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RESEARCH ARTICLE/KLİNİK ÇALIŞMA

FLORA 2022;27(2):227-240 • doi: 10.5578/flora.20229806

Molecular Epidemiological Evaluation of *Acinetobacter baumannii* Isolates Isolated As the Agent of Hospital Infections in Türkiye

Türkiye'de Hastane İnfeksiyon Etkeni Olarak İzole Edilen *Acinetobacter baumannii* İzolatlarının Moleküler Epidemiyolojik Değerlendirilmesi

Arzu KAYIŞ[1](İD), Murat ARAL[2](İD)

[1] Program of Medical Laboratory Techniques, Kahramanmaraş Sütçü İmam University Vocational School of Health Services, Kahramanmaraş, Türkiye

[2] Clinic of Medical Microbiology, Kahramanmaraş Sütçü İmam University Research and Practice Hospital, Kahramanmaraş, Türkiye

Cite this article as: *Kayış A, Aral M. Candidemia in older adults: Molecular epidemiological evaluation of Acinetobacter baumannii isolates isolated as the agent of hospıtal infectıons in Türkiye FLORA 2022;27(2):227-40.*

ABSTRACT

Introduction: *Acinetobacter baumannii is a major nosocomial pathogen which can cause infections with high morbidity and mortality in hospitalized patients. The aim of this study was to evaluate antibiotic susceptibility of nosocomial Acinetobacter baumannii isolates and to determine, by using the rep-PCR method, the clonal relationship between these isolates.*

Materials and Methods: *A total of 70 nosocomial Acinetobacter baumannii isolates identified by BD Phoenix automated microbiology system and isolated by standard bacteriologic methods from various clinical samples that was sent to Medical Microbiology Laboratory of a university research and practice hospital at the period of June 2014-October 2016 were used in this study. The sensitivity of Acinetobacter baumannii isolates to different antibiotics was determined by BD Phoenix automated microbiology system.*

Results: *Antibiotic resistance rates obtained from isolates of Acinetobacter baumannii by BD Phoenix method; ertapenem 100%; amoxicillin-clavulanate, ampicillin, ceftriaxon, cefuroxime 98.6%; aztreonam, ceftazidime, ciprofloxacin, imipenem, piperacillin and piperacillin-tazobactam 97.1%; cefepime, gentamicin, meropenem and netilmicine 95.7%; amikacine 91.4%; trimethoprim-sulfamethoxazole 88.5%; tigecycline 45.7%; colistine 4.3% respectively. As a result of the clonal correlation analysis with Rep-PCR; 10 clones were identified, one being the main clone. The similarity rate between isolates was 95.8%. Clone 1 was found to be the dominant type. The time interval between the first and last isolate was eighteen months in dominant clone.*

Conclusion: *It was concluded that Acinetobacter baumannii isolates were scattered as a result of cross transmission and patient transfer among clinics in the hospital. The clonal relationship of resistant isolates in the hospital environment once again showed the importance of infection control measures.*

Key Words: *Acinetobacter baumannii; Antibiotic resistance; Clonal relationship; Rep-PCR; Nosocomial infection*

Received/Geliş Tarihi: 06/12/2021 - Accepted/Kabul Ediliş Tarihi: 31/03/2022

Available Online Date: 08.06.2022

ÖZ

Türkiye'de Hastane İnfeksiyon Etkeni Olarak İzole Edilen *Acinetobacter baumannii* İzolatlarının Moleküler Epidemiyolojik Değerlendirilmesi

Arzu KAYIŞ[1], Murat ARAL[2]

[1] Kahramanmaraş Sütçü İmam Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu, Tıbbi Laboratuvar Teknikleri Programı, Kahramanmaraş, Türkiye

[2] Kahramanmaraş Sütçü İmam Üniversitesi Uygulama ve Araştırma Hastanesi, Tıbbi Mikrobiyoloji Kliniği, Kahramanmaraş, Türkiye

Giriş: *Hastanede yatan hastalarda morbidite ve mortalitesi yüksek infeksiyonlara yol açan Acinetobacter baumannii, önemli bir hastane kökenli patojendir. Bu çalışmada, hastane kökenli Acinetobacter baumannii izolatlarının antibiyotik duyarlılıklarının değerlendirilmesi ve bu izolatların klonal ilişkilerinin rep-PCR yöntemiyle belirlenmesi amaçlanmıştır.*

Materyal ve Metod: *Bu çalışmada Haziran 2014-Ekim 2016 tarihleri arasında bir üniversite araştırma ve uygulama hastanesi tıbbi mikrobiyoloji laboratuvarına çeşitli kliniklerden gönderilen örneklerden, standart bakteriyolojik yöntemlerle izole edilen ve BD Phoenix otomatik mikrobiyoloji sistemi ile tanımlanan hastane kökenli 70 Acinetobacter baumannii izolatı kullanılmıştır. Acinetobacter baumannii izolatlarının farklı antibiyotiklere duyarlılıkları BD Phoenix otomatik mikrobiyoloji sistemi ile belirlenmiştir.*

