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Original Article

High prevalence of TEM, VIM, and OXA-2 beta-lactamases and clonal diversity among *Acinetobacter baumannii* isolates in Turkey

Nergis Asgin[1] Baris Otlu[2], Elcin Kal Cakmakliogullari[1], Betul Celik[2]

[1] *Department of Medical Microbiology, Faculty of Medicine, Karabuk University, Karabuk, Turkey*
[2] *Department of Medical Microbiology, Faculty of Medicine, Inonu University, Malatya, Turkey*

Abstract

Introduction: *Acinetobacter baumannii* is an opportunistic pathogen that causes nosocomial infections with high mortality. Treatment options are limited owing to its resistance to numerous antibiotics. Here, we sought to determine the antibiotic susceptibilities of *A. baumannii* isolates, investigate clonal relationship among the strains, and determine the frequency of beta-lactamase resistance genes.

Methodology: The identification and antibiotic susceptibilities of 69 *A. baumannii* strains were determined using a BD-Phoenix automated system. The presence of *bla*$_{OXA-2}$, *bla*$_{OXA-10}$, *bla*$_{OXA-23}$, *bla*$_{OXA-24/40}$, *bla*$_{OXA-51}$, *bla*$_{OXA-58}$, *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{IMP}$, *bla*$_{VIM}$, and *bla*$_{GIM}$ genes were investigated using polymerase chain reaction (PCR), and clonal relatioships among the isolates were determined using pulsed-field gel electrophoresis (PFGE).

Results: All strains were resistant to ampicillin–sulbactam, gentamicin, cefepime, ciprofloxacin, and ceftriaxone. While 65 of the 69 strains (94.2%) were resistant to piperacillin-tazobactam, amikacin, imipenem, and meropenem, all strains were susceptible to tigecycline and colistin. The frequencies of *bla*$_{OXA-51}$, *bla*$_{OXA-23}$, *bla*$_{TEM}$, *bla*$_{OXA-2}$, *bla*$_{VIM}$, *and bla*$_{SHV}$ were 100%, 94.2%, 53.6%, 21.7%, 14.5%, and 2.9%, respectively. Based on PFGE results, 56 of the 69 strains were clonally related, and the clustering rate was 81.2%. No common outbreak isolate was detected.

Conclusions: The most prevalent OXA genes were *bla*$_{OXA-51}$, *bla*$_{OXA-23}$, and *bla*$_{OXA-2}$. Furthermore, *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{VIM}$, which are common in Enterobacterales and *Pseudomonas* spp, were detected, suggesting horizontal gene transfer had occurred between bacteria. No single clone outbreak was detected by PFGE. However, multiclonal spread and the high clustering rate suggest cross-contamination. Therefore, in future, more effective infection control measures must be implemented.

Key words: *Acinetobacter baumannii*; beta-lactamase genes; outbreak; OXA-2; OXA-23; pulsed-field gel electrophoresis.

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Introduction

Acinetobacter baumannii is a gram-negative coccobacillus that is strictly aerobic, motile and non-fermentative. As it is resistant to dryness and disinfectants, it can survive in hospital equipment and fabrics for long periods [1-3]. Therefore, in the last three decades, *A. baumannii* has been reported as a cause of nosocomial infections and outbreaks. It can cause healthcare-associated infections such as meningitis, sepsis, and ventilator-associated pneumonia, which have high mortality rates, particularly in patients that are hospitalized for long periods, are undergoing invasive procedures, or are attached to mechanic ventilators [1,2,4]. Treatment options are limited as the bacteria are resistant to a large number of antibiotics, including carbapenems. Therefore, in 2009, the Infectious Diseases Society of America identified *A. baumannii* as one of the ESKAPE pathogens *(Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), which show multidrug resistance and can escape the biocidal effects of antibiotics via diverse resistance mechanisms, including drug inactivation/alteration, modification of drug binding sites/targets, and changes in cell permeability [5]. Similarly, in 2017, the World Health Organization defined *A. baumannii* as a critical priority pathogen requiring urgent development of novel antibiotics [6].

The increase in antibiotic resistance has considerably limited the treatment options that are available against nosocomial pathogens worldwide. Therefore, in addition to antimicrobial treatment, effective control measures must be put into place to prevent nosocomial infections. Moreover, it is crucial to understand the molecular characterization and epidemiology of the nosocomial pathogen.

In this study, we aimed to determine the antibiotic susceptibility, the resistance genes, and the clonal relationship among *A. baumannii* strains isolated from a Turkish hospital.

Methodology

Ethical approval

The study protocol was approved by the Non-interventional Clinical Research Ethics Board of Karabuk University.

Bacterial Strains

The study included 69 non-repetitive *A. baumannii* strains that were isolated from clinical samples from inpatients at Karabuk University Training and Research Hospital from 2014 to 2015. The strains were stored in tryptic soy broth containing 10% (v/v) glycerol at −80°C until use.

Strain Identification and Determination of Antibiotic Susceptibility

All clinical samples were inoculated on Columbia agar enriched with 5% sheep blood [Becton Dickinson (BD, Sparks, MD, USA)], Eosin Methylene Blue agar (BD) and chocolate agar (BD) followed by incubation for 18–24 hours at 35°C. Identification and antibiotic susceptibility of the strains were determined using the Phoenix 100 Automated Microbiology System (BD, Sparks, MD, USA). Strain identifications were also confirmed using the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) method using the VITEK®-MS device (Biomerieux, Marcy-l'Étoile, France). The susceptibility of isolates to imipenem, meropenem, tigecycline, and colistin was determined using the gradient minimum inhibitory concentration (MIC) method (Liofilchem, Roseto degli Abruzzi, Italy). The antibiotic susceptibility test results were evaluated according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [7]. The Food and Drug Administration tigecycline breakpoints for Enterobacterales were applied to *Acinetobacter* spp. as breakpoint criteria are not outlined in the CLSI guidelines [8]. *Escherichia coli (E. coli)* ATCC 29212 and *P. aeruginosa* ATCC 27853 were used as quality control strains.

