

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

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## Research

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

**Posted Date:** November 17th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-103545/v1>

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

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## Abstract

**Background:** Researching carbapenem-resistant isolates and the use of antibiotics and following infection control policies enable the identification of carbapenemase-producing bacteria and prevent their spread.

**Methods:** *P. aeruginosa* isolates were recovered from Medicine Faculty of Recep Tayyip Erdoğan University between April 2015 and October 2016 and identified by conventional methods and the automated Vitek 2 Compact (BioMerieux, France) 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  $\beta$ -lactamase resistance genes. PFGE typing was performed to show clonal relation among samples.

**Results:** Seventy *P. aeruginosa* strains were isolated from seventy patients. The median age of 70 cases was found 66 with minimum 17 and maximum 92 years old. 67.1% of the patients 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. The most common comorbidity was cardiovascular diseases. Twenty-four (34.3%) strains were carbapenem resistant, 2 strains were multidrug-resistant except colistin, and none of the samples had colistin resistance. The gene encoding  $\beta$ -lactamase or metallo- $\beta$ -lactamase was found in a total of 36 strains. The *bla<sub>VEB</sub>* gene was identified in only 1 strain alone, but in combination with other resistance genes in a total of 17 strains. While the *bla<sub>PER</sub>* gene was detected in 5 samples alone, it was found in 13 samples in combination with other genes. Among the genes encoding metallo- $\beta$ -lactamase, the most *bla<sub>NDM</sub>* positive was detected (n=22), followed by 14 positive samples of *bla<sub>KPC</sub>*. *bla<sub>IMP</sub>* and *bla<sub>VIM</sub>* were detected in 5 and 1 samples, respectively. Also, the association of *bla<sub>VEB</sub>*-*bla<sub>PER</sub>* and *bla<sub>VEB</sub>*-*bla<sub>KPC</sub>*-*bla<sub>NDM</sub>* 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. Most of the community-associated strains were collected in the F2 clade, while most of the hospital-associated strains were collected in the G1 clade. However, no difference was found between the community and hospital-associated strains according to PFGE results. Simultaneously, other microorganisms were also isolated from patients from which these 6 *P. aeruginosa* strains were isolated. Of these patients, 5 patients died, except the number 70.

**Conclusions:** The median length of stay (days) was found to be significantly higher in the group with HAI than in the group with CAI. Compared to sample 28 and 37, which carried 5  $\beta$ -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.

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

## Background

*Pseudomonas 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 causative pathogenities, including community and health-associated infections [1, 2]. *P. aeruginosa* is very difficult to control in the hospital environment due to its versatile and ubiquitous bacterium feature [3]. *P.aeruginosa* infections are significantly increased in exposure to healthcare units in chronic diseases (eg cystic fibrosis), immunocompromised patients (eg burns, hematological malignancies) [1, 3].

Infections caused by *P. aeruginosa* are difficult to treat because of the intrinsic resistance of the bacterium to many antipseudomonal agents and its ability to easily acquire antibiotic resistance [4]. In hospital infections caused by *P. aeruginosa* strains 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- $\beta$ -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* strains 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 (the intensive care burn unit, 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) and community-associated (CAI) was evaluated. The isolates were identified by conventional methods and the automated Vitek 2 Compact (BioMerieux, France) system. This study was approved by the Scientific and Ethical Committee of Rize Recep Tayyip Erdogan University Clinical Research Ethics Committee (Rize,

Turkey) (40465587-32 /2018-32).

### **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), aztreonam (ATM), meropenem (MEM), cefepime (FEP) and imipenem (IPM) sensitivity was evaluated. *P. aeruginosa* ATCC 27853 was used as a quality control strain.