Bulgular: *Acinetobacter baumannii izolatlarındaki antibiyotik direnç oranları BD Phoenix yöntemi ile sırasıyla; ertapenem %100, amoksisilin/klavulanat, ampisilin, seftriakson, sefuroksim %98.6, aztreonam, seftazidim, siprofloksasin, imipenem, piperasilin ve piperasilin-tazobaktam %97.1, sefepim, gentamisin, meropenem ve netilmisin %95.7, amikasin %91.4, trimetoprim-sulfametoksazol %88.5, tigesiklin %45.7, kolistin %4.3 olarak bulunmuştur. Rep-PCR ile yapılan klonal ilişki analizi sonucunda; biri baskın klon olmak üzere 10 klon tespit edilmiştir. İzolatlar arasındaki benzerlik oranı %95.8 olarak saptanmıştır. Birinci klon baskın klon olarak belirlenmiştir. Baskın klondaki ilk ve son izolatın izolasyon tarihleri arasında 18 aylık süre olduğu belirlenmiştir.*

Sonuç: *Acinetobacter baumannii izolatlarının servisler arası transfer edilen hastalar ve çapraz bulaşlar sonucu yayıldığı düşünülmüştür. Dirençli izolatların hastane ortamındaki dağılımının klonal ilişki göstermesi, infeksiyon kontrol önlemlerinin önemini bir kez daha göstermiştir.*

Anahtar Kelimeler: *Acinetobacter baumannii; Antibiyotik direnci; Klonal ilişki; Rep-PCR; Hastane İnfeksiyonu*

INTRODUCTION

Since the 1970s, *Acinetobacter baumannii* (*A. baumannii*) has been defined as an important hospital infection agent, and is one of the most frequently isolated pathogens in current hospital infections worldwide, especially in intensive care units and burns units[1,2]. In the 2016 Turkish National Healthcare Services-Related Infections Agent Distribution and Antibiotic Resistance Network Survey Report, *A. baumannii* was the leading agent at the rate of 21.5% in the ranking of all the agents of all hospital infections[3]. *A. baumannii* strains resistant to multiple drugs (MDR) have been isolated in hospital infections associated with interventions in sterile areas such as burns infections, surgical wound infections, blood circulation infections, and especially ventilator-related pneumonia[1,4]. In many molecular-based observational studies, it has been shown that certain carbapenem-resistant clones show a tendency to clonal expansion by persisting within hospitals throughout the world[1,5,6]. The aim of this study was to evaluate antibiotic susceptibility of nosocomial *A. baumannii* isolates isolated from various clinical samples and to determine by using rep-PCR method the clonal relationship between these isolates.

MATERIALS and METHODS

This descriptive, cross-sectional study was conducted between June 2014 and October 2016 in the Medical Microbiology Laboratory of a university research and practice hospital. A total of 70 *A. baumannii* isolates identified using standard microbiology methods and the

BD Phoenix automatic microbiology system were defined by the Hospital Infection Control Committee as "hospital infection agent". These 70 isolates were examined in respect of antibiotic sensitivity and clonal relationships.

The clinical samples sent to the Medical Microbiology Laboratory were inoculated to 5% sheep blood agar and Eosin Methylene Blue (EMB) agar and were incubated at 37°C. Taking the colony morphology into consideration of the bacteria produced in a pure state in the medium, *A. baumannii* potential bacteria with gram negative, aerobic, diplococcus or coccobacillus morphology, catalase positive, oxidase negative, and those that can not ferment glucose and lactose were included for identification tests in the BD Phoenix automatic microbiology system. Of the isolates determined as *A. baumannii*, the isolates defined as hospital infection agent in collaboration with the Infection Control Committee were stored at -30°C in tryptone soya broth with 10% glycerine added until molecular assay.

The *A. baumannii* isolates underwent sensitivity tests to 18 antibiotics (amikacin, amoxicillin/clavulanate(f), ampicillin, aztreonam, cefepime, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, ertapenem, gentamicin, imipenem, meropenem, netilmicin, piperasilin, piperacillin/tazobactam, tigecycline, trimethoprim-sulfamethoxazole) with the colistin-resistance microdilution method in the BD Phoenix automatic microbiology system according to the manufacturer's instructions. Minimal inhibitor concentration (MIC) values were interpreted according to Clinical And Laboratory Standards Institute (CLSI) 2013 criteria until July 2015, and The European Committee On Antimicrobial Susceptibility Testing (EUCAST) criteria after July 2015. MIC values not interpreted in EUCAST interpreted according to CLSI criteria. In this study, isolates resistant to at least three different classes of antibiotics: aminoglycosides, anti-pseudomonas penicillins, carbapenems, cephalosporins and quinolones were defined as MDR, and isolates resistant to all antibiotics except one or two antibiotic groups were defined as extensively drug-resistant (XDR) [7].

Evaluation of the rep-PCR Fingerprint Relationships of the Isolates and Statistical Analysis

Clonal relationships between *A. baumannii* isolates were examined with the Diversilab® system Rep-PCR (Biomeriйux, France) method which provides the opportunity for rapid molecular epidemiological diagnosis. Calculation of the rep-PCR profile similarities was made using the Pearson correlation coefficient test and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method on the Diversilab software. In the data analysis, the gel profile appearance, the similarity percentage, and a dendogram report were created for each isolate. Interpretation of the rep-PCR fingerprint profile similarities was made with reference to the criteria given in the Diversilab guide. Taking the similarity coefficients into consideration, the isolates were classified as-an not be differentiated (similarity> 97%), similar (similarity 95-97%, 1-2 bands difference), and different (similarity< 95%, >2 bands difference). Those which were indistinguishable and similar in hospital infections and outbreaks were accepted. These definitions were made on the band interpretations[8].

