagen, Hilden, Germany). The DNA extracts were kept at -80 °C until use. *blaKPC, blaNDM, blaIMP*, *blaVIM*, and *blaOXA-48* genes were examined by multiplex PCR method as previously described by Poirel et al.[13]. The presence of *blaOXA-2, blaOXA-10, blaGES*, *blaPER*, and *blaVEB* genes was determined by multiplex PCR method as described by Kiratisin et al.[14]. DNA amplification was carried out by a thermocycler, GeneAmp PCR System 9700 (Applied Biosystems, Waltham, MA, USA).

The amplicons were electrophoresed for 1 h at 100V in 1.5% agarose gel and stained by ethidium bromide before their UV images were taken using a Kodak Gel Logic 200 (1708x1280, Kodak Company, Rochester, USA).

Molecular Epidemiological Analysis

Isolates were stored at –80°C refrigerator until genotyping. Pulsed field gel electrophoresis (PFGE) method was performed as previously described by Selim et al.[15], with minor modifications. Agarose plugs prepared for each of the 38 *P. aeruginosa* isolates were incubated with 30 units of SpeI restriction enzyme. Band profiles were analyzed using the GelCompar II software system (version 6.5; Applied Maths, Sint-Martens-Latem, Belgium). Dice correlation coefficient was used to make similarity calculations for band analysis and UPGMA method was used for cluster analysis (optimization 1.0, tolerance 1.0). Isolates with band similarities over 85% were considered to be the same clone.

Statistical Analysis

Antibiotic susceptibility results and distribution of resistance genes are given as numbers and percentages.

Results

A total of 38 CZA-resistant *P. aeruginosa* strains were isolated during the study period. Isolates were obtained mostly from endotracheal aspirate cultures (n=22, 57.9%), followed by blood (n=6, 15.8%), tissue biopsy (n=6, 15.8%) and urine cultures (n=4, 10.5%), respectively. All but one of the strains were detected in clinical samples of patients hospitalized in the ICU.

The inhibition zone diameters obtained by the disk diffusion method in the isolates ranged between 0-13 mm, and no inhibition zone was formed in 84.2% (n=32) (Table 1). MIC50 and MIC90 values determined by the gradient diffusion method for CZA were found to be ≥256 mg/l and ≥256 mg/l, respectively, and the MIC range was 32-256 mg/l. It was determined that the gradient method and disc diffusion methods were 100% compatible with each other.

Of 38 CZA resistant *P. aeruginosa*, 92.1% (35/38) were resistant to amikacin, 76.3% (29/38) to ciprofloxacin, 73.6% (28/38) to levofloxacin, 100% (38/38) to imipenem and meropenem. All isolates were susceptible to colistin, and MIC values were found to be between 0.5-2 mg/l.

Resistance Genes

Among a total of 38 isolates, 11 (28.9%) isolates were positive for MBL genes, 10 of which were *blaVIM* (26.3%) and one was *blaIMP* (2.6%). The *blaOXA-10* gene was found to be positive together in seven of the *blaVIM* positive isolates and 1 *blaIMP* positive isolate. Additionally, *blaOXA-10* alone was detected in nine of the isolates (23.6%), *blaPER* in two (5.2%) and *blaGES* in one (2.6%). None of the other *blaNDM*, *blaKPC*, *blaOXA-48* and *blaVEB* genes, which were the subject of the study, were found in 15 (39.4%) isolates (Table 1).

Molecular Epidemiology and Clonality

Fifteen different genotypes were detected among a total of 38 *P. aeruginosa* isolates, and isolates showing clustering were collected in eight different clusters (tolerance 1.0, optimization 1.0, cutoff 85%). Thirty-one of the thirty-eight *P. aeruginosa* isolates were located in any cluster, and the clustering rate was found to be 81.5%. The largest cluster was genotype 1 cluster with nine isolates, and the second largest clusters were genotype 4 and 5 clusters with five isolates each. Other clusters were genotypes 2 and 8, with three isolates each, and genotypes 9, 10 and 12, with two isolates each (Figure 1).

