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ORIGINAL RESEARCH

Screening of Antimicrobial Resistance Genes and Epidemiological Features in Hospital and Community-Associated Carbapenem-Resistant *Pseudomonas aeruginosa* Infections

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Introduction: Researching carbapenem-resistant isolates enables the identification of carbapenemase-producing bacteria and prevents their spread.

Methods: *P. aeruginosa* isolates were recovered from Medicine Faculty of Recep Tayyip Erdoğan University and identified by conventional methods and the automated Vitek 2 Compact system. Antimicrobial susceptibility experiments were performed in accordance with CLSI criteria and the automated Vitek 2 Compact system. The PCR method was investigated for the presence of β-lactamase resistance genes. PFGE typing was performed to show clonal relation among samples.

Results: Seventy *P. aeruginosa* isolates were isolated from seventy patients. Of the patients, 67.1% had contact with the health service in the last 90 days and 75.7% of the patients had received antimicrobial therapy in the previous 90 days. Twenty-four isolates were carbapenem resistant, 2 isolates were multidrug-resistant except colistin, and none of the samples had colistin resistance. The gene encoding β-lactamase or metallo-β-lactamase was found in a total of 36 isolates. The bla_{VEB} and bla_{PER} genes were identified in 1 and 5 isolates alone or 17 and 13 isolates in combination with other resistance genes, respectively. The bla_{NDM} was the most detected metallo-β-lactamase encoding gene (n=18), followed by bla_{KPC} (n=12). bla_{IMP} and bla_{VIM} were detected in 5 and 1 isolates, respectively. Also, the association of bla_{VEB}-bla_{PER} and bla_{VEB}-bla_{KPC}-bla_{NDM} was found to be very high. Much more resistance genes and co-occurrence were detected in hospital-acquired samples than community-acquired samples. No difference was found between the community and hospital-associated isolates according to PFGE results. Simultaneously from 6 patients, other microorganisms were also isolated and 5 of them died.

Conclusion: The average length of stay (days) was found to be significantly higher in HAI group than CAI group. The death of 5 patients with fewer or no resistance genes showed that the co-existence of other microorganisms in addition to resistance genes was important on death.

Keywords: *Pseudomonas aeruginosa*, antibiotic resistance genes, epidemiology, PFGE

Introduction

P. aeruginosa is an opportunistic gram-negative bacteria commonly found on many surfaces. Its rapid colonization on living and non-living areas leads to a wide range of diseases, including community and health-associated infections.[1,2] *P. aeruginosa* is very difficult to control in the hospital environment due to its versatile and

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ubiquitous features.[3] Infections caused by P. aeruginosa have been increased significantly in healthcare units, affecting specially patient with chronic diseases or with immunocompromised system.[1,3]

Infections caused by *P. aeruginosa* are difficult to treat because of its intrinsic resistance to many antipseudomonal agents and the ability to easily acquire antibiotic resistance.[4] In hospital infections caused by *P. aeruginosa* isolates with multiple drug resistance, mortality, hospitalization, and treatment costs increase, especially in intensive care units. Mortality decreases significantly if early and appropriate antipseudomonal treatment is initiated.[5,6] Carbapenems are powerful broad-spectrum-β-lactam antibiotics commonly used in the treatment of *P. aeruginosa*. However, increased carbapenem resistance among these organisms has been found worldwide, and carbapenem-resistant *P. aeruginosa* ranges from 10% to 50% in most countries. Few antibiotic options are available for treatment, and multidrug resistance is much more common in patients infected with carbapenem-resistant *P. aeruginosa*.[7,8]

In this study, it was aimed to determine the antimicrobial resistance profile of *P. aeruginosa* isolates isolated from culture samples sent from various polyclinics and services in a university hospital and thought to be an infectious agent of community (CAI) and hospital-associated (HAI), to screen the resistance genes and to investigate the clonal relationship between isolates.

