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Original Article

Molecular Characterization of Carbapenem-Resistant *Acinetobacter baumannii* Blood Culture Isolates from Three Hospitals in Turkey

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SUMMARY: We aimed to investigate the clonal relationships, common sequence types, and carbapenemase genes in 177 non-repetitive blood culture isolates of *Acinetobacter baumannii* collected from patients at three university hospitals in Turkey in 2016. Molecular epidemiological characteristics of the isolates were examined using pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing (MLST) (Pasteur scheme-*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, and *rpoB*). Multiplex PCR was used to investigate the carbapenemase genes, including $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-48-like}}$, $bla_{\text{OXA-58-like}}$, bla_{IMP}, bla_{VIM}, and bla_{NDM}. PFGE genotyping yielded 92 pulsotypes with a clustering ratio of 69.7%. As per a ≥85% similarity coefficient, 159 (90.9%) isolates were found to be clonally related. The $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$ genes were identified in 100% and 28.2% of the isolates, respectively. The bla_{NDM} gene was identified in two isolates. The MLST analysis included 54 isolates with different pulsotypes, and 29 sequence types (STs). Most of the isolates (n = 36) belonged to the clonal complex (CC)2, one isolate belonged to CC1, and one isolate belonged to CC164. Sixteen new STs (ST1235–ST1250) were identified. Identifying both global ST2 and a large number of new STs, revealed high genetic diversity in *A. baumannii* isolates in the study population.

INTRODUCTION

Acinetobacter baumannii is an increasing cause of multidrug-resistant hospital-acquired infections, particularly in intensive care units (ICUs). The most common infection caused by *A. baumannii* is hospital-acquired pneumonia, particularly ventilator-associated pneumonia and bacteremia (1). *A. baumannii* infection has mortality rates as high as 58%, among bloodstream infections caused by hospital-acquired gram-negative bacteria (2).

The widespread use of carbapenem antibiotics in recent years has caused an increase in the frequency of multidrug-resistant *A. baumannii* isolates (3,4). The resistance of *A. baumannii* to carbapenem, fluoroquinolone, third-generation cephalosporins, aminoglycosides, and colistin was found in blood culture isolates at rates of 91.8%, 89%, 93.8%, 70.9%, and 2.1%, respectively, in a Turkish study (2). The emergence and dissemination of several clonal lineages have been reported to contribute to the increasing prevalence of carbapenem resistance in *A. baumannii*, and these clonal lineages are named international clones (ICs) I-III (5).

Using molecular typing methods, it is possible to identify the clonal relationships among *A. baumannii* isolates and understand the local and global spreading dynamics of these bacteria. These investigations are crucial to develop infection control strategies and overcome nosocomial infection outbreaks. Multilocus sequence typing (MLST) (6,7) and pulsed-field gel electrophoresis (PFGE) (8) are among the techniques developed for this purpose. The aim of this study was to investigate the clonal relationships, common sequence types, and carbapenemase genes in carbapenem-resistant *A. baumannii* isolates obtained from blood

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Table 1. Primer sequences used in the study

Gene	Primer sequence (5′-3′)	Product size (bp)	Reference
$bla_{\text{OXA-24-like}}$	*Forward* -GGTTAGTTGGCCCCCTTAAA *Reverse* -AGTTGAGCGAAAAGGGGATT	246	14
$bla_{\text{OXA-48-like}}$	*Forward* -TTGGTGGCATCGATTATCGG *Reverse* -GAGCACTTCTTTTGTGATGGC	744	15
$bla_{\text{OXA-23-like}}$	*Forward* -CTTGCTATGTGGTTGCTTCTC *Reverse* -ATCCATTGCCCAACCAGTC	650	16
$bla_{\text{OXA-58-like}}$	*Forward* -AAGTATTGGGGCTTGTGCTG *Reverse* -CCCCTCTGCGCTCTACATAC	599	15
$bla_{\text{NDM-1}}$	*Forward* -GTAGTGCTCAGTGTCGGCAT *Reverse* -GGGCAGTCGCTTCCAACGGT	476	17
$bla_{\text{VIM-like}}$	*Forward* -GTGTTTGGTCGCATATCGC *Reverse* -CGCAGCACCAGGATAGAAG	380	18
bla_{IMP}	*Forward* -GGAATAGAGTGGCTTAATTC *Reverse* -CCAAACCACTACGTTATC	188	19
cpn60	*Forward* -ACTGTACTTGCTCAAGC *Reverse* -TTCAGCGATGATAAGAAGTGG	-	22
fusA	*Forward* -ATCGGTATTTCTGCKCACATYGAT *Reverse* -CCAACATACKYTGWACACCTTTGTT	-	22
gltA	*Forward* -AATTTACAGTGGCACATTAGGTCCC *Reverse* -GCAGAGATACCAGCAGAGATACACG	-	22
pyrG	*Forward* -GGTGTTGTTTCATCACTAGGWAAAGG *Reverse* -ATAAATGGTAAAGAYTCGATRTCACCMA	-	22
recA	*Forward* -CCTGAATCTTCYGGTAAAAC *Reverse* -GTTTCTGGGCTGCCAAACATTAC	-	22
rplB	*Forward* -GTAGAGCGTATTGAATACGATCCTAAC *Reverse* -CACCACCACCRTGYGGGTGATC	-	22
rpoB	*Forward* -GGCGAAATGGC(AGT)GA(AG)AACC A *Reverse* -GA(AG)TC(CT)TCGAAGTTGTAAC C	-	22

culture samples of patients, who were treated in the intensive care units of three hospitals in different regions of Turkey.