When examined in terms of molecular epidemiological data, it was seen that 37 of 38 isolates were obtained from patients in the ICU and one was obtained from a patient in the general surgery ward. Although the isolate of the patient hospitalized in general surgery ward and the isolates of two patients hospitalized in ICU had similar genotypes (genotype 8), it was determined that the patient hospitalized in general surgery ward did not receive treatment in the ICU during his hospitalization. When the isolation dates of the strains belonging to the remaining 37 patients hospitalized in ICU were compared, no clusters that would suggest a cross-contamination were observed.

Discussion

Shortly after CZA became available for use in carbapenem-resistant *P. aeruginosa* infections, the reporting of resistant isolates worldwide emerged as a clinical challenge

Table 1. Distribution of resistance genes and antimicrobial activity in ceftazidime-avibactam-resistant *P. aeruginosa* clinical isolates

Sample no	Isolate no	CZA MIC (mg/l)	CZA zone diameter (mm)	Resistance genes									
				blaVIM	*blaIMP*	*blaNDM*	*blaKPC*	*blaOXA-48*	*blaOXA-10*	*blaPER*	*blaGES*	*blaVEB*	*blaOXA-2*
1	114	≥256	0	+	-	-	-	-	+	-	-	-	-
2	110	≥256	0	+	-	-	-	-	+	-	-	-	-
3	111	>256	0	+	-	-	-	-	+	-	-	-	-
4	54	≥256	0	-	-	-	-	-	+	-	-	-	-
5	57	≥256	0	-	+	-	-	-	+	-	-	-	-
6	118	≥256	0	+	-	-	-	-	+	-	-	-	-
7	123	≥256	0	+	-	-	-	-	+	-	-	-	-
8	115	≥256	0	+	-	-	-	-	+	-	-	-	-
9	175	32	11	-	-	-	-	-	+	-	-	-	-
10	37	≥256	0	-	-	-	-	-	-	-	-	-	-
11	160	≥256	0	-	-	-	-	-	-	-	-	-	-
12	46	≥256	0	-	-	-	-	-	-	+	-	-	-
13	113	≥256	0	-	-	-	-	-	+	-	-	-	-
14	139	≥256	0	-	-	-	-	-	+	-	-	-	-
15	147	≥256	0	-	-	-	-	-	+	-	-	-	-
16	96	≥256	0	-	-	-	-	-	-	-	-	-	-
17	107	≥256	0	-	-	-	-	-	+	-	-	-	-
18	93	≥256	0	-	-	-	-	-	+	-	-	-	-
19	62	≥256	0	-	-	-	-	-	+	-	-	-	-
20	69	≥256	0	-	-	-	-	-	+	-	-	-	-
21	81	≥256	0	-	-	-	-	-	-	-	-	-	-
22	171	≥256	0	-	-	-	-	-	-	-	-	-	-
23	172	≥256	0	-	-	-	-	-	-	-	-	-	-
24	82	≥256	0	-	-	-	-	-	-	-	-	-	-
25	99	32	10	-	-	-	-	-	-	-	-	-	-
26	84	≥256	0	+	-	-	-	-	-	-	-	-	-
27	86	>256	0	+	-	-	-	-	+	-	-	-	-
28	74	≥256	0	+	-	-	-	-	-	-	-	-	-
29	103	128	9	-	-	-	-	-	-	-	+	-	-
30	122	32	13	-	-	-	-	-	-	-	-	-	-
31	154	32	12	-	-	-	-	-	-	-	-	-	-
32	159	32	11	-	-	-	-	-	-	-	-	-	-
33	42	32	13	-	-	-	-	-	-	+	-	-	-
34	70	≥256	0	-	-	-	-	-	-	-	-	-	-
35	72	≥256	0	-	-	-	-	-	-	-	-	-	-
36	117	≥256	0	-	-	-	-	-	-	-	-	-	-
37	141	≥256	0	-	-	-	-	-	-	-	-	-	-
38	40	≥256	0	+	-	-	-	-	-	-	-	-	-