Materials and Methods

Bacterial Isolates and Identification

Seventy clinical *P. aeruginosa* isolates were recovered from different units and samples (blood, urine, wound swab sample, sputum, tracheal aspirate, cerebrospinal fluid (CSF), bronchoalveolar lavage) in Medicine Faculty of Recep Tayyip Erdoğan University between April 2015 and October 2016. The clinical data of the patients were obtained retrospectively from file records and medical tables. One sample of each patient classified as a hospital (HAI, n=27) and community-associated (CAI, n=43) was evaluated. The isolates were identified by conventional methods and the automated Vitek 2 Compact (BioMerieux, France) system.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility experiments were performed in accordance with CLSI (Clinical and Laboratory Standards Institute) criteria using both the Kirby–Bauer disc diffusion method and the automated Vitek 2 Compact (BioMerieux, France) system. In this context, amikacin (AK), gentamicin (GN), ciprofloxacin (CIP), ceftazidime (CAZ), piperacillin (PRL), piperacillin-tazobactam (TZP), cefoperazone (CEP), cefoperazone/sulbactam (CES) aztreonam (ATM), meropenem (MEM), cefepime (FEP), imipenem (IPM), levofloxacin (LEV) and colistin (CT) sensitivity was evaluated. *P. aeruginosa* ATCC 27853 was used as a quality control isolate.

Detection of β-Lactamase Encoding Resistance Genes

The presence of β-lactamase coding genes was investigated by the PCR method (Table 1). To obtain template DNA, a single colony bacterial isolates were inoculated into 3 mL of Luria–Bertani (LB) broth medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl, pH 7.4) at 37 °C for 16 hours at 200 rpm. One and half mL of the grown culture was transferred to eppendorf tubes and centrifuged at 10,000 rpm for 5 minutes in microcentrifuge. The supernatant part was poured and 1 mL of sterile distilled water was added to the pellet and vortexed (twice). One mL of sterile distilled water was added to the pellet and the bacterial suspension was boiled at 95 °C for 10 minutes. After boiling, the bacteria suspension was centrifuged at 13,000 rpm for 5 minutes. Three µL of the supernatant part was used as template DNA in PCR. Standard PCR mixtures were prepared as follows: 1.5 units *Taq* DNA polymerase I (Solis Biodyne), 5 µL DNA, 10 µL 5X DNA polymerase buffer (Solis Biodyne), 3 µL of 25 mM MgCl2, 2.5 µL of 4 mM each dNTP and 1 µL each primer stock (10 pmol/µL) and sterile deionized water was added to complete 50 µL of final volume. PCR reactions occurred with the following amplification conditions: 3 min 94 °C initial denaturation, followed by 34 cycles of denaturation at 94 °C 45 sec, annealing at 55 °C 1 min, extension at 70 °C 3 min, and final extension step 5 min at 70 °C. Amplification products were run in 1.5–2% agarose gel and visualized with UV light. The oligonucleotides used in PCR and their melting temperature (Tm) are given in Table 1.

Molecular Typing of the *P. aeruginosa* Isolates

PFGE typing was performed on 70 isolates to show clonal relation among samples. Isolation and deproteinization of the genomic DNA were done following the protocol of Durmaz et al with minor modifications.[16] Briefly, *P. aeruginosa* colonies on Mueller Hinton agar were

Table 1 Primers Used in the Amplification of Selected Genes

Primer	5′-3′ Sequence	Amplicon Size (bp)	Tm (°C)	Reference
VEB	F- ATTTCCCGATGCAAAGCGT R- TTATTCCGGAAGTCCCTGT	542	55	[9]
PER	F- ATGAATGTCATCACAAAAT R- TCAATCCGGACTCACT	927	50	[10]
IMP	F:CATGGTTTGGTGGTTCTTGT R:ATAATTTGGCGGACTTTGGC	488	56	[11]
VIM	F:ATTGGTCTATTTGACCGCGTC R:TGCTACTCAACGACTGAGCG	780	58	[11]
NDM	F:TGGAATTGCCCAATATTATGC R:TCAGCGCAGCTTGTCGGCCATGC	813	54	[12]
KPC	F: CGTTCTTGTCTCTCATGGCC R: CCTCGCTGTGCTTGTCATCC	796	52	[13]
OXA-48	F:CAGTCAAGTTCAACCCAACCG R:GCGTGGTTAAGGATGAACAC	438	55	[14]
OXA- 51	F: TAATGCTTTGATCGGCCTTG R:TGGATTGCACTTCATCTTGG	353	52	[15]
OXA–23	F:GATCGGATTGGAGAACCAGA R:ATTTCTGACCGCATTTCCAT	501	52	[15]

Abbreviations: Tm, melting temperature; bp, base pairs.

