ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Multicenter Research of the Molecular Epidemiology of Carbapenem-Resistant *Acinetobacter baumannii* Infections

Karbapenem Dirençli Acinetobacter baumannii Enfeksiyonlarının Moleküler Epidemiyolojisinin Çok Merkezli Araştırması

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ABSTRACT

Aim: *Acinetobacter baumannii is an important pathogen causing concern worldwide with increasing antibiotic resistance. We aimed to provide epidemiological data and molecular differences between multidrug resistant A.baumannii isolates obtained from 7 different centers in Türkiye.*

Material and Method: *One hundred sixty-six multidrug-resistant A.baumannii strains were included in the study. All the isolates were tested for antibiotic susceptibility according to EUCAST criteria. To molecularly confirm the carbapenem-resistant A.baumannii isolates, the specific blaOXA-51-like gene region was detected by polymerase chain reaction (PCR) method. A possible phylogenetic relationship between multi-drug resistant isolates was investigated using Pulsed-field Gel Electrophoresis method.*

Results: *One hundred sixty-six carbapenem-resistant A.baumannii strains were evaluated by the ApaI-PFGE (Pulsed-field gel electrophoresis –PFGE) method using the CHEF-DRII electrophoresis system. A total of 51 clusters were grouped into 142 pulsotypes. Twenty clusters had single members. Thirty-one clusters had multiple members and were identified with the capital letters of the alphabet such as A, B, …, and Z26. It was observed that 40 PFGE profiles showed 100% related.*

Conclusion: *More intense contamination is in question in the provinces with many patients. In four of the seven different centers, more innocent results were obtained in terms of the number of samples, antibiotic resistance and clonal relationship when compared to the other three centers. However, this may be caused by the low population zone and inadequate health services. Regional or multicenter studies are minimal. Thus, further studies involving more centers and larger populations are needed. Consequently, the present study will shed light on future studies and create thriving literature on border regions.*

Key words: *A.baumannii; $_{blaOXA-51-like}$ gene; carbapenem-resistant; pulsed-field gel electrophoresis; PCR*

ÖZET

Amaç: *Acinetobacter baumannii, antibiyotik direnci giderek artan ve tüm dünyada endişe yaratan önemli bir patojendir. Türkiye'nin yedi farklı merkezinden temin edilen çoklu ilaca dirençli A.baumannii izolatları arasındaki epidemiyolojik verileri ve moleküler farklılıkları sağlamayı amaçladık.*

Materyal ve Metot: *Yüz altmış altı çoklu ilaca dirençli A.baumannii suşu çalışmaya dâhil edildi. Tüm izolatlar EUCAST kriterlerine göre antibiyotik duyarlılığı açısından test edildi. Karbapenem dirençli A.baumannii izolatlarının moleküler olarak doğrulanması amacıyla polimeraz zincir reaksiyonu (PCR) yöntemi ile spesifik blaOXA-51 benzeri gen bölgesi saptanmıştır. Çoklu ilaca dirençli izolatlar arasındaki olası filogenetik ilişki, Pulsed-field jel elektroforez (PFGE) yöntemi kullanılarak araştırıldı.*

Bulgular: *166 karbapenem dirençli A.baumannii suşu, CHEF-DRII elektroforez sistemi kullanılarak ApaI-PFGE yöntemi ile değerlendirildi. Toplam 51 küme 142 pulsotipte gruplandı. Yirmi kümenin tek üyeleri vardı. Otuz bir küme birden fazla üyeye sahipti ve A, B, … ve Z26 gibi alfabenin büyük harfleriyle tanımlanıyordu. Kırk PFGE profilinin %100 ilişkili olduğu görüldü.*

Tartışma: *Hasta sayısı fazla olan illerde daha yoğun bulaşma söz konusudur. Yedi farklı merkezden dördünde örnek sayısı, antibiyotik direnci ve klonal ilişki açısından diğer üç merkeze göre daha masum sonuçlar elde edildi. Ancak bunun nedeni nüfusun az olması ve sağlık hizmetlerinin yetersiz olması olabilir. Bölgesel ya da çok merkezli çalışmalar oldukça sınırlıdır. Bu nedenle daha fazla merkezi ve daha geniş popülasyonları içeren çalışmalara ihtiyaç vardır. Sonuç olarak bu çalışmanın sınır bölge olarak ileride yapılacak çalışmalara ışık tutacağı ve iyi bir literatür oluşturacağı kanaatindeyiz.*

Anahtar kelimeler: *A.baumannii, blaOXA-51 benzeri gen, karbapenem dirençli, Pulsed-field jel elektroforez, PCR*