Criteria Defining Hospital Infections (Healthcare Services-Related Infections)

For the diagnosis of hospital infections (healthcare services-related infections), the diagnostic criteria used by the Infection Control Committee of the University Research and Practice Hospital were the criteria for specific infection types and definitions of healthcare services-related infection of the Centre for Disease Control and Prevention (CDC)/NHSN updated in 2008 and translated into Turkish and published by the Turkish Ministry of Health Refik Saydam Hifsisihha Centre Directorate, and the 2013 criteria updated by the CDC/NHSN[9-11].

RESULTS

Seventy *A. baumannii* isolates included in the study, 5 (7.1%), 20 (28.6%), and 45 (64.3%) of 70 isolates were recovered in 2014, 2015, and 2016 respectively. Clinic samples from which these isolates were isolated, and the hospital

Table 1. Distribution of the clinical samples from which *Acinetobacter baumannii* isolates were obtained and hospital infection diagnoses

Sample type	**n**	**%**
DTA	19	27.1
Blood	18	25.7
Wound	13	18.6
CSF	9	12.9
Urine	5	7.1
Sputum	2	2.9
Pleural fluid	2	2.9
Tracheal cathater end	1	1.4
Intravenous cathater	1	1.4
Infection diagnosis	**n**	**%**
VIP	16	22.9
CRBCI	16	22.9
Meningitis after surgical intervention	9	12.8
Deep incisional primary SSI	6	8.6
Superficial incisional primary SSI	5	7.1
CRUSI	4	5.7
Strong probability of ventilator-related pneumonia	3	4.3
Laboratory proven BCI	3	4.3
Clinically identified pneumonia	3	4.3
Decubitus ulcer infection	2	2.9
Organ/space SSI, other LRTI	1	1.4
Other infections of the respiratory system	1	1.4
IAI following surgical intervention	1	1.4
Total	70	100

DTA: Deep tracheal aspirate, CSF: Cerebrospinal fluid, S: Surgery, D: Diseases, Inf: Infection, SSI: Surgical site infection, BCI: Blood circulation infection, LRTI: Lower respiratory tract infection, IAI: Intra-abdominal infection, VIP: Ventilator-related pneumonia, CRBCI: Catheter-related BCI, CRUSI: Catheter-related urinary system infection.

infections caused by these isolates are shown in Table 1.

Of the total isolates included in the study, 72.9% (n= 51) were isolated in the Intensive Care Units (ICU), and 27.1% (n= 19) in clinics. The distribution of isolates according to clinics is shown in Table 2. The patients from which *A. baumannii* isolates were obtained comprised 44 (62.86%) males and 26 (37.14%) females.

The antibiotic sensitivity of the isolates is shown in Table 3. The isolates were found to have resistance to ertapenem at 100%, to amoxicillin/clavulanate, ampicillin, ceftriaxone, and cefuroxime at 98.6%, to aztreonam, ceftazidime, ciprofloxacin, imipenem, piperacillin and piperacillin-tazobactam at 97.1%, to cefepime, gentamicin, meropenem and netilmicin at 95.7%, to amikacin at 91.4%, to trimethoprim-sulfamethoxazole at 88.5%, to tigecycline at 45.7%, and to colistin at 4.3%. When the sensitivity of the isolates to antibiotics was evaluated, the most sensitive antibiotic was colistin, and the greatest resistance was determined to ertapenem, amoxicillin/clavulanate, ampicillin, ceftriaxone, and cefuroxime. Of the total isolates, 97.1% were MDR and carbapenem resistant, and 77.14% (54 isolates) were XDR.

Table 2. The clinics from which the *Acinetobacter baumannii* isolates were obtained

Clinics	n	%
Intensive Care Unit	51	72.9
Anaesthesia and reanimation ICU	22	31.4
Neonatal ICU	11	15.7
Paediatric ICU	6	8.5
Brain surgery ICU	5	7.1
Neurology ICU	3	4.2
Chest diseases ICU	1	1.4
Hematology ICU	1	1.4
Gastroenterology ICU	1	1.4
General surgery ICU	1	1.4
Clinic	19	27.1
Nephrology clinic	4	5.7
General surgery clinic	4	5.7
Chest diseases clinic	3	4.2
Plastic surgery clinic	2	2.8
Orthopedics clinic	2	2.8
Paediatric diseases clinic	1	1.3
Thoracic surgery clinic	1	1.4
Urology clinic	1	1.4
Infectious diseases clinic	1	1.4
Total	**70**	**100**

ICU: Intensive care unit.