suspended in 1 mL HST (100mm Tris-HCl, 100mm EDTA, pH 8.0) and the optical density was adjusted to 0.8 (590 nm). The cells were embedded into low (2%) melting agarose. Molds were prepared with the help of agarose and kept in the refrigerator at 4 ° C for 10 minutes. The prepared isolate molds were added HLS-1 (50 mm Tris-HCl, 50 mm EDTA, pH 8.0, 2.5 mg/mL Lysozyme, 1.5 mg/mL Proteinase K) and incubated at 37 °C for 1 hour in a water bath. At the end of the period, HLS-1 was removed. HLS-2 (0.5 M EDTA, 1% sarcosyl, 400 µg/mL Proteinase K) was placed on molds and incubated in a water bath at 55 °C for 2 hours. After the lysis step, the molds were washed 3 times each with dH_2O and 1XTE (Tris-EDTA). After digestion of the cells and washing of the plugs, genomic DNA in the agarose plugs was restricted by 10 U of *Spe*I (Thermo) for 3 hours at 37 °C in a incubator. DNA fragments were separated on 1% pulse-field certified agarose (BIO-RAD) gels run in 0.5X Tris-borate-EDTA buffer (44.5 mM/LTris, 44.5 mM/L boric acid, 1 mM/L EDTA [pH8.0]) by using a CHEF-DR II system (Bio-Rad Laborato-ries). The electrophoresis conditions were 14°C at 6V/cm^2 for 18 hours. The initial and final switch times were 5 seconds and 20 seconds, respectively. The gel was stained with ethidium bromide (5mg/mL) for 30 mins and photographed under UV light. The DNA band profiles were analyzed with Gel Compar software (version 3.0; Applied Maths, Sint-Martens-Latem, Belgium) dendrogram and Dice. According to the interpretative criteria of Tenover et al, the clinical isolates were aligned according to PFGE profile, where the profiles sharing 100% similarity is grouped in clusters.[17]

Statistical Analysis

SPSS 21.0 (NY IBM Corp., 2012) program was used. SPSS 21.0 (NY IBM Corp., 2012) program was used. Categorical variables were expressed in terms of frequency (n) and percentage (%).The $\chi 2$ test and Fisher's exact test were used to compare categorical data. $P < 0.05$ significance level was accepted.

Results and Discussion

Epidemiological Results

One sample from each patient was accepted into the study and seventy *P. aeruginosa* isolates were obtained from seventy patients (Table 2).

Table 2 Distribution of Cases According to Service and Intensive Care Units

	Total (n=70)	HAI (n=27)	CAI (n=43)
Services, n (%)			
IMICU	14 (20.0)	11 (40.7)	3 (7.0)
SICU	12 (17.2)	8 (29.6)	4 (9.3)
ARICU	4 (5.7)	3 (11.1)	1 (2.3)
CVSICU	3 (4.3)	1 (3.7)	2 (4.7)
CICU	2 (2.8)	1 (3.7)	1 (2.3)
Other services	35 (50.0)	3 (11.1)	32 (74.4)

Abbreviations: IMUCU, Internal Medicine Intensive Care Unit; SICU, Surgery Intensive Care Unit; ARICU, Anesthesia and Reanimation Intensive Care Unit; CVSICU, Cardiovascular Surgery Intensive Care Unit; CICU, Coronary Intensive Care Unit.

One sample from each patient was accepted into the study and seventy *P. aeruginosa* isolates were obtained from seventy patients (Table 2). The average age of 70 cases was 66 with minimum 17 and maximum 92 years old. The average age of HAI and CAI was 38.6% (n=27) and 61.4% (n=43) respectively. The two groups were similar in terms of age (z=−1.829, p=0.067) and gender distribution (χ^2 (1)= 0.509, p=0.476). The average length of stay (days) was found to be significantly higher in the group with HAI than in the group with CAI (22 vs 18 days). Thirty-three isolates (47.1%) were isolated from respiratory secretions and most of them were respiratory infections (n=16 community-acquired pneumonia (CAP) and n=13 ventilator-associated pneumonia (VAP)). The ratio of the patients contacted with the health service in the last 90 days was 67.1% and 75.7% of the patients had received antimicrobial therapy in the previous 90 days. The most common comorbidity was cardiovascular diseases (38.6%). Respiratory pathologies constituted 27.1% (n=19) of the patients in the distribution of the first (preliminary) diagnosis at the time of admission to the hospital, other diagnoses were urinary system infections (18.6%, n=13), neurological pathologies (12.9%, n=9) Gastro Intestinal System (GIS) pathologies (11.4%, n=8), Cardiovascular System (CVS) pathologies (8.6% n=6), trauma (8.6% n=6) skin-soft tissue pathologies (7.1% n=5) and oncological pathologies (5.7% n=4) (Table 3). When the two groups were evaluated in terms of the distribution of hospitalization indications, it was found that the most complaint was respiratory failure (n=14) and the presence of mental change was significant in the HAI group (χ2 (1) =6.756, Fisher's exact test, p=0 0.019). The history of hospital contact and use of antibiotics in the last 3 months was high in all patients (81.5% and 72.1% in HAI and CAI, respectively). While 33.3% (n=9) of patients with HAI had an operative history in the last one month, this rate was 14% (n=6) in cases with TAI (χ^2 (1) =3.700, p=0.054). The detailed descriptions of demographic and pathological characteristics of HAI and CAI could be found in Table 3.