MATERIALS AND METHODS

The study included 180 carbapenem-resistant *Acinetobacter calcoaceticus–A. baumannii* complex (Acb complex) isolates, isolated from the blood samples of patients who had been treated in the intensive care units of university hospitals located in three different regions (Ankara, Istanbul, and Malatya) of Tukey in 2016. *A. baumannii* isolates were identified by amplified ribosomal DNA restriction analysis (ARDRA) (9,10) and PCR amplification of the $bla_{\text{OXA-51-like}}$ gene (11,12). Only one isolate isolated from the blood sample of each patient was included in the study.

Antibiotic susceptibility tests were initially performed in participating hospitals using the Kirby-Bauer disc diffusion method, MicroScan WalkAway Plus System (Beckman Coulter, Brea, CA, USA), or Phoenix test (BD Diagnostics, USA). To confirm carbapenem resistance, the imipenem and meropenem MIC values for all *A. baumannii* isolates were determined using the E-test (Liofilchem, Roseto degli Abruzzi, Italy) according to the criteria of the European Committee on Antimicrobial Susceptibility Testing (13).

Investigation of carbapenemase genes by multiplex PCR: The carbapenemase-encoding genes, including $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-48-like}}$, $bla_{\text{OXA-58-like}}$, bla_{IMP}, bla_{VIM}, and bla_{NDM}, were identified by multiplex PCR using the primers listed in Table 1 (14–19,22). DNA extraction from all isolates and standard isolates was performed using the boiling method (20). The PCR amplification was carried out in a 20 µL reaction mixture, containing 2.5 µL 10× *Taq* DNA polymerase reaction buffer, 1.25 mM $MgCl_2$, 200 mM dNTP mix (Fermentas/Thermo Fisher Scientific, Waltham, MA, USA), 10 pmol of each primer, 2.5 U Taq DNA polymerase (Fermentas/Thermo Fisher Scientific), and 2 µL genomic DNA. The PCR cycles were performed in the following order: initial denaturation at 94°C for 5 min, 35 cycles of denaturation (1 min, 95°C), annealing (30 s, 60°C), elongation (1.5 min, 72°C), and final

elongation (10 min, 72°C). PCR products were run on 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light.

PFGE typing: PFGE was performed as previously described (8). The bacterial cells were embedded in 1% SeaKem Gold Agarose (Lonza Rockland, Rockland, ME, USA) with 1% sodium dodecyl sulfate. A lysis buffer containing 25 µL proteinase K was used to lyse the cells in the agarose plugs. The lysis step was performed at 55°C in a shaking water bath for 2 h. After the lysis step, the plugs were washed in a shaking water bath five times at 55°C, twice with 10 mL of sterile ultrapure water, and three times with 10 mL of TE buffer. Each washing step lasted for 15 min. After the washing step, the chromosomal DNA was digested with 30 U of *ApaI* (Fermentas/Thermo Fisher Scientific). Then, the fragmented DNA samples were subjected to electrophoresis on 1% pulsed-field certified agarose (Bio-Rad Laboratories, Belgium), using the CHEF-DR III system (Bio-Rad Laboratories), applying 5–20 pulse times for 19 h at a temperature of 14°C and at 6 V/cm^2. Ethidium bromide (0.1%) was used to stain the DNA samples. The Gel Logic 2200 imaging system (Kodak Company, NY, USA) was used to photograph the stained DNA samples, which were examined under UV light. To analyze the DNA band profiles, BioNumerics version 7.5 software (Applied Maths, Sint-Martens-Latem, Belgium) was used. A Dice coefficient at 1.5% tolerance and 1% optimization was used for the comparisons (21).