A total of 10 different clones were obtained as one main clone from the 70 *A. baumannii* isolates typed using the rep-PCR Diversilab method. The rate of similarity between the isolates was found to be 95.8%, and the clustering rate was 94.3%. The first six clones were indistinguishable from each other or similar, and the single remaining clones[7-10] were different. Clone 1 included the isolates with key numbers 1-48. The first 48 isolates were similar. Clone 2 included key numbers 50-52. The three isolates were indistinguishable. Clone 3 included key numbers 53 and 54. The isolates included were 97.4% similar. Clone 4 included key numbers 55-62, and the isolates in this clone were similar and indistinguishable. Clone 5 included key numbers 63 and 64, and these isolates were indistinguishable at the rate of 99.1%. Clone 6 included key numbers 66-68, and these isolates were indistinguishable at the rate of 99.2%. Clones 7, 8, 9, and 10 included single isolates and were different. The key numbers of the isolates of these clones were 49, 65, 69, and 70 (Figures 1,2).

The isolates in Clone 1 constituted 68.6% (48/70) of all the isolates, and 68.7% (33/48) of these isolates were obtained from ICUs and 31.3% (15) from clinics (Table 4). Of these 48 isolates, 5 (10.4%) were obtained in 2014, 13 (27.1%) in 2015, and 30 (62.5%) in 2016. Of the 30 isolates in 2016, 17 (56.7%) were isolated in March. When the distribution of the hospital infection diagnoses was examined, 15 (31.2%) of the isolates were determined to be the agent of ventilator-related pneumonia (Table 4). The first isolate in this clone was isolated from a deep tracheal aspirate sample in the paediatric ICU on 23.06.2014, and a diagnosis of ventilator-related pneumonia was made. The last isolate in this clone was isolated from a

Table 3. Antibiotic sensitivity percentages of the *Acinetobacter baumannii* isolates

	S n (%)	I n (%)	R n (%)
Amikacin	3 (4.3)	3 (4.3)	64 (91.4)
Amoxicillin/Clavulanate	-	-	69 (98.6)
Ampicillin	-	-	69 (98.6)
Aztreonam	-	2 (2.9)	68 (97.1)
Cefepime	2 (2.9)	1 (1.4)	67 (95.7)
Ceftazidime	2 (2.9)	-	68 (97.1)
Ceftriaxone	-	-	69 (98.6)
Cefuroxime	-	-	69 (98.6)
Ciprofloxacin	2 (2.9)	-	68 (97.1)
Colistin	67 (95.7)	-	3 (4.3)
Ertapenem	-	-	70 (100)
Gentamicin	3 (4.3)	-	67 (95.71)
Imipenem	2 (2.9)	-	68 (97.1)
Meropenem	1 (1.4)	2 (2.9)	67 (95.71)
Netilmicin	3 (4.3)	-	67 (95.71)
Piperacillin	2 (2.9)	-	68 (97.1)
Piperacillin/Tazobactam	2 (2.9)	-	68 (97.1)
Tigecycline	34 (48.6)	3 (4.3)	32 (45.7)
Trimethoprim-Sulfamethoxazole	5 (7.1)	2 (2.9)	62 (88.6)

S: Sensitive, I: Intermediate sensitive, R: Resistant.

blood sample in the anaesthesia and reanimation ICU on 17.09.2016, and a diagnosis of central venous catheter-related blood circulation infection was made. The effective activity of Clone 1 was determined to have continued in our hospital for a period of 18 months.

The isolates in Clone 2 were determined to be effective in different ICUs and clinics of our hospital for a period of four months, the isolates in Clone 3 for three months, the isolates in Clone 4 for 13 months, and the isolates in Clone 6 for four months. The two isolates in Clone 5 were isolated in March 2016 from wound samples in two separate clinics (plastic surgery clinic and infectious diseases clinic) at an interval of 10 days and the diagnoses were made of deep incisional surgical wound site infection.

DISCUSSION

In 2009, the American Infectious Diseases Association reported "ESKAPE" as the pathogens most problematic in respect of treatment and resistance to hospital and intensive care (ESKAPE: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumanii*, *Pseudomonas aeruginosa*, Enterobacter strains)[12]. Of these, *A. baumannii* is a non-fermentative, gram negative bacteria, which has increased in importance in recent years, is widely found in the hospital environment, and has developed resistance to many antibiotics[13].

Of the isolates in this study, 72.9% were isolated from ICUs, and 27.1% from clinics, and 44% of those isolated from ICUs, and 31.4% of the total number of isolates were obtained from the anaesthesia and reanimation ICU. The reason that the *A. baumannii* isolates were isolated more often in ICUs, primarily the anaesthesia and reanimation ICU is that critical patients are followed up in this unit. As these patients are more often applied with invasive interventions such as mechanical ventilation, tracheostomy,