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Introduction

Acinetobacter baumannii is an important pathogen causing concern around the world with an increasing antibiotic resistance. It is one of the leading healthcare-associated infection (HAI) agents, especially in intensive care units. It is most frequently encountered with nosocomial infections that can cause epidemics[1]. As *A.baumannii* has all antimicrobial resistance mechanisms, treatment with multi-drug resistance is also a major clinical difficulty in recent years[2]. Especially, the resistance of carbapenem reaches up to 90% throughout the world[3]. Thus, carbapenem-resistant *A.baumannii* (CRAB) is associated with a high mortality rate in healthcare institutions[4]. In addition, it is one of the critical priority pathogens in the list of World Health Organization (WHO). Today, cholistin is preferred in the treatment of high CRAB infection[2]. This is an extremely important public health problem due to increased incidence of HAIs, prolonged hospitalization, increased mortality rate, impaired quality of life, and economic losses[5]. Hospital-acquired infections are defined as one of the most serious patient safety problems in healthcare services around the world[6]. In recent years, surveillance and control programs are developed to prevent the increasing incidence of HAI and multiple antibiotic resistance. Each country and even each region has started to develop its own surveillance systems. In virtue of these strategies, significant reductions have been reported in the incidence of HAI[7]. Epidemiologic studies conducted on the clinical and molecular features of CDAB may aid infection control strategies. Pulsed-field gel electrophoresis (PFGE) technique is accepted as the golden standard for the genotyping of *A.baumannii*[8].

The primary aim of the current study is to determine the clonal relationships between *A.baumannii* strains, isolated from 7 centers (Adana, Ağrı, Ardahan, Iğdır, Kars, Mersin, and Van) in Türkiye, via PFGE method and to contribute to the surveillance system. There is no literature and surveillance data on this subject including the centers of study. Thus, the present study will be an important data source for the literature. Also, demographic characteristics between two regions will be compared.

Materials and Methods

Sample Collection

In the study, 166 multidrug-resistant (MDR) *Acinetobacter baumannii* strains from 7 centers (Adana, Ağrı, Ardahan, Iğdır, Kars, Mersin, and Van) in 2 regions (Çukurova and Eastern Anatolia) of Türkiye between 2019 and 2020 were included in the study. Of the total 166 strains belonging to different patients, 84 were isolated from the Eastern Anatolia region and 82 from the Çukurova region.

The clinical materials from which *A.baumannii* strains were obtained consisted of 32 (19.2%) blood samples, 42 (25.3%) sputum samples, 46 (27.7%) tracheal aspirate samples, 22 (13.2%) urine samples, 19 (11.4%) wound samples, and 5 (3%) pleural fluid samples. Among total samples, the most frequent isolation was obtained from tracheal aspirate samples with 46 (27.7%) strains and the lowest isolation was obtained from pleural fluid with 5 (3%) strains (Table 1).

Table 1. *Distribution of clinical materials according to the centers*

Centers	Sputum (n=42) (%)	Tracheal aspirate (n=46) (%)	Blood (n=32) (%)	Urine (n=22) (%)	Lesion (n=19) (%)	Pleural fluid (n=5) (%)	Total (n=166) (%)
Adana City Hospital	5 (11.9%)	14 (30.4%)	7 (21.8%)	4 (18.1%)	7 (36.8%)	2 (40%)	39
Mersin University Hospital	9 (21.4%)	13 (28.2%)	7 (21.8%)	7 (21.8%)	7 (21.8%)	0 -	43
Van State Hospital	11 (26.1%)	13 (28.2%)	9 (28.1%)	5 (22.7%)	1 (5.2%)	3 (60%)	42
Kars State Hospital	13 (30.9%)	0 -	5 (15.6%)	3 (13.6%)	0 -	0 -	21
Iğdır State Hospital	2 (4.7%)	4 (8.6%)	2 (6.2%)	3 (13.6%)	3 (15.7%)	0 -	14
Agri State Hospital	0 -	2 (4.3%)	2 (6.2%)	0 -	1 (5.2%)	0 -	5
Ardahan State Hospital	2 (4.7%)	0 -	0 -	0 -	0 -	0 -	2

Culture and Identification

The isolates were inoculated on 5% sheep blood agar and MacConkey agar media and incubated overnight at 37˚C for 18–24 hours. Phenotypic identification at species level was confirmed by colony morphology, microscope image, catalase, oxidase test, and biochemical tests[9]. The isolates were stored in blood glycerol broth at -20°C.