PCR detection of antibiotic resistance genes

Genomic DNA was isolated using the QIAsymphony DSP Virus/Pathogen kit in the QIAsymphony system according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). DNA samples were stored at −25°C until use. Previously defined primers were used for the detection of beta-lactamase genes (Table 1) [9-12].

Presence of the $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24/40}}$, $bla_{\text{OXA-51}}$, and $bla_{\text{OXA-58}}$ genes was investigated using the multiplex PCR method, and $bla_{\text{OXA-2}}$, $bla_{\text{OXA-10}}$, bla_{TEM}, bla_{SHV}, bla_{IMP}, bla_{VIM}, and bla_{GIM} genes by the in-house PCR

Table 1. Primer sequences used for the amplification of the target genes.

Target gene	Primer sequences	Amplicon size	Reference
bla_{OXA-2}	F 5'AAGAAACGCTACTCGCCTGC 3' R 5' CCACTCAACCCATCCTACCC 3'	478	[10]
bla_{OXA-10}	F 5' GTCTTTCGAGTACGGCATTA 3' R 5' ATTTTCTTAGCGGCAACTTAC 3'	720	[10]
bla_{OXA-23}	F 5' GATCGGATTGGAGAACCAGA 3' R 5' ATT TCTGACCGCATT TCCAT 3'	501	[8]
bla_{OXA-24}	F 5' GGTTAGTTGGCCCCCTTAAA 3' R 5' AGTTGAGCGAAAAGGGGATT 3'	246	[8]
bla_{OXA-51}	F 5' TAATGCTTTGATCGGCCTTG 3' R 5' TGGATTGCACTTCATCTTGG 3'	353	[8]
bla_{OXA-58}	F 5' AAGTATTGGGGCTTGTGCTG 3' R 5' CCCCTCTGCGCTCTACATAC 3'	599	[8]
bla_{IMP}	F 5' GGAATAGAGTGGCTTAAYTCTC 3' R 5' CCA AAC YACTASGTTATCT 3'	188	[9]
bla_{VIM}	F 5' GATGGTGTTTGGTCGCATA 3' R 5' CGAATGCGCAGCACCAG 3'	309	[11]
bla_{GIM}	F5' TCGACACACCTTGGTCTGAA 3' R 5' AATTCCAACTTTGCCATGC 3'	477	[9]
bla_{TEM}	F 5' TCCGGTCATGAGACAATAACC 3' R 5' TTGGTCTGACAGTTACCAATGG 3'	931	[10]
bla_{SHV}	F 5' TGGTTATGCGTTATATTCGCC 3' R 5' GGTTAGCGTTGCCAGTGCT 3'	868	[10]

method [10]. The Gene Amp PCR System 9700 system (Applied Biosystems, Waltham, MA, USA) was used for amplification. The amplification conditions for $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24/40,}}$ $bla_{\text{OXA-51}}$, and $bla_{\text{OXA-58}}$ were the following: initial denaturation for 3 minutes at 94°C, followed by 35 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. The following conditions were used to amplify $bla_{\text{OXA-2}}$, bla_{OXA10}, $bla_{\text{TEM,}}$ bla_{SHV}, bla_{IMP}, bla_{VIM}, and bla_{GIM}: initial denaturation for 10 minutes at 95°C, followed by 35 cycles consisting of denaturation at 94°C for 1 minute, annealing at 57°C for 1 minute, and extension at 72°C for 1.5 minutes, and a final extension at 72°C for 3 minutes.

Amplification products were analyzed by electrophoresis for 1 hour at 100V in a 1.5% agarose gel followed by ethidium bromide staining. Imaging was performed using the Gel Logic 2200 imaging system (Kodak Co., Rochester, NY, USA) under ultraviolet light. Band sizes for each gene were measured by comparison with a 100-bp DNA ladder (New England Biolabs, Ipswich, MA, USA).

Molecular Typing by pulsed-field gel electrophoresis (PFGE)

The clonal relationship among *A. baumannii* isolates was determined using a method described by Durmaz *et al*. [13]. Genomic DNA was cut with 10 u/µl of *Apa*I restriction endonuclease enzyme (Fermentas, Vilnius, Lithuania) after which PFGE was performed by clamped homogeneous electric field (CHEF) electrophoresis on a 1% agarose gel with a CHEF-DR II system (Bio-Rad Lab, Nazareth, Belgium). The fragments were separated over a period of 24 hours at 14°C with a gradient of 6 V/cm, a reorientation angle of 120° and an initial switch time of 5 seconds and a final switch time of 30 seconds. For band analysis, similarities were calculated using the Dice similarity coefficient (DSC) and cluster analysis was performed using the unweighted pair group using arithmetic means (UPGMA). If the DSC was < 90%, isolates were considered to be of different genotypes, and if DSC was ≥ 90%, isolates were classed as the same genotype. A dendrogram was created from the PFGE data using the UPGMA-based clustering algorithm.

Statistical Analysis

The data were analyzed using the MINITAB -17 statistical software program (Minitab Inc., State College, PA, USA). The Anderson-Darling test was performed to determine whether the data were normally distributed. Descriptive statistics are presented as numbers and percentages. The mean age of the patients is presented as mean ± standard deviation. For comparisons of beta-lactamase genes and isolated units, the chi-square test was performed, and a p-value < 0.05 was considered as statistically significant.