### **Detection of $\beta$ -lactamase encoding resistance genes**

The PCR method was investigated for the presence of resistance genes. To obtain template DNA, a single colony bacterial strains 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 10000 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 13000 rpm for 5 minutes. Three  $\mu$ l of the supernatant part was used as template DNA in PCR. Standard PCR mixtures were 50  $\mu$ l final volume; 1,5 units Taq DNA polymerase I (Solis Biodyne), 5  $\mu$ l DNA, 10  $\mu$ l 5X DNA polymerase buffer (Solis Biodyne), 3  $\mu$ l of 25 mM MgCl<sub>2</sub>, 2.5  $\mu$ l of 4 mM each dNTP and 1  $\mu$ l each primer stock (10 pmol/ $\mu$ l) and final volume of sterile deionized water was prepared by completing. Amplification conditions; 3 min 94 °C initial denaturation, 94 °C 45 sec denaturation, 55 °C 1 min binding, 3 min 70 °C elongation (34 cycles) and final synthesis 5 min 70 °C. Amplification products were run in 1.5-2% agarose gel and visualized with UV. The oligonucleotides used in PCR and their T<sub>m</sub> are given in the Table 1.

**Table 1**

### ***Molecular typing of the P. aeruginosa strains***

PFGE typing was performed on 70 strains 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 muller hilton agar were 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 strain 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<sub>2</sub>O 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 frag-ments 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 Laboratories). The electrophoresis conditions were 14°C at 6V/cm<sup>2</sup> for 18 hours. The initial and final switch timeswere 5 seconds and 20 seconds, respectively. The gel was stained with ethidium bromide (5mg/mL) for 30 mins and pho-tographed under UV light. The DNA band profiles were analyzed with Gel Compar software (version 3.0; Applied Maths, Sint-Martens-Latem, Belgium) dendogram and Dice Accordingto the interpretative criteria of Tenover et al, isolates were classified as indistinguishable (cluster), closely related, possibly related, or different [17].

### ***Statistical analysis***

SPSS 21.0 (NY IBM Corp., 2012) program was used. Categorical variables were expressed in terms of frequency (n) and percentage (%). The fitness of continuous variables to normal distribution was tested with Kolmogorov-Simirnov and expressed in terms of arithmetic mean, standard deviation, median, minimum and maximum values. While Mann-Whitney U test was used in paired comparison of continuous variables, categorical variables were analyzed with Pearson  $\chi^2$  and Fisher's exact tests. P<.05 significance level was accepted.

## **Results and Discussion**

### ***Epidemiological results***

One sample from each patient was accepted into the study. The median age of 70 cases was 66 with minimum 17 and maximum 92 years old. The median age of HAI and CAI were 38.6% (n=27) and

61.4% (n=43) respectively (**Table 2**). The two groups were similar in terms of age distribution ( $z=-1.829$ ,  $p=0.067$ ) and the two groups were found to be similar in terms of gender distribution ( $\chi^2(1)=.509$ ,  $p=0.476$ ). The median 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,  $z=-2.192$ ,  $p=0.003$ ). Thirty three strains (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)). 67.1% of the patients 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. The most common comorbidity was cardiovascular diseases (38.6%).

**Table 2**

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 ( $\chi^2(1)=6.756$ , Fisher's exact test,  $p=0.019$ ). The history of hospital contact and use of antibiotics in the last 3 months was high in all patients (67.1%, 75.7%, 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 ( $\chi^2(1)=3.700$ ,  $p=0.054$ ). The detailed descriptions of demographic and pathological characteristic of HAI and CAI could be found in Table 3.

**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, peripheral 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.

**Table 4**

***Antibiotic susceptibility***

Thirteen antimicrobials 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 strain. Twenty-four (34.3%) strains were carbapenem resistant.