Considering the invasive procedures and applications during the *P. aeruginosa* isolation period, the presence of decubitus with hemodialysis, endotracheal tube application, nasogastric tube application, peripheric artery catheter application, urinary catheter and mechanical ventilator applications was found to be significantly higher in the HAI group compared to the CAI group (p <0.01). Bronchoscopy was performed on HAI patients but it was not performed on CAI patients. It was found to be significantly higher as a risk factor in patients with HAI (p = 0.038). The rate of performing cystostomy was similar in both groups. The detailed descriptions of diagnosis, clinical features and risk factors of HAI and CAI groups could be found in Table 4.

Antibiotic Susceptibility

Thirteen antibiotics were used to determine the resistance phenotype of 70 *P. aeruginosa* in this study (Table 5). While the highest resistance was observed against piperacillin (n = 36), colistin resistance was not observed in any isolate. Twenty-four (34.3%) isolates were carbapenem resistant.

A meta-analysis compared the period between 2007 and 2016, evaluated the pooled resistance prevalence of *P. aeruginosa* to piperacillin-tazobactam, ceftazidime, cefepime, meropenem, imipenem, ciprofloxacin, gentamicin, amikacin, tobramycin, and colistin were 33.9%, 38.6%, 35.6%, 30.1%, 28.0%, 30.7%, 28.2%, 17.8%, 15.7%, and 2.2%, respectively.[18] They found the resistance rates of piperacillin, piperacillin–tazobactam, imipenem, meropenem, amikacin and colistin significantly increased between 2012 and 2016; however, gentamicin, tobramycin and ciprofloxacin resistance rates significantly decreased. Similarly, our study showed that resistance to gentamicin and ciprofloxacin decreased, whereas resistance to piperacillin-tazobactam, piperacilline, meropenem, imipenem, and amikacin increased. The same meta–analysis[18] showed that antibiotic resistance rates vary according to geographical areas and colistin resistance for the black sea region is between 0% and 4%. In

Table 3 Comparison of Demographic and Pathological Characteristics of HAI (n=27) and CAI (n=43) Groups

	Total	HAI	CAI	Statistics	
	n=70	n=27	n=43	z, $\chi 2$	p value
Age (year)	66 (17–92)	73 (18–92)	64 (17–89)	−1.829	0.067
Over 65 years old		16 (81±6.9)	20 (75±6.6)	(%95 confidal)	0.0013
Duration of stay (days)	19.5 (1–72)	22 (14–72)	18 (1–39)	−2.921	0.003
Gender					
Women	27 (38.6)	9 (33.3)	18 (41.9)	0.509	0.476
Male	43 (61.4)	18 (66.7)	25 (58.1)		
Surgery history (last 1 month)	15 (21.4)	9 (33.3)	6 (14.0)	3.700	0.054
Hospital history (last 3 months)	47 (67.1)	16 (59.3)	31 (72.1)	1.238	0.266
Antibiotic utilization (last 3 months)	53 (75.7)	22 (81.5)	31 (72.1)	0.795	0.373
First (Pre) diagnosis, n (%)				–	–
Respiratory pathologies	19 (27.1)	10 (37.0)	9 (20.9)		
Urinary pathologies	13 (18.6)	0	13 (30.2)		
Neurological pathologies	9 (12.9)	5 (18.5)	4 (9.3)		
GIS pathologies	8 (11.4)	5 (18.5)	3 (7.0)		
CVS pathologies	6 (8.6)	2 (7.4)	4 (9.3)		
Trauma	6 (8.6)	3 (11.1)	3 (7.0)		
Soft-tissue pathologies	5 (7.1)	0	5 (11.6)		
Oncological pathologies	4 (5.7)	2 (7.4)	2 (4.7)		
Hospitalization indication, n (%)					
Respiratory Failure	14 (20.0)	6 (22.2)	8 (18.6)	0.136	0.713
Mental changes	4 (5.7)	4 (14.8)	0	6.756*	0.019
Hemodynamic instability	4 (5.7)	1 (3.7)	3 (7.0)	0.330*	0.498
Post CPR	7 (10.0)	4 (14.8)	3 (7.0)	1.132*	0.253

Note: *Fisher's exact test.

this context, it is possible to say that there is no change in colistin resistance for the black sea region.