Results

Patients' demographic data

Of the 69 patients in this study, 42 (60.9%) were male and 27 (39.1%) were female. The mean age of the patients was 70.2 ± 12.64 (age range: 39–93 years). The female to male ratio was 1:1.55. All of the patients had at least one underlying disease. The most common underlying diseases were cerebrovascular disease (36%), chronic obstructive pulmonary disease (35%), and diabetes mellitus (33%). The most common risk factors were intubation (71%), peripheral arterial catheterization (64%), and central venous catheterization (58%). A total of 69 *A. baumannii* strains were obtained from endotracheal aspirates (ETA) in 24 cases (34.8%), blood in 18 cases (26%), wounds in 13 cases (18.8%), urine in seven cases (10.2%), sputum in four cases (5.8%), and from broncho-alveolar lavage fluid in three cases (4.4%). The strains were isolated from 54 patients (78.3%) in intensive care units and from 15 patients (21.7%) in the wards. More than half (52%) of the 69 *A. baumannii* isolates were obtained from two intensive care units: the surgical intensive care unit (36%) and reanimation intensive care unit (16%).

Antibiotic susceptibilities of the isolated strains

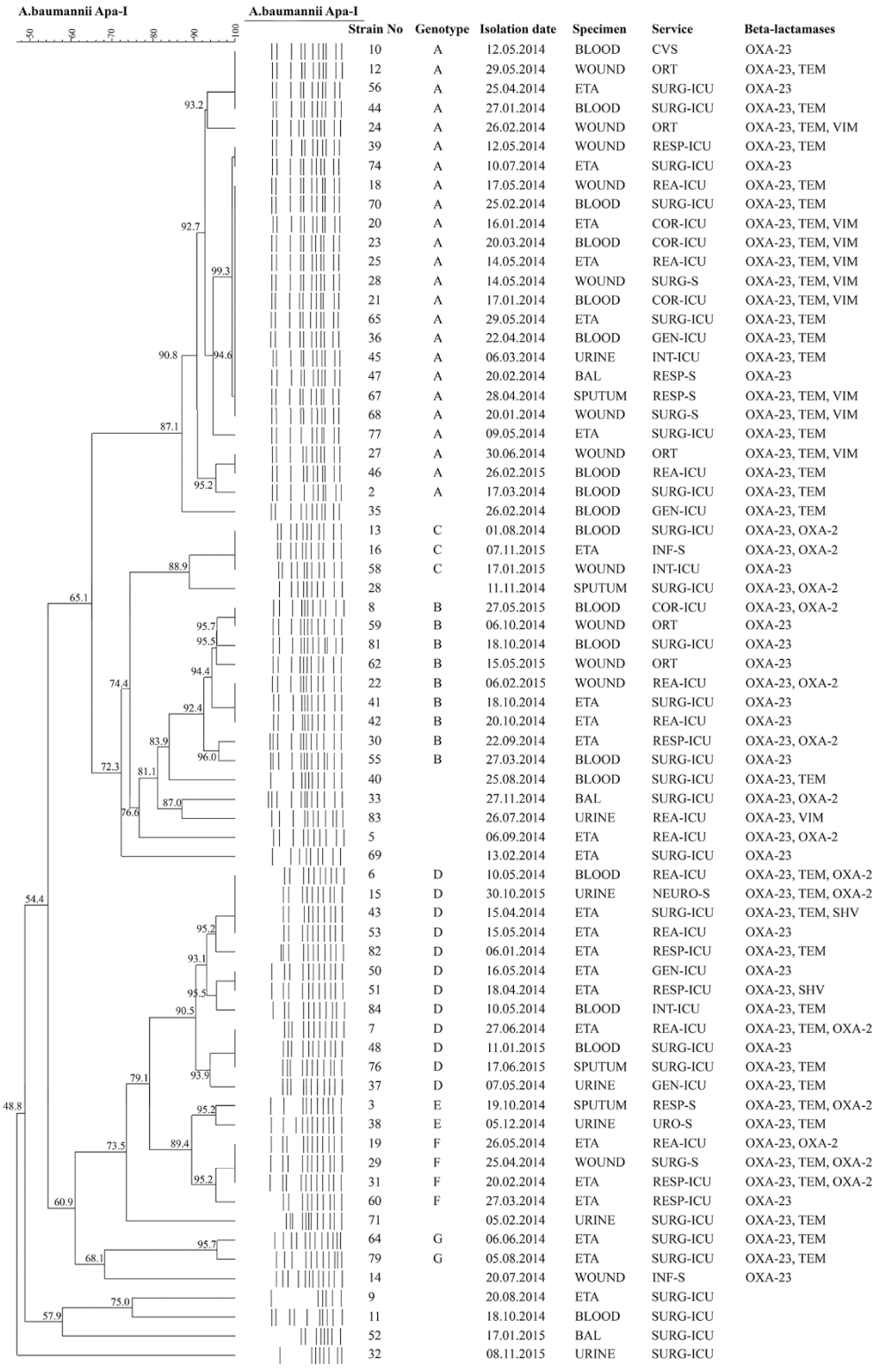
All 69 strains (100%) were resistant to ampicillin–sulbactam, gentamicin, cefepime, ciprofloxacin, and ceftriaxone but were susceptible to tigecycline and colistin.

Table 2. MIC values of imipenem, meropenem, tigecycline, and colistin against *A. baumannii* strains.

Antibiotic	*MIC 50 (µg/mL)	**MIC 90 (µg/mL)	***MIC range (µg/mL)	Concentration range (µg/mL)
Imipenem	32	≥ 32	(0,5 - ≥ 32)	(0,002 – 32)
Meropenem	16	≥ 32	(0,25 - ≥ 32)	(0,002 – 32)
Tigecycline	0.05	1	(0,125 - 2)	(0,016 – 256)
Colistin	0.75	2	(0,064 - 2)	(0,016 – 256)

*MIC 50: MIC value for 50% of isolates; **MIC 90: MIC value for 90% of isolates; ***MIC: Minimum Inhibitory Concentration.

Figure 1. Dendrogram based on pulsed-field gel electrophoresis pattern analysis of the isolated *A. baumannii* strains.