MLST: MLST studies were performed on 54 *A. baumannii* isolates, selected from the isolates representing different pulsotypes. A total of 7 housekeeping gene regions were sequenced, including *cpn60* (60-KDa chaperonin), *fusA* (elongation factor EF-G), *gltA* (citrate synthase), *pyrG* (CTP synthase), *recA* (homologous recombination factor), *rplB* (50S ribosomal protein L2), and *rpoB* (RNA polymerase subunit B). The primers used for amplification are listed in Table 1 (22). Each PCR reaction mixture had a volume of 50 µL, which included 25 µL of Thermo Scientific DreamTaq Green PCR Master Mix (2×) (ThermoFisher Scientific/Life Technologies, USA), 10 pmol primary pairs (each), and 5 µL template DNA. PCR cycles were performed as in the following order: initial denaturation (5 min, 95°C), 35 cycles of denaturation (30 s, 95°C), annealing (30 s, 50°C), elongation (30 s, 72°C), and final elongation (5 min, 72°C) (http://pubmlst.org/abaumannii/info/primers_Pasteur.shtml). The PCR products were examined by running the products in a 2% agarose gel containing 0.5 µg/mL ethidium bromide. The PCR products were purified according to the manufacturer's instructions using the Agencourt AMPure XP kit (Beckman Coulter). The total volume of the mixture undergoing the sequence reaction was 10 µL, which included 4 µL of Dye Term Inator Cycle Sequencing Quick Start Kit (Beckman Coulter), 1 µL primer (5 pmol), and 1–5 µL of purified DNA. The cycle sequence started with denaturation (3 min, 94˚C) and was followed by 30 cycles of denaturation (20 s, 96°C), annealing (30 s, 55˚C), and elongation (4 min, 60°C). Following the purification of the sequence products using the sodium acetate precipitation method, the Beckman Coulter Ceq8000 (Beckman Coulter) instrument was used for sequencing the purified product. After obtaining the sequence products, they were examined using the following online resources: The National Center for Biotechnology Information (NCBI) GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and Clustal W interface (www.ebi.ac.uk/clustal). *A. baumannii* was examined using the online tools at the MLST website (http://pubmlst.org/abaumannii/).

Clonal complexes are defined to include any ST that matches the central genotype at four or more loci. In other words, a founder clone and its single locus variants (SLV), double locus variants (DLV), and triple locus variants (TLV) together form a clonal complex. Here, the ST number that identifies the founder allelic profile is also the clonal complex (clonal group) number.

Ethical considerations: This study was approved by the Yildirim Beyazıt University Ethics Committee (33/17.02.2016).

Availability of data and material: The following accession numbers were assigned by PubMLST to the 54 Turkish isolates of the *Acinetobacter baumanni* MLST ids: 3906, 3907, 3908, 3909, 3910, 3911, 3912, 3913, 3914, 3915, 3916, 3917, 3918, 3919, 3920, 3921, 3922, 3923, 3924, 3925, 3926, 3927, 3928, 3929, 3930, 3931, 3932, 3933, 3934, 3935, 3936, 3937, 3938, 3939, 3940, 3941, 3942, 3943, 3944, 3945, 3946, 3947, 3948, 3949, 3950, 3951, 3952, 3953, 3954, 3955, 3956, 3957, 3958, 3959. http://pubmlst.org/abaumannii/.

RESULTS

Out of the 180 carbapenem-resistant Acb complex isolates, 177 were found to be *A. baumannii* species, using the $bla_{\text{OXA-51-like}}$ gene and ARDRA methods. The MIC values for imipenem and meropenem were determined to be ≥8 mg/L for *A. baumannii* (177) isolates, confirming that all isolates were carbapenem-resistant.

Carbapenemase genes: The results of multiplex PCR indicated that all isolates harbored the $bla_{\text{OXA-23-like}}$ gene. Of the 177 carbapenem-resistant isolates, 50 (28.2%) were $bla_{\text{OXA-58-like}}$ gene-positive. All the isolates were found to be negative for carbapenemase-encoding genes $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-48-like}}$, bla_{IMP}, and bla_{VIM}. Two isolates were found to be positive for the bla_{NDM} gene. These two *A. baumannii* isolates belonged to two different patients treated in the same center, in Malatya province.

PFGE: Two isolates showed faint bands and were therefore excluded from PFGE interpretation. In the remaining 175 isolates, 92 different PFGE types (pulsotypes) were identified. Of these, 53 were unique, as they were represented by only one isolate. The remaining 39 pulsotypes were clusters with sizes ranging from 2 to 15 isolates. A total of 122 isolates were clustered in 39 pulsotypes, with a clustering rate of 69.7% (122/175) (Fig. 1).

Based on a similarity coefficient ≥85%, 32 PFGE groups were identified. As shown in Fig. 1, while 16 of these groups were uniquely observed in only one isolate, the remaining 16 groups from I to XVI comprised of a total of 159 (90.9%) isolates. PFGE group V was the largest group, containing 64 isolates, followed by

Table 2. Molecular typing results of the 54 *Acinetobacter baumannii* isolates studied both MLST and PFGE