Antibiotic Susceptibility Tests

All the isolates were tested for antibiotic susceptibility against Amikacin, Ciprofloxacin, Gentamicin, Imipenem, Levofloxacin, Meropenem, Trimethoprim/Sulfomethoxazole, Ceftazidime, Piperacillin/Tazobactam (Sigma-Aldrich, USA) via liquid microdilution method according to EUCAST criteria. Kirby Bauer Disk Diffusion (KBDD) test was performed for carbapenem resistance[10,11].

Molecular Examinations

In order to molecularly confirm the CRAB isolates, the specific bla$_{OXA\text{-}51\text{-}like}$ gene region was detected by PCR method[12]. Possible phylogenetic relationship between multi-drug resistant isolates was investigated by using PFGE method.

Polymerase Chain Reaction (PCR)

Carbapenem resistance of *A.baumannii* strains was verified by identifying specific bla$_{OXA\text{-}51\text{-}like}$ gene region (Thermo Scientific™). O51-GD2M-F (5'–GACCGAGTATGTACCTG CTTCGACC-3') and O51-GD2M-R (5'–GAGGCTGAACAACCCATCCAGTTAACC-3') (497 bp) primary sequences were used.

DNA extraction

Pure bacterial colonies were incubated in 1 ml Luria Broth (10 g peptone, 5 g yeast extract, 5 g NaCl, 1000 ml distilled water) that was shared in eppendorfs, for 18 hours at 37°C. After incubation, eppendorfs were centrifuged at 13000 rpm for 5 min. Supernatant was discarded and 300 µl sterile water was added to the pellet at the bottom and pipetted. It was boiled at 100°C for 10 minutes, and then, centrifuged at 13000 rpm for 10 minutes. Of the supernatant, 200–250 µl was stored at -20°C in order to be used as template DNA.

Amplification

To the total reaction mixture prepared for amplification, 12.5µl Master Mix (Thermo Scientific™), 3µl template DNA, and 0.5µl of each primer (100 pmol/µl of stock solution) and sterile distilled water were added to reach a total reaction volume of 25µl. Thermal cycling (APPLIED BIOSYSTEMS 2720 Termal Cycler) parameters were applied as follows: initial denaturation at 94°C for 4 min; denaturation at 30 cycles of 94°C for 30 s; primer binding at 55°C for 30 s; chain elongation at 72°C for 1 min; and final elongation at 72°C for 7 min. The bands obtained as a result of the PCR products run on a 2% agarose gel in an electrophoresis tank were evaluated in a gel imaging device.

Pulsed-Field Gel Electrophoresis (PFGE) method

A.baumannii, identified by PCR at species level was cultured as a single colony on blood agar medium. After incubation at 37°C for one night, the purity of the culture was checked. The single colony forming in the medium was passed back to blood agar broth medium and left to incubate at 37°C for one night. The clonal relationship between the colonies in the pure culture obtained was investigated by PFGE method using ApaI restriction enzyme according to Pulsenet KEPA protocol[13]. Electrophoresis was performed on a CHEFF-DR II device (Bio-Rad Laboratories, Nazareth, Belgium) with an initial pulse time of 5 sec, end pulse time of 30 sec, current of 6V, and temperature of 14°C for 20 hours and DNA patterns were evaluated via the GelCompar II software. Dendogram of PFGE profiles was created by using UPGMA and clustering analysis was performed. According to the "Dice" similarity coefficient depending on bands, the relationship between strains with 80% or more similarity was determined. Clustering analyses were shown by capital letters (A, B, C, ...)[14].

Results

In the study, 166 CDAB strains were included between 2019 and 2020. Of the isolates, 84 belonged to Eastern Anatolia region and 82 to Çukurova Region. All of 166 *A.baumannii* strains isolated from 7 hospitals had specific *bla*$_{OXA\text{-}51\text{-}like}$ gene regions. In the distribution of clinical material of CDAB strains according to provinces, tracheal aspirate took place at the top at 4 centers. Among the total samples, the clinical material containing maximum *A.baumannii* was tracheal aspirate (27.7%) and pleural fluid (3%) contained minimum *A.baumannii* (Table 1).