Strain No	Genotype	Isolation date	Specimen	Service	Beta-lactamases
10	A	12.05.2014	BLOOD	CVS	OXA-23
12	A	29.05.2014	WOUND	ORT	OXA-23, TEM
56	A	25.04.2014	ETA	SURG-ICU	OXA-23
44	A	27.01.2014	BLOOD	SURG-ICU	OXA-23, TEM
24	A	26.02.2014	WOUND	ORT	OXA-23, TEM, VIM
39	A	12.05.2014	WOUND	RESP-ICU	OXA-23, TEM
74	A	10.07.2014	ETA	SURG-ICU	OXA-23
18	A	17.05.2014	WOUND	REA-ICU	OXA-23, TEM
70	A	25.02.2014	BLOOD	SURG-ICU	OXA-23, TEM
20	A	16.01.2014	ETA	COR-ICU	OXA-23, TEM, VIM
23	A	20.03.2014	BLOOD	COR-ICU	OXA-23, TEM, VIM
25	A	14.05.2014	ETA	REA-ICU	OXA-23, TEM, VIM
28	A	14.05.2014	WOUND	SURG-S	OXA-23, TEM, VIM
21	A	17.01.2014	BLOOD	COR-ICU	OXA-23, TEM, VIM
65	A	29.05.2014	ETA	SURG-ICU	OXA-23, TEM
36	A	22.04.2014	BLOOD	GEN-ICU	OXA-23, TEM
45	A	06.03.2014	URINE	INT-ICU	OXA-23, TEM
47	A	20.02.2014	BAL	RESP-S	OXA-23
67	A	28.04.2014	SPUTUM	RESP-S	OXA-23, TEM, VIM
68	A	20.01.2014	WOUND	SURG-S	OXA-23, TEM, VIM
77	A	09.05.2014	ETA	SURG-ICU	OXA-23, TEM
27	A	30.06.2014	WOUND	ORT	OXA-23, TEM, VIM
46	A	26.02.2015	BLOOD	REA-ICU	OXA-23, TEM
2	A	17.03.2014	BLOOD	SURG-ICU	OXA-23, TEM
35		26.02.2014	BLOOD	GEN-ICU	OXA-23, TEM
13	C	01.08.2014	BLOOD	SURG-ICU	OXA-23, OXA-2
16	C	07.11.2015	ETA	INF-S	OXA-23, OXA-2
58	C	17.01.2015	WOUND	INT-ICU	OXA-23
28		11.11.2014	SPUTUM	SURG-ICU	OXA-23, OXA-2
8	B	27.05.2015	BLOOD	COR-ICU	OXA-23, OXA-2
59	B	06.10.2014	WOUND	ORT	OXA-23
81	B	18.10.2014	BLOOD	SURG-ICU	OXA-23
62	B	15.05.2015	WOUND	ORT	OXA-23
22	B	06.02.2015	WOUND	REA-ICU	OXA-23, OXA-2
41	B	18.10.2014	ETA	SURG-ICU	OXA-23
42	B	20.10.2014	ETA	REA-ICU	OXA-23
30	B	22.09.2014	ETA	RESP-ICU	OXA-23, OXA-2
55	B	27.03.2014	BLOOD	SURG-ICU	OXA-23
40		25.08.2014	BLOOD	SURG-ICU	OXA-23, TEM
33		27.11.2014	BAL	SURG-ICU	OXA-23, OXA-2
83		26.07.2014	URINE	REA-ICU	OXA-23, VIM
5		06.09.2014	ETA	REA-ICU	OXA-23, OXA-2
69		13.02.2014	ETA	SURG-ICU	OXA-23
6	D	10.05.2014	BLOOD	REA-ICU	OXA-23, TEM, OXA-2
15	D	30.10.2015	URINE	NEURO-S	OXA-23, TEM, OXA-2
43	D	15.04.2014	ETA	SURG-ICU	OXA-23, TEM, SHV
53	D	15.05.2014	ETA	REA-ICU	OXA-23
82	D	06.01.2014	ETA	RESP-ICU	OXA-23, TEM
50	D	16.05.2014	ETA	GEN-ICU	OXA-23
51	D	18.04.2014	ETA	RESP-ICU	OXA-23, SHV
84	D	10.05.2014	BLOOD	INT-ICU	OXA-23, TEM
7	D	27.06.2014	ETA	REA-ICU	OXA-23, TEM, OXA-2
48	D	11.01.2015	BLOOD	SURG-ICU	OXA-23
76	D	17.06.2015	SPUTUM	SURG-ICU	OXA-23, TEM
37	D	07.05.2014	URINE	GEN-ICU	OXA-23, TEM
3	E	19.10.2014	SPUTUM	RESP-S	OXA-23, TEM, OXA-2
38	E	05.12.2014	URINE	URO-S	OXA-23, TEM
19	F	26.05.2014	ETA	REA-ICU	OXA-23, OXA-2
29	F	25.04.2014	WOUND	SURG-S	OXA-23, TEM, OXA-2
31	F	20.02.2014	ETA	RESP-ICU	OXA-23, TEM, OXA-2
60	F	27.03.2014	ETA	RESP-ICU	OXA-23
71		05.02.2014	URINE	SURG-ICU	OXA-23, TEM
64	G	06.06.2014	ETA	SURG-ICU	OXA-23, TEM
79	G	05.08.2014	ETA	SURG-ICU	OXA-23, TEM
14		20.07.2014	WOUND	INF-S	OXA-23
9		20.08.2014	ETA	SURG-ICU	
11		18.10.2014	BLOOD	SURG-ICU	
52		17.01.2015	BAL	SURG-ICU	
32		08.11.2015	URINE	SURG-ICU	

The clusters were named with upper case letters (A–G). The distribution of the beta-lactamase genes among the isolated strains and other demographic data (specimen, isolation date, and services) are presented. (ETA: endotracheal aspirate, BAL: broncho-alveolar lavage fluid, ICU: intensive care unit, ORT: orthopedic surgery, REA: reanimation, CVS: cardiovascular surgery, S: service, RESP.: respiratory, GEN: general, COR: coronary, SURG: surgical, INT: internal, INF: infection, NEURO: neurology service, URO: urology service).