CZA: Seftazidime-avibactam, MIC: Minimal inhibitory concentration, VIM: Verona integron-encoded metallo-beta-lactamase, IMP: Imipenemase, NDM: New Delhi metallo-beta-lactamase, KPC: *Klebsiella pneumoniae* carbapenemase, OXA-48: Oxacillinase-48, OXA-10: Oxacillinase-10, OXA-2: Oxacillinase-2, PER: *Pseudomonas* extended resistant, GES: Guiana extended-spectrum beta-lactamase, VEB: Vietnam extended spectrum beta-lactamase

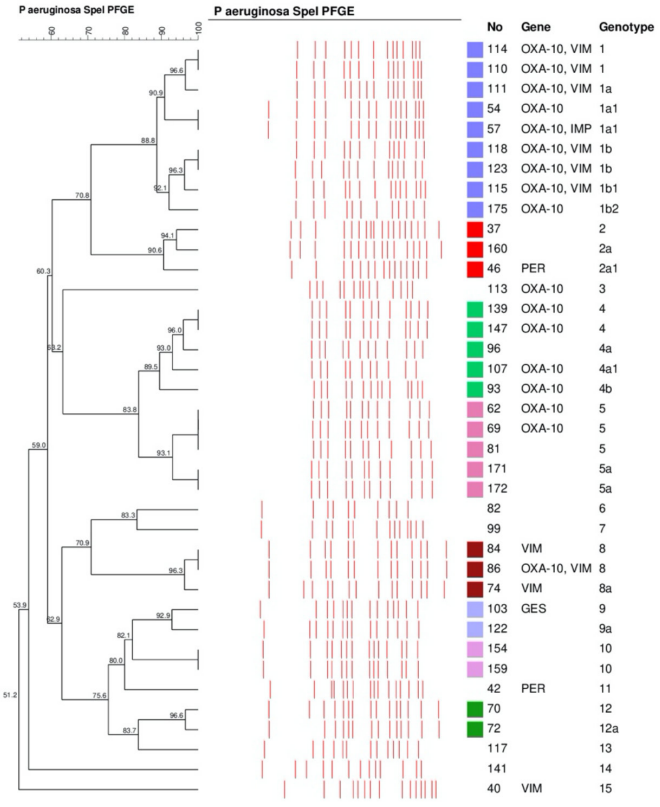

Figure 1. PFGE dendogram of ceftazidime-avibactam resistant *P. aeruginosa* isolates

PFGE: Pulsed field gel electrophoresis

and increased concerns about the global spread of this microorganism[1,8]. Therefore, research on understanding the mechanisms that cause CZA resistance has become even more important. In these studies on the molecular causes of resistance, it has been reported that the presence of MBL, which can be carried between microorganisms via plasmids, has a significant rate[5,9,16]. In the large-scale INFORM surveillance study, *P. aeruginosa* isolates collected from 42 medical centers in nine countries in the Asia-Pacific region were evaluated and 7.4% (151/2038) were found to be resistant to CZA, and MBL-positive strains accounted for 48.3% (73/151) of resistant isolates[16]. When previous studies were examined, it was observed that the type and frequency of MBL showed regional variations, and VIM enzyme was generally dominant, but in some countries IMP ranked first[3,9,7,17]. In the study by Mojica et al.[3], which included data from five Latin American countries, MBL positivity was found at a rate of 38.5% (n=42) in 109 CZA resistant *P. aeruginosa* isolates. Among the isolates, VIM (28.4%) was the most common, followed by IMP+KPC (6.4%), IMP (2.7%) and SPM (0.9%) positivity. In China, Hu et al.[9] detected MBL in 42.5% of 80 CZA resistant *P. aeruginosa* isolates, 16.2% of which were VIM and 26.3% were IMP. Weber et al.[7] found 36.3% of the isolates to be VIM positive and did not detect IMP or NDM. Schaumburg et al.[17] found MBL (mostly IMP) positivity in 49.3% and they stated that MBL positivity was the most likely cause of CZA resistance in *P. aeruginosa*. Similarly, Sid Ahmed et al.[11] detected the presence of MBL in 64.9% of 37 CZA resistant *P. aeruginosa* isolates. However, unlike most studies mentioned above, Castenheira et al.[18] found MBL positivity in CZA resistant *P. aeruginosa* isolates to be only 4.3%, while Babouee Flury et al.[10] in Switzerland did not detect MBL in any isolate. Although there are publications reporting that VIM and IMP constitute the majority of MBL detected in carbapenem-resistant *P. aeruginosa* isolates in our country[19,20], there is very little information about CZA-resistant *P. aeruginosa* isolates. In the study by Hoşbul et al.[21], VIM enzyme was found in only one of 10 CZA resistant *P. aeruginosa* isolates, while NDM and IMP were not detected. We detected the MBL positivity rate as 28.9% including IMP, mostly VIM in our isolates, which was consistent with the general literature.