Table 2. *Antibiotic resistance rates*

Antibiotics	Adana City Hosp. (n=39) (%)	Mersin Univ. Hosp. (n=43) (%)	Van State Hosp. (n=40) (%)	Kars State Hosp. (n=24) (%)	Iğdır State Hosp. (n=13) (%)	Ağrı State Hosp. (n=5) (%)	Ardahan State Hosp. (n=2) (%)	Total (n=166) (%)
Amikacin	35 94.5%	39 90.6%	30 75%	15 %62.5	5 38.4%	2 40%	2 100%	128 78%
Ciprofloxacin	39 100%	39 90.6%	39 97.5%	20 83.3%	10 76.9%	5 100%	2 100%	152 92.6%
Gentamicin	32 86.4%	39 90.6%	7 17.5%	18 75%	11 84.6%	5 100%	2 100%	114 69.5%
İmipenem	39 100%	43 100%	40 100%	24 100%	13 100%	5 100%	2 100%	166 100%
Levofoxacin	39 100%	39 90.6%	38 95%	19 79.1%	10 76.9%	1 20%	2 100%	146 89%
Meropenem	39 100%	43 100%	40 100%	24 100%	13 100%	5 100%	2 100%	166 100%
Trimethoprim/ Sülfometoksazol	34 91.8%	38 88.3%	30 75%	11 45.8%	5 38.4%	2 40%	1 50%	121 73.7%
Ceftazidime	39 100%	39 90.6%	38 95%	19 79.1%	6 46.1%	5 100%	2 100%	146 89%
Piperacillin/ tazobactam	39 100%	39 90.6%	38 95%	19 79.1%	6 46.1%	5 100%	1 50%	145 88.4%

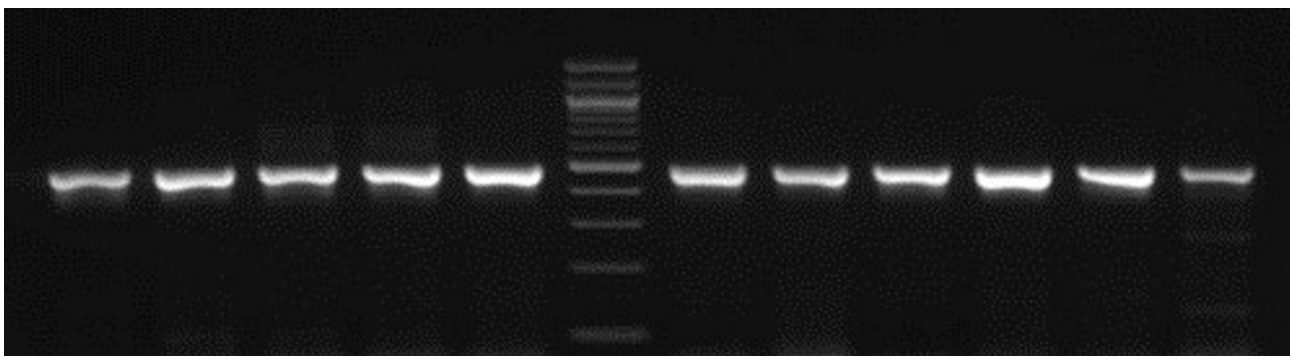

Figure 1. *PCR image of blaOXA-51-like gene regions in CRAB isolates.*

A.baumannii isolates, which were identified to species level from hospitals and whose antibiotic susceptibilities were determined, were verified by phenotypic and molecular tests again. Kirby Bauer Disk Diffusion test was performed to verify the resistance of carbapenem[10,11]. Carbapenem resistance was detected in all *A.baumannii* strains. As a result of the liquid microdilution method, Amikacin (78%), Ciprofloxacin (92.6%), Gentamicin (69.5%), Imipenem (100%), Levofloxacin (89%), Meropenem (100%), Trimethoprim/ Sulfamethoxazole (73.7%), Ceftazidime (89%), and Piperacillin/Tazobactam (88.4%) were found to be resistant with the rates given in parentheses. Except for carbapenem, the highest resistance was found in ciprofloxacin with the rate of 92.6%. The lowest resistance was found in gentamicin with a rate of 69.5% (Table 2). When the number of samples collected between the centers was compared, it was found that resistance rates were much higher in the provinces with high populations such as Adana, Mersin, and Van.

The presence of a specific $bla_{OXA\text{-}51\text{-}like}$ gene region was demonstrated by PCR for both carbapenem resistance and species identification in *A.baumannii* isolates. One hundred sixty-six isolates containing carbapenemase gene, i. e., specific $bla_{OXA\text{-}51\text{-}like}$ gene were included in the study (Fig. 1).