The resistance rates to piperacillin-tazobactam, amikacin, imipenem, and meropenem were 94.2% (n = 65). The MIC data for imipenem, meropenem, tigecycline and colistin against *A. baumannii* strains are presented in Table 2.

Distributions of antibiotic resistance genes

The PCR results indicated that the intrinsic $bla_{\text{OXA-51}}$ gene specific for *A. baumannii* was detected in all of the isolates (100%), $bla_{\text{OXA-23}}$ in 65 isolates (94.3%) and $bla_{\text{OXA-2}}$ in 15 isolates (21.7%). In addition, bla_{TEM} was detected in 37 of the 69 isolates (53.6%). No significant relationship was determined between the source of the isolated strain and the frequency of bla_{TEM} ($p = 0.28$). Although bla_{VIM} was detected in 10 (14.5%) strains and bla_{SHV} in two (2.9%) strains, bla_{GIM}, bla_{IMP}, $bla_{\text{OXA-10}}$, $bla_{\text{OXA-24/40}}$, and $bla_{\text{OXA-58}}$ were not detected in any of the isolates. Of the 65 carbapenem-resistant strains, 16 (24.6%) possessed multiple beta-lactamase genes in addition to $bla_{\text{OXA-51}}$ and $bla_{\text{OXA-23}}$. Of these strains, nine contained bla_{TEM} and bla_{VIM}, six contained bla_{TEM} and $bla_{\text{OXA-2}}$, and only one had bla_{TEM} and bla_{SHV}. Furthermore, four carbapenem-susceptible strains contained only $bla_{\text{OXA-51}}$.

PFGE typing

A dendrogram was constructed based on the PFGE results. The distribution of the beta-lactamase genes among the strains and other demographic data are illustrated in Figure 1. The 69 *A. baumannii* isolates revealed 20 different profiles. We found that 56 of the 69 strains were clonally related, and these strains were gathered in seven clusters (a tolerance of 1.0 and a cutoff value of 90% similarity were applied). The clusters were named with upper case letters (A–G), and 56 of the 69 strains were found to be within a cluster, corresponding to a clustering rate of 81.2%. The numbers of strains in each of the clusters ranged from 2 to 24. The largest cluster was clone A that was formed from 24 isolates (34.8%), followed by clone D (12 isolates; 17.4%), and clone B (9 isolates; 13%) All of the clonally related strains possessed the $bla_{\text{OXA-23}}$ gene, and $bla_{\text{OXA-23}}$ negative strains (n = 4) were located in a sporadic group.

Of the 24 isolates present in clone A, 20 (83.3%) carried bla_{TEM} in addition to $bla_{\text{OXA-51}}$ and $bla_{\text{OXA-23}}$, and nine (37.5%) carried the bla_{VIM} gene. In nine strains, both bla_{TEM} and bla_{VIM} were detected. Furthermore, in four strains, only $bla_{\text{OXA-51}}$ and $bla_{\text{OXA-23}}$ were detected and other beta-lactamase genes were not found.

We isolated the clone A strains over a 12-month period. Of the 24 strains present in clone A, 17 were obtained from the intensive care unit (ICU) and seven from service patients. Two of the three strains obtained from the coronary ICU were isolated at one-day intervals. One of these isolates was obtained from ETA and the other from blood. Both strains had $bla_{\text{OXA-23}}$ in addition to bla_{TEM} and bla_{VIM}. Eight strains were isolated from blood, seven from wounds, six from ETA, and three from other samples. Four of the seven wound isolates were obtained from different ICUs and services in May 2014 over 15 days. Two of the three strains isolated from wound cultures of orthopedic surgery patients were obtained over one month.

Of the 12 isolates present in clone D, eight strains carried bla_{TEM}, three strains carried $bla_{\text{OXA-2}}$, and two strains carried the bla_{SHV} gene. In three strains, $bla_{\text{OXA-2}}$ and bla_{TEM} were both detected, and one strain possessed both $bla_{\text{TEmetalloM}}$ and bla_{SHV}.

The clone D strains were isolated over 22 months. Eleven of the strains were obtained from ICUs and one from the neurology service. Six strains were isolated from ETA, three from blood, two from urine, and one from sputum. Five of the six ETA strains were isolated from different ICUs over two months. Two of the three blood isolates were obtained from the reanimation ICU and the internal ICU on the same day (10 May 2014).

All nine strains present in clone B harbored $bla_{\text{OXA-23}}$, while three strains possessed $bla_{\text{OXA-2}}$ in addition to $bla_{\text{OXA-23}}$. Other beta-lactamases were not detected in any of these strains. The clone B strains were isolated over 14 months. Three of these were obtained from blood, three from ETA, and three from wound cultures. Three ETA strains were isolated from three different ICUs over one month. Two of the three strains were isolated from two different patients in the surgical ICU on the same day (18 October2014). One strain was obtained from ETA and one from blood. Both strains had only $bla_{\text{OXA-23}}$. Two of the strains in clone G were obtained from the ETA samples of the surgical ICU over two months. Both strains had $bla_{\text{OXA-23}}$ and bla_{TEM}. All four carbapenem-susceptible strains were isolated from the surgical ICU on different dates.

Discussion

The increase in multidrug resistance among *A. baumannii* strains is a global health threat. Long-term, low-dose, or unnecessary consumption of antibiotics leads to the emergence of multidrug-resistant strains [14,15]. Moreover, treatment options are limited by the ability of *A. baumannii* to develop novel drug resistance mechanisms and the spread of resistance via mobile genetic elements [1,4,14]. In the last two decades, carbapenem resistance in *A. baumannii* isolates has

reached high levels worldwide. In this study, the rate of carbapenem resistance among the clinical isolates was 95%. Similarly, data reported by the Central Asia and Eastern European Surveillance of Antimicrobial Resistance revealed that the carbapenem resistance rate was 93% in Turkey [16]. According to the 2017 report of The European Antimicrobial Resistance Surveillance Network (EARS-Net), the rate of carbapenem resistance was 52.5% in Hungary, 78.7% in Italy, 68.2% in Spain, and 96.2% in Croatia [17]. Tigecycline and colistin are often used for the treatment of carbapenem-resistant *A. baumannii* infections. In this study, we found that all the isolated strains were susceptible to tigecycline and colistin. However, there have been many reports of tigecycline and colistin resistance, which range from 0.6% to 45.8% [18-21] and 0 to 30.6%, respectively, around the world [22-25]. Furthermore, colistin resistance was reported to be of 4% in the EARS-Net 2016 report [26].