					Pasteur's MLST								
Isolate	Origin	$bla_{OXA-58-like}$ gene	Pulso Group	Pulso Type	MLST Ids[1)]	*cpn60*	*fusA*	*gltA*	*pyrG*	*recA*	*rplB*	*rpoB*	STs	CCs
TR15	Ankara		Unique	7	3906	2	2	2	2	2	2	2	2	2
TR20	Ankara		V	32	3907	2	2	2	2	2	2	2	2	2
TR50	Ankara		Unique	9	3908	2	2	2	2	2	2	2	2	2
TR65	Malatya		XVI	91	3909	2	2	2	2	2	2	2	2	2
TR95	Malatya		V	24	3910	2	2	2	2	2	2	2	2	2
TR117	Malatya	Positive	X	69	3911	2	2	2	2	2	2	2	2	2
TR121	Istanbul	Positive	IV	10	3912	2	2	2	2	2	2	2	2	2
TR126	Istanbul		VIII	62	3913	2	2	2	2	2	2	2	2	2
TR143	Istanbul	Positive	V	19	3914	2	2	2	2	2	2	2	2	2
TR153	Istanbul		V	17	3915	2	2	2	2	2	2	2	2	2
TR158	Istanbul		IV	12	3916	2	2	2	2	2	2	2	2	2
TR161	Istanbul	Positive	Unique	79	3917	2	2	2	2	2	2	2	2	2
TR162	Istanbul	Positive	V	18	3918	2	2	2	2	2	2	2	2	2
TR176	Istanbul		Unique	61	3919	2	2	2	2	2	2	2	2	2
TR179	Istanbul	Positive	IV	13	3920	2	2	2	2	2	2	2	2	2
TR78	Malatya	Positive	XI	75	3921	1	1	1	1	1	1	1	8	1
TR101	Malatya		Unique	86	3922	25	3	6	2	28	1	29	78	S
TR47	Ankara		Unique	83	3923	40	3	7	2	40	4	4	164	164
TR94	Malatya		V	25	3924	2	2	2	25	2	2	2	254	2
TR42	Ankara		X	71	3925	2	2	2	2	99	2	2	523	2
TR34	Ankara		Unique	8	3926	2	2	2	55	2	2	2	604	2
TR149	Istanbul		V	42	3927	2	2	2	55	2	2	2	604	2
TR140	Istanbul		XV	87	3928	2	2	2	2	2	2	96	632	2
TR105	Malatya		Unique	69	3929	1	2	1	1	5	1	30	718	S
TR131	Istanbul	Positive	VIII	63	3930	2	2	2	2	2	2	101	745	2
TR146	Istanbul	Positive	I	2	3931	2	2	2	2	2	2	49	996	2
TR9	Ankara		X	72	3932	2	156	2	2	2	2	2	1108	2
TR16	Ankara		II	3	3933	2	156	2	2	2	2	2	1108	2
TR177	Istanbul		VIII	64	3934	2	156	2	2	2	2	2	1108	2
TR41	Ankara		V	72	3935	2	2	2	2	2	2	148	1154	2
TR60	Ankara	Positive	IX	65	3936	2	2	2	2	2	2	148	1154	2
TR90	Malatya		V	22	3937	2	2	2	2	2	2	148	1154	2
TR102	Malatya	Positive	VII	53	3938	2	2	2	2	2	2	148	1154	2
TR113	Malatya	Positive	III	6	3939	2	2	2	2	2	2	148	1154	2
TR116	Malatya	Positive	XII	78	3940	2	2	2	2	2	2	148	1154	2
TR133	Istanbul		XIV	85	3941	2	2	2	2	2	2	148	1154	2
TR147	Istanbul		VII	54	3942	2	2	2	2	2	2	148	1154	2
TR170	Istanbul	Positive	VI	50	3943	2	2	2	2	2	2	148	1154	2
TR123	Istanbul		XV	88	3944	1	3	2	1	137[2)]	24	137[2)]	1235[3)]	S
TR167	Istanbul		VII	60	3945	2	2	2	53[2)]	2	2	99[2)]	1236[3)]	S
TR142	Istanbul		V	38	3946	1	2	1	1	13	1[2)]	148[2)]	1237[3)]	S
TR31	Ankara	Positive	Unique	90	3947	17	45[2)]	23	10	81[2)]	13	20	1238[3)]	S
TR58	Ankara		I	1	3948	2	156	98[2)]	2	2	2	2	1239[3)]	2
TR81	Malatya	Positive	VII	57	3949	2	2	2	2	2	50[2)]	148	1240[3)]	2
TR112	Malatya		XIII	80	3950	41	42	13	1	9[2)]	4	14	1241[3)]	S
TR163	Istanbul	Positive	Unique	92	3951	23	2[2)]	23	16	25	18	26	1242[3)]	S
TR110	Malatya		XII	77	3952	1	2	1	1	9[2)]	1	2	1243[3)]	S
TR61	Malatya		V	41	3953	2	2	23[2)]	55	2	2	2	1244[3)]	S
TR68	Malatya		X	70	3954	2	2	2	2	114[2)]	2	96	1245[3)]	S
TR111	Malatya	Positive	XI	74	3955	5[2)]	1	1	1	9	1	1	1246[3)]	S
TR165	Istanbul		Unique	84	3956	1	142[2)]	2	1	137[2)]	4	117[2)]	1247[3)]	S
TR174	Istanbul		XV	89	3957	1[2)]	160[2)]	2	1	137[2)]	24	2	1248[3)]	S
TR99	Malatya		Unique	14	3958	2	2	150[2)]	2	171[2)]	2	57[2)]	1249[3)]	S
TR166	Istanbul		Unique	82	3959	1	158[2)]	2	1	137[2)]	1	148[2)]	1250[3)]	S

STs, sequence types; CCs, clonal complexes; S, singleton.