**Table 5**

***Detection of MBLs***

Carbapenems are used as the last resort for the treatment of infections caused by these pathogens. However, *P. aeruginosa* is capable of producing enzymes, such as MBL, that inactivate carbapenems. When the resistance genes of the isolates were scanned, the gene encoding  $\beta$ -lactamase or metallo- $\beta$ -lactamase was found in a total of 36 strains. The *bla<sub>VEB</sub>* gene was identified in only 1 strain alone, but in combination with other resistance genes in a total of 17 strains (**Table 6**). While the *bla<sub>PER</sub>* gene was detected in 5 samples alone, it was found in 13 samples in combination with other genes. Neither *bla<sub>VEB</sub>* nor *bla<sub>PER</sub>* was detected in 9 samples. Among the genes encoding metallo- $\beta$ -lactamase, the most *bla<sub>NDM</sub>* positive was detected (n=22), followed by 14 positive samples of *bla<sub>KPC</sub>*. *bla<sub>IMP</sub>* and *bla<sub>VIM</sub>* were detected in 5 and 1 samples, respectively. In 2 strains, *bla<sub>VEB</sub>-bla<sub>PER</sub>-bla<sub>KPC</sub>-bla<sub>NDM</sub>-bla<sub>OXA-48</sub>* combination was found. Also, the association of *bla<sub>VEB</sub>-bla<sub>PER</sub>* and *bla<sub>VEB</sub>-bla<sub>KPC</sub>-bla<sub>NDM</sub>* 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 (Fig. 1). When looked clonally, 43 strains 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% Tenover criteria. Group A, 2 (A1-A2); Group F, 2 (F1-F2); Group G is divided into 2 (G1-G2) subgroups. In particular, six strains



(65, 66, 67, 68, 69, 70 and 6 strains) were included in the group of 20 strains classified as F2 in the major F group. The 6 strains mentioned above are 100% associated with 14 strains in the F2 major group (51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64). Most community-associated strains were collected in the F2 clade, while most hospital-associated strains were collected in the G1 clade. However, no difference was found between the community and hospital-associated strains according to PFGE results.

### Fig. 1

Simultaneously, other microorganisms were also isolated from patients from which these 6 *P. aeruginosa* strains were isolated. Of these patients, 5 patients died, except the number 70. *Acinetobacter* spp., / *K. pneumoniae*, *Acinetobacter* spp., *Candida* spp., *Acinetobacter* spp., / *K. pneumoniae*, *Acinetobacter* spp. and *Klebsiella* spp., microorganisms were also detected in patients with numbers 65, 66, 67, 68, 69 and 70 respectively. 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*<sub>PER</sub>/*bla*<sub>VEB</sub>, *bla*<sub>KPC</sub>, *bla*<sup>OXA-51</sup> and *bla*<sub>NDM</sub> 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 strains were isolated are as in Table 7. Compared to sample 28 and 37, which carried 5  $\beta$ -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.

### Table 7

Since *P. aeruginosa* and *Acinetobacter* species producing extended-spectrum  $\beta$ -lactamase (ESBL) are resistant to most  $\beta$ -lactam antibiotics, monitoring and control of its spread is important when such  $\beta$ -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 [18]. It is essential to define the main risk groups as well as the genetic data of the strains [17]. 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 [19] resistance rates of PA 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 strains reported to the ECDC were carbapenem resistant (CR), but this had risen to over 20% by 2016 [20, 21]. Although the main trigger associated with CR in *P. aeruginosa* is the production of plasmid-mediated  $\beta$ -lactamases / carbapenemases, mutational resistance mechanisms in chromosomal genes - for example, altered expression of outer membrane porins or flux system and increased chromosomal cephalosporinase (AmpC) activity may all be affected.

## Conclusion

In Turkey, the period of 2011-2016, the overall carbapenem resistance has varied between 42.9% - 30.26-%. In 2016, T.C. Ministry of Health Public Health Agency of Turkey National Nosocomial Infections Surveillance Network Summary Report was published. Carbapenem resistance in *P. aeruginosa* across Turkey was determined to be 35.65%. In the University hospitals where our study was conducted, it was 36.55% (50-75 percentile), with a median of 35.71% and 45.28%, respectively. In our study, carbapenem resistance was found 34.3% with compatible of the country's Median value (50%) [22]. The median 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  $\beta$ -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.