In recent studies, it has been stated that OXA-10 and OXA-2 enzymes may be associated with CZA resistance in *P. aeruginosa* isolates[11,22,23]. Arca-Suárez et al.[23] determined that CZA resistance, which developed *in vivo* during treatment in a patient infected with extensively drug-resistant (XDR) *P. aeruginosa*, was caused by a modification in the OXA-10 enzyme. Sid Ahmed et al.[11] also detected OXA-10 in 15% of CZA resistant *P. aeruginosa* isolates and stated that the presence of OXA-10 was associated with the development of resistance. In our study, in addition to OXA-10 positivity alone in 26.3% of the isolates (n=9), we also detected OXA-10 enzyme in seven of the VIM-positive isolates and one IMP-positive isolate. Accordingly, considering the previous data, it is thought that the OXA-10 enzyme may cause CZA resistance, especially in isolates where it is found alone. Fraile-Ribot et al.[22] found that duplication of residue D149 in OXA-2 (OXA-539) led to resistance to CZA *in vivo*. Mojica et al.[3] detected OXA-2 positivity in 17 of 109 resistant isolates, but did not find any duplication in the whole genome sequence analysis. We did not observe OXA-2 positivity in any of our isolates. Apart from all these, similar to some other studies, OXA-48 and KPC enzymes were not found in any of our isolates[17,18].

It has been stated that PER and GES enzymes, which are extended-spectrum beta-lactamases that are common worldwide, may also be the source of CZA resistance in *P. aeruginosa* isolates[3,24,25]. Ortiz de la Rosa et al.[24] demonstrated that *blaPER-1* alone can confer resistance to CZA when expressed at high level in the recombinant plasmid in PAO1. Babouee Flury et al.[10] also detected PER positivity accompanied by other resistance mechanisms in a small number of *P. aeruginosa* isolates. Mojica et al.[3] found the PER positivity to be 2.8%. In our study, we found PER positivity in two isolates (5.2%). In a recent study by Li et al.[25], it was stated that overexpression of the *blaGES-1*

gene carried in the class 1 integron of the Tn6584 complex transposon contributed to CZA resistance, and furthermore, such overexpression of *blaGES-1* combined with an efflux pump caused high CZA resistance. Similarly, Recio et al.[26] reported that they isolated a large number of CZA resistant *P. aeruginosa* isolates carrying only GES enzymes in their hospitals in a short time. Weber et al. detected GES positivity in 0.9% of CZA resistant isolates[7], Hu et al.[9] in 1.25%, and Mojica et al.[3] in 4.6%. In our study, we detected GES enzyme in 2.6% of our isolates, consistent with these rates.

Metallo-beta-lactamases, which are one of the causes of CZA resistance, are important because they can be easily transported between bacteria through mobile genetic elements, thus causing cross-contamination within the hospital. Therefore, it is necessary to be careful about large-scale spread and possible epidemics that may occur with multidrug-resistant bacteria in units where broad-spectrum antibiotic pressure is intense, such as ICUs[4]. The fact that all but one of the CZA resistant *P. aeruginosa* that we isolated consecutively during the study period were obtained from patients in the ICU was a warning for us in terms of a possible cross-contamination and epidemic. Thereupon, in the PFGE analysis we conducted to determine whether the strains were clonally related to each other, we detected 15 different genotypes among 38 *P. aeruginosa* isolates. We found that the isolates showing clustering among them were collected in eight different clusters. The largest cluster was genotype 1 cluster with nine isolates, and the second largest clusters were genotype 4 and 5 clusters with five isolates each. Other clusters were genotypes 2 and 8, with three isolates each, and genotypes 9, 10 and 12, with two isolates each. This result we obtained suggested that there was a polyclonal diversity among our isolates.