Detection of MBLs

Carbapenems are used as the last resort for the treatment of infections caused by pathogens. However, *P. aeruginosa* clinical isolates are capable of producing enzymes, such as MBL, that inactivate carbapenems. When the resistance genes of the isolates were scanned, the gene encoding β-lactamase or metallo-β-lactamase was found in a total of 36 isolates. The bla_{VEB} gene was identified in only 1 isolate alone, but in combination with other resistance genes in a total of 17 isolates (Table 6). While the bla_{PER} gene was detected in 5 samples alone, it was found in 13 samples in combination with other genes. Neither bla_{VEB} nor bla_{PER} was detected in 9 samples. Among the genes encoding metallo-β-lactamase, the most bla_{NDM} positive was detected (n=18), followed by 12 positive samples of bla_{KPC}. bla_{IMP} and bla_{VIM} were detected in 5 and 1 samples, respectively. In 2 isolates, bla_{VEB}-bla_{PER}-bla_{KPC}-bla_{NDM}-bla_{OXA-48} combination was found. Also, the association of bla_{VEB}-bla_{PER} and bla_{VEB}-bla_{KPC}-bla_{NDM} was found to be very high. Much more resistance genes and associations were detected in hospital-acquired samples than community-acquired samples, both proportionally and in terms of co-occurrence (Table 6).

Clonal Relationship

Clonal association was investigated with a total of 6 gel images and 7 major PFGE profiles were identified (Figure 1). When looked clonally, 43 isolates were found to be related under major F group. The F1 subgroup is divided into 3 patterns (F1a, F1b, F1c). When the relationship with the F1 subgroup is examined, it is also closely related to this group according to the 85% Tennover criteria. Group A, 2 (A1-A2); Group F, 2 (F1-F2); Group G is divided into 2 (G1-G2) subgroups. In particular, six isolates (65, 66,

Table 4 Diagnosis, Clinical Features and Risk Factors of HAI (n =27) and CAI (n =43) Groups

	Total	HAI	CAI	Statistics	
	n=70	n=27	n=43	z, χ^2	p value
Final diagnosis on the PA isolation date (%)					
VAP (ventilator-associated pneumonia)	13 (18.6)	13 (48.2)	0	-	-
KSI (blood stream infection)	6 (8.6)	6 (22.2)	0		
HP (hospital pneumonia)	6 (8.6)	6 (22.2)	0		
HUTI (hospital urinary tract infection)	1 (1.4)	1 (3.7)	0		
UTI (urinary tract infection)	16 (22.9)	0	16 (37.2)		
CAP (community-associated pneumatic)	16 (22.9)	0	16 (37.2)		
STI (soft tissue infection)	6 (8.5)	0	6 (14.0)		
IAI (intra-abdominal infection)	3 (4.2)	0	3 (6.9)		
CBSI (community-bloodstream infection)	2 (2.9)	0	2 (4.7)		
Meningitis	1 (1.4)	1 (3.7)	0		
Comorbid patients, n (%)					
CVS diseases	27 (38.6)	12 (44.4)	15 (34.9)	0.640	0.424
KOAH	12 (17.1)	5 (18.5)	7 (16.3)	0.059*	0.526
Neurological diseases	9 (12.9)	5 (18.5)	4 (9.3)	1.257*	0.292
Diabetes mellitus	9 (12.9)	3 (11.1)	6 (14.0)	0.120*	0.517
Malignant diseases	7 (10.0)	3 (11.1)	4 (9.3)	0.060*	0.554
Renal diseases	6 (8.6)	1 (3.7)	5 (11.6)	1.329*	0.394
Skin-soft tissue pathologies	5 (7.1)	2 (7.4)	3 (7.0)	0.005*	0.645
Invasive procedures and applications during the PA isolation period, n (%)					
Hemodialysis	24 (34.3)	19 (70.4)	5 (11.6)	25.402	0.000
Endotracheal application	27 (38.6)	23 (85.2)	4 (9.3)	40.307	0.000
Enteral nutrition	49 (70.0)	16 (59.3)	33 (76.7)	2.415	0.120
Peripheral venous catheter	48 (68.6)	18 (66.7)	30 (69.8)	0.074	0.786
Nasogastric	26 (37.1)	16 (59.3)	10 (23.3)	9.209	0.002
Peripheral artery catheter	24 (34.3)	19 (70.4)	5 (11.6)	25.402	0.000
Decubitus	22 (31.4)	17 (63.0)	5 (11.6)	20.281	0.000
Central venous catheter	21 (30.0)	11 (40.7)	10 (23.3)	2.415	0.120
Urinary catheter	26 (37.1)	16 (59.3)	10 (23.3)	9.209	0.002
Mechanical ventilator	21 (30.0)	17 (63.0)	4 (9.3)	22.742	0.000
Total parenteral nutrition	14 (20.0)	3 (11.1)	11 (25.6)	2.171	0.141
Transfusion	14 (20.0)	6 (22.2)	8 (18.6)	0.136	0.713
Cystostomy	13 (18.6)	6 (22.2)	7 (16.3)	0.387	0.534
Bronchoscopy	7 (10.0)	7 (16.3)	0	4.884*	0.038
Steroid	7 (10.0)	3 (11.1)	4 (9.3)	0.060*	0.554
Tracheotomy	3 (4.3)	1 (3.7)	2 (4.7)	0.036*	0.671
Chest tube	3 (4.3)	0	3 (7.0)	1.968*	0.279