Beta-lactamase enzymes, in particular oxacillinases (bla_{OXA}), which are defined as Ambler Class D, play a key role in antimicrobial resistance among *Acinetobacter* species [3,14]. The first OXA enzymes, OXA-1, OXA-2, and OXA-3, were originally identified by Sykes and Matthew in 1976 [4]. OXA-2 type beta-lactamases hydrolyze oxacillin and cloxacillin at a high level and are weakly inhibited by clavulanic acid, and they were first observed in *P. aeruginosa* from France in 2002 and in *E. coli* from Israel in 2005 [27-28]. In this study we detected the bla_{OXA-2} gene in 15 of the 69 (21.7%) isolated *A. baumannii* strains. To the best of our knowledge, this is the first report of the presence of the bla_{OXA-2} gene among *A. baumannii* strains in Turkey. Moreover, there are few reports concerning bla_{OXA-2} in the literature. Rahimzadeh *et al*. reported that the frequency of the bla_{OXA-2} gene among *A. baumannii* isolates was 11.6% in Iran [29]. In addition, Maurya *et al*. reported that bla_{OXA-2} is located within class I and class II integrons among different species including *E. coli*, *K. pneumoniae,* and *P. aeruginosa* that were isolated in India [30]. The presence of bla_{OXA-2} in different bacteria and its presence in integrons indicate horizontal gene transfer.

In this study, bla_{OXA-23} was detected in 65 of the 69 isolates (94.2%). This encodes the first known carbapenem-resistant OXA-enzyme that was initially reported in Scotland in 1985 [4,14]. Prior to the discovery of $bla_{OXA-23,}$ the OXA enzymes were thought to be a small group of enzymes encoded by plasmids that affected only penicillins; the migration of these genes via transposons caused them to become the dominant mechanism of antibiotic resistance [4]. The frequency of bla_{OXA-23} in *A. baumannii* strains has been reported to range from 32% to 100% in Turkey [21,31,32] and from 28% to 96% worldwide [33-36].

The metallo-beta-lactamases (MBL), which are known as Ambler Class B, are the second most commonly identified beta-lactamases in *A. baumannii* isolates [22]. MBLs are so-called as they utilize a Zn^{2+} cofactor [14,22]. They are commonly isolated from *Pseudomonas* species [14]. In this study, bla_{VIM} was detected in 14.5% of the isolates, whereas bla_{GIM} and bla_{IMP} were not detected in any of the isolates. In a study of 519 isolates in Turkey, bla_{VIM} was detected at the rate of 0.2% (n = 1), whereas bla_{GIM} and bla_{IMP} were not found [18]. In this study, the high rate of bla_{VIM} detection (14.5%) suggests that this resistance gene, which is most often found in *Pseudomonas* spp., had been transferred to Acinetobacters via plasmids over time. The frequency of bla_{VIM} has been reported as 10.1% in Korea [37] and 34.2% in India [22]. Among the Class A beta-lactamases, bla_{TEM} and bla_{SHV} were detected in 53.6% and 2.9% of the isolates, respectively. Similarly, Beriş *et al*. reported that the frequency of bla_{TEM} and bla_{SHV} in Turkey was 55.7% and 7.7%, respectively [18]. Conversely, Coşkun *et al*. reported a low frequency of bla_{TEM} (2%) [21]. The frequency of bla_{TEM} has been reported to be 2.9%–71% worldwide [19,38,39].

To prevent the spread of *Acinetobacter baumannii* within the hospital, it is essential to determine the potential origins of the microorganism and its transmission routes. Therefore, it is important to determine whether a clonal relationship exists among the isolates. In this study, the clonal relationship among *A. baumannii* isolates was investigated using the PFGE, which is considered to be a gold standard method. The PFGE results indicated that there was no single clone outbreak in our hospital, and, instead, polyclonal spread had taken place. Three dominant clones carrying bla_{OXA-23}, clones A, B and D, were observed to circulate between units within the hospital setting. In addition, a small clonal spread was observed between some units. The main reservoir of the strains was the surgical ICU and the reanimation ICU. Some clone A and clone B isolates were obtained from the same unit over 1–2 days. For instance, two of the three clone A strains isolated from the coronary ICU were obtained with a one-day interval, and two of the three clone B strains isolated from the surgical ICU were obtained on the same day.

Nine strains in genotype B were isolated over a period of 14 months, of which five strains were obtained from different units within one month. Twelve

isolates from clone D were obtained over 18 months, while eight isolates were obtained from different ICUs over a period of two months. This suggests that the dominant clone strains were able to circulate among different units, sometimes causing a small spread. Based on these findings, it is clear that cross-transmission is widespread in our hospital, and we are facing a huge infection control problem. Therefore, a comprehensive infection control program is required to be initiated. For instance, as a simple and cost-effective measure, we must ensure that all health care workers (HCWs) use hand disinfectant before and after contact with patients and monitor their compliance. Furthermore, to reduce cross-transmission and infection rates, contact precautions for colonized/infected patients should be used, and hospital equipment and surfaces must be cleaned with approved disinfectants at appropriate concentrations and times [3,40]. Also, no-touch disinfection methods such as ultraviolet light or vaporized hydrogen peroxide may be included in this program.