One hundred sixty-six CRABs were evaluated by ApaI-PFGE method using CHEF-DR II electrophoresis system. Clustering rate was calculated as 46.5%. Using the Gel COMPARE-II software system, a total of 51 clusters were grouped into 142 pulsotypes based on 80% or more similarity. Twenty clusters had single members. Thirty-one clusters had multiple members and identified with the capital letters of the alphabet such as A, B, ..., and Z26. Thirty-one clusters were distributed into pulsotypes identified as A1, A2, A3, ...Z26z1 (Fig. 2) in themselves.

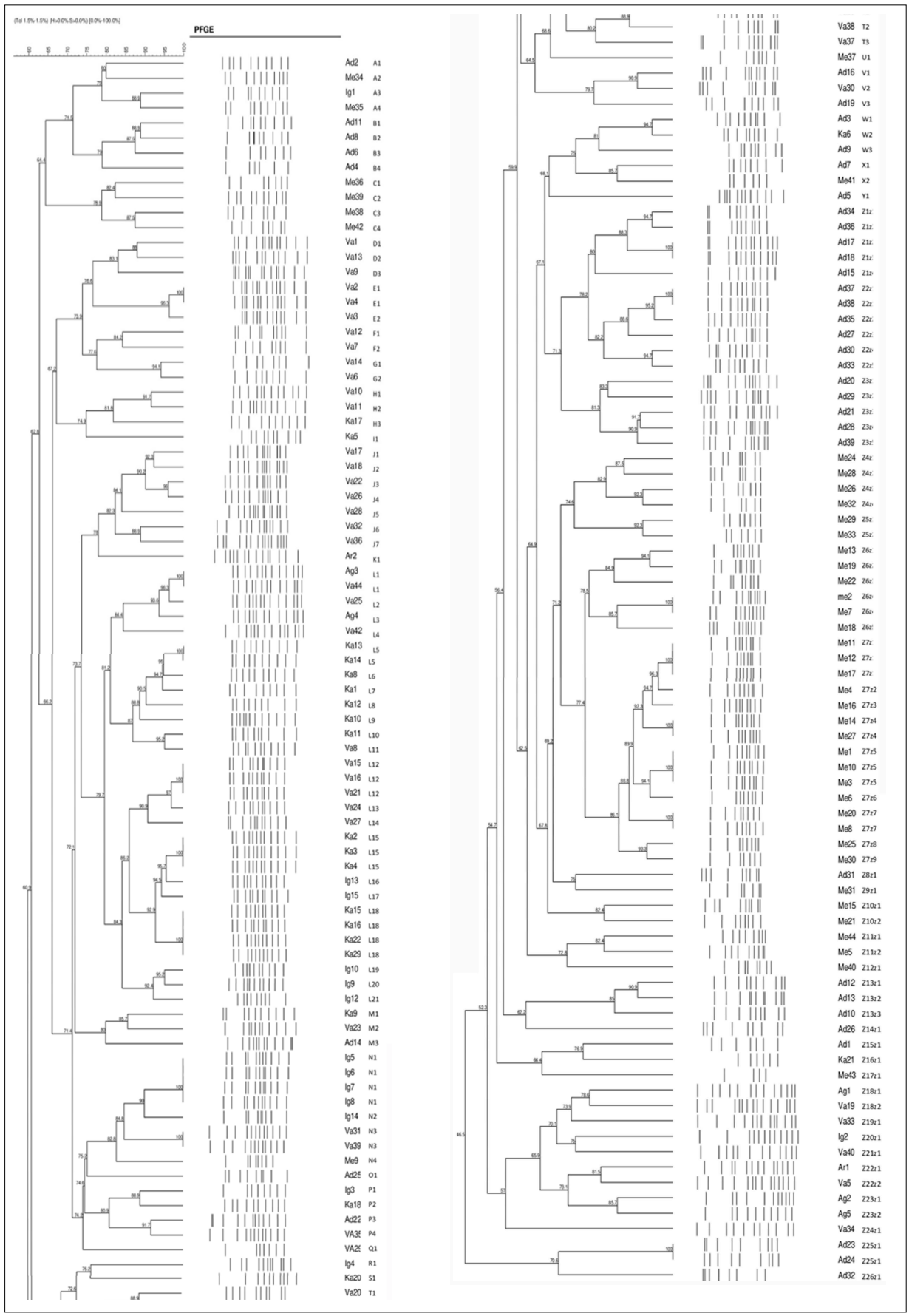

Figure 2. *Pulsed-field gel electrophoresis dendrogram image.*

Table 3. *100% related isolates by PFGE profiles*

Küme	Pulsotip	İzolat numarası
E	E1	Va2, Va4
L	L1	Ag3, Va44
	L5	Ka13, Ka14
	L12	Va15, Va16, Va21
	L15	Ka2, Ka3, Ka4
	L18	Ka15, Ka16, Ka22, Ka29
N	N1	Ig5, Ig6, Ig7, Ig8
	N3	Va31, Va39
Z1	Z1z3	Ad17, Ad18
Z2	Z2z1	Ad37, Ad38
Z6	Z6z4	Me2, Me7
Z7	Z7z1	Me11, Me12, Me17
	Z7z4	Me14, Me27
	Z7z5	Me1, Me3, Me10
	Z7z7	Me20, Me8
Z25	Z25z1	Ad23, Ad24