[1)]: New ids (aliases) are given PubMLST curations.

[2)]: Variant genes locus shown as single locus variants (TR58, 61, 68, 81, 110, 111, 112, 163), double locus variants (TR31, 123, 142, 167), and triple locus variants (TR99, 165, 166, 174).

[3)]: New STs were given Pub MLST curations. TR31 (ST1238) and TR163 (ST1242) were identified as *A. pittii* by MLST typing.

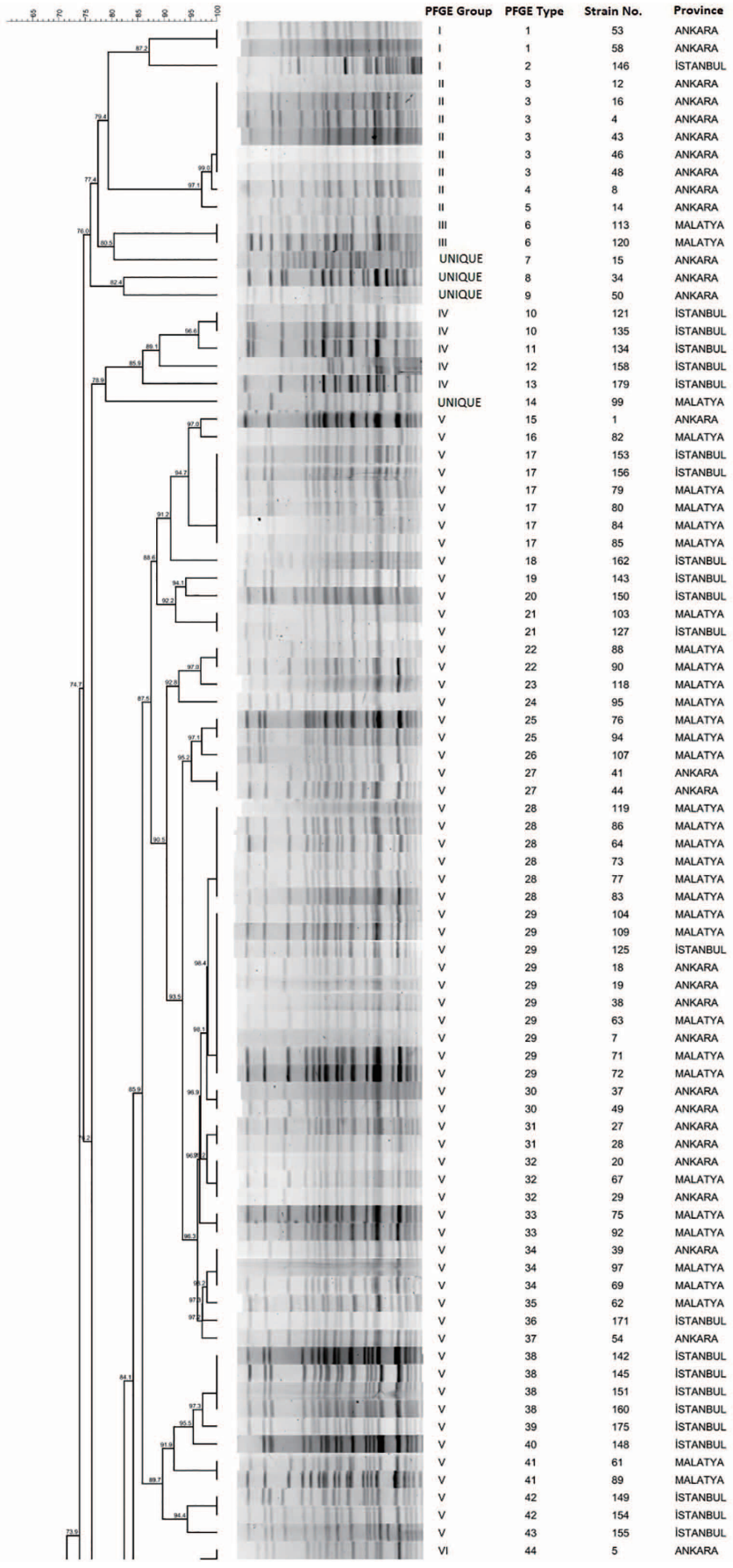

Fig. 1. (continued on next page).