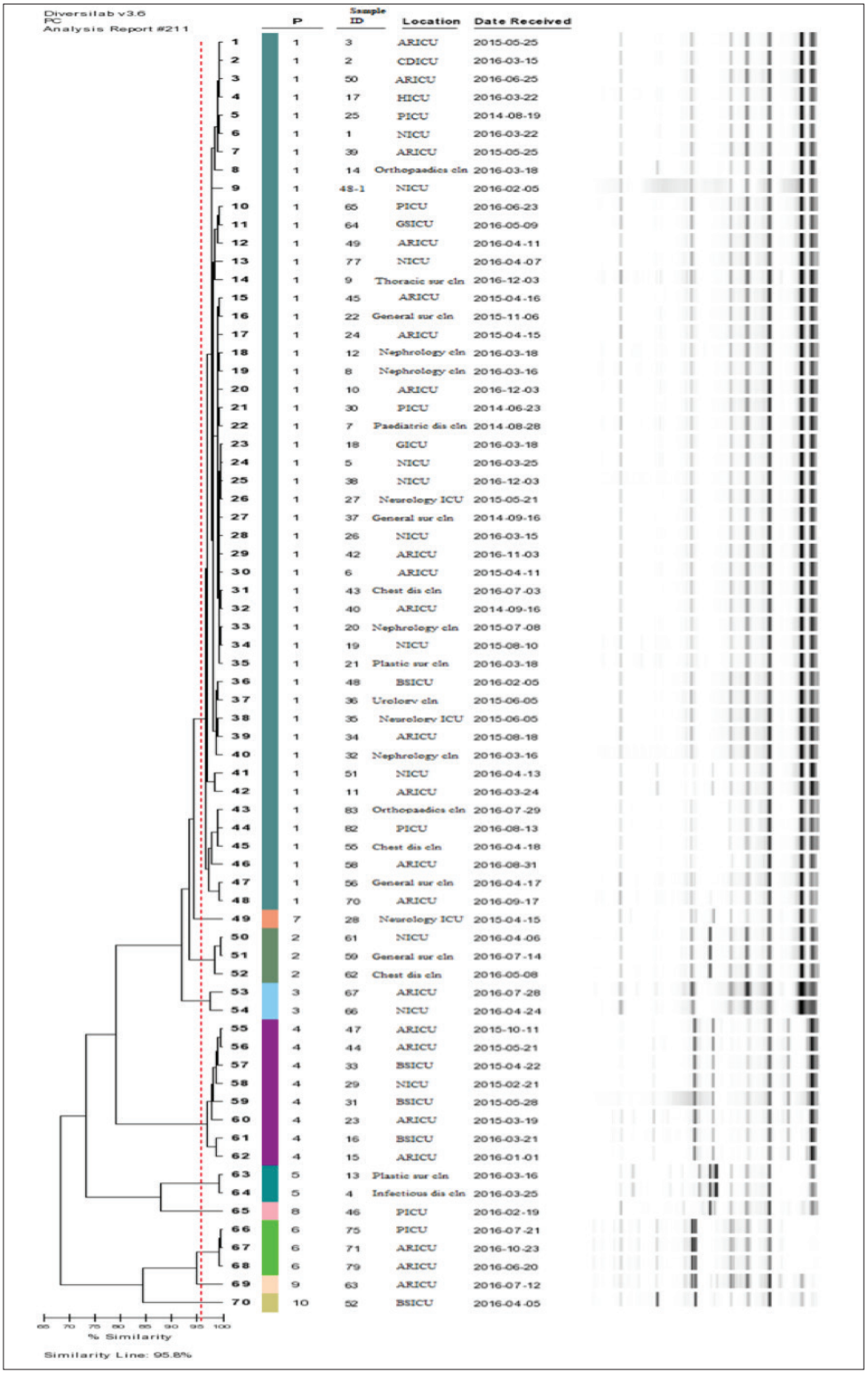

Figure 1. Dendrogram of the 70 *Acinetobacter baumannii* isolates.
ICU: Intensive care unit, PICU: Paediatric ICU, ARICU: Anaesthesia and reanimation ICU, NICU: Neonatal ICU, BSICU: Brain surgery ICU, CDICU: Chest diseases ICU, GICU: Gastroenterology ICU, HICU: Hematology ICU, GSICU: General surgery ICU, S: Surgery, D: Diseases, Inf: Infection, Cln: Clinic.