## Abbreviations

IMICU: Internal Medicine Intensive Care Unit; SICU: Surgical Intensive Care Unit; CVSICU: Cardio Vascular Surgery Intensive Care Unit; CICU: Coronary Intensive Care Unit; ARICU: Anesthesia and Reanimation Intensive Care Unit; OS; GIS: Gastro Intestinal System; CVS: Cardiovascular System; other services. CSF: cerebrospinal fluid, ARICU: Anesthesia and Reanimation Intensive Care Unit, IMICU: Internal Medicine Intensive Care Unit, PCU: Palliative Care Unit, IDS: Infectious Diseases Service, IVTA: In-Vehicle Traffic Accident, TSH: Traumatic Subarachnoid Hemorrhage, CRI: Chronic Renal Insufficiency, PTC: Percutaneous Transhepatic Cholangiography, TAC: Trekeal Aspiration Culture, NBS: Neurogenic Bladder with Sringomy; VIM: Verona integron-encoded MBL; NDM: New Delhi metallo- $\beta$ -lactamase; MBLs: metallo- $\beta$ -lactamases; Bla:  $\beta$ -lactamase; MHT: modified Hodge test; PCR: polymerase chain reaction; SIM: Seoul imipenemase; GIM: German

imipenemase; SMB: Serratia metallo- $\beta$ -lactamase; ESBL: extended spectrum  $\beta$ -lactamase; 5CS & 3CS: 5' & 3'-conserved segments; ISCR: insertion sequence common region; MIC: minimum inhibitory concentration; PFGE: pulse field gel electrophoresis; UTI: urinary tract infection; CLSI: Clinical and Laboratory Standards Institute

### **Authors' contributions**

A.E, AÇÇ and A.K: Designed and drafted the manuscript. AE, AÇÇ, UK, İEY, NJ: Performed the experiments. SÖ, ES, AK: analyzed the data. AE: AÇÇ, NE: AE, AÇÇ, NE: Collected samples. AE, AÇÇ, AK: Provided analysis methods.

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### **Acknowledgements**

This work was supported by Recep Tayyip Erdogan University Research Fund Grants (BAP).

### **Competing interests**

The authors declare that they have no competing interests.

### **Availability of data and materials**

Our data are available on request by contacting the corresponding author.

### **Funding**

This study was supported by the Recep Tayyip Erdogan University-Scientific Research Projects Coordination Unit [Project number: TSA-2017-801].

### **Ethics approval and consent to participate**

This study was approved by Scientific and Ethical Committee of Rize Recep Tayyip Erdogan University Clinical Research Ethics Committee (Rize, Turkey) (40465587-32 /2018-32).

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22. URL-4, <https://inline.saglik.gov.tr/login.aspx>

**Table 1** Primers used in the amplification of selected genes

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

**Table 2** Distribution of cases according to service and intensive care units

	Total (n=70)	HAI (n=27)	CAI (n=43)
<b>Services, n (%)</b>			
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)

**Table 3** Comparison of demographic and pathological characteristics of HAI (n=27) and CAI (n=43) groups

	Total n=70	HAI n=27	CAI n=43	Statistics	
				z, $\chi^2$	p value
Age (year)	66 (17-92)	73 (18-92)	64 (17-89)	-1.829	.067
<b>Over 65 years old</b>		16(81±6.9)	20(75±6.6)	(%95 confidal)	.0013
<b>Duration of stay (days)<sup>a</sup></b>	19.5 (1-72)	22 (14-72)	18 (1-39)	-2.921	.003
<b>Gender</b>					
Women	27 (38.6)	9 (33.3)	18 (41.9)	.509	.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	.054
Hospital history (last 3 months)	47 (67.1)	16 (59.3)	31 (72.1)	1.238	.266
Antibiotic utilization (last 3 months)	53 (75.7)	22 (81.5)	31 (72.1)	.795	.373
<b>First (Pre) diagnosis, n (%)</b>				-	-
Respiratory pathologies	19 (27.1)	10 (37.0)	9 (20.9)		
Urinary pathologies	13 (18.6)	0	13 (30.2)		
Urinary pathologies	9 (12.9)	5 (18.5)	4 (9.3)		
GIS problem	8 (11.4)	5 (18.5)	3 (7.0)		
CVS pathology	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)		
<b>Hospitalization indication, n (%)</b>					
Respiratory Failure	14 (20.0)	6 (22.2)	8 (18.6)	.136	.713
Mental changes	4 (5.7)	4 (14.8)	0	6.756*	.019
Hemodynamic instability	4 (5.7)	1 (3.7)	3 (7.0)	.330*	.498
Post CPR	7 (10.0)	4 (14.8)	3 (7.0)	1.132*	.253

<sup>a</sup>: Median (minimum-maximum), \*: Fisher's exact test.

