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*OXA-23-like*, *bla*OXA-24-like*, *bla*OXA-48-like*, *bla*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*OXA-23-like and *bla*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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**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 universities located in three different regions (Ankara, Istanbul, and Malatya) in Turkey in 2016. *A. baumannii* isolates were identified by amplified ribosomal DNA restriction analysis (ARDRA) (9,10) and PCR amplification of the *bla*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*OXA-23-like, *bla*OXA-48-like, *bla*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 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

| Gene | Primer sequence (5’-3’) | Product size (bp) | Reference |
|------|-------------------------|-------------------|-----------|
| *bla*OXA-23-like | **Forward** - GTGTTAGTGGCCCTTATAAA **Reverse** - AGTTGAGCGAAGAGGGATT | 246 | 14 |
| *bla*OXA-48-like | **Forward** - TTTAGCTCATGATCTCGGATG **Reverse** - CTGCAATGATCTCGGATG | 744 | 15 |
| *bla*OXA-58-like | **Forward** - CTTGCTATGTTGCTTTCG **Reverse** - ATCCATGCCCAACCATGTC | 650 | 16 |
| *bla*NDM-1 | **Forward** - GTAGTGCTCGATGTCGGCATG **Reverse** - GGGCAATCGCTTCCACAGGT | 599 | 15 |
| *bla*IMP | **Forward** - GAAATAGAATGGGCTTTATTC **Reverse** - CCAACCACATTACCATGTC | 476 | 17 |
| *cpn60* | **Forward** - ACTGTACATCGGCTAAGC **Reverse** - TTACAGGACGATAGAAAGATGG | 380 | 18 |
| fusA | **Forward** - ATCCGATTATTCTGCTCACATYGGAT **Reverse** - CCAACATACKYTGWACACCTTTGTT | - | 22 |
| gltA | **Forward** - AATTTACGTGCGCATTAGGTCGCC **Reverse** - GCCAGAATACGAGAGACATACGAC | - | 22 |
| pyrG | **Forward** - GTGGTGTTTTCATCAGATGWWAAAGG **Reverse** - AAAAAATGTTTAAAGAYTCGTGRTACCCMA | - | 22 |
| recA | **Forward** - CTGGAATCTTCTCGGTTTTAACC **Reverse** - GTTTTCTGGGCGCTCACCACATTAC | - | 22 |
| rplB | **Forward** - GTAGAGCTTGATTAATCGACCATACTAC **Reverse** - CACCCACCACRTGYGGGTGATC | - | 22 |
| rpoB | **Forward** - GGGCAATGGCAGATGA(AG)ACC A **Reverse** - GAA(AG)TC(CT)TGCTGTTGCAACC | - | 22 |
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 (Lonzia 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 Apal (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 (Bio-Rad Laboratories). Ethidium bromide (0.1%) was used to stain the 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), ftsA (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/primer_list_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 Beyazit 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 baumannii species, 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/).

**RESULTS**

Out of the 180 carbapenem-resistant Acb complex isolates, 177 were found to be A. baumannii species, using the blaOXA-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 blaOXA-23-like gene. Of the 177 carbapenem-resistant isolates, 50 (28.2%) were blaOXA-51-like gene-positive. All the isolates were found to be negative for carbapenemase-encoding genes blaOXA-24-like, blaOXA-48-like, blalam, and blavtm. Two isolates were found to be positive for the blalam 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
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Table 2. Molecular typing results of the 54 *Acinetobacter baumannii* isolates studied both MLST and PFGE