Va: Van; Ag: Ağrı; Ka: Kars; Ig: Iğdır; Ad: Adana; Me: Mersin.

According to the results of the analysis, it was observed that 40 PFGE profiles showed 100% related profiles in different clusters. The clusters were formed of a total of 16 pulsotypes as 10 clusters with 2 members, 4 clusters with 3 members, and 2 clusters with 4 members (Fig. 3).

It was observed that E1, L1, L5, N3, Z1z3, Z2z1, Z6z4, Z7z4, Z7z7 and Z25z1 pulsotypes were gathered as 2-member; L12, L15, Z7z1, Z7z5 pulsotypes as 3-member, and L18 and N1 pulsotypes as 4-member (Table 3).

The largest cluster was found to be the 30-member cluster L, which was aggregated in 21 pulsotypes identified as L1, L2, L3, ...L21. Only isolates from the Eastern Anatolia region were found in cluster L. This was followed by the 15-member cluster Z7, which was grouped in 9 pulsotypes (Z7z1-Z7z9). The second largest cluster, cluster Z7, belonged to Mersin isolates only. Subsequent 8-member cluster N consisted of 4 pulsotypes grouped in N1-N4. Cluster J had 7 members and 7 pulsotypes, with each member having a different pulsotype. Clusters Z2 and Z6 consisted of 6 members grouped in 5 pulsotypes. Cluster Z3 was grouped in 5 pulsotypes, Z3z1-Z3z5 with 5 members. The smallest clusters were 2-membered F, G, X, Z5, Z10, Z11, Z18, Z22, Z23 and Z25 clusters. D, E, H, M, T, V, W and Z13 clusters were gathered in 3 pulsotypes which were 3-membered (Table 4).

Discussion

Acinetobacter baumannii has become one of the most serious threats especially in intensive care units where antibiotic treatment does not respond. In addition, they are also encountered as HAIs causing the most frequent reason of morbidity and mortality in the world. Multidrug-resistant *A.baumannii,* threatening community health, is between 3% and 17% among HAIs around the world. In Türkiye, this rate varies between 5% and 20%[15,16]. With surveillance studies, it is thought to be able to prevent both pandemic and infection by 70% all over the world. The studies based on

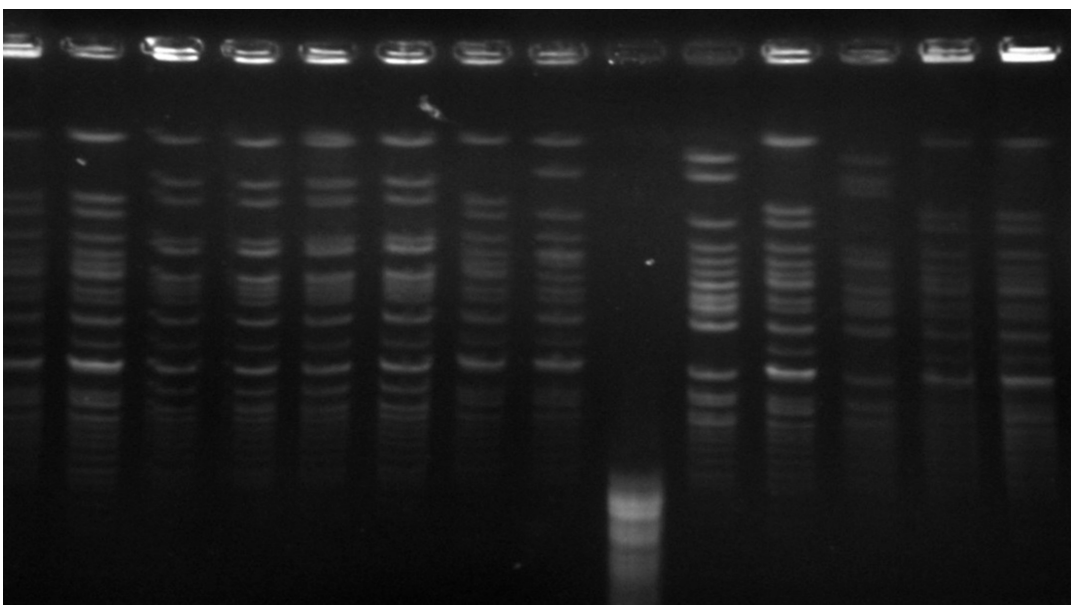

Figure 3. *Pulsed-field gel electrophoresis results image of 100% clonal related CRAB strains.*