Conclusion

In this study, $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-2}}$ were determined in 65 (94.2%) and 15 (21.7%) of 69 isolates, respectively. The frequencies of bla_{TEM} and bla_{SHV}, which are of plasmid origin and more common in Enterobacterales, were 53.6% and 2.9% respectively. The frequency of bla_{VIM}, which is of plasmid origin and is often seen in *Pseudomonas* spp, was 14.5%. These findings indicate horizontal gene transfer between bacteria. Although we determined that no single clone outbreak had taken place in our hospital, a multiclonal spread had occurred. Three dominant clones carrying $bla_{\text{OXA-23}}$ had circulated within the hospital setting. According to our findings, the surgical ICU is the main reservoir for the cross-transmission of strains. This suggests that there is a serious infection control problem in our hospital. Therefore, we should implement a comprehensive infection prevention and control program. In addition, compliance of HCWs to hand hygiene, usage of contact precautions for colonized/infected patients and cleaning of hospital equipment and surfaces with approved disinfectant should be closely monitored [3,40].

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References

1. Howard A, O'Donoghue M, Feeney A, Sleator RD (2012) *Acinetobacter baumannii*: an emerging opportunistic pathogen. Virulence 3: 243-250.
2. Bassetti M, Righi E, Esposito S, Petrosillo N, Nicolini L (2008) Drug treatment for multidrug resistant *Acinetobacter baumannii* infections. Future Microbiol 3: 649-660.
3. Philips M (2015) *Acinetobacter* Species. In Bennett JE, Dolin R,Blaser MJ editors. In Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. PA: Philadelphia, PA: Elsevier Health Sciences. 2552-2558.
4. Evans BA, Amyes SGB (2014) OXA β-lactamases. Clin Microbiol Rev 27: 241-263.
5. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J (2009) Bad bugs, no drugs: No ESKAPE! An update from the infectious diseases society of America.Clin Infect Dis 48: 1-12.
6. World Health Organization (WHO) (2017) WHO publishes list of bacteria for which new antibiotics are urgently needed. Geneva, Switzerland. Available: https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed. Accessed:18 April 2019.
7. Clinical and Laboratory Standart Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing, 27th informational supplement.CLSI document M100-S27 (ISBN 1-56238-839-8) . Wayne, PA,US.
8. United States Food and Drug Administration (FDA) (2013) TYGACIL (tigecycline).Susceptibility test result interpretive criteria for tigecycline.s.21-22. Available: https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021821s026s031lbl.pdf Accessed: 10 May 2019.
9. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, Amyes SGB, Livermore DM (2006) Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents 27: 351-353.
10. Ellington MJ, Kistler J, Livermore DM, Woodford N (2007) Multiplex PCR for rapid detection of genes encoding acquired metallo-β-lactamases. J Antimicrob Chemother 59: 321- 322.
11. Kiratisin P, Apisarnthanarak A, Laesripa C, Saifon P (2008) Molecular characterization and epidemiology of extended-spectrum-β- lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic. Antimicrob Agents Chemother 52: 2818-2824.
12. Kock MM, Bellomo AN, Storm N, Ehlers MM (2017) Prevalence of carbapenem resistance genes in *Acinetobacter baumannii* isolated from clinical specimens obtained from an academic hospital in South Africa. South African J Epidemiol Infect 28: 28-32.
13. Durmaz R, Otlu B, Koksal F, Hosoglu S, Ozturk R, Ersoy Y, Aktas E, Gursoy NC, Caliskan A (2009) The optimization of a rapid pulsed-field gel electrophoresis protocol for the typing of *Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella* spp. Jpn J Infect Dis 62: 372-377.
14. Peleg AY, Seifert H, Paterson DL (2008) *Acinetobacter baumannii*; emergence of a successful pathogen. Clin Microbiol Rev 21: 538-582.
15. Doi Y, Murray GL, Peleg AY (2015) *Acinetobacter baumannii*:evolution of antimicrobial resistance-treatment options. Semin Respir Crit Care Med 36: 85-98.
16. Central Asian and Eastern European Surveillance of Antimicrobial Resistance (CAESAR) (2018) Annual report 2017. Available: http://www.euro.who.int/en/health-topics/disease-prevention/antimicrobial-resistance/publications/2017/central-asian-and-eastern-