Note: *Fisher's exact test.

67, 68, 69, 70 and 6 isolates) were included in the group of 20 isolates classified as F2 in the major F group. The 6 isolates mentioned above are 100% associated with 14 isolates in the F2 major group (51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64). Most community-associated isolates were collected in the F2 clade, while most hospital-associated isolates were collected in the G1 clade. However, no difference was found between the community and hospital-associated isolates according to PFGE results.

Simultaneously, other microorganisms were also isolated from patients from which these 6 *P. aeruginosa* isolates were isolated. Six of the 70 patients were also co-infected with other microorganisms, namely with: *Acinetobacter* spp.,/*Klebsiella pneumoniae* (patients 65

Table 5 Resistance Rates of *P. aeruginosa* Isolates

Antibiotics	Resistant	Sensitive	Medium Sensitive
Ceftazidim	30 (42.9)	34 (48.6)	6 (8.6)
Cefepim	34 (48.6)	29 (41,4)	7 (10.0)
Cefoperazone	12 (17.1)	58 (82.9)	0
Cefoperazone-sulbactam	10 (14.3)	60 (85.7)	0
Piperacillin	36 (51.4)	30 (42.9)	4 (5.7)
Piperacillin-tazobactam	34 (48.6)	29 (41.4)	7 (10.0)
Amikacin	10 (14.4)	54 (77.0)	6 (8.6)
Gentamicin	12 (17.1)	54 (77.0)	4 (5.7)
Ciprofloxacin	18 (25.7)	47 (67.2)	5 (7.1)
Levofloxacin	28 (40.0)	38 (54.3)	4 (5.7)
Colistin	0	70 (100)	0
Imipenem	13 (18.6)	56 (80.0)	1 (1.4)
Meropenem	18 (25.7)	49 (70.0)	3 (4.3)
Carbapenem resistance	24 (34.3)	46 (65.7)	0

Table 6 β-Lactamase Genes Identified in *P. aeruginosa* Isolates

Genes	Isolate Numbers	Positive Number (%)
bla_{VEB}	3, 6, 16, 23*, 26, 28, 30, 33, 37, 38*, 46, 48, 50*, 53*, 56, 60, 61*, 65*	18 (25.71)
bla_{PER}	3, 8, 4, 6, 7*, 13, 20*, 28, 29, 36*, 37, 55*, 56, 61*, 64*, 65*, 66*, 67*	18 (25.71)
bla_{KPC}	3, 4, 6, 16, 28, 36*, 37, 38*, 43, 50*, 56, 66*	12 (17.14)
bla_{NDM}	6, 7*, 9, 10, 11, 26, 27*, 28, 29, 30, 36*, 37, 38*, 43, 46, 53*, 56, 68	18 (25.71)
bla_{IMP}	7*, 23*, 30, 33, 36*	5 (7.14)
bla_{VIM}	26	1 (1.42)
bla_{OXA-23}	60	1 (1.42)
bla_{OXA-48}	6, 7*, 9, 20*, 26, 28, 37, 38*, 46, 59, 60, 67*	12 (17.14)

Note: *Indicates community-associated samples.