When the isolates were examined in terms of epidemiological relationship, it was seen that the isolate of the patient in general surgery ward and the isolates of two patients in ICU had similar genotypes (genotype 8) and were isolated on the same date. However, the fact that the general surgery patient did not be treated in the ICU during his hospitalization suggested that contamination might occur through healthcare workers. It was determined that strains belonging to genotype 1, which included the most isolates, were first seen in 2 patients in January 2022, and re-emerged 6 months later and were isolated in 7 more patients. When the isolation dates of other strains were examined, it was observed that there was no chronological clustering in isolates with the same genotype that would suggest a cross contamination. However, it was found remarkable that certain genotypes continued to exist, albeit intermittently, throughout the study period of approximately 1.5 years. Apart from this, we should also point out that the enzymes responsible for resistance differ in some isolates with the same genotype.

Study Limitations

Our study had some limitations. First, other potential resistance mechanisms that might cause CZA resistance, such as efflux pumps, loss of porins, overexpression of *blaPDC*, decreased permeability, and overproduction of AmpC, were not investigated. Secondly, in molecular epidemiological studies conducted with CZA resistant *P. aeruginosa* isolates, it was observed that the multilocus sequence typing (MLST) method was used, which allowed comparison with international clones[7,11]. Due to technical limitations, MLST could not be studied and a literature comparison could not be made because there were differences in methods in the evaluation of clonal relationship.

Conclusion

As a result, as stated in previous studies, CZA resistance mechanisms observed in *P. aeruginosa* isolates are quite diverse and complex[3,10]. In our study, MBL production, especially VIM, was found to be the most common cause of CZA resistance. Apart from this, OXA-10, PER and GES positivity were also found to be remarkable. In the molecular epidemiological examination, it was observed that the strains isolated predominantly from ICU patients had different genotypes and exhibited polyclonal diversity. Close monitoring of resistance to CZA, one of the alternative antibiotics, in multidrug-resistant *P. aeruginosa* infections is important for effective treatment strategies. It is thought that our data will make a significant contribution to the national and global literature. However, it is obvious that more comprehensive studies are needed to determine the mechanisms responsible for CZA resistance in *P. aeruginosa*.

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Ethics

Ethics Committee Approval: The study was approved by the Haydarpaşa Numune Training and Research Hospital Clinical Research Ethics Committee on 22.05.2023 with the decision number HNEAH-KAEK 2023/KK/96.

Informed Consent: Informed consent was not obtained since the study was conducted with bacterial isolates.

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Authorship Contributions

Surgical and Medical Practices: N.A., E.S.T., Concept: N.A., E.S.T., Design: N.A., E.S.T., Data Collection or Processing: N.A., E.S.T., Analysis or Interpretation: N.A., E.S.T., Literature Search: N.A., Writing: N.A., E.S.T.