Table 4. *Relationship status of PFGE pulsotypes*

Cluster	Pulsotype	Related pulsotypes			
		≥95	≥90	≥85	≥80
A	A1-A4		A3/A4		A1/A2/(A3-A4)
B	B1-B4		B1/B2	(B1-B2)/B3	(B1-B3)/B4
C	C1-C4			C3/C4	C1/C2/(C3-C4)
D	D1-D3			D1/D2	D3/(D1-D2)
E	E1-E2	E1/E2			
F	F1-F2				F1/F2
G	G1-G2	G1/G2			
H	H1-H3		H1/H2		(H1-H2)/H3
J	J1-J7	J3/J4	J1/J2/(J3-J4) J6/J7	(J1-J4)/J5	(J1-J5)/(J6-J7)
L	L1-L21	L1/L2/L3 L5/L6/L7 L10/L11 L12/L13 L15/L16/L17 L19/L20	 (L5-L7)/L8/L9 (L12-L13)/L14 (L15-L17)/L18 (L19-L20)/L21	(L1-L3)/L4 (L5-L9)/(L10,L11) (L12-L14)/(15-L18)/(L19-L21)	(L1-L4)/(L5-L11)/(L12-L21)
M	M1-M3			M1/M2	(M1-M2)/M3
N	N1-N4		N1/N2	(N1-N2)/N3	(N1-N3)/N4
P	P1-P4		P1/P2 P3/P4		(P1-P2)/(P3-P4)
T	T1-T3		T1/T2		(T1-T2)/T3
V	V1-V3		V1/V2		(V1-V2)/V3
W	W1-W3	W1/W2			(W1-W2)/W3
X	X1-X2			X1/X2	
Z1	Z1z1-Z1z4	Z1z1/Z1z2	(Z1z1-Z1z2)/Z1z3		(Z1z1-Z1z3)/Z1z4
Z2	Z2z1-Z2z5	Z2z1/Z2z2 Z2z4/Z2z5	(Z2z1-Z2z2)/Z2z3		(Z2z1-Z2z3)/(Z2z4-Z2z5)
Z3	Z3z1-Z3z5		Z3z3/Z2z4/Z3z5		Z3z1/Z3z2/(Z3z3-Z3z5)
Z4	Z4z1-Z4z4		Z4z3/Z4z4	Z4z1/Z4z2	(Z4z1-Z4z2)/(Z4z3-Z4z4)
Z5	Z5z1-Z5z2		Z5z1/Z5z2		
Z6	Z6z1-Z6z5	Z6z1/Z6z2		(Z6z1-Z6z2)/Z6z3 Z6z4/Z6z5	(Z6z1-Z6z3)/(Z6z4-Z6z5)
Z7	Z7z1-Z7z9	Z7z1/Z7z2/ /Z7z3 Z7z5/ Z7z6	(Z7z1-Z7z3)/Z7z4/ /(Z7z5- Z7z6)/Z7z7 Z7z8/Z7z9	(Z7z1-Z7z7)/(Z7z8-Z7z9)	
Z10	Z10z1-Z10z2				Z10z1/Z10z2
Z11	Z11z1-Z11z2				Z11z1/Z11z2
Z13	Z13z1-Z13z3		Z13z1/Z13z2	(Z13z1,Z13z2)/Z13z3	
Z18	Z18z1-Z18z2				Z18z1/Z18z2
Z22	Z22z1-Z22z2				Z22z1/Z22z2
Z23	Z23z1-Z23z2			Z23z1/Z23z2	

infection control and surveillance programs reported that HAI decreased by 32% in 5 years, and infection increased by 18% in the hospitals without surveillance programs[17,18]. According to surveillance studies covering Türkiye, Romania, Greece, Italy, Spain, Spain, and United Kingdom, carbapenem resistance is an important problem encountered in the treatment *of A.baumannii*[8,19].