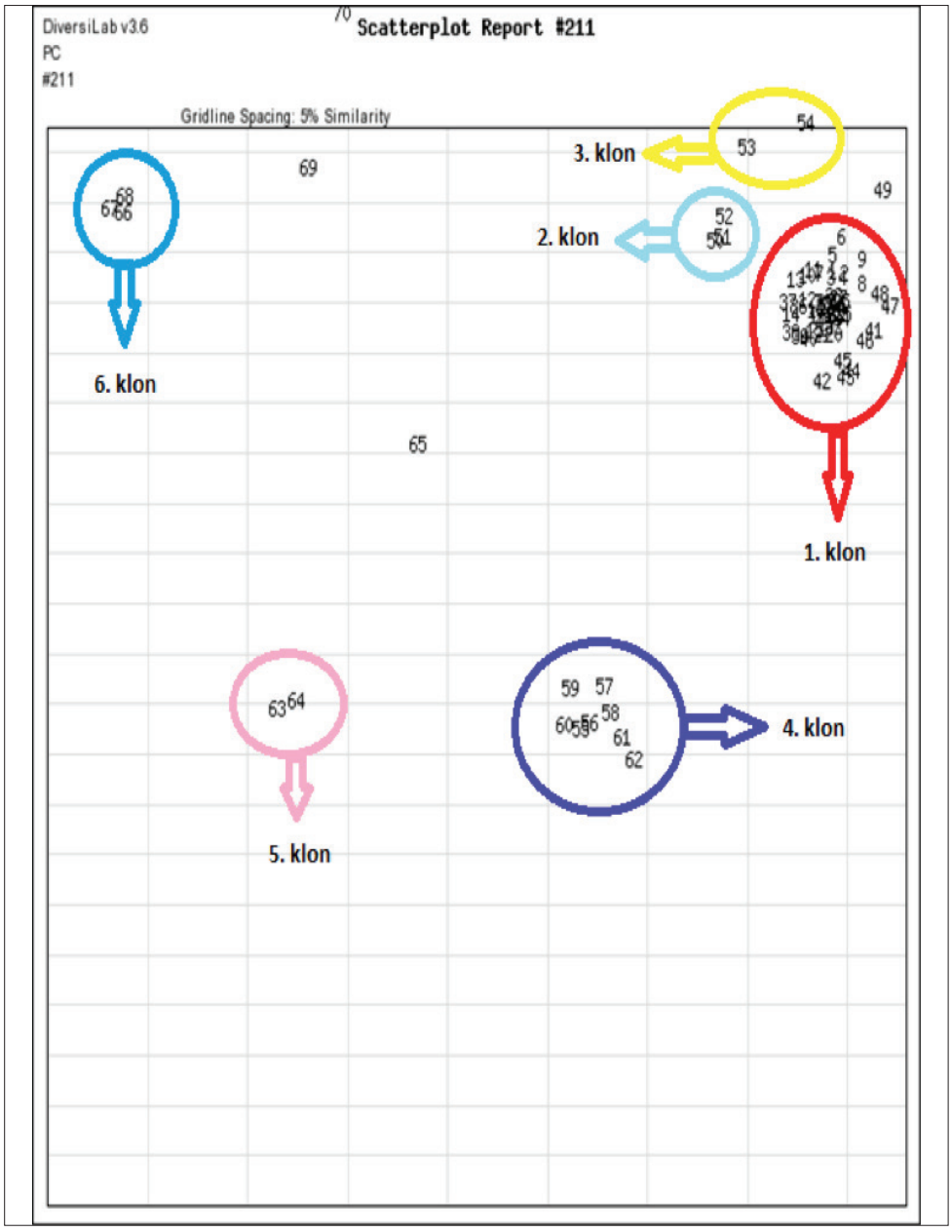

Figure 2. Scatterplot analysis of the 70 *Acinetobacter baumannii* isolates.

intubation, vascular catheterisation and urinary catheterisation, underwent surgery, and had severe comorbidities, they had risk factors for *A. baumannii* colonisation and the development of infection. It has been reported that contaminated humidifiers and ventilator components are often the cause of infections caused by Acinetobacter strains, and ICUs are the centres where this equipment is widely used[14-16].

The results of the current study showed that the leading diagnosis in hospital infections was respiratory tract infection (34.3%), followed by blood circulation infections (27.1%), skin and wound infections (20%) (Table 3). Although differences may be seen from one centre to another in the distribution of *A. baumannii* isolates according to the clinical samples, respiratory system samples are usually ranked first followed by blood and

Table 4. The clinics from which 48 *Acinetobacter baumannii* isolates in Clone 1 were obtained and infection diagnoses

Clinics	n	%		
Intensive Care Unit				
Anaesthesia and reanimation ICU	14	29.1		
Neonatal ICU	8	16.6		
Paediatric ICU	4	8.3		
Brain surgery ICU	2	4.2		
Neurology ICU	1	2.1	33	%68.7
Chest diseases ICU	1	2.1		
Hematology ICU	1	2.1		
Gastroenterology ICU	1	2.1		
General surgery ICU	1	2.1		
Clinic				
Nephrology clinic	4	8.3		
General surgery clinic	3	6.2		
Chest diseases clinic	2	4.2		
Orthopedics clinic	2	4.2		
Paediatric diseases clinic	1	2.1	15	%31.3
Thoracic surgery clinic	1	2.1		
Urology clinic	1	2.1		
Plastic surgery clinic	1	2.1		
Infection Diagnosis				
VIP	15	31.2		
CRBCI	10	20.8		
Deep incisional primary SSI	4	8.3		
Superficial incisional primary SSI	4	8.3		
CRUSI	4	8.3		
Meningitis after surgical intervention	3	6.3		
Laboratory proven BCI	3	6.3		
Strong probability of ventilator-related pneumonia	2	4.2		
Organ/space SSI, other LRTI	1	2.1		
Clinically identified pneumonia	1	2.1		
Other infections of the respiratory system	1	2.1		
Total	48	**100**		

wound samples. Respiratory system samples (deep tracheal aspirate, sputum, pleural fluid, tracheal catheter end) were ranked first in the current study at the rate of 34.3%, followed by blood samples (25.7%) and wound samples (18.6%) [14,15,17]. There are also studies in the literature that have not shown respiratory system samples ranked first[18,19]. Differences in these studies can be due to the selection of a specific infection type or sample type.