european-surveillance-of-antimicrobial-resistance.-annual-report-2017-2018.Accessed:28 March 2019.
17. ECDC. European Centre for Disease Prevention and Control (2018) Surveillance of antimicrobial resistance in Europe 2017 Available: https://ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2017. Accessed:21 April 2019.
18. Beris FS, Budak EE, Gülek D, Uzun A, Cizmeci Z, Mengeloglu FZ, Direkel S, Cetinkol Y, Altintop Y., Iraz M, Dal T, Coskun USS, Balci PO, Kayman T, Caliskan A, Yazici Y, Tosun İ, Ertürk A, Cicek AC (2016) Investigation of the Frequency and Distribution of Beta- Lactamase Genes in the Clinical Isolates of *Acinetobacter baumannii* collected from different regions of Turkey: A multicenter study. Mikrobiyol Bul 50: 511-521.
19. Alkasaby NM, El M, Zaki S (2017) Molecular study of *Acinetobacter baumannii* isolates for genes in intensive care unit, Mansoura University Hospital, Egypt. Hindawi- Int J Microbiol 2017: 1-6.
20. Modarresi F, Azizi O, Shakibaie MR, Motamedifar M, Valibeigi B, Mansouri S (2015) Effect of iron on expression of efflux pump (adeABC) and quorum sensing (luxI, luxR) genes in clinical isolates of *Acinetobacter baumannii*. Apmis 123: 959-968.
21. Coskun USS, Caliskan E, Cicek AC, Sandalli C, Turumtay H (2019) β-lactamase genes in carbapenem resistance *Acinetobacter baumannii* isolates from a Turkish university hospital. J Infect Dev Ctries 13: 50-55. doi: 10.3855/jidc.10556.
22. Girija SA, Jayaseelan VP, Arumugam, P (2018) Prevalence of VIM- and GIM-producing *Acinetobacter baumannii* from patients with severe urinary tract infection. Acta Microbiol Immunol Hung 65: 539-550.
23. Vali L, Dashti K, Opazo-Capurro AF, Dashti AA, Al Obaid K, Evans, BA (2015) Diversity of multi-drug resistant *Acinetobacter baumannii* population in a major hospital in Kuwait. Front Microbiol 6: 1-8.
24. Queenan AM, Pillar CM, Deane J, Sahm DF, Lynch AS, Flamm RK, Peterson J, Davies TA (2012) Multidrug resistance among *Acinetobacter* spp. in the USA and activity profile of key agents: Results from CAPITAL Surveillance 2010. Diagn Microbiol Infect Dis 73: 267- 270.
25. Ko KS, Suh JY, Kwon KT, Jung SI, Park KH, Kang CI, Chung DR, Peck KR, Song JH (2007) High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. J Antimicrob Chemother 60**:** 1163-1167.
26. European Centre for Disease Prevention and Control (ECDC) (2017) Surveillance of antimicrobial resistance in Europe 2016. EARS-Net surveillance data. Available: https://ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2016 Accessed: 23 April 2019.
27. Bert F (2002) Identification of PSE and OXA beta-lactamase genes in *Pseudomonas aeruginosa* using PCR-restriction fragment length polymorphism. J Antimicrob Chemother 50: 11-18.
28. Chmelnitsky I, Carmeli Y, Leavitt A, Schwaber MJ,Venezia SN (2005) CTX-M-2 and a new CTX-M-39 enzyme are the major extended-spectrum beta-lactamases in multiple *Escherichia coli* clones isolated in Tel Aviv, Israel. Antimicrob Agents Chemother 49: 4745-4750.
29. Rahimzadeh A, Farajnia S, Pourbabaee AA, Ansarin KH, Zolfaghari MR, Masoudi N (2012) Detection of prevalence of OXA-2 and OXA-10 type ESBL and class I Integron among *Acinetobacter bumanii* strains isolated from patients of Tabriz City (Iran) by PCR technique. J Babol Univ Med Sci 14: 56-63.
30. Maurya AP, Talukdar AD, Dhar CD, Chakravarty A, Bhattacharjee, A (2015) Genetic environment of OXA-2 beta-lactamase producing Gram-negative bacilli from a tertiary referral hospital. Indian J Med Res 141: 368- 369.
31. Altun S, Kocak ZT, Altun B, Onde U, Kinikli S, Demiröz AP (2016) Growing OXA-23 type strains among carbapenem-resistant *Acinetobacter baumannii* and tigecycline as an alternate combination therapy. Turkish J Med Sci 46: 1894-1899.
32. Cicek AC, Saral A, Iraz M, Ceylan A, Duzgun AO, Peleg AY, Sandalli C (2014) OXA- and GES-type β-lactamases predominate in extensively drug-resistant *Acinetobacter baumannii* isolates from a Turkish University Hospital. Clin Microbiol Infect 20: 410-415.
33. Nowak P, Paluchowska PM, Budak A (2014) Co-occurrence of carbapenem and aminoglycoside resistance genes among multidrug-resistant clinical isolates of *Acinetobacter baumannii* from Cracow, Poland. Med Sci Monit Basic Res 20: 9-14.
34. Merkier A, Catalano M, Ramirez M, Quiroga C, Orman B, Ratier L, Famiglietti A, Vay C, Di Martino A, Kaufman S, Centrón D (2008) Polyclonal spread of blaOXA-23 and blaOXA-58 in *Acinetobacter baumannii* isolates from Argentina. J Infect Dev Ctries 2: 235-240. doi: 10.3855/jidc.269.
35. Kanj S, Tayyar R, Shehab M, El-Hafi B, Rasheed S, Kissoyan KA, Kanafani Z, Wakim R, Kara Zahreddine N, Araj G, Dbaibo G, Matar G (2018) Increased blaOXA-23-like prevalence in *Acinetobacter baumannii* at a tertiary care center in Lebanon (2007-2013). J Infect Dev Ctries 12: 228-234. doi: 10.3855/jidc.9642.
36. Gholami M, Moshiri M, Ahanjan M, Salimi AC, Hasannejad BM, Asadi A, Eshaghi M, Pournaja A, Abbasian S, Kouhsari E, Irajian G (2018) The diversity of class B and class D carbapenemases in clinical *Acinetobacter baumannii* isolates. Infez Med 26: 329-335.
37. Lee K, Yong D, Jeong, SH, Chong Y (2011) Multidrug-resistant *Acinetobacter* spp.: Increasingly problematic nosocomial pathogens. Yonsei Med J 52: 879-891.
38. Odewale G, Adefioye OJ, Ojo J, Adewumi FA, Olowe OA (2016) Multidrug resistance of *Acinetobacter baumannii* in Ladoke Akintola University Teaching Hospital, Osogbo, Nigeria. Eur J Microbiol Immunol 6: 238-243.
39. Alyamani, EJ, Khiyami MA, Booq RY, Alnafjan BM, Altammami MA, Bahwerth FS (2015) Molecular characterization of extended-spectrum beta-lactamases (ESBLs) produced by clinical isolates of *Acinetobacter baumannii* in Saudi Arabia. Ann Clin Microbiol Antimicrob 14: 38.
40. Weber DJ, Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E (2010) Role of hospital surfaces in the transmission of emerging health care-associated pathogens: Norovirus, *Clostridium difficile*, and *Acinetobacter* species. Am J Infect Control 38: 25-33.

Corresponding author

Assistant professor Nergis Asgin, MD.
Karabuk University, Faculty of Medicine, Department of Medical Microbiology
Baliklarkayasi mevkii Demir çelik kampusu 78050 Karabuk, Turkey.
Tel: +90 370 4158700/1852
Fax: +90 370 4187160
E mail: nergisasgin@karabuk.edu.tr

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