Carbapenem-resistant *A.baumannii* is an important problem in the fight against infection in healthcare facilities. The World Health Organization reported in the Central Asian and Eastern European Surveillance of Antimicrobial Resistance Annual Report 2020 (CAESAR) that the rate of CRAB was less than 1% in Belgium, Denmark, Finland, Malta, the Netherlands, and Norway; whereas, in retrospective studies conducted in Southern and Eastern Europe, the rate of CAESAR was reported to be more than 50% in 290 countries.

Data of the study conducted for the Latin American surveillance study showed that the highest imipenem resistance was detected in Argentina (20%), Colombia

(14%), and Brazil (8.5%). Although it was reported that carbapenem resistance decreased periodically, it was reported that the rate of resistance increased up to 80% between 2008 and 2010[20]. In USA, 48% carbapenem resistance was reported[21]. In a recent study, it is worrying that the prevalence of carbapenem-resistant Acinetobacter was found as 50%, 85%, and 62–100% in Singapore, India and Pakistan, respectively[22]. Although the study groups and dates are different, increasing carbapenem resistance cannot be neglected. Carbapenem resistance was reported to be 71–72% between 2007 and 2011 at our country border (Van), which is adjacent to Iran and located in the eastern Anatolia of Türkiye[23]. Also in 2014, it was reported that the rate of carbapenem resistance in MDR *A.baumannii* was 98% upon the detection of the $bla_{OXA\text{-}51\text{-}like}$ gene region at the same center[24]. Over years, the increase in carbapenem resistance reaching 100%, was also supported by the current study. The rate of MDR *A.baumannii* isolated from another center in the same region (Kars) was reported as 39%[25]. In a study conducted in Iran at our border, *A.baumannii* was reported as 61.97% and carbapenem resistance as 38.03% in HAI[26]. Cholistin resistance is also reported in the resistance profiles of 50 CRAB strains collected from training hospitals in Iran[27]. Again, in an analysis performed on karst, it was reported that MDR *A.baumannii* isolates were among the gram (-) isolates most frequently isolated in the intensive care unit[28]. When 7 centers were examined in terms of antibiotic resistance in the present study, it was observed that the highest resistance belonged to Adana, Mersin, and Van centers. Resistance was found to be lower in rural areas with low population density. It is promising that no cholistin resistance was observed in any center. Such multi-center studies are needed regarding the epidemiology of antibiotic resistance, which is spreading rapidly today.

Pulsed-field gel electrophoresis is a standard method for the typing of the isolated MDR *A.baumannii* strains as a source of HAI. It is quite important to determine the clonal relationship, especially in multi-center studies. There are only three centers where PFGE typing is conducted among seven centers included in the study. Previously, no data were found at Ağrı, Ardahan, Kars, and Iğdır centers. Apart from these, in a study conducted with 69 CRAB isolates from Van province, it was reported that 62 (89.9%) of these strains were present in clusters and these strains were included in 16 clusters[29]. It is also reported that 9 out of 10 Acinetobacter strains were clonally related. In the present study, it was observed that 8 of 42 isolates that belonged to the same region had the same clone within 4 clusters and 28 of them were 80% related strains. In time, the increasing isolation of MDR *A.baumannii* and the high number of strains with similar genomes has become an endemic infection in the region. It also supports cross-contamination between the patients.

It was determined that 40 of 166 CRAB strains had the same genotype in 16 pulsotypes. Isolates of each center were similar among themselves. Eight isolates from Van province took place in 4 different pulsotypes, 9 isolates from Kars province in 3 pulsotypes, and 4 isolates from Iğdir province in a single cluster.

Among the strains isolated from Ağrı and Ardahan, 2 isolates in 1 cluster, 6 isolates in 3 different clusters from Adana, and 12 isolates in 5 pulsotypes from Mersin were 100% clonally related. Analysis data regarding the eastern Anatolia border regions of Türkiye are limited. When compared with the accessible epidemiological data, more intense contamination is in question in the provinces with a high number of patients. When compared with the studies conducted in different regions of Türkiye, it was found that both isolated *A.baumannii* strains and antibiotic resistance were at a lower incidence than other regions.

In 4 of the 7 different centers (Ağri, Ardahan, Iğdır, and Kars), more innocent results were obtained in terms of the number of samples, antibiotic resistance and clonal relationship when compared to other 3 centers (Adana, Mersin, Van). However, this may be caused by the low population zone and inadequate health services. Thus, further studies involving more centers and larger populations are needed. Consequently, we conclude that the present study will shed light on future studies, contribute to the surveillance system and create a well literature as a border region.

Conflict of Interest

The authors declare no conflict of interest.

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