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References

1. Gaibani P, Giani T, Bovo F, Lombardo D, Amadesi S, Lazzarotto T, Coppi M, Rossolini GM, Ambretti S. Resistance to Ceftazidime/Avibactam, Meropenem/Vaborbactam and Imipenem/Relebactam in Gram-Negative MDR Bacilli: Molecular Mechanisms and Susceptibility Testing. Antibiotics (Basel). 2022;11:628.
2. World Health Organization (WHO). Prioritization of Pathogens to Guide Discovery, Research And Development Of New Antibiotics For Drug-Resistant Bacterial Infections Including Tuberculosis. Geneva: World Health Organization, 2017. Available from: http://www.who.int
3. Mojica MF, De La Cadena E, García-Betancur JC, Porras J, Novoa-Caicedo I, Páez-Zamora L, Pallares C, Appel TM, Radice MA, Castañeda-Méndez P, Gales AC, Munita JM, Villegas MV. Molecular Mechanisms of Resistance to Ceftazidime/Avibactam in Clinical Isolates of *Enterobacterales* and *Pseudomonas aeruginosa* in Latin American Hospitals. mSphere. 2023;8:e0065122.
4. Çekin ZK, Dabos L, Malkoçoğlu G, Fortineau N, Bayraktar B, Iorga BI, Naas T, Aktaş E. Carbapenemase -producing *Pseudomonas aeruginosa* isolates from Turkey: first report of *P. aeruginosa* high-risk clones with VIM-5- and IMP-7-type carbapenemases in a tertiary hospital. Diagn Microbiol Infect Dis. 2021;99:115174.
5. Xiong L, Wang X, Wang Y, Yu W, Zhou Y, Chi X, Xiao T, Xiao Y. Molecular mechanisms underlying bacterial resistance to ceftazidime/avibactam. WIREs Mech Dis. 2022;14:e1571.
6. Wang Y, Wang J, Wang R, Cai Y. Resistance to ceftazidime-avibactam and underlying mechanisms. J Glob Antimicrob Resist. 2020;22:18-27.
7. Weber C, Schultze T, Göttig S, Kessel J, Schröder A, Tietgen M, Besier S, Burbach T, Häussler S, Wichelhaus TA, Hack D, Kempf VAJ, Hogardt M. Antimicrobial Activity of Ceftolozane-Tazobactam, Ceftazidime-Avibactam, and Cefiderocol against Multidrug-Resistant *Pseudomonas aeruginosa* Recovered at a German University Hospital. Microbiol Spectr. 2022;10:e0169722.
8. Gill CM, Aktaþ E, Alfouzan W, Bourassa L, Brink A, Burnham CD, Canton R, Carmeli Y, Falcone M, Kiffer C, Marchese A, Martinez O, Pournaras S, Satlin M, Seifert H, Thabit AK, Thomson KS, Villegas MV, Nicolau DP; ERACE-PA Global Study Group. The ERACE-PA Global Surveillance Program: Ceftolozane/tazobactam and Ceftazidime/avibactam in vitro Activity against a Global Collection of Carbapenem-resistant *Pseudomonas aeruginosa*. Eur J Clin Microbiol Infect Dis. 2021;40:2533-41.
9. Hu Y, Chen J, Huang L, Liu C, Zhou H, Zhang R. Antimicrobial susceptibility study and molecular epidemiology of ceftazidime/avibactam against *Pseudomonas aeruginosa* collected from clinical patients in PR China (2004-2021). J Med Microbiol. 2023;72.
10. Babouee Flury B, Bösch A, Gisler V, Egli A, Seiffert SN, Nolte O, Findlay J. Multifactorial resistance mechanisms associated with resistance to ceftazidime-avibactam in clinical *Pseudomonas aeruginosa* isolates from Switzerland. Front Cell Infect Microbiol. 2023;13:1098944.
11. Sid Ahmed MA, Khan FA, Hadi HA, Skariah S, Sultan AA, Salam A, Al Khal AL, Söderquist B, Ibrahim EB, Omrani AS, Jass J. Association of blaVIM-2, blaPDC-35, blaOXA-10, blaOXA-488 and blaVEB-9 β-Lactamase Genes with Resistance to Ceftazidime-Avibactam and Ceftolozane-Tazobactam in Multidrug-Resistant *Pseudomonas aeruginosa*. Antibiotics (Basel). 2022;11:130.
12. European Committee on Antimicrobial Susceptibility Testing (EUCAST). MIC and zone diameter distributions and ECOFFs. V.12.0. 2022. Available from: https://www.eucast.org/mic_distributions_and_ecoffs
13. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 2011;70:119-23.