441 **Table 4** Diagnosis, clinical features and risk factors of HAI (n =27) and CAI (n =43) groups

	Total n=70	HAI n=27	CAI n=43	Statistics z, $\chi^2$ p value	
<b>Final diagnosis on the PA isolation date (%)</b>					
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		
<b>Comorbid patients, n (%)</b>					
CVS diseases	27 (38.6)	12 (44.4)	15 (34.9)	.640	.424
KOAH	12 (17.1)	5 (18.5)	7 (16.3)	.059*	.526
Neurological diseases	9 (12.9)	5 (18.5)	4 (9.3)	1.257*	.292
Diabetes mellitus	9 (12.9)	3 (11.1)	6 (14.0)	.120*	.517
Malignant diseases	7 (10.0)	3 (11.1)	4 (9.3)	.060*	.554
Renal diseases	6 (8.6)	1 (3.7)	5 (11.6)	1.329*	.394
Skin-soft tissue pathologies	5 (7.1)	2 (7.4)	3 (7.0)	.005*	.645
<b>Invasive procedures and applications during the PA isolation period, n (%)</b>					
Hemodialysis	24 (34.3)	19 (70.4)	5 (11.6)	25.402	.000
Endotracheal application	27 (38.6)	23 (85.2)	4 (9.3)	40.307	.000
Enteral nutrition	49 (70.0)	16 (59.3)	33 (76.7)	2.415	.120
Peripheral venous catheter	48 (68.6)	18 (66.7)	30 (69.8)	.074	.786
Nasogastric	26 (37.1)	16 (59.3)	10 (23.3)	9.209	.002
Peripheral artery catheter	24 (34.3)	19 (70.4)	5 (11.6)	25.402	.000
Decubitus	22 (31.4)	17 (63.0)	5 (11.6)	20.281	.000
Central venous catheter	21 (30.0)	11 (40.7)	10 (23.3)	2.415	.120
Urinary catheter	26 (37.1)	16 (59.3)	10 (23.3)	9.209	.002
Mechanical ventilator	21 (30.0)	17 (63.0)	4 (9.3)	22.742	.000
Total parenteral nutrition	14 (20.0)	3 (11.1)	11 (25.6)	2.171	.141
Transfusion	14 (20.0)	6 (22.2)	8 (18.6)	.136	.713
Cystostomy	13 (18.6)	6 (22.2)	7 (16.3)	.387	.534
Bronchoscopy	7 (10.0)	7 (16.3)	0	4.884*	.038
Steroid	7 (10.0)	3 (11.1)	4 (9.3)	.060*	.554
Tracheotomy	3 (4.3)	1 (3.7)	2 (4.7)	.036*	.671
Chest tube	3 (4.3)	0	3 (7.0)	1.968*	.279

442 \*: Fisher's exact test.



**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)
Gentamycin	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**  $\beta$ -lactamase genes identified in *P. aeruginosa* strains

Genes	Strain numbers	Positive number (%)
<i>bla</i> <sub>VEB</sub>	3, 6, 16, 23*, 26, 28, 30, 33, 37, 38*, 46, 48, 50*, 53*, 56, 60, 61*, 65*	18 (25.71)
<i>bla</i> <sub>PER</sub>	3, 8, 4, 6, 7*, 13, 20*, 28, 29, 36*, 37, 55*, 56, 61*, 64*, 65*, 66*, 67*	18 (25.71)
<i>bla</i> <sub>KPC</sub>	3, 4, 6, 16, 28, 36*, 37, 38*, 43, 50*, 56, 66*	13 (18.57)
<i>bla</i> <sub>NDM</sub>	6, 7*, 9, 10, 11, 26, 27*, 28, 29, 30, 36*, 37, 38*, 43, 46, 53*, 56, 68	21 (30)
<i>bla</i> <sub>IMP</sub>	7*, 23*, 30, 33, 36*	5 (7.14)
<i>bla</i> <sub>VIM</sub>	26	1 (1.42)
<i>bla</i> <sub>OXA-23</sub>	60	1 (1.42)
<i>bla</i> <sub>OXA-48</sub>	6, 7*, 9, 20*, 26, 28, 37, 38*, 46, 59, 60, 67*	12 (17.14)
<i>bla</i> <sub>OXA-51</sub>	59	