and 68), *Acinetobacter* spp., (patients 66 and 69); *Candida* spp., (patient 67); *Klebsiella* spp., (patient 70). In opposition to patient nº70, the remained patient has deceased. While samples numbered 65, 66, and 67 of these patients were community-associated, samples 68, 69 and 70 were hospital-associated. Among these six samples, bla_{PER} /bla_{VEB}, bla_{KPC}, bla_{OXA-51} and bla_{NDM} resistance genes were detected in 65, 66, 67 and 68, respectively, while no resistance gene was detected in sample 69 and 70. Other information about the patients from whom 6 isolates are isolated as in Table 7. Compared to sample 28 and 37, which carried 5 β-lactamase coding genes, the death of these 5 patients with fewer or no resistance genes showed that the coexistence of other factors - especially other microorganisms in addition to resistance genes, was important.

Since *P. aeruginosa* and *Acinetobacter* species producing extended-spectrum β-lactamase (ESBL) are resistant to most β-lactam antibiotics, monitoring and control of its spread is important when such β-lactamase occurs. Since a limited number of antibiotics such as antipseudomonal penicillins and cephalosporins, aminoglycosides, fluoroquinolones and carbapenems are effective on *P. aeruginosa*, it is important to monitor and control the spread of genes that cause resistance to these drugs.[19] It is essential to define the main risk groups as well as the genetic data of the isolates.[2] According to the European Center for Disease Prevention and Control (ECDC) in 2016, *P. aeruginosa* resistance in most European countries exceeded 10% of all antimicrobials studied. Moreover, the prevalence of MDR- *P. aeruginosa* is increasing globally, mainly due to nosocomial outbreaks and transferable resistance mechanisms, a phenomenon associated with the spread of high-risk clones, especially horizontally acquired (by multiple loci sequence types ST235, ST111, ST175). Despite its low antibiotic consumption comparable to other Northern European countries[20] resistance rates of *P. aeruginosa* in Estonia, especially to carbapenems, are much higher than in other low-end use countries, and trends are becoming alarming. In 2012, 12.5% of the isolates reported to the ECDC were carbapenem resistant, but this had risen to over 20% by 2016.[21,22] Although the main trigger associated with carbapenem resistant in *P. aeruginosa* is the production of plasmid-mediated β-lactamases/carbapenemases, mutational resistance mechanisms in chromosomal genes– for example, altered expression of outer membrane porins or efflux system and increased chromosomal cephalosporinase (AmpC) activity may all be affected.