14. Kiratisin P, Apisarnthanarak A, Laesripa C, Saifon P. Molecular characterization and epidemiology of extended-spectrum-beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic. Antimicrob Agents Chemother. 2008;52:2818-24.
15. Selim S, El Kholy I, Hagagy N, El Alfay S, Aziz MA. Rapid identification of *Pseudomonas aeruginosa* by pulsed-field gel electrophoresis. Biotechnol Biotechnol Equip. 2015;29:152-6.
16. Karlowsky JA, Kazmierczak KM, Bouchillon SK, de Jonge BLM, Stone GG, Sahm DF. In Vitro Activity of Ceftazidime-Avibactam against Clinical Isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa* Collected in Asia-Pacific Countries: Results from the INFORM Global Surveillance Program, 2012 to 2015. Antimicrob Agents Chemother. 2018;62:e02569-17.
17. Schaumburg F, Bletz S, Mellmann A, Becker K, Idelevich EA. Comparison of methods to analyse susceptibility of German MDR/XDR *Pseudomonas aeruginosa* to ceftazidime/avibactam. Int J Antimicrob Agents. 2019;54:255-60.
18. Castanheira M, Doyle TB, Smith CJ, Mendes RE, Sader HS. Combination of MexAB-OprM overexpression and mutations in efflux regulators, PBPs and chaperone proteins is responsible for ceftazidime/avibactam resistance in *Pseudomonas aeruginosa* clinical isolates from US hospitals. J Antimicrob Chemother. 2019;74:2588-95.
19. Malkoçoğlu G, Aktaş E, Bayraktar B, Otlu B, Bulut ME. VIM-1, VIM-2, and GES-5 Carbapenemases Among *Pseudomonas aeruginosa* Isolates at a Tertiary Hospital in Istanbul, Turkey. Microb Drug Resist. 2017;23:328-34.
20. Hazırolan G. Özkul C. Evaluation of *in vitro* activity of ceftolozane/tazobactam and ceftazidime/avibactam against carbapenem-resistant *Pseudomonas aeruginosa* strains and mechanisms of carbapenem resistance: data from tertiary care hospital. Jundishapur J Microbiol. 2023;16:e134090.
21. Hoşbul T, Aydoğan CN, Kaya S, Bedir O, Gümral R, Albay A. Karbapenem Dirençli *Klebsiella pneumoniae* Klinik İzolatlarına Karşı Seftazidim-avibaktam ve Kolistinin *In Vitro* Etkinliği [*In Vitro* Activity of Ceftazidime-avibactam and Colistin Against Carbapenem-Resistant *Klebsiella pneumoniae* Clinical Isolates]. Mikrobiyol Bul. 2022;56:218-29.
22. Fraile-Ribot PA, Mulet X, Cabot G, Del Barrio-Tofiño E, Juan C, Pérez JL, Oliver A. *In Vivo* Emergence of Resistance to Novel Cephalosporin-β-Lactamase Inhibitor Combinations through the Duplication of Amino Acid D149 from OXA-2 β-Lactamase (OXA-539) in Sequence Type 235 *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2017;61:e01117-17.
23. Arca-Suárez J, Lasarte-Monterrubio C, Rodiño-Janeiro BK, Cabot G, Vázquez-Ucha JC, Rodríguez-Iglesias M, Galán-Sánchez F, Beceiro A, González-Bello C, Oliver A, Bou G. Molecular mechanisms driving the *in vivo* development of OXA-10-mediated resistance to ceftolozane/tazobactam and ceftazidime/avibactam during treatment of XDR *Pseudomonas aeruginosa* infections. J Antimicrob Chemother. 2021;76:91-100.
24. Ortiz de la Rosa JM, Nordmann P, Poirel L. ESBLs and resistance to ceftazidime/avibactam and ceftolozane/tazobactam combinations in *Escherichia coli* and *Pseudomonas aeruginosa*. J Antimicrob Chemother. 2019;74:1934-9.
25. Li X, Zhang X, Cai H, Zhu Y, Ji J, Qu T, Tu Y, Zhou H, Yu Y. Overexpression of blaGES-1 due to a strong promoter in the class 1 integron contributes to decreased ceftazidime-avibactam susceptibility in carbapenem-resistant *Pseudomonas aeruginosa* ST235. Drug Resist Updat. 2023;69:100973.
26. Recio R, Villa J, González-Bodí S, Brañas P, Orellana MÁ, Mancheño-Losa M, Lora-Tamayo J, Chaves F, Viedma E. Genomic Analysis of Ceftazidime/Avibactam-Resistant GES-Producing Sequence Type 235 *Pseudomonas aeruginosa* Isolates. Antibiotics (Basel). 2022;11:871.