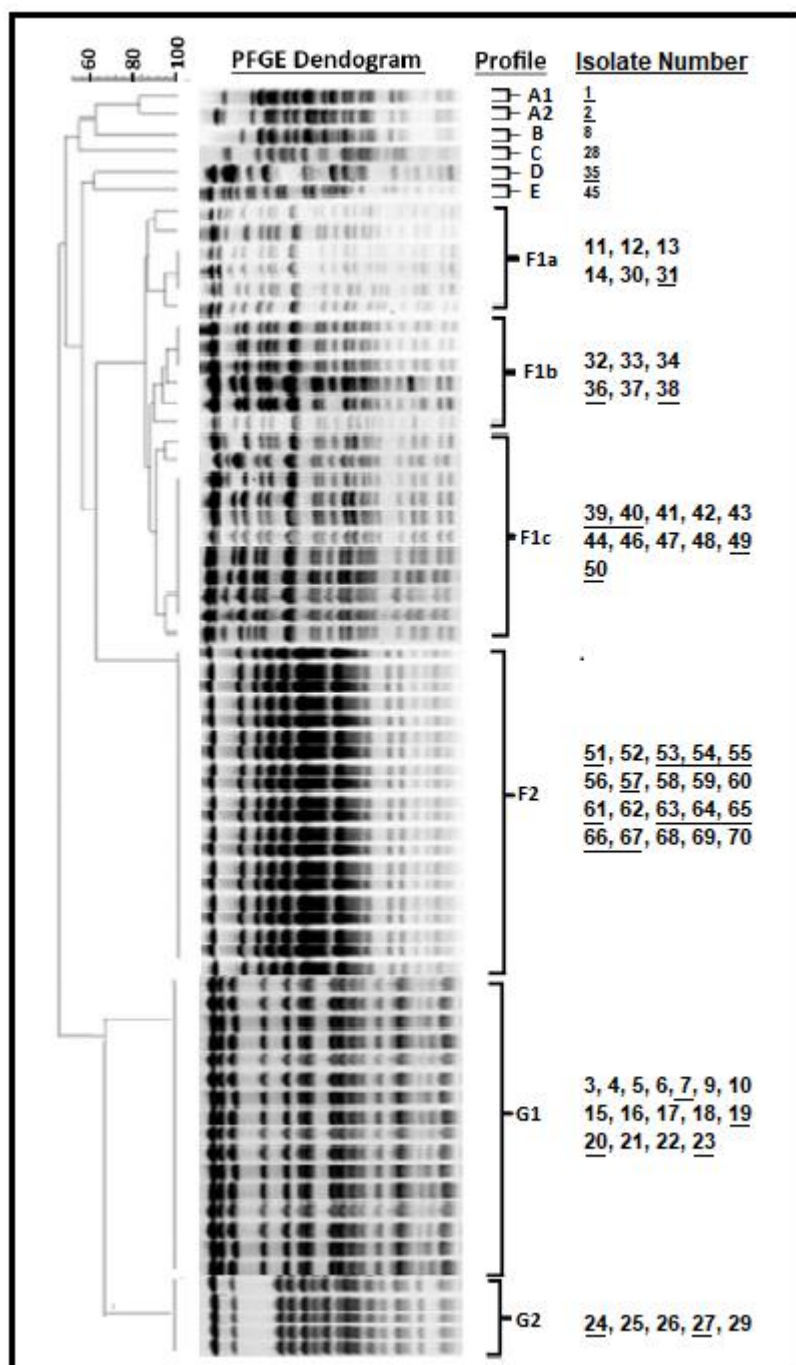
\*, indicates community-associated samples.