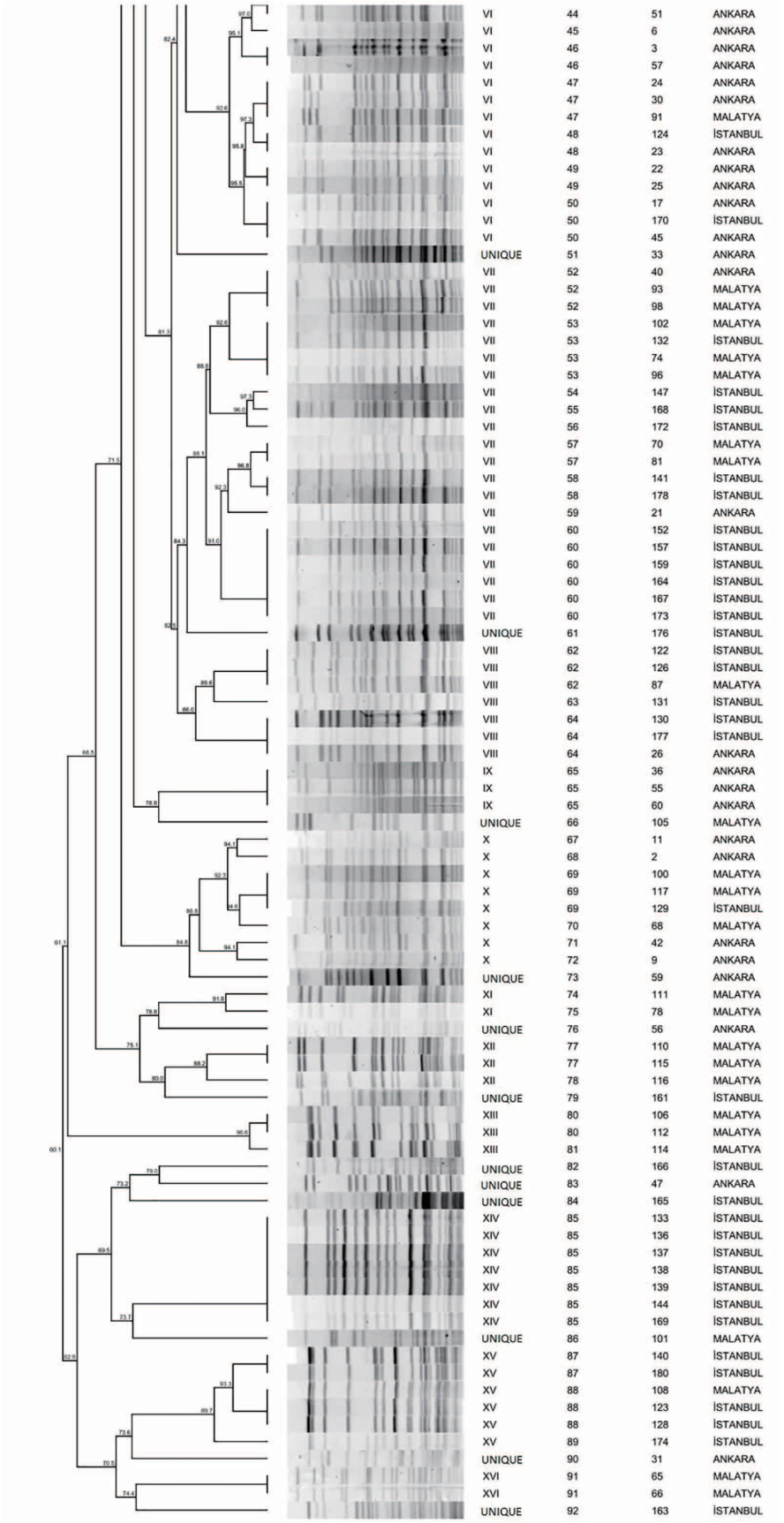

Fig. 1. (Color online) Dendrogram of pulsed field gel electrophoresis (PFGE) of the 175 multidrug-resistant *Acinetobacter baumannii* clinical isolates recovered from bloodstream infections of the patients from three different provinces (Ankara, İstanbul and Malatya). Each pulsotype was indicated as different numbers from 1 to 92, ≥2 strains having indistinguishable PFGE profiles were classified in the same pulsotype. PFGE groups indicating Roman numerals comprised the strains having a similarity coefficient ≥85%. Numbers given on branches indicate cophenetic correlation coefficient values.