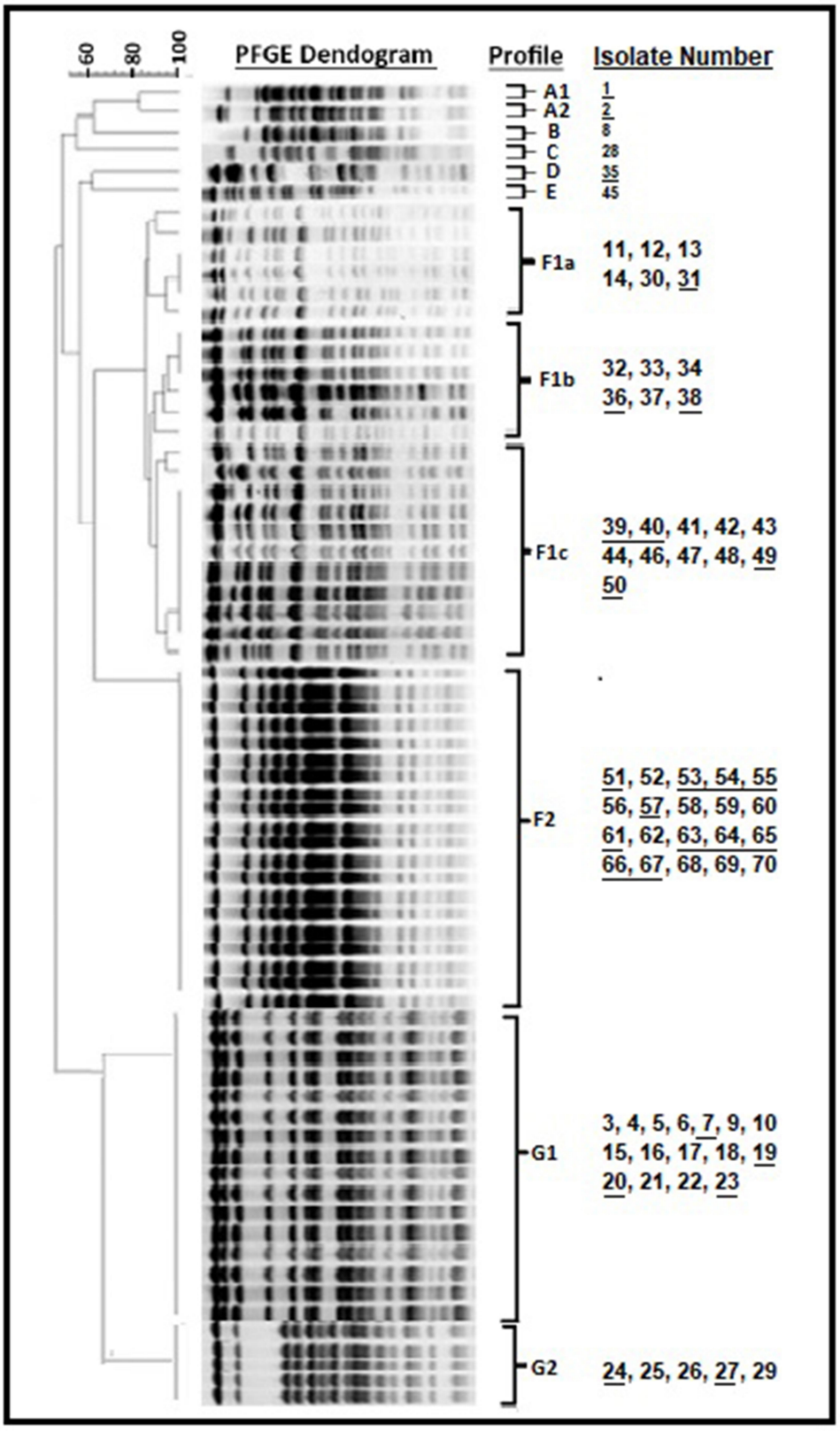

Figure 1 PFGE analysis of *P. aeruginosa* isolates generated seven major clusters (profile 1 (A1, A2, B, C, D, E), F1a, F1b, F1c, F2, G1 and G2). Community-associated samples were shown as underlined.

Table 7 Epidemic Characteristics of 6 Patients in Which Other Microorganisms Were Isolated in Addition to *P. aeruginosa*

Sample No	**65**	**66**	**67**	**68**	**69**	**70**
Sample	CSF	TAC	Blood	Blood	TAC	Urine
Age	42	82	84	88	87	47
Gender	Man	Man	Man	Women	Man	Man
Holding dates	30.07.2016	15.05.2016	08.04.2016	06.09.2016	11.07.2016	22.06.2016
	22.12.2016	16.08.2016	24.04.2016	06.11.2016	01.11.2016	24.08.2016
Holding time (day)	145	93	16	61	113	62
Service	ARICU - IMICU PCU	IMICU	IMICU	IMICU	IMICU	IDS
Comorbidity or additional disease	IVTA – TSH	CRI	PTC	PTC	KOAH	NBS
Positive simultaneous breeding: another example	Blood -TAC	Blood	TAC	Blood - Urine	TAC	Urine
Positive simultaneous growth: other microorganism.	*Acinetobacter* spp. *Klebsiella pneumoniae*	*Acinetobacter* spp.	*Candida* spp.	*Acinetobacter* spp. *Klebsiella* spp.	*Acinetobacter* spp.	*Klebsiella* spp.
History of using antibiotics in the last 3 months	NO	YES	YES	YES	YES	YES
The fate of the patient	EX	EX	EX	EX	EX	Following

Conclusion

In Turkey, the period of 2011–2016, the overall carbapenem resistance has varied between 42.9% - 30.26-%. In the University hospitals where our study was conducted, this ratio was similarly 36.55. The average length of stay (days) was significantly higher in the group with HAI than in the group with CAI. Compared to sample 28 and 37, which carried 5 β-lactamase coding genes, the death of these 5 patients with fewer or no resistance genes showed that the coexistence of other factors - especially other microorganisms in addition to resistance genes, was important.

Ethical Approval

This study was reviewed and approved by the Scientific and Ethical Committee of Rize Recep Tayyip Erdogan University Clinical Research Ethics Committee (Rize, Turkey) (40465587-32/2018-32). The data of patients' clinical variables were collected from their medical records and did not contain name, address, or other personal information. The patients' written informed consent was obtained from individual or guardian participants. This study was also in line with the guidelines outlined in the Declaration of Helsinki.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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