**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 22.12.2016	15.05.2016 16.08.2016	08.04.2016 24.04.2016	06.09.2016 06.11.2016	11.07.2016 01.11.2016	22.06.2016 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.	<i>Acinetobacter</i> spp. <i>Klebsiella</i> <i>pneumoniae</i>	<i>Acinetobacter</i> spp.	<i>Candida</i> spp.	<i>Acinetobacter</i> spp. <i>Klebsiella</i> spp.	<i>Acinetobacter</i> spp.	<i>Klebsiella</i> 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

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**Fig. 1** PFGE analysis of *P. aeruginosa* strains 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.

# Figures

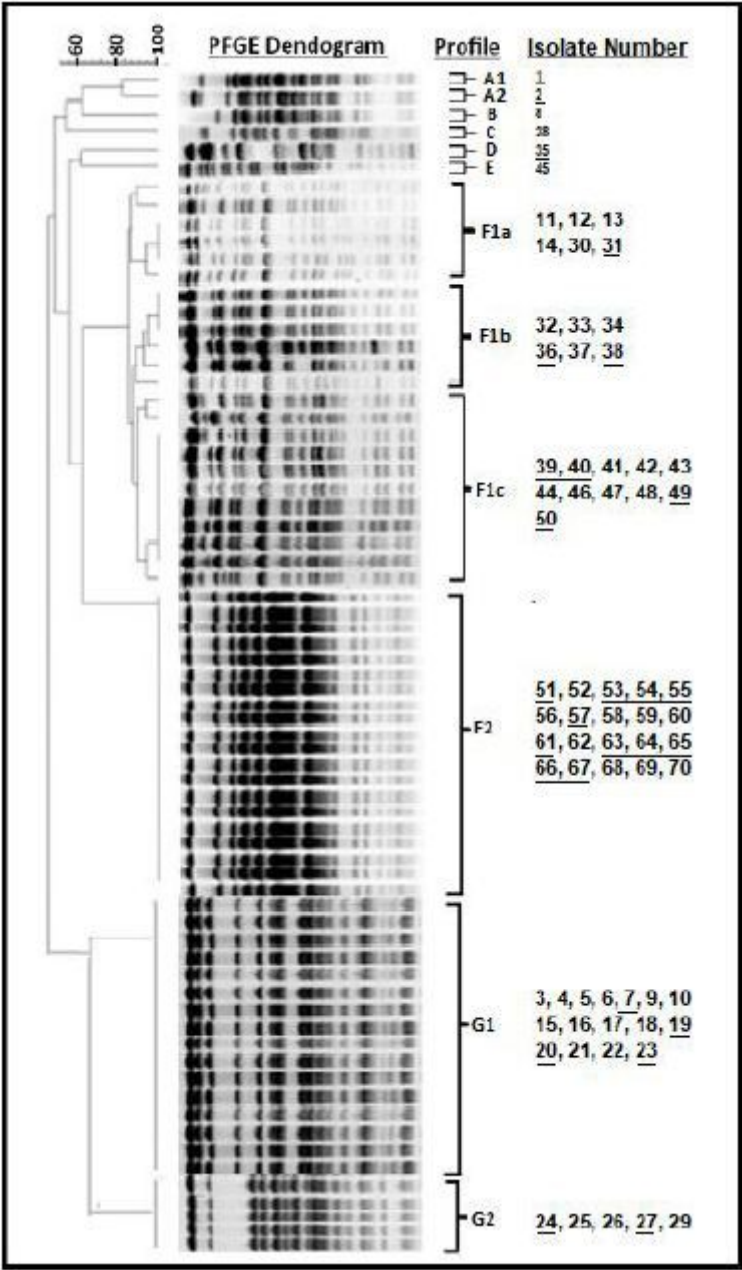


Figure 1

PFGE analysis of *P. aeruginosa* strains 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.