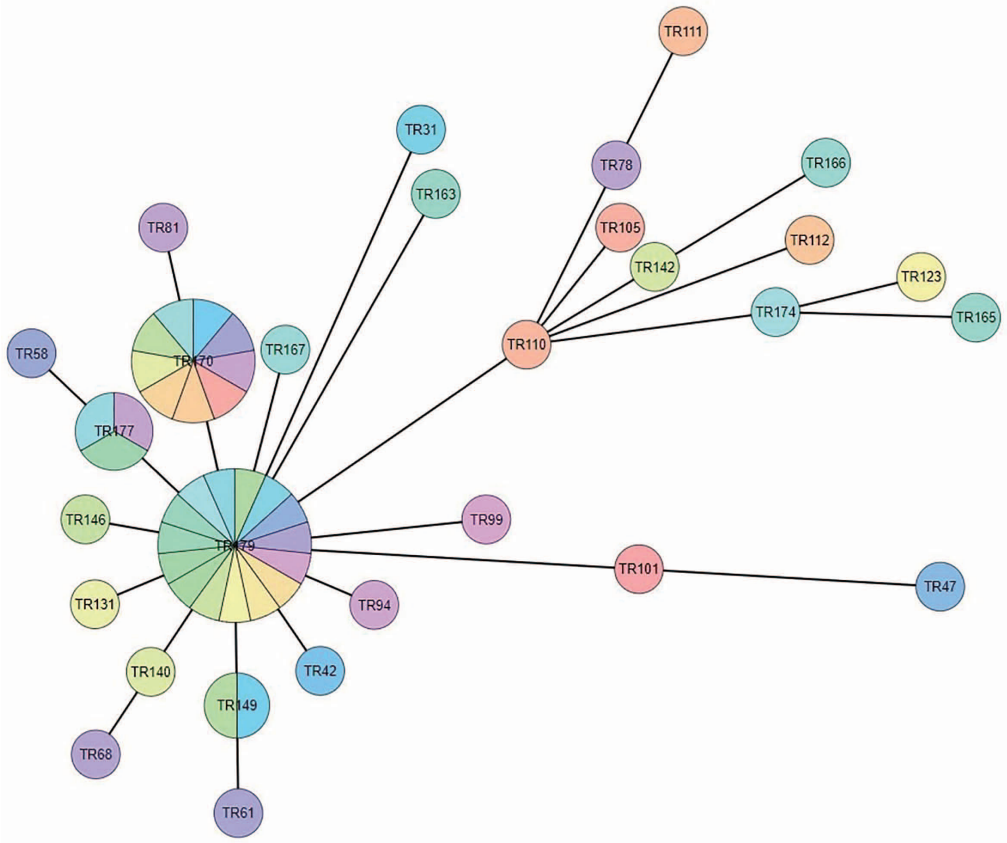

Fig. 2. (Color online) Minimum spanning tree analysis. The 54 *Acinetobater* isolates undergoing MLST analysis were shown in different colors (On each circle is indicated which strain it describes, eg. TR 99 lilac colored). Circle size grown as the number of isolates with the same ST increases. The number of divisors in the circle showed how many isolates were in the same ST. For example, the large circle in the middle was 15 divisors and contains 15 strains with ST2. This figure was made using the minimum spanning tree tool in the PubMLST database (https://pubmlst.org/).

groups VII (n = 21 isolates) and VI (n = 15 isolates). These groups included isolates from all three provinces.

MLST: Based on the quality and length of the sequences, seven loci were evaluated in 54 isolates, which were submitted to the MLST database (https://pubmlst.org/) (Table 2, MLST ids: 3906-3959). MLST typing of these 54 isolates revealed 29 different STs. ST2 comprised of the largest number of isolates (n = 15), followed by ST1154 (n = 9), ST1108 (n = 3), and ST604 (n = 2). Other STs were found in only one isolate each. Sixteen new STs (ST1235-ST1250) were identified. Based on the presence of SLVs, DLVs, and TLVs, the newly identified STs were related to their predecessors as follows: 1235 (DLV157), 1236 (DLV2), 1237 (DLV704), 1238 (DLV63–*A. pittii*), 1239 (SLV1108), 1240 (SLV1154), 1241 (SLV158), 1242 (SLV205–*A. pittii*), 1243 (SLV493), 1244 (SLV604), 1245 (SLV632), 1246 (SLV642), 1247 (TLV10), 1248 (TLV157), 1249 (TLV2), and 1250 (TLV325) (Table 2).

The minimum spanning tree (MST) analysis revealed the relationships among the genotypes (Fig. 2), disclosing 3 clonal complexes (CC), most of which belonged to the international clone II (ICII). Clonal complex II included a total of 36 isolates: 15 in ST2, 9 in ST1154, 3 in ST1108, 2 in ST604, and one each in ST254, ST523, ST632, ST745, ST996, and newly discovered STs, ST1239 and 1240. It was also found that one isolate belonged to CC1, another one belonged to CC164, and 16 isolates were singletons (S). The most probable founder genotype of CC2 was ST2, from which the other STs evolved through a single allelic change. Among the identified STs, the highest frequency was that of ST2, which indicated clonal expansion in this sequence type. The results of the MLST analyses show the genomic evolution of the isolates obtained in studied provinces (Fig. 2).

DISCUSSION

Carbapenem-resistant *A. baumannii* infection is a common problem worldwide, especially in intensive care units. The genes responsible for carbapenem resistance in dominant *A. baumannii* clones are $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$, bla_{VIM} and bla_{NDM} (5). The $bla_{\text{OXA-23-like}}$ gene is the most commonly identified gene worldwide, and is often expressed in IC I or II (23).

Studies carried out in Turkey, and published between 2006 and 2010, reported that the $bla_{\text{OXA-58-like}}$ gene was the most commonly found gene responsible for carbapenem resistance in *A. baumannii* isolates, followed by the $bla_{\text{OXA-23-like}}$ gene (24,25). However, recent studies have shown an increase in the incidence of $bla_{\text{OXA-23-like}}$ gene producing *A. baumannii* isolates (26,27). A multicenter study reported that this was accompanied by increased rates of doripenem resistance

(70.8% in 2009 and 96.4% in 2011) (27).