A. baumannii outbreaks caused by strains resistant to multiple drugs and carbapenem have often been reported throughout the world in general. This makes the treatment of *A.*

Table 5. Resistance rates (%) of *Acinetobacter baumannii* isolates in this study and some studies

	AK	AMC	AMP	ATM	FEP	CAZ	CRO	CXM	CIP	CT	ETP	GN	IPM	MEM	NET	PRL	TZP	TG	SXT
22*Gülbudak ve ark. (2014)	85.3	-	-	-	97.3	89.3	-	-	97.3	-	-	66.7	96	94.6	-	96	96	-	89.3
23*Rezaee ve ark. 2014)	21	-	-	97	93	93	-	-	93	-	-	-	15	15	-	93	-	68	-
24*Korkmaz ve ark. (2015)	53.7	-	-	-	-	93.5	96	-	93.1	7.2	-	52.2	90.3	89.7	18	96.7	95.3	14.7	83.2
16*Şahin ve ark. (2016)	59	-	-	-	96	97	-	-	99	0	-	56	99	99	56	100	100	5	71
17*Goudarzi ve ark. (2016)	81.6	-	-	-	57.5	50	93.3	-	91.6	0	-	85.8	93.3	65	40.8	-	75	-	73.3
15*Ren ve ark. (2016)	23	-	-	85	78	81	95	-	82	-	-	81	66	-	-	-	68	-	-
25*Şafak ve ark. (2016)	63	-	-	-	-	94.3	-	-	93.4	3.5	-	69	84	86.9	-	-	92.6	6.1	96.1
26*Xiao ve ark. (2016)	83	-	-	-	-	100	-	-	98	2	-	-	100	89	-	100	100	-	93
27*Chen ve ark. (2017)	42.18	-	-	-	65.01	68.98	-	-	74.94	0	-	65.75	58.95	59.45	-	-	65.33	8.53	71.28
28*Sarhaddi ve ark. (2017)	90.7	-	-	-	96.3	100	-	-	98.1	-	-	50	100	-	-	-	100		96.3
29*Çelik ve ark. (2017)	77.4	-	96.2	100	97.9	97.9	100	-	97.4	1.46	69	74.4	96.7	98.6	100	98.1	-	100	66.5
Current study	**91.4**	**98.6**	**98.6**	**97.1**	**95.7**	**97.1**	**98.6**	**98.6**	**97.1**	**4.3**	**100**	**95.71**	**97.1**	**95.71**	**95.71**	**97.1**	**97.1**	**45.7**	**88.6**

AK: Amikacin, AMC: Amoxicillin/clavulanate (F), AMP: Ampicillin, ATM: Aztreonam, FEP: Cefepime, CAZ: Ceftazidime, CRO: Ceftriaxone, CXM: Cefuroxime, CIP: Ciprofloxacin, CT: Colistin, ETP: Ertapenem, GN: Gentamicin, IPM: Imipenem, MEM: Meropenem, NET: Netilmicin, PRL: Piperacillin, TZP: Piperacillin/tazobaktam, TG: Tigecycline, SXT: Trimethoprim-sulfamethoxazole.

baumannii one of the most difficult within the hospital-acquired gram-negative pathogen group[16,20]. In a multicentre study in Turkey of 165 patients (59 cases, 109 control subjects), *A. baumannii* strains were determined in 51.8% (29/56) of resistant isolates[21]. Sensitivity to antibiotics can show differences between countries, centres, and even hospital departments. These differences can be thought to reflect different epidemiology conditions, and different policies of antibiotic use and control. Antibiotic resistance rates of the current study and other studies are shown in Table 5. From this table it can be seen that in the majority of studies, including the current study, the antibiotic resistance rates have been evaluated as high.

In the guidelines for the research, discovery, and development of new antibiotics published by the World Health Organization (WHO) in 2017, carbapenem-resistant *A. baumannii* was ranked first globally of the antibiotic-resistant bacteria[30]. According to the summary report of the National Hospital Infections Survey Network in Turkey in 2014, in Turkey in general, *A. baumannii* was resistant to carbapenem at the rate of 91.55%, and to colistin at 5.55%. According to the 2015 data of the same report, *A. baumannii* was resistant to carbapenem at the rate of 68.39%, and to colistin at 4.43%, and in 2016, these rates were 72.38% and 3.02%, respectively[31-33]. In the 2016 Turkish National Healthcare Services-Related Infections Agent Distribution and Antibiotic Resistance Network Survey Report, resistance patterns were defined according to some hospital infection strains, and imipenem and meropenem resistance was reported as higher in that report. Imipenem resistance was reported as 97.39%, and meropenem resistance as 97.14% for a diagnosis of hospital-acquired pneumonia, imipenem resistance was reported as 96.33%, and meropenem resistance as 97.74% for a diagnosis of hospital-acquired urinary system infection, imipenem resistance was reported as 94.34%, and meropenem resistance as 93.60% for a diagnosis of hospital-acquired blood circulation infection, and imipenem resistance was reported as 91.53%, and meropenem resistance as 91.67% for a diagnosis of hospital-acquired surgical site infection[3]. Although the carbapenem resistance rates were seen to decrease in Turkey in general in the period 2014-2017, when evaluated on the basis of infections, it can be understood that high rates continued.

It has been reported that if comparisons with clones cannot be made with genotyping methods of multiple drug-resistant strains obtained as a result of sensitivity tests in local regions, the same type of epidemiologially predominant multiple drug-resistant strains show a tendency to increase the level of resistance[22]. Molecular typing methods are important tools in determining the infection source of epidemic origins. Although there are various molecular typing methods, rep-PCR is at the forefront as it easy to use, provides rapid results, has high data selection and has the differentiation power to be able to compare with PFGE[34].