| Isolate Origin | Isolate | Origin | blaOXA-58-like gene(s) | Pasteur's MLST |
|----------------|---------|--------|----------------------|----------------|
|                |          |        |                      | Ids (STs, CCs) |
| TR15 Ankara    | Unique   | 7      | 3906                 | 2 2 2 2 2     |
| TR20 Ankara    | V        | 32     | 3907                 | 2 2 2 2 2     |
| TR50 Ankara    | Unique   | 9      | 3908                 | 2 2 2 2 2     |
| TR65 Malatya   | XVI      | 91     | 3909                 | 2 2 2 2 2     |
| TR95 Malatya   | V        | 24     | 3910                 | 2 2 2 2 2     |
| TR117 Malatya  | Positive | X      | 3911                 | 2 2 2 2 2     |
| TR121 Istanbul | Positive | IV     | 3912                 | 2 2 2 2 2     |
| TR126 Istanbul | VIII     | 62     | 3913                 | 2 2 2 2 2     |
| TR143 Istanbul | Positive | V      | 3914                 | 2 2 2 2 2     |
| TR153 Istanbul | V        | 17     | 3915                 | 2 2 2 2 2     |
| TR158 Istanbul | IV       | 12     | 3916                 | 2 2 2 2 2     |
| TR161 Istanbul | Positive | Unique | 3917                 | 2 2 2 2 2     |
| TR162 Istanbul | Positive | V      | 3918                 | 2 2 2 2 2     |
| TR176 Istanbul | Unique   | 61     | 3919                 | 2 2 2 2 2     |
| TR179 Istanbul | Positive | IV     | 3920                 | 2 2 2 2 2     |
| TR177 Istanbul | Unique   | 75     | 3921                 | 2 2 2 2 2     |
| TR101 Malatya  | Unique   | 86     | 3922                 | 2 2 2 2 2     |
| TR47 Ankara    | Unique   | 83     | 3923                 | 40 3 7 2 2     |
| TR94 Malatya   | V        | 25     | 3924                 | 2 2 2 2 2     |
| TR42 Ankara    | X        | 71     | 3925                 | 2 2 2 2 2     |
| TR34 Ankara    | Unique   | 8      | 3926                 | 2 2 2 55 2     |
| TR149 Istanbul | V        | 42     | 3927                 | 2 2 2 55 2     |
| TR140 Istanbul | XV       | 87     | 3928                 | 2 2 2 55 2     |
| TR105 Malatya  | Unique   | 69     | 3929                 | 2 2 2 55 2     |
| TR131 Istanbul | Positive | VIII   | 3930                 | 2 2 2 55 2     |
| TR146 Istanbul | Positive | I      | 3931                 | 2 2 2 55 2     |
| TR9 Ankara     | X        | 72     | 3932                 | 2 2 2 55 2     |
| TR16 Ankara    | II       | 3      | 3933                 | 2 2 2 55 2     |
| TR177 Istanbul | Unique   | 64     | 3934                 | 2 2 2 55 2     |
| TR41 Ankara    | V        | 72     | 3935                 | 2 2 2 55 2     |
| TR60 Ankara    | Positive | IX     | 3936                 | 2 2 2 55 2     |
| TR9 Malatya    | V        | 22     | 3937                 | 2 2 2 55 2     |
| TR102 Malatya  | Positive | VII    | 3938                 | 2 2 2 55 2     |
| TR113 Malatya  | Positive | III    | 3939                 | 2 2 2 55 2     |
| TR167 Istanbul | XV       | 60     | 3945                 | 2 2 2 55 2     |
| TR47 Ankara    | Unique   | 83     | 3948                 | 2 2 2 55 2     |
| TR131 Istanbul | Positive | VIII   | 3950                 | 2 2 2 55 2     |
| TR146 Istanbul | Positive | I      | 3951                 | 2 2 2 55 2     |
| TR9 Ankara     | X        | 72     | 3952                 | 2 2 2 55 2     |
| TR60 Malatya   | Positive | IX     | 3953                 | 2 2 2 55 2     |
| TR95 Malatya   | V        | 24     | 3954                 | 2 2 2 55 2     |
| TR167 Istanbul | Unique   | 84     | 3956                 | 2 2 2 55 2     |
| TR31 Ankara    | Positive | Unique | 3957                 | 2 2 2 55 2     |
| TR58 Ankara    | I        | 1      | 3948                 | 2 2 2 55 2     |
| TR81 Malatya   | Positive | VII    | 3949                 | 2 2 2 55 2     |
| TR112 Malatya  | XIII     | 80     | 3950                 | 2 2 2 55 2     |
| TR163 Istanbul | Positive | unique | 3951                 | 2 2 2 55 2     |
| TR110 Malatya  | Positive | III    | 3952                 | 2 2 2 55 2     |
| TR61 Malatya   | V        | 41     | 3953                 | 2 2 2 55 2     |
| TR68 Malatya   | X        | 70     | 3954                 | 2 2 2 55 2     |
| TR111 Malatya  | Positive | XI     | 74                  | 2 2 2 55 2     |
| TR165 Istanbul | Unique   | 84     | 3956                 | 2 2 2 55 2     |
| TR174 Istanbul | XV       | 89     | 3957                 | 2 2 2 55 2     |
| TR99 Malatya   | Unique   | 14     | 3958                 | 2 2 2 55 2     |
| TR166 Istanbul | Unique   | 82     | 3959                 | 2 2 2 55 2     |

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, Istanbul, 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.
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*$_{OXA-23-like}$, *bla*$_{OXA-24-like}$, *bla*$_{OXA-58-like}$, *bla*$_{VIM}$ and *bla*$_{NDM}$ (5). The *bla*$_{OXA-23-like}$ 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*$_{OXA-58-like}$ gene was the most commonly found gene responsible for carbapenem resistance in *A. baumannii* isolates, followed by the *bla*$_{OXA-23-like}$ gene (24,25). However, recent studies have shown an increase in the incidence of *bla*$_{OXA-23-like}$ gene producing *A. baumannii* isolates (26,27). A multicenter study reported that this was accompanied by increased rates of doripenem resistance.
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(70.8% in 2009 and 96.4% in 2011) (27).

The intrinsic bla\textsubscript{OXA-23-like} and bla\textsubscript{OXA-24-like} genes were identified in all A. baumannii isolates included in our study. The bla\textsubscript{OXA-58} 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\textsubscript{OXA-23} was the most prevalent gene (69.3%), followed by bla\textsubscript{OXA-58} (19.2%) and bla\textsubscript{OXA-24} (7.1%) (28).

Interestingly, we identified the bla\textsubscript{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\textsubscript{NDM} positive isolates were isolated. To the best of our knowledge, this is the first study to identify A. baumannii isolates with bla\textsubscript{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 carbapenemase 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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