The intrinsic $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-23-like}}$ genes were identified in all *A. baumannii* isolates included in our study. The $bla_{\text{OXA-58-like}}$ gene was the second most common gene in the isolates (28.2%). In agreement with our results, a multicenter study on doripenem-resistant Acb complex isolates from Greece, a neighboring country, reported that $bla_{\text{OXA-23}}$ was the most prevalent gene (69.3%), followed by $bla_{\text{OXA-58}}$ (19.2%) and $bla_{\text{OXA-24/40}}$ (7.1%) (28).

Interestingly, we identified the bla_{NDM} gene in two *A. baumannii* isolates isolated from the same center. These isolates were in different pulsotypes (pulsotypes 69 and 91). However, MLST analysis was not performed for either isolate. Molecular typing results do not indicate cross-contamination among patients, from whom bla_{NDM} positive isolates were isolated. To the best of our knowledge, this is the first study to identify *A. baumannii* isolates with bla_{NDM} genes isolated from blood cultures in our country.

Pulsed-field gel electrophoresis is a standard method widely used in outbreaks and local epidemiological studies to investigate clonal relatedness in hospital settings (29–32). In our study, of the 175 isolates typed using the PFGE method, 159 (90.9%) were classified into 16 clonally related pulsogroups, as many studies indicated that the isolates were related (considered the same clones), if their Dice similarity index was ≥85% (33,34). Pulsotype analysis revealed that the clustering rate was 69.7% (122/175). It was determined that the high clustering rate was not limited to only one region. Moreover, the dominant V, VI, and VII pulsogroups harbored isolates from three different provinces. These findings indicate cross-transmission between provinces and suggest that more effective infection control measures should be implemented in the provinces.

Recent studies performed in different hospitals reported high clustering rates among *A. baumannii* isolates, similar to the findings of our study (31,32). A multicenter study in Turkey found that the clonal relationship rate among multidrug-resistant *A. baumannii* isolates was 91.2%, encompassing a wide range of PFGE groups of isolates isolated from several centers (31). These findings show that the dominant clones in drug-resistant *A. baumannii* isolates represent a global problem, continuing to affect an increasing number of centers in many countries.

MLST is the gold standard for population structure typing, and the investigation of the global epidemiology of specific bacterial species. MLST analysis enables investigators to determine the distribution of STs and international clonal complexes worldwide (5). In the present study, 29 different STs were detected in 54 isolates analyzed using MLST. Of these STs, 16 were new and 13 matched one of the 4374 STs listed in Pasteur's MLST database for *A. baumannii*. Of the 54 isolates, 27.7% were in ST2, which was dominant in many countries (35–37). Other STs detected in two or more isolates were ST1154 (n = 9), ST1108 (n = 3), and ST604 (n = 2). Most of the isolates (63%) belonged to CC2, which is a common observation worldwide (5,38). The distribution of the remaining isolates showed that one isolate belonged to CC1 and one belonged to CC164. Of the 54 isolates, 16 were singletons. These findings indicate a high diversity in the *A. baumannii* isolates studied. This high diversity was further supported by the findings of the MST analysis.

A multicenter study showed that *A. baumannii* isolates isolated from Turkey belonged to ST15, ST83, and ST84, whereas the isolates isolated from other Mediterranean countries belonged to ST1 (CC1) and ST2 (CC2) (38). Another study, conducted on doripenem-resistant Acb complex isolates collected from European and Mediterranean countries, found that the majority of the isolates belonged to ST2 (CC2) and ST15. In that study, in addition to the predominance of ST2, other STs such as ST10, ST15, ST84, ST97, ST157, and ST158 were also identified in the isolates from Turkey (27). A third study, carried out on multidrug-resistant *A. baumannii* clinical isolates collected from 21 hospitals in Greece, Italy, Lebanon, and Turkey, showed that most of the tested isolates belonged to CC2 (ST2 and ST45). In that study, Turkish isolates were identified as CC15 (ST15 and ST84) and CC83 (ST83) (39). Finally, Metan et al. found that CC15 (ST84) was the dominant clonal complex, followed by CC2 (ST2), CC1 (new STs), and ST19 in 98 *A. baumannii* isolates from Turkey (40). In agreement with our results, the majority of the studies report that the ST2 sequence type is dominant, and that global STs are identified in the country isolates besides the newly identified STs.

In conclusion, the results of the present study showed that OXA-23 carbapenamase was the primary enzyme responsible for carbapenem resistance in *A. baumannii* isolates tested. PFGE typing showed that dominant clones had the potential to spread not only in one hospital, but also in hospitals located in different provinces. These findings indicate that control measures, including identification of the patients at risk of nosocomial infections, monitoring hand hygiene, and following standard precautions with effective surveillance activities, are necessary to prevent the spread of resistant dominant clones. Furthermore, the high number of new STs, in addition to the globally widespread ST2, indicates that the investigated *A. baumannii* isolates exhibit highly variable population dynamics.

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Conflict of interest None to declare.

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