In a study by Elmas-Dal of adult ICU patients, 96 *A. baumannii* isolates have been examined using rep-PCR Diversilab, and 83 of the isolates have been found to be within 24 different clusters. The clustering rate of the isolates was determined as 86%. The largest cluster, encoded as P5 included 24 isolates, followed by P6 (13 isolates), P17 (13 isolates), P8 (12 isolates), P4 (7 isolates), P1 (3 isolates), P2 (2 isolates), P9 (2 isolates), P13 (2 isolates) and P19 (2 isolates). The clone with the highest number of isolates was determined to have maintained a presence in the hospital for approximately 14 months[35]. Reszaee et al examined 75 *A. baumannii* isolates, and showed that rep-PCR separated the predominant genotypes of resistant *A. baumannii* isolates into three clones[23]. Gülbudak et al. have studied 75 Acinetobacter isolates, and as a result of clonal relationship analysis with rep-PCR Diversilab, determined two main clones (A-7 subtypes and B-3 subtypes) and a total of 8 (A-H) different clones. Clone A was determined as the predominant type, with 72% (n= 54) of the isolates. Clone B included 13 isolates, Clones C and D, two in each, and Clones E, F, G, and H, one in each. Clone A was isolated from 71% (20/28) of the samples from the reanimation ICU, from 70% (n= 28) of the samples from surgical wards, and from 100%

(6/6) of the samples from the Internal Medicine ICU. An interval of eight months was determined between the isolation of the first and last isolates. It was concluded in that study that the resistance rates of Acinetobacter isolates increased and this increase was in parallel with the spread of the isolates in the same clone[22]. Pasanen et al. have evaluated 55 Acinetobacter isolates with rep-PCR Diversilab to analyse clonalities and two large clones were determined. The majority of the isolates in these clones were from patients receiving major burns treatment or from those in ICU[36].

Shoja et al. have investigated clonal relationships between 40 *A. baumannii* isolates using the rep-PCR method and determined four different clones. Clone A included 12.5% (5/40) isolates, Clones B and C 32.5%(13/40), and Clone D 22.5% (9/40). The isolates in Clones B and C were determined to lead to infections in burns patients most often and were the epidemic isolates which spread most between wards[37]. Sarhaddi et al. have investigated clonal relationships between 54 *A. baumannii* isolates using the rep-PCR method and four different clones were obtained formed from two or more isolates defined at an 85% similarity level. The majority (31/54) of the isolates were in Clone[28].

According to the samples in the currrent study and the relationship results that emerged, there was determined to have been an outbreak of *A. baumannii* strain in our hospital in 2016. In molecular epidemiological studies conducted to show the clonal relationships between the bacteria isolates, although different numbers of clones have been obtained, generally four or more clones have been determined[16,22,28,35]. The 10 clones determined in the current study were evaluated as a similar result to findings in literature.

The most isolates were contained in Clone 1 (48/70), which was accepted as the predominant clone because of the activity maintained in the hospital for a period of 18 months. Previous studies have reported that generally a predominant clone is epidemiologically predominant in outbreaks associated with *A. baumannii*. A predominat clone was similarly determined in the current study[38]. As 97.1% of the isolates in the current study were multiple drug-resistant, evaluation could not be made in respect of the antibiotic resistance of the clones.

The results of this molecular epidemiological study, which was the first to be conducted in our hospital, showed the distribution of the clonal relationships of resistant isolates in the hospital environment, that the same clone remained in the hospital for a long period, and demonstrated again the importance of infection control precautions. The polyclonal result of this study suggests that the source of infection is not a single focus, but similar to other studies that have determined polyclonal sources, several factors are responsible for the spread of infection, such as transfer of patients between departments or hospitals and patient cross-contamination. The need to increase training and supervision programs was shown by reviewing the infection control practices in our hospital and the policies of antibiotic use.

These results showed the need to prevent the formation of endemic clones by monitoring the distribution of clones, particularly of multiple drug-resistant *A. baumannii* isolates with rapid molecular epidemiological diagnosis methods in routine laboratory tests, and by comparisons with previous clones.

Acknowledgements

The author would like to thank Dr. Zerife ORHAN (Faculty Member) and Assoc. Prof. Dr. Mehtap OMAÇ SÖNMEZ for their support in the laboratory studies, and in the writing and evaluation of this article.

ETHICS COMMITTEE APPROVAL

This study was approved by Kahramanmaraş Sütçü İmam University Faculty of Medicine, Scientific Research Ethics Committee (Decision No: 08, Date: 10.11.2014). Therefore, written informed consent form was not obtained from the patients for this reason.

CONFLICT of INTEREST

None of the authors had conflict of interest.

AUTHORSHIP CONTRIBUTIONS

Concept and Design: AK, MA

Data Collection or Processing: AK, MA

Analysis/Interpretation: AK, MA

Literature Search: AK, MA

Writing: AK

Final Approval: AK, MA

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Address for Correspondence/Yazışma Adresi

Dr. Arzu KAYIŞ

Program of Medical Laboratory Techniques,
Kahramanmaraş Sütçü İmam University Vocational School of Health Services,
Kahramanmaraş-Türkiye

E-posta: arzu_kayis@hotmail.com