Araştırma Makalesi/Original Article
Makale Dili "İngilizce"/Article Language "English"

Türk Hijyen ve Deneysel Biyoloji Dergisi

Investigation of phenotypic and genotypic characteristics of *Acinetobacter baumannii* isolated from clinical samples

Klinik örneklerden izole edilen *Acinetobacter baumannii*'nin fenotipik ve genotipik özelliklerinin araştırılması

Nezahat KOŞAR[1] (ID), Djursun KARASARTOVA[2] (ID), Ayşe Semra GÜRESER[3] (ID), Ayşegül TAYLAN ÖZKAN[4] (ID)

ABSTRACT

Objective: *Acinetobacter baumannii* strains are common nosocomial pathogens worldwide. Our study aimed to determine the antibiotic resistance rate, bla_{OXA} and ISAba1 genes, metallo-beta-lactamases production, biofilm formation, and clonal classification of *A. baumannii* isolated from clinical samples in Çorum Erol Olçok Training and Research Hospital, Türkiye. It was also aimed to describe the plasmid profile and analyze the association between genes, clones, and plasmids.

Methods: Ninety-eight *A. baumannii* isolated between 2018 and 2019 were included in the study. Antibiotic susceptibility tests were determined with Vitek 2. The reference broth microdilution method was used to assess colistin susceptibility. These results were compared with those obtained from the Vitek 2. A multiplex polymerase chain reaction detected bla_{OXA-23}, $bla_{OXA-24/40}$, bla_{OXA-51}, and bla_{OXA-58} genes. The ISAba1/ bla_{OXA-23} and ISAba1/ bla_{OXA-51} genes were analyzed separately via PCR. Genotypes and subtypes of the isolates were determined with the Repetitive Extragenic Palindromic

ÖZET

Amaç: *Acinetobacter baumannii* dünya çapında yaygın bir nozokomiyal patojendir. Çalışmamızda Çorum Erol Olçok Eğitim ve Araştırma Hastanesine başvuran hastaların klinik örneklerinden izole edilmiş olan *A. baumannii* izolatlarının antibiyotik direnç oranı, bla_{OXA} ve ISaba1 genleri, metallo-beta-laktamaz üretimi, biyofilm oluşumu ve klonal sınıflandırmasının belirlenmesi amaçlanmıştır. Ayrıca plazmid profilini tanımlamak ve genler, klonlar ve plazmidler arasındaki ilişkiyi analiz etmek de amaçlanmıştır.

Yöntem: 2018 ve 2019 yılları arasında izole edilen 98 *A. baumannii* çalışmaya dahil edilmiştir. Antibiyotik duyarlılık testleri Vitek 2 otomatize sistemi ile belirlenmiştir. Kolistin duyarlılığı için referans yöntem olan sıvı mikrodilüsyon yöntemi kullanılmıştır. Sıvı mikrodilüsyon ile Vitek 2'den elde edilen sonuçlar karşılaştırılmıştır. Bla_{OXA-23}, $bla_{OXA-24/40}$, bla_{OXA-51} ve bla_{OXA-58} genleri multipleks polimeraz zincir reaksiyonu ile tespit edilmiştir. ISAba1/bla_{OXA-23} ve ISAba1/bla_{OXA-51} genleri PCR ile saptanmıştır. İzolatların genotipleri ve alt tipleri

[1]Erbaa State Hospital, Microbiology Laboratory, Tokat, Türkiye
[2]Hitit University, Faculty of Medicine, Department of Medical Microbiology, Çorum, Türkiye
[3]University of Health Sciences Turkey, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Clinic of Medical Microbiology, Ankara, Türkiye
[4]International Cyprus University, Faculty of Medicine, Nicosia, North Cyprus

İletişim / Corresponding Author : Nezahat KOŞAR
Erbaa Devlet Hastanesi, Mikrobiyoloji Laboratuvarı, Tokat - Türkiye
E-posta / E-mail : nezahatkosar@hotmail.com

Geliş Tarihi / Received : 25.10.2024
Kabul Tarihi / Accepted : 25.01.2025

DOI ID : 10.5505/TurkHijyen.2025.94752

Koşar N, Karasartova D, Güreser AS, Taylan Özkan A. Investigation of phenotypic and genotypic characteristics of *Acinetobacter baumannii* isolated from clinical samples.. Turk Hij Den Biyol Derg, 2025; 82(2): 209 - 230

PCR method. International clones were investigated by multiplex PCR. The plasmid profiles of the isolates were analyzed using alkaline lysis. Phenotypic methods were preferred for determining biofilm formation and metallo-beta-lactamase production.

Results: *A. baumannii* was identified in deep tracheal aspirate samples (32%), wounds (21%), blood (17%), sputum (15%), urine (9%), tissue biopsy samples (4%), pleural fluid (1%), and cerebrospinal fluid (1%). *A. baumannii* was detected in patient samples from the intensive care unit at a frequency of 58%. Of these isolates, 13% were susceptible to all antibiotics tested, while 45% were found to be extensively drug resistant. The ISAba1/bla_{OXA-23}, ISab1/bla_{OXA-51}, bla_{OXA-23}, and bla_{OXA-51} genes were found in 98.8% of the carbapenem-resistant isolates. $Bla_{OXA-24/40}$ and bla_{OXA-58} genes were not detected. The epidemiological distribution of the isolates revealed seven genotypes and 21 subtypes. Genotype D was found, and international clone 2 was classified in the hospital epidemic isolates. It was shown that 73.4% of isolates carried plasmids, which were not identical among isolates. Different biofilm levels were measured in 88% of the isolates. However, more vigorous biofilm formation was detected in isolates sensitive to ceftazidime, imipenem, meropenem, trimethoprim-sulfamethoxazole, and ciprofloxacin.

Conclusion: The bla_{OXA-23} genotype was associated with carbapenem resistance in *A. baumannii* isolates. Genotype D and international clone 2 were defined as endemic isolates in our hospital, and no similarity was found between susceptible and resistant isolates in terms of plasmid profiles, genotypes, and clonality. Stronger biofilm formation was detected in isolates susceptible to ceftazidime, imipenem, meropenem, trimethoprim-sulfamethoxazole, and ciprofloxacin, and more comprehensive studies are needed on the relationship between biofilm formation and antibiotic resistance.

Key Words: *Acinetobacter baumannii*, antibiotic resistance, REP-PCR, plasmid, biofilm

Repetitive Extragenic Palindromic PCR yöntemi ile belirlenmiştir. Uluslararası klonlar multipleks PCR ile araştırılmıştır. İzolatların plazmid profilleri ise alkali lizis yöntemi ile saptanmıştır. Biyofilm oluşumu ve metallo-beta-laktamaz üretiminin belirlenmesinde ise fenotipik yöntemler kullanılmıştır.

Bulgular: *A. baumannii* derin trakeal aspirat örneklerinde (%32), yarada (%21), kanda (%17), balgamda (%15), idrarda (%9), doku biyopsi örneklerinde (%4), plevral sıvıda (%1) ve beyin omurilik sıvısında (%1) saptanmıştır. Yoğun bakım ünitesinden alınan hasta örneklerinde *A. baumannii* %58 sıklıkta tespit edilmiştir. İzolatların %13'ü test edilen tüm antibiyotiklere duyarlı iken %45'inde yaygın antibiyotik direnci olduğu bulunmuştur. Karbapenem dirençli izolatların %98,8'inde ISAba1/bla_{OXA-23}, ISAba1/ bla_{OXA-51}, bla_{OXA-23}, bla_{OXA-51} genleri tespit edilmiştir. $Bla_{OXA-24/40}$ ve bla_{OXA-58} genleri saptanmamıştır. Epidemiyolojik sınıflandırmada yedi genotip ve 21 alt tip saptanmıştır. Hastane endemik izolatı olarak Genotip D ve uluslararası klon 2 tanımlanmıştır. İzolatların %73,4'ünün plazmid taşıdığı ve bu plazmidlerin izolatlar arasında farklılık gösterdiği belirlenmiştir. İzolatların %88'inde farklı biyofilm seviyeleri ölçülürken seftazidim, imipenem, meropenem, trimetoprim-sülfametoksazol ve siprofloksasine duyarlı izolatlarda daha güçlü biyofilm oluşumu tespit edilmiştir.

Sonuç: *A. baumannii* izolatlarında bla_{OXA-23} genotipi karbapenem direnci ile ilişkilidir. Genotip D ve uluslararası klon 2 hastanemizin endemik izolatı olarak tanımlanmış olup izolatların plazmid profilleri, genotipler ve klonalite açısından duyarlı ve dirençli izolatlar arasında benzerlik saptanmamıştır. Seftazidim, imipenem, meropenem, trimetoprim-sülfametoksazol ve siprofloksasine duyarlı izolatlarda daha güçlü biyofilm oluşumu tespit edilmiştir ve biyofilm oluşumu ile antibiyotik direnci arasındaki ilişki konusunda daha geniş kapsamlı çalışmaların yapılmasına ihtiyaç olduğu kanaatine varılmıştır.

Anahtar Kelimeler: *Acinetobacter baumannii*, antibiyotik direnci, REP-PCR, plazmid, biyofilm

INTRODUCTION

Acinetobacter baumannii is a gram-negative coccobacillus and a crucial nosocomial pathogen rapidly developing antibiotic resistance (1). Minimum inhibitory concentration (MIC) against imipenem, the safest treatment option, has reached an alarming level. In addition, colistin-resistant isolates have been reported, and treatment options for *A. baumannii* have become very limited (2). OXA-type ß-lactamases and metallo-beta-lactamases (MBLs) produced by bacteria are mainly responsible for carbapenem resistance (CR) (3). Beta-lactamases (bla) of the bla_{OXA-51} group are naturally produced in *Acinetobacter* species (2). *A. baumannii* can also produce bla_{OXA-23}, $bla_{OXA-24/40}$, bla_{OXA-58}, $bla_{OXA-143}$, and $bla_{OXA-235}$. Global epidemiological surveillance of dominant drug-resistant strains is necessary, and *A. baumannii* strains (international clone, IC) were identified following the European outbreak (3). Some studies have shown that plasmids carried by *A. baumannii* are essential in acquiring resistance genes, and plasmids are used as markers in epidemiological studies (4). Our study aimed to determine the antibiotic resistance rate, blaOXA and ISAba1 genes, metallo-beta-lactamases production, biofilm formation, and IC classification of *A. baumannii* isolated from clinical samples in Çorum Erol Olçok Training and Research Hospital, Türkiye. It was also aimed to describe the plasmid profile and analyze the association between genes, clones, and plasmids.

MATERIAL and METHOD

Bacterial strains

The study was conducted from April 1, 2018, to April 30, 2019, at the Medical Microbiology Laboratory of Çorum Erol Olçok Training and Research Hospital. Ninety-eight *A. baumannii* strains isolated from various patient samples (the first isolate from each patient) were included in the study. After identifying and antibiotic susceptibility testing of the strains by both manual and automated techniques (Vitek 2 Compact, bioMérieux, Marcy l'Étoile, France), isolates were suspended in 15% glycerol lysogeny broth (LB) and stored at −80°C for further research.

Antimicrobial susceptibility tests

The Vitek 2 Compact system was used to detect the antibiotic susceptibility of isolates according to the European Committee on Antimicrobial Resistance Testing (EUCAST) standards (5). Both the automated system and the reference broth microdilution (BMD) method were used to detect colistin susceptibility (6). Categorical agreement (CA) of test results was defined using the definitions of the International Organization for Standardization (ISO) 20776-2:2021 (7). Isolates detected as colistin-sensitive by Vitek 2 but resistant by BMD were categorized as a significant error (VME). In contrast, colistin-resistant isolates detected by Vitek 2 but susceptible by BMD were classified as a substantial error (ME). Standardized international terminology was used for the various resistance patterns in *A. baumannii* isolates, categorized as multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) (8). MDR was defined as acquired resistance to at least one agent across three or more antimicrobial categories, XDR is defined as resistance to at least one agent in all but two or fewer antimicrobial categories (indicating that bacterial isolates remain susceptible to only one or two categories), and PDR is defined as resistance to all agents in all antimicrobial categories (8).

PCR of class D- β lactamases genes

DNAs were extracted by Tissue δ Bacterial DNA Purification Kit (The EURx Gene MATRIX) and then stored at −20°C. The gene regions of bla_{OXA-23}, $bla_{OXA-24/40}$, bla_{OXA-51}, and bla_{OXA-58} were studied using multiplex polymerase chain reaction (mPCR) (9). The ISAba1/ bla_{OXA-23} and ISAba1/ bla_{OXA-51} genes were investigated separately with the PCR (10).

Metallo-beta-lactamases combination disk diffusion test

0.5 McFarland bacterial suspensions were

inoculated on Mueller-Hinton agar (MHA) plates. Two imipenem (10 µg) disks were placed 22 mm apart on the surface of the medium. 10 µL of ethylenediaminetetraacetic acid (EDTA) (0.5 M, pH: 8) was dropped in one of the imipenem disks. The plates were incubated for 18 hours at 35°C. Isolates were classified as MBL producers when the inhibition zone diameter of the imipenem/EDTA disc was above that of the imipenem disc by 7 mm or more (11).

International clone identification

Two groups of PCR primers were utilised to identify ICs. (12). The PCR methods, target genes, and primer sequences utilized for genotyping *A. baumannii* are presented in Table 1. ICs were determined by evaluating the bands whose molecular size was determined with Group 1 and Group 2 PCR analysis of each sample (13). According to Reboli et al.'s method, the genotypes and subtypes of the isolates were determined with the Repetitive Extragenic Palindromic PCR (REP-PCR) method (14).

Table 1. PCR methods, target genes and primer sequences used for genotyping of *A. baumannii*

Target gene	Method	Primer sequence	Product size (bp)	References
bla-Oxa-51-like-F	Multiplex PCR	5'-TAA TGC TTT GAT CGG CCT TG-3'	353	Woodford et al.,2006
bla-Oxa-51-like-R		5'-TGG ATT GCA CTT CAT CTT GG-3'		
bla-Oxa-58-like-F		5'-AAG TAT TGG GGC TTG TGC TG-3'	599	
bla-Oxa-58-like-R		5'-CCC CTC TGC GCT CTA CAT AC-3'		
bla-Oxa-23-like-F		5'-GAT CGG ATT GGA GAA CCA GA-3'	501	
bla-Oxa-23-like-R		5'-ATT TCT GAC CGC ATT TCC AT-3'		
bla-Oxa-24-like-F		5'-GGT TAG TTG GCC CCC TTA AA-3'	246	
bla-Oxa-24-like-R		5'-AGT TGA GCG AAA AGG GGA TT-3'		
ISAba-1-F	PCR	5'-TGA GAT GTG TCA TAG TAT TC-3'	314	Bahador et al., 2015
OXA-23-R		5'-AGA GCA TTA CCA TAT AGA TT-3'		
ISAba-1-F		5'-AAG CAT GAT GAG CGC AAA G-3'	227	
OXA-51-R		5'-GGT GAG CAG GCT GAA ATA AAA-3'		
Group1ompAF306	Multiplex PCR	5'-GAT GGC GTA AAT CGT GGT A-3'	355	Turton et al., 2007
Group1and2ompAR660		5'-CAA CTT TAG CGA TTT CTG G-3'		
Group1csuEF		5'-CTT TAG CAA ACA TGA CCT ACC-3'	702	
Group1csuER		5'-TAC ACC CGG GTT AAT CGT-3'		
Gp1OXA66F89		5'-GCG CTT CAA AAT CTG ATG TA-3'	559	
Gp1OXA66R647		5'-GCG TAT ATT TTG TTT CCA TTC-3'		
Group2ompAF378	Multiplex PCR	5'-GAC CTT TCT TAT CAC AAC GA-3'	343	Turton et al., 2007
Group1and2ompAR660		5'-CAA CTT TAG CGA TTT CTG G-3'		
Group2csuEF		5'- GGC GAA CAT GAC CTA TTT-3'	580	
Group2csuER		5'-CTT CAT GGC TCG TTG GTT-3'		
Gp2OXA69F169		5'-CAT CAA GGT CAA ACT CAA-3'	162	
Gp2OXA69R330		5'-TAG CCT TTT TTC CCC ATC-3'		
REP1R-I REP2-I	REP-PCR	5'- IIIICGICGICATCIGGC-3' 5'- ICGICTTATCIGGCCTAC-3'	-	Reboli et al., 1994

REP-PCR, Repetitive Extragenic Palindromic polymerase chain reaction

Plasmid DNA extraction

The Miniprep plasmid DNA isolation method was used for plasmid DNA extraction (15). The following solutions were added to freshly prepared bacterial cultures: Alkaline Lysis Solution (ALS) I (10 mM EDTA, pH:8.0, 50 mM glucose/ 25mM Tris-Cl, 13 µL of RNAase added to 1 mL solution), ALS II (1% SDS/ 0.2 M NaOH), and ALS III (5 M potassium acetate/glacial acetic acid). DNA products were purified and then visualized using a UV transilluminator. The Fragment Size Calculator was used to calculate plasmid sizes. (http://www.basic.northwestern.edu/biotools/SizeCalc.html) (16).

Biofilm formation

A single colony of *A. baumannii* grown on LB agar was inoculated into brain-heart infusion broth containing 0.25% glucose (G-BHIB). After incubation at 37°C for 24 hours, the medium was 1/20 diluted with G-BHIB, and 200 µL of the mixture of each strain was dispensed into polystyrene plates in three adjacent wells. The microplates were washed with phosphate-buffered saline (PBS) X1 after 48 hours of incubation at 37°C in a shaker water bath and dried at room temperature. The residue was stained for 15 minutes with 1% crystal violet, washed with PBS, and dried at room temperature for 20 minutes. 200 µL of ethanol/acetone solution (80/20) was added to each well. Optical density (OD) was measured using a spectrophotometer at a wavelength of 590 nm. Based on the OD measurement, biofilm formation was classified as follows: OD < 1 negative; 1 < OD < 2 low level (+); 2 < OD < 3 moderate level (++); and OD > 3 high level (+++). As a quality control measure, the *Staphylococcus aureus* ATCC 25923 strain was used (17).

Biofilm imaging with scanning electron microscopy

Five isolates with high, medium, low, and negative OD values were selected for imaging using scanning electron microscopy (SEM). These isolates and the positive control strain (*S. aureus* ATCC 25923) were tested on ceramic surfaces by modifying Patenge's method (18).

Approximately 30 fields of view of each sample were scanned with SEM. Bacterial adhesion and aggregation in each area were evaluated. In the SEM imaging, if the bacteria in the 10,000 × magnification covered more than 80% of the field, it was classified as high OD; bacteria covering 50–80% of the field it was classified as medium OD; bacteria covering 10-50% of the field was classified as low OD; and bacteria covering less than 10% of the field was classified as negative OD.

Statistical analysis

SPSS version 21.0 (IBM Corp, Armonk, NY, USA) was used for data analysis. P values <0.05 were determined as statistically significant. For continuous variables with a normal distribution, results were presented as the mean ± standard deviation, Student's t-test was used to compare means between two groups, and analysis of variance (ANOVA) was used to compare means between three or more groups. For continuous variables with a non-normal distribution, results were reported as the median and range, and the Mann-Whitney U test was used to compare means between two groups, and the Kruskal-Wallis test was used to compare means between three or more groups. Chi-squared or Fisher's exact tests were used for intergroup comparisons of categorical variables. Spearman's correlation coefficient was used to assess the correlation between two continuous variables with a non-normal distribution.

The study was approved by the Hitit University Non-Interventional Clinical Research Ethics Committee (Date: 25.10.2018 and Number: 2018-173).

RESULTS

A. baumannii was identified in deep tracheal aspirate samples (DTA) (32%), wounds (21%), blood (17%), sputum (15%), urine (9%), tissue biopsy samples (4%), pleural fluid (1%), and cerebrospinal fluid (1%). *A. baumannii* was detected in patient samples from the intensive care unit (ICU) at a frequency of 58%.

Antimicrobial susceptibility results

According to the antibiotic resistance profiles of the isolates, 45% were XDR, and 42% were MDR. Antibiotic resistance levels for ceftazidime (CAZ), imipenem (IPM), meropenem (MEM), and ciprofloxacin (CIP) have been recorded at 86.7% each. The resistance rates to other antibiotics were determined as follows: gentamicin (CN), 73.4%; amikacin (AK), 60.2%; trimethoprim-sulfamethoxazole (SXT), 60.2%; tigecycline (TGC), 2%; and colistin (CT), 8.1%.

There were eight colistin-resistant isolates with BMD. Using Vitek 2, six had MICs ≤ 0.5 mg/L, and they gave false-susceptible results. The rate of VMEs was 75%, but no MEs were observed in the susceptibility test of *A. baumannii* isolates. The positive predictive value (PPV) of the Vitek 2 for detecting resistant isolate was 25% (2/8), while the negative predictive value (NPV) was 100% (90/90). The results of antibiotic susceptibility tests of isolates are shown in Table 2.

Distribution of carbapenemase genes

Ninety-three (94.8%) isolates were bla_{OXA-51} carbapenemase genotype, while 84 (85.7%) were bla_{OXA-23} genotype. 84 (85.7%) isolates were ISAba1/bla_{OXA-51} + ISAba1/bla_{OXA-23} genotype. The genes linked to the bla_{OXA-24}/40 and bla_{OXA-58} groups were undetected.

The bla_{OXA-23}, bla_{OXA-51}, ISAba1/bla_{OXA-23}, and ISAba1/bla_{OXA-51} genes were detected in 98.8% of CR isolates, and they were detected as significantly higher in the CR groups (all $p < 0.001$).

Table 3 shows the distribution of bla genes based on the isolates' carbapenem susceptibility.

Table 2. Antimicrobial resistance rates of *A. baumannii* isolates

Antimicrobial Agent	Antimicrobial Resistance Rates	
	R% (n/N)	I % (n)
Imipenem	86.7 (85/98)	0
Meropenem	86.7 (85/98)	0
Ceftazidime	86.7 (85/98)	-
Ciprofloxacin	86.7 (85/98)	1 (1/98)
Gentamicin	73.4 (72/98)	-
Amikacin	60.2 (59/98)	-
Trimethoprim-Sulfamethoxazole-	60.2 (59/98)	0
Tigecycline	2 (2/98)	20.4 (20/98)
Colistin (Vitek 2)	2 (2/98)	-
Colistin (BMD)	8.1 (8/98)	-

Table 3. Distribution of *bla* genes and the carbapenem susceptibility of isolates

Genes	CRAB n=85 %(n)	CSAB n=13 % (n)	All isolates n=98 % (n)
blaOXA-51	98.8 (84)	69.2 (9)	94.8 (93)
blaOXA-23	98.8 (84)	0 (0)	85.7 (84)
blaOXA-24/40	0 (0)	0 (0)	0 (0)
blaOXA-58	0 (0)	0 (0)	0 (0)
ISAba1/blaOXA-51	98.8 (84)	38.4 (5)	90.8 (89)
ISAba1/blaOXA-23	98.8 (84)	0 (0)	85.7 (84)

Carbapenem-resistant *A. baumannii* (CRAB), Carbapenem-susceptible *A. baumannii* (CSAB)

Clusters, clones, plasmids, and biofilm formation

The isolates' local epidemiological distributions and band patterns were identified by repetitive-element PCR (REP-PCR). In our study, seven genotypes and 21 subtypes were detected. These subtypes belonged to eight different clusters, and 13 isolates were classified under a single pattern and did not show similarities to the other isolates. Genotype D was detected in 52 (53%) of the isolates and was determined to be the endemic strain of our hospital. Although seven different clone groups were detected by multiplex PCR, five isolates did not belong to a clone. IC 2 was the dominant international clone in our hospital, constituting 71 isolates (73%) of the isolates. The distribution characteristics of IC strains of the isolates are summarized in Supplementary Table 1.

Plasmid DNA extraction showed that 73.4% of *A. baumannii* isolates carried one to six plasmids varying between 1.3 and 50.2 Kb, and these plasmids were heterogeneously distributed among the isolates. Plasmids were detected in 74.1% (63/85) of the CR isolates and 69.2% (9/13) of the susceptible isolates.

All colistin-resistant isolates had plasmids, and only two isolates had identical plasmid profiles, while the remaining isolates displayed distinct patterns. All isolates' genotypes, IC distribution, and plasmid characterisation information are included in Supplementary Table 2.

It was defined that 20% (20/98) of the isolates formed a high-level biofilm, 33% (32/98) a medium level of biofilm, 35% (34/98) a low level of biofilm, and 12% (12/98) did not form biofilm.

The distribution of biofilm measurement results of the isolates according to the resistance group is shown in Figure 1. SEM images of biofilm formation are shown in Figure 2. A statistically significant difference was seen between the resistance groups and the level of biofilm formation (p= 0.024). The strains susceptible to CAZ, IPM, MEM SXT, and CIP formed stronger biofilms than those resistant to these antibiotics. (p = 0.007, p = 0.007, p = 0.007, p < 0.001, and p = 0. 012, respectively) (Table 4).

Table 4. The relationship between biofilm formation level and antibiotic resistance

	Biofilm OD (590 nm)		*P*
	Resistance Median (25p-75p)	Sensitive Median (25p-75p)	
Ceftazidime	1.98 (1.18-2.75)	2.86 (2.06-3.14)	0.007
Gentamicin	1.98 (1.35-2.70)	2.64 (1.77-3.22)	0.059
Amikacin	2.01 (1.4-2.8)	2.3 (1.42-2.92)	0.689
Imipenem	1.98 (1.18-2.75)	2.86 (2.06-3.14)	0.007
Meropenem	1.98 (1.18-2.75)	2.86 (2.06-3.14)	0.007
SXT	1.85 (1.12-2.46)	2.86 (1.98-3.27)	<0.001
Ciprofloxacin	1.98 (1.18-2.75)	2.88 (2.04-3.17)	0.012
Tigecycline	2.17 (0.95-)	2 (2.82-1.35)	0.973
Colistin	2.61 (1.51-3.43)	2.16 (1.4-2.81)	0.695

SXT, sulfamethoxazole-trimethoprim

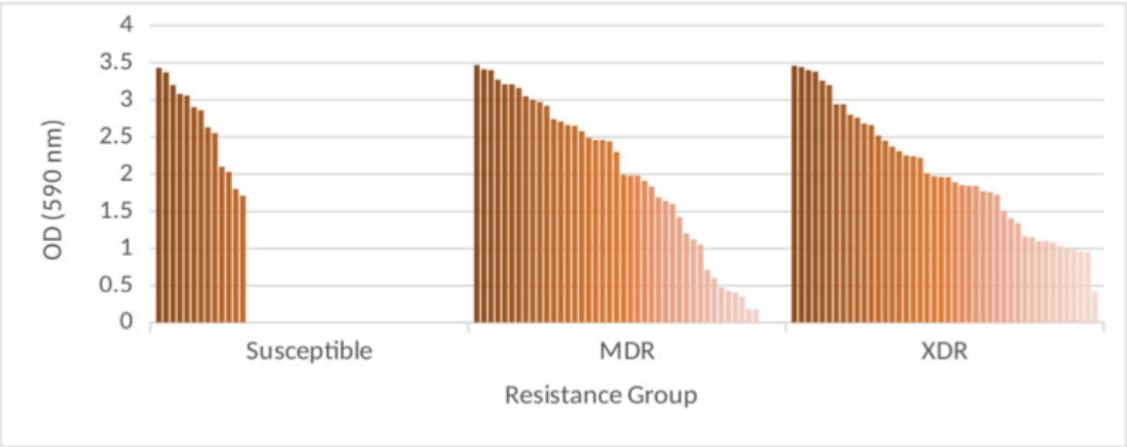

Figure 1. Biofilm formation levels of *A. baumannii* strains in different resistance groups

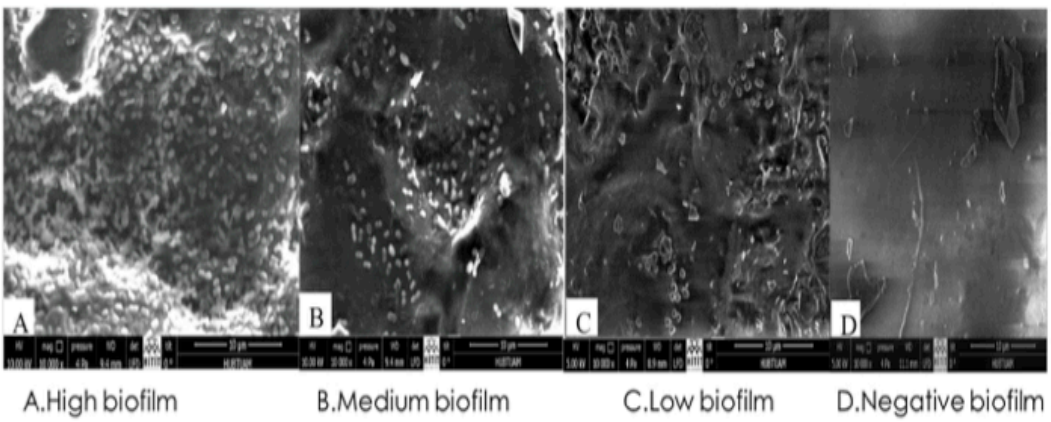

Figure 2. Biofilm formation of *A. baumannii* on ceramic surface, imaging by SEM

Supplementary Table 1. Epidemiological data, phenotypic characteristics and resistance genes of *A. baumannii*

Epidemiological Data					Phenotypic Characteristics			Resistance Genes				
Strain	Cluster	IC	Sample	Ward	Carbapenem Resistance	MBL	Biofilm	*blaOXA-51*	*blaOXA-23*	*blaOXA-24/40-58*	*ISAba1/blaOXA-51*	*ISAba1/blaOXA-23*
5	A	IC1	DTA	ICU	+	–	–	+	+	–	+	+
6	D	IC2	wound	ICU	+	–	++	+	+	–	+	+
7	E	IC2	DTA	ICU	+	+	–	+	+	–	+	+
8	D	-	blood	ICU	+	–	–	–	–	–	–	–
9	D	IC2	urine	ICU	+	+	+	+	+	–	+	+
10	D	IC2	urine	Out	+	+	-	+	+	–	+	+
11	A	IC1	blood	ICU	+	+	-	+	+	–	+	+
12	D	IC2	urine	PACA	+	+	+	+	+	–	+	+
13	D	IC2	wound	TH	+	+	+	+	+	–	+	+
14	A	IC1	blood	TH	+	–	+	+	+	–	+	+
15	E	IC2	sputum	ICU	+	+	+	+	+	–	+	+
17	A	IC1	wound	ICU	+	+	-	+	+	–	+	+
18	D	IC2	blood	ICU	+	+	+	+	+	–	+	+
19	D	IC2	wound	ORTO	+	+	+	+	+	–	+	+
20	G	-	blood	ONCO	–	–	+	–	–	–	–	–
21	E	IC2	urine	BS	+	+	+	+	+	–	+	+
22	B	G13	urine	IM	–	–	++	+	–	–	+	–
23	E	IC2	wound	TH	+	+	++	+	+	–	+	+
24	E	IC2	wound	INF	+	+	+	+	+	–	+	+
25	A	IC1	sputum	ICU	+	+	+	+	+	–	+	+
26	D	IC2	blood	ICU	+	+	++	+	+	–	+	+
27	F	-	blood	ICU	–	–	++	–	–	–	–	–
28	D	IC2	pleural fluid	TH	+	+	++	+	+	-	+	+
29	C	G5	sputum	ICU	–	–	++	+	–	–	+	–
30	C	G5	sputum	ICU	–	–	+++	+	–	–	+	–
31	E	IC2	sputum	TH	+	+	+	+	+	–	+	+
32	E	IC2	wound	TH	+	+	+	+	+	–	+	+
33	E	IC2	wound	TH	+	+	++	+	+	–	+	+
34	D	IC2	sputum	TH	+	+	+	+	+	–	+	+

Supplementary Table 1 (cont.). Epidemiological data, phenotypic characteristics and resistance genes of *A. baumannii*

Epidemiological Data					Phenotypic Characteristics			Resistance Genes				
Strain	Cluster	IC	Sample	Ward	Carbapenem Resistance	MBL	Biofilm	*blaOXA-51*	*blaOXA-23*	*blaOXA-24/40-58*	*ISAba1/blaOXA-51*	*ISAba1/blaOXA-23*
35	A	IC1	Tissue biopsy	PACA	+	+	-	+	+	–	+	+
36	D	IC2	blood	ICU	+	+	++	+	+	–	+	+
37	A	IC1	DTA	ICU	+	+	+++	+	+	–	+	+
38	A	IC1	blood	ICU	+	+	+++	+	+	–	+	+
39	D	G13	urine	PEDI	–	–	++	+	–	–	+	-
41	D	IC2	blood	ICU	+	+	++	+	+	–	+	+
42	A	IC1	DTA	ICU	+	+	-	+	+	–	+	+
44	D	IC2	blood	ICU	+	+	++	+	+	–	+	+
45	E	IC2	sputum	TH	+	+	+	+	+	–	+	+
46	E	IC2	sputum	TH	+	+	+	+	+	–	+	+
47	D	IC2	blood	ICU	+	+	++	+	+	–	+	+
48	D	IC2	DTA	PACA	+	+	+	+	+	–	+	+
49	F	IC2	DTA	ICU	+	+	+	+	+	–	+	+
50	D	IC2	DTA	ICU	+	+	++	+	+	–	+	+
51	E	IC2	sputum	CA	+	+	++	+	+	–	+	+
52	D	IC2	DTA	ICU	+	+	++	+	+	–	+	+
53	D	IC2	Tissue biopsy	ORTHO	+	+	++	+	+	–	+	+
54	D	IC2	wound	ORTHO	+	+	+	+	+	-	+	+
55	E	IC2	DTA	ICU	+	+	++	+	+	-	+	+
56	G	IC2	DTA	ICU	+	+	++	+	+	-	+	+
57	D	IC2	DTA	ICU	+	-	+	+	+	-	+	+
58	E	IC2	DTA	ICU	+	-	++	+	+	-	+	+
59	D	IC2	DTA	ICU	+	-	+	+	+	-	+	+
60	D	IC2	wound	ORTHO	+	-	+	+	+	-	+	+
61	D	IC2	DTA	ICU	+	-	+	+	+	-	+	+
62	D	IC2	DTA	ICU	+	-	+	+	+	-	+	+
63	D	IC2	DTA	ICU	+	-	+	+	+	-	+	+
64	D	IC2	blood	ICU	+	+	++	+	+	-	+	+

Supplementary Table 1(cont.). Epidemiological data, phenotypic characteristics and resistance genes of *A. baumannii*

Epidemiological Data					Phenotypic Characteristics			Resistance Genes				
Strain	Cluster	IC	Sample	Ward	Carbapenem Resistance	MBL	Biofilm	*blaOXA-51*	*blaOXA-23*	*blaOXA-24/40-58*	*ISAba1/blaOXA-51*	*ISAba1/blaOXA-23*
65	D	G5	wound	FTR	–	–	++	+	–	–	–	–
66	B	G6	wound	ORTHO	–	–	+	+	–	–	–	–
67	A	IC1	Tissue biopsy	DIFO	+	+	+	+	+	-	+	+
68	E	G5	blood	ICU	–	–	+++	+	–	–	–	–
70	B	-	sputum	TH	–	–	+++	–	–	–	–	–
72	D	IC2	blood	ICU	+	+	++	+	+	–	+	+
73	D	IC2	sputum	PACA	+	-	++	+	+	–	+	+
74	A	IC1	Tissue biopsy	DIFO	+	+	++	+	+	–	+	+
75	F	IC2	sputum	PACA	+	–	++	+	+	–	+	+
77	E	IC2	DTA	ICU	+	+	+++	+	+	–	+	+
78	D	IC2	wound	GS	+	+	–	+	+	–	+	+
79	A	IC1	DTA	ICU	+	+	+	+	+	–	+	+
80	F	IC2	sputum	TH	+	+	+++	+	+	–	+	+
81	D	IC2	sputum	PACA	+	–	+	+	+	–	+	+
82	D	IC2	sputum	GS	+	+	++	+	+	–	+	+
83	D	IC2	DTA	ICU	+	+	++	+	+	–	+	+
84	D	IC2	wound	ORTHO	+	+	+++	+	+	–	+	+
85	E	G4	wound	ICU	+	+	-	+	+	–	+	+
87	D	IC2	DTA	ICU	+	+	+	+	+	–	+	+
88	B	G8	DTA	ICU	–	–	++	+	–	–	+	–
89	B	G4	urine	PACA	–	–	+++	+	–	–	–	–
91	E	-	DTA	ICU	–	–	+++	–	–	–	–	–
92	D	IC2	wound	PACA	+	+	+++	+	+	-	+	+
93	D	IC2	DTA	ICU	+	+	+++	+	+	–	+	+
94	D	IC2	DTA	ICU	+	+	++	+	+	–	+	+
95	E	IC2	DTA	ICU	+	+	+++	+	+	–	+	+
96	D	IC2	CSF	ICU	+	+	+++	+	+	–	+	+
97	D	IC2	DTA	ICU	+	+	+++	+	+	–	+	+

Supplementary Table 1 (cont.). Epidemiological data, phenotypic characteristics and resistance genes of *A. baumannii*

Epidemiological Data					Phenotypic Characteristics			Resistance Genes				
Strain	Cluster	IC	Sample	Ward	Carbapenem Resistance	MBL	Biofilm	*blaOXA-51*	*blaOXA-23*	*blaOXA-24/40-58*	*ISAba1/blaOXA-51*	*ISAba1/blaOXA-23*
98	E	IC2	wound	ORTHO	+	+	+++	+	+	–	+	+
99	E	IC2	wound	ICU	+	+	+++	+	+	–	+	+
100	D	IC2	DTA	ICU	+	+	++	+	+	–	+	+
101	D	IC2	blood	ICU	+	+	+++	+	+	–	+	+
103	D	IC2	urine	ICU	+	+	+++	+	+	–	+	+
104	D	IC2	DTA	ICU	+	+	+++	+	+	–	+	+
105	D	IC2	urine	ICU	+	+	-	+	+	–	+	+
106	D	IC2	DTA	ICU	+	+	+	+	+	–	+	+
107	D	IC2	blood	ICU	+	+	+	+	+	–	+	+
108	E	IC2	wound	GS	+	+	-	+	+	–	+	+
109	D	IC2	DTA	ICU	+	+	+	+	+	–	+	+
110	D	IC2	DTA	ICU	+	+	+	+	+	–	+	+
111	D	IC2	wound	GS	+	+	++	+	+	–	+	+

BS, Brain Surgery; CA, Cardiology; CSF, cerebrospinal fluid; DIFO, diabetic foot; DTA, deep tracheal aspiration; GS, General Surgery; ICU, intensive care unit; IM, Internal Medicine; INF, infection; IC, international clone; MBL, metallo-beta-lactamase; ONCO, Oncology; ORTHO, Orthopedics; Out, outpatient; PACA, Palliative Care; PEDI, Pediatrics; TH, Thoracic.

Supplementary Table 2. Genotype,IC distribution and plasmid characterization of *A. baumannii*

Isolate No	Antibiotic resistance	Number of plasmids	Size of plasmids (Kb)	IC	Genotype
5	CAZ, IPM, MEM, CN, CIP, SXT	4	38.5, 32.2, 1.7, 1.3	IC1	A
6	CAZ, IPM, MEM, AK, CN, CIP, SXT	-	-	IC2	D
7	CAZ, IPM, MEM, CIP, SXT	-	-	IC2	E
8	CAZ, IPM, MEM, AK, CN, CIP, CT	5	50.2, 23.1, 3.7, 2.6, 2.3	-	D
9	CAZ, IPM, MEM, AK, CN, CIP, SXT	5	50.2, 23.1, 3.7, 2.6, 2.3	IC2	D
10	CAZ, IPM, MEM, AK, CN, CIP	-	-	IC2	D
11	CAZ, IPM, MEM, CN, CIP, SXT	4	49, 33.3, 1.9, 1.4	IC1	A
12	CAZ, IPM, MEM, AK, CN, CIP	-	-	IC2	D
13	CAZ, IPM, MEM, AK, CN, CIP, SXT	3	26.2, 14, 5.1	IC2	D
14	CAZ, IPM, MEM, CN, CIP, SXT	4	49, 33.3, 1.9, 1.4	IC1	A
15	CAZ, IPM, MEM, CIP, SXT	2	22.4, 6	IC2	E
17	CAZ, IPM, MEM, CN, CIP, SXT	4	49, 33.3, 1.9, 1.4	IC1	A
18	CAZ, IPM, MEM, AK, CN, CIP, CT	2	19, 6.1	IC2	D
19	CAZ, IPM, MEM, AK, CN, CIP, SXT	-	-	IC2	D
20	-	-	-	-	G
21	CAZ, IPM, MEM, CIP, SXT	-	-	IC2	E
22	-	2	42.3, 5.4	G13	B
23	CAZ, IPM, MEM, AK, CN, CIP, SXT	4	49.2, 26, 2.1, 1.5	IC2	E
24	CAZ, IPM, MEM, AK, CN, CIP, SXT	-	-	IC2	E
25	CAZ, IPM, MEM, CN, CIP, SXT	4	38.5, 32.2, 1.7, 1.3	IC1	A
26	CAZ, IPM, MEM, AK, CN, CIP	-	-	IC2	D
27	-	4	41.1, 10.9, 6.1, 4	-	F
28	CAZ, IPM, MEM, CN, CIP	-	-	IC2	D
29	-	2	6.3, 5.1	G5	C
30	-	2	6.3, 5.1	G5	C
31	CAZ, IPM, MEM, AK, CN, CIP, SXT	1	27.4	IC2	E
32	CAZ, IPM, MEM, CN, CIP, SXT	1	25	IC2	E
33	CAZ, IPM, MEM, AK, CN, CIP, SXT	-	-	IC2	E
34	CAZ, IPM, MEM, AK, CN, CIP, SXT	-	-	IC2	D
35	CAZ, IPM, MEM, AK, CN, CIP, SXT, TGC	-	-	IC1	A
36	CAZ, IPM, MEM, AK, CN, CIP	-	-	IC2	D

Supplementary Table 2 (cont.). Genotype,IC distribution and plasmid characterization of *A. baumannii*

Isolate No	Antibiotic resistance	Number of plasmids	Size of plasmids (Kb)	IC	Genotype
37	CAZ, IPM, MEM, CIP	4	49, 33.3, 1.9, 1.4	IC1	A
38	CAZ, IPM, MEM, CIP	4	49, 33.3, 1.9, 1.4	IC1	A
39	-	2	6.3, 5.1	G13	D
41	CAZ, IPM, MEM, AK, CN, CIP	-	-	IC2	D
42	CAZ, IPM, MEM, CIP	1	25.1	IC1	A
44	CAZ, IPM, MEM, CIP	5	38.5, 28.4, 15.4, 2.5, 1.6	IC2	D
45	CAZ, IPM, MEM, AK, CN, CIP, SXT	4	27.4, 23.5, 2.5, 1.6	IC2	E
46	CAZ, IPM, MEM, AK, CN, CIP, SXT	4	27.4, 23.5, 2.5, 1.6	IC2	E
47	CAZ, IPM, MEM, AK, CN, CIP, SXT	5	15.4, 6.1, 3.71, 2.5, 1.6	IC2	D
48	CAZ, IPM, MEM, AK, CN, CIP	2	21.5, 6.3	IC2	D
49	CAZ, IPM, MEM, CN, CIP, SXT	2	21.5, 6.3	IC2	F
50	CAZ, IPM, MEM, CN, CIP, SXT	5	44, 28, 12.9, 2.4, 1.6	IC2	D
51	CAZ, IPM, MEM, CN, CIP, SXT	2	17.6, 6.3	IC2	E
52	CAZ, IPM, MEM, CN, CIP, SXT	5	44, 28, 12.9, 2.4, 1.6	IC2	D
53	CAZ, IPM, MEM, CN, CIP, SXT	5	44, 28, 12.9, 2.4, 1.6	IC2	D
54	CAZ, IPM, MEM, CN, CIP, SXT	5	44, 28, 12.9, 2.4, 1.6	IC2	D
55	CAZ, IPM, MEM, AK, CN, CIP, SXT	2	17.6, 6.3	IC2	E
56	CAZ, IPM, MEM, AK, CN, CIP, SXT	4	24, 14.7, 6.5, 1.4	IC2	G
57	CAZ, IPM, MEM, AK, CN, CIP, SXT	-	-	IC2	D
58	CAZ, IPM, MEM, AK, CN, CIP, SXT	2	17.6, 6.3	IC2	E
59	CAZ, IPM, MEM, CN, CIP, SXT	4	29.1, 28.1, 16.6, 4.8	IC2	D
60	CAZ, IPM, MEM, AK, CN, CIP, SXT	1	6.1	IC2	D
61	CAZ, IPM, MEM, AK, CN, CIP, SXT	6	29.1, 28.1, 16.6, 4.8, 2.4, 1.5	IC2	D
62	CAZ, IPM, MEM, AK, CN, CIP, SXT, CT	6	29.1, 28.1, 16.6, 4.8, 2.4, 1.5	IC2	D
63	CAZ, IPM, MEM, AK, CN, CIP, SXT	6	29.1, 28.1, 16.6, 4.8, 2.4, 1.5	IC2	D
64	CAZ, IPM, MEM, AK, CN, CIP, SXT	-	-	IC2	D
65	-	1	9.4	G5	D
66	-	-	-	G6	B
67	CAZ, IPM, MEM, AK, CIP	6	6.1, 4.4, 1.9, 1.7, 1.4, 1.3	IC1	A
68	-	-	-	G5	E
70	-	-	-	-	B

Supplementary Table 2 (cont.). Genotype,IC distribution and plasmid characterization of *A. baumannii*

Isolate No	Antibiotic resistance	Number of plasmids	Size of plasmids (Kb)	IC	Genotype
72	CAZ, IPM, MEM, AK, CN, CIP, SXT	3	17.6, 6.3, 1.6	IC2	D
73	CAZ, IPM, MEM, CN, CIP, SXT	-	-	IC2	D
74	CAZ, IPM, MEM, AK, CIP	6	6.1, 4.4, 1.9, 1.7, 1.4, 1.3	IC1	A
75	CAZ, IPM, MEM, AK, CN, CIP, SXT	1	6.1	IC2	F
77	CAZ, IPM, MEM, AK, CIP	3	26.2, 14, 5.1	IC2	E
78	CAZ, IPM, MEM, AK, CN, CIP	-	-	IC2	D
79	CAZ, IPM, MEM, CIP	4	49, 3.3, 1.9, 1.4	IC1	A
80	CAZ, IPM, MEM, CN, CIP, SXT	-	-	IC2	F
81	CAZ, IPM, MEM, AK, CN, CIP, SXT	4	14.4, 8.2, 5.5, 1.6	IC2	D
82	CAZ, IPM, MEM, AK, CN, CIP, SXT	3	17.6, 6.3, 1.6	IC2	D
83	CAZ, IPM, MEM, CN, CIP, SXT	3	17.6, 6.3, 1.6	IC2	D
84	CAZ, IPM, MEM, AK, CN, CIP, SXT	6	29.1, 28.1, 16.6, 4.8, 2.4, 1.5	IC2	D
85	CAZ, IPM, MEM, CIP, SXT	-	-	G4	E
87	CAZ, IPM, MEM, AK, CN, CIP, SXT	3	17.6, 6.3, 1.6	IC2	D
88	-	1	39.6	G8	B
89	-	1	6.1	G4	B
91	-	1	10	-	E
92	CAZ, IPM, MEM, AK, CN, CIP, CT	2	21.5, 6.2	IC2	D
93	CAZ, IPM, MEM, AK, CN, CIP	2	22.6, 6.3	IC2	D
94	CAZ, IPM, MEM, AK, CN, CIP, SXT	6	29.1, 28.1, 16.6, 4.8, 2.4, 1.5	IC2	D
95	CAZ, IPM, MEM, AK, CIP, SXT, CT	4	28.5, 15.1, 6, 2	IC2	E
96	CAZ, IPM, MEM, AK, CN, CIP, TGC, CT	2	22.4, 6	IC2	D
97	CAZ, IPM, MEM, AK, CN, CIP, CT	2	22.4, 6	IC2	D
98	CAZ, IPM, MEM, AK, CN, CIP	2	17.6, 6.3	IC2	E
99	CAZ, IPM, MEM, AK, CN, CIP, SXT	1	6.1	IC2	E
100	CAZ, IPM, MEM, AK, CN, CIP, SXT	-	-	IC2	D
101	CAZ, IPM, MEM, AK, CN, CIP	-	-	IC2	D
103	CAZ, IPM, MEM, AK, CN, CIP	2	22.4, 6	IC2	D
104	CAZ, IPM, MEM, AK, CN, CIP	2	22.4, 6	IC2	D
105	CAZ, IPM, MEM, AK, CN, CIP, SXT	6	44.7, 28.5, 8, 5.1, 2.5, 1.6	IC2	D
106	CAZ, IPM, MEM, AK, CN, CIP, SXT	6	44.7, 28.5, 8, 5.1, 2.5, 1.6	IC2	D

Supplementary Table 2 (cont.). Genotype,IC distribution and plasmid characterization of *A. baumannii*

Isolate No	Antibiotic resistance	Number of plasmids	Size of plasmids (Kb)	IC	Genotype
107	CAZ, IPM, MEM, AK, CN, CIP, SXT, CT	3	34, 5.1, 1.6	IC2	D
108	CAZ, IPM, MEM, AK, CN, CIP, SXT	2	22.4, 6	IC2	E
109	CAZ, IPM, MEM, AK, CN, CIP, SXT	4	45.3, 28.4, 6, 1.6	IC2	D
110	CAZ, IPM, MEM, AK, CN, CIP, SXT	6	44.7, 28.5, 8, 5.1, 2.5, 1.6	IC2	D
111	CAZ, IPM, MEM, AK, CN, CIP, SXT	6	44.7, 28.5, 8, 5.1, 2.5, 1.6	IC2	D
E. coli ATCC 25922	-	6	48, 40.4, 30.4, 3.7, 2.1, 1.4	-	-

AK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; CN, gentamicin; CT, colistin; IC, international clone; IPM, imipenem; MEM, meropenem; SXT, sulfamethoxazole-trimethoprim, TGC, tigecycline.

DISCUSSION

Numerous studies demonstrate that MDR *A. baumannii* is frequently isolated from the respiratory tract and blood samples of patients in ICU, attributed to extended hospitalization and mechanical ventilation support. In this study, *A. baumannii* was primarily isolated from DTAs (32%) of patients in the ICU (58%), corresponding to the literature (19). Of the isolates with resistance profiles, 45% were XDR, and 42% were MDR.

In our investigation, the highest antibiotic resistance levels for ceftazidime (CAZ), imipenem (IPM), meropenem (MEM), and ciprofloxacin (CIP) have been recorded at 86.7% each. The resistance rates of these antibiotics in this study were above those reported by the Antimicrobial Resistance in the European Union/European Economic Area Network (EARS-Net) in 2020. However, they are consistent with the rates reported by the Central Asian and European Surveillance of Antimicrobial Resistance Network (CAESAR-Net) in 2020 (20, 21).

The colistin resistance frequency was 8.1% using gold standard BMD, whereas it was 2% using Vitek 2. Several studies in the literature research VME and ME rates by comparing the gold standard BMD method with the Vitek 2 automated system for detecting colistin susceptibility (22). In our study, eight isolates were resistant to colistin when tested with BMD, while six of them had a MIC of less than 0.5 mg/L and were susceptible when tested with Vitek 2. VME rate was 75%, but ME was not detected. VME rates have been documented in the literature, ranging from 6.7% to 55.8% (23,24). In our study, the VME rate was 75%, which is above what has been reported in the existing literature, and we concluded that Vitek 2 did not meet the criteria required for method approval due to the high VME rate and low PPD (25%) of the test for detecting resistant isolates.

MBL and Class D ß-lactamases production are the most common CR mechanisms in *Acinetobacter* spp. Research on Class D ß-lactamases identified the bla_{OXA-51} gene in all CR isolates; however, the bla_{OXA-23} gene was the most frequently linked with resistance (25). In the present study, 94.8% of the isolates were the bla_{OXA-51} genotype, 85.7% were bla_{OXA-23} genotype, and no isolates with $bla_{OXA-24/40}$ or bla_{OXA-58} genotypes were found. Researchers indicate that the expression of bla_{OXA-51} and bla_{OXA-23} genes improves CR when stimulated by insertion sequences (IS) like ISAba1 (10). Our study found that the bla_{OXA-23} and ISAba1/bla_{OXA-23} gene regions were harboured in 98.8% of CR isolates, whereas these genes were absent in all carbapenem-susceptible isolates. Our results are consistent with previous studies, and the bla_{OXA-23} genotype was associated as a predictor of CR in the MDR and XDR isolates ($p< 0.001$). Bla_{OXA-51} is a natural beta-lactamase gene that functions as a genotype-related marker for *A. baumannii* and is responsible for CR when insertion sequence (IS) elements, such as ISaba1, are expressed. While bla_{OXA-51} genes are found in most *A. baumannii* isolates, there is some debate about whether these genes are present in all isolates. (26). Detection of the bla_{OXA-51} gene is an easy and handy method for identifying *A. baumannii*, whether they are consistently present, and is also specific for this species (27). However, we detected one CR isolate and four susceptible isolates without the bla_{OXA-51} gene.

A study by Bahador et al., demonstrated that the ISAba/bla_{OXA-51} and ISAba/bla_{OXA-23} genotypes of *A. baumannii* had significantly higher CR rates (10). On the contrary, these high-resistance genotypes were susceptible to imipenem. Turton et al., found that only ISAba/bla_{OXA-51} genotypes were CR (28). In our study, bla_{OXA-51}, bla_{OXA-23}, and related ISAab1/bla_{OXA-51} and ISAba1/bla_{OXA-23} genes were present in 98.8% of the CR isolates, consistent with the literature. ISAab1/bla_{OXA-51} and ISAba1/bla_{OXA-23} genes were detected together in 84 (85.7%) strains.

The MBL production rate of *A. baumannii* isolates is up to 99% worldwide, depending on differences in the tests used in studies (29). In our study, 83.5% of CR strains were MBL producers. In addition, the fact that phenotypic test results could not be confirmed

with genotypic tests is a limitation of our study.

REP-PCR, which is easy to apply, fast, reliable, and highly distinctive, is frequently used in the laboratory to examine the clonal proximity of resistant isolates, determine the source of the epidemic, and control the spread (30). In our study, seven genotypes (A-G) and 21 subtypes of *A. baumannii* were classified. Among the 21 subtypes, eight different clusters were observed, whereas 13 isolates were classified as a single pattern, showing no similarity to other isolates. Genotype D was detected in 53% of the isolates and was found to be the endemic genotype at our hospital.

In a study, IC 2 continued to spread in IMP-resistant *A. baumannii* isolates from 15 hospitals in Italy, Greece, and Spain and was reported to be the dominant clonal lineage in Europe (31). Caldart et al., found that 27 CR *A. baumannii* isolates belonged to IC 1, IC 5, and IC 6 (32). The significant determinants of CR in the IC 1 and IC 5 strains were bla_{OXA-23}, associated with ISAba1 and ISAba3, respectively. In our study, IC 2 was commonly detected in our hospital, consistent with the global data. Genotypes IC 1 and IC 2 tended to be generally MDR and XDR, while genotypes G5, G6, G8, and G13 were susceptible isolates. The majority of IC 2 (49/71) were grouped into Genotype D, and a significant relationship was found between Genotype D and IC 2 ($p < 0.001$).

Studies on plasmids in *A. baumannii* are very limited. In a bioinformatics study conducted by Salgado-Camargo et al., 173 plasmid profiles of *A. baumannii* were analyzed using the GenBank database (NCBI) (33). They determined that the plasmid sizes ranged from 1.1 Kb to 216.7 Kb, with up to six plasmids in one isolate. Only 35.2% of the plasmids carried the resistance gene. In that study, plasmid resistance genes were thought to be acquired by secondary acquisition after the emergence of the lineage, as only a few members of a plasmid lineage have resistance genes. Our study revealed that 73.4% of *A. baumannii* isolates carried plasmids distributed heterogeneously among the isolates. Additionally, no correlation was observed between susceptible and resistant isolates' genotypes, IC, and plasmid profiles. These findings support the theory that not all *A. baumannii* plasmids carry specific resistance genes and that plasmid conjugation is limited. In addition, the fact that all colistin-resistant strains carried plasmids suggests there may be plasmid-mediated resistance, but plasmid-mediated resistance has not been defined in *A. baumannii* (34).

Numerous studies have examined the correlation between biofilm formation and antibiotic resistance, demonstrating differences in their findings. Some previous studies have found a positive relationship between the biofilm formation capacity of bacteria and antibiotic resistance. A study indicated that 76.9% (173/225) of MDR *A. baumannii* isolates showed biofilm formation; however, no significant correlation was found between biofilm formation and antibiotic resistance (35). Another study reported that susceptible *A. baumannii* strains formed stronger biofilms than the MDR and XDR strains (36). That study also underlined that biofilm formation was higher in isolates sensitive to aminoglycosides, carbapenems, fluoroquinolones, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, tetracycline, and penicillin than in isolates resistant to these antibiotics. Another study reported a negative correlation between antibiotic resistance and biofilm formation by *A. baumannii* (37). In our research, strains susceptible to CAZ, IPM, MEM SXT, and CIP formed stronger biofilms than the strains that were resistant to these antibiotics ($p = 0.007$, $p = 0.007$, $p = 0.007$, $p < 0.001$, and $p = 0.012$, respectively). Further research is required to elucidate the connection between biofilm formation and the development of antibiotic resistance.

In hospital settings, *A. baumannii* is one of the most persistent and opportunistic pathogens that are difficult to control. The survival of *A. baumannii* is due to multifactorial and combined strategies. In our study, CR-related genes and bla_{OXA-23} genes in hospital-acquired *A. baumannii* isolates were encountered as the main determinants

of CR. Resistant bacteria have the potential to cause nosocomial outbreaks, and the selection and application of an appropriate method is needed to determine the dominant genotype. *A. baumannii* can form biofilms; this could be an adaptive mechanism developed for survival in the hospital environment. Our results underline the need for appropriate infection control measures to reduce *A. baumannii* survival and prevent resistance development.

As a result, Genotype D and IC 2 were defined as endemic isolates in our hospital, and no similarity was found between susceptible and resistant isolates regarding plasmid profiles, genotypes, and clonality. More vigorous biofilm formation was detected in isolates susceptible to ceftazidime, imipenem, meropenem, trimethoprim-sulfamethoxazole, and ciprofloxacin, and more comprehensive studies are needed on the relationship between biofilm formation and antibiotic resistance.

ACKNOWLEDGMENTS

* This work was funded by the Hitit University Scientific Research Projects Unit, Grant/Award Number: TIP19001.19.004.

ETHICS COMMITTEE APPROVAL

* The study was approved by the Hitit University Non-Interventional Clinical Research Ethics Committee (Date: 25.10.2018 and Number: 2018-173).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Almasaudi SB. Acinetobacter spp. as nosocomial pathogens: Epidemiology and resistance features. Saudi J Biol Sci, 2018; 25: 586-96.
2. Doi Y, Murray G, Peleg AY. Acinetobacter baumannii: evolution of antimicrobial resistance-treatment options. Semin Respir Crit Care Med, 2015; 36: 85-98.
3. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multi-resistant Acinetobacter baumannii. Antimicrob Agents Chemother, 2007; 51: 3741-84.
4. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev, 2008; 21 (3): 538-82.
5. EUCAST (The European Committee on Antimicrobial Susceptibility Testing). Breakpoint tables for interpretation of MICs and zone diameters Version 10.0, 2020. Date of access: 01 January 2020. Available from: http://www.eucast.org.
6. EUCAST (The European Committee on Antimicrobial Susceptibility Testing). Recommendations for MIC determination of colistin (polymyxin E) as recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group. Date of access 22 March 2016. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Recommendations_for_MIC_determination_of_colistin_March_2016.pdf.
7. ISO - ISO 20776-2:2021- Clinical laboratory testing and in vitro diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Part 2: Evaluation of performance of antimicrobial susceptibility test devices against reference broth micro-dilution. Date of access: December 2021. Available from: https://www.iso.org/standard/79377.html.
8. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect, 2012; 18 (3): 268-81.
9. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimikrob Agents, 2006; 27 (4):351-3.
10. Bahador A, Raoofian R, Pourakbari B, Taheri M, Hashemizadeh Z, Hashemi FB. Genotypic and Antimikrobial Susceptibility of Carbapenem-resistant Acinetobacter baumannii: Analysis of ISAba Elements and bla OXA 23-like Genes Including a New Variant. Front Microbiol, 2015; 6: 1249.
11. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA Disk Method for Differentiation of Metallo-Beta-Lactamase-Producing Clinical Isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Mikrobiol, 2002; 40: 3798-801.
12. Turton JF, Gabriel SN, Valderrey C, Kaufmann ME, Pitt TL. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of Acinetobacter baumannii. Clin Microbiol Infect, 2007; 13: 807–15.
13. Karah N, Sundsfjord A, Towner K, Samuelsen Ø. Insights into the global molecular epidemiology of carbapenem non-susceptible clones of Acinetobacter baumannii. Drug Resist Updat, 2012; 15 (4): 237-47.
14. Reboli AC, Houston ED, Monteforte JS, Wood CA, Hamill RJ. Discrimination of epidemic and sporadic isolates of Acinetobacter baumannii by repetitive element PCR-mediated DNA fingerprinting. J Clin Mikrobiol, 1994; 32 (11): 2635-40.
15. Sambrook J, Fritsch ER, & Maniatis T. Molecular Cloning: A Laboratory Manual 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989.
16. Huang XZ, Frye JG, Chahine MA, Glenn LM, Ake JA, Su W, et al. Characteristics of plasmids in multi-drug-resistant Enterobacteriaceae isolated during prospective surveillance of a newly opened hospital in Iraq. PLoS One, 2012; 7 (7): e40360.

17. Can F, Kaya M, Bayindir Bilman F, Uncu H, Demirbilek M, Yazici AC. Activity of tigecycline on planktonic and sessile cells of Acinetobacter baumannii. Mikrobiyol Bul, 2009; 43 (4): 587-95.

18. Patenge N, Arndt K, Eggert T, Zietz C, Kreikemeyer B, Bader R, et al. Evaluation of antimicrobial effects of novel implant materials by testing the prevention of biofilm formation using a simple small scale medium-throughput growth inhibition assay. Biofouling, 2012; 28 (3): 267-77.

19. Caliskan A, Kirisci Ö. Antibiotic sensitivity and multiple antibiotic resistance of Acinetobacter infections in a state hospital in the period 2013-2018. Turk Bull of Hyg and Exp Bio, 2020; 77 (4): 487-92.

20. EARS-Net (European Antimicrobial Resistance Surveillance Network). Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report 2019. Date of access: 18 November 2020. Available from: https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2019.

21. CAESAR-Net (Central Asian and European Surveillance of Antimicrobial Resistance Network). Central Asian and European Surveillance of Antimicrobial Resistance –Annual report 2020. Date of access: 01 October 2020. Available from: https://www.euro.who.int/data/assets/pdf_file/0003/469200/Central-Asian-and-European-Surveillance-of-Antimicrobial-Resistance.-Annual-report-2020-eng.pdf.

22. Vourli S, Dafopoulou K, Vrioni G, Tsakris A, Pournaras S. Evaluation of two automated systems for colistin susceptibility testing of carbapenem-resistant Acinetobacter baumannii clinical isolates. JAntimicrob Chemother, 2017; 72 (9): 2528-30.

23. Tanriverdi Caycı Y, Çinar C, Bilgin K, Gur Vural D, Birinci A. Comparison of the Results of the Microdilution and Automated System in Determination of Colistin Resistance. Journal of Duzce Uni Inst of Health Science, 2020; 10 (3): 297-301.

24. Şafak B, Tombak Ö, Eren Topkaya A. Kolistin Antimikrobiyal Duyarlılık Testinin Vıtek-2 Ve Sıvı Mikrodilüsyon Yöntemleriyle Karşılaştırmalı Olarak Değerlendirilmesi. Namık Kemal Tıp Dergisi, 2020; 8 (1): 73-8.

25. Mugnier PD, Poirel L, Naas T, Nordmann P. Worldwide dissemination of the blaOXA-23 carbapenemase gene of Acinetobacter baumannii. Emerg Infect Dis, 2010; 16: 35-40.

26. Brown S, Amyes S. OXA (beta)-lactamases in Acinetobacter: the story so far. J Antimicrob Chemother, 2006; 57 (1): 1-3.

27. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of Acinetobacter baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. J Clin Microbiol, 2006; 44 (8): 2974-6.

28. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, et al. The role of ISAba1 in expression of OXA carbapenemase genes in Acinetobacter baumannii. FEMS microbial Lett, 2006; 258: 72–7.

29. Safari M, Mozaffari Nejad AS, Bahador A, Jafari R, Alikhani MY. Prevalence of ESBL and MBL encoding genes in Acinetobacter baumannii strains isolated from patients of intensive care units (ICU). Saudi J Biol Sci, 2015; 22 (4): 424-9.

30. Bou G, Cerveró G, Domínguez MA, Quereda C, Martínez-Beltrán J. PCR-based DNA fingerprinting (REP-PCR, AP-PCR) and pulsed-field gel electrophoresis characterization of a nosocomial outbreak caused by imipenem- and meropenem-resistant Acinetobacter baumannii. Clin Microbiol Infect, 2000; 6 (12): 635-43.

31. Nowak J, Zander E, Stefanik D, Higgins PG, Roca I, Vila J, et al. High incidence of pandrug-resistant Acinetobacter baumannii isolates collected from patients with ventilator-associated pneumonia in Greece, Italy and Spain as part of the MagicBullet clinical trial. J Antimicrob Chemother, 2017; 72 (12): 3277-82.

32. Caldart RV, Fonseca EL, Freitas F, Rocha L, Vicente AC. Acinetobacter baumannii infections in Amazon Region driven by extensively drug resistant international clones, 2016-2018. Mem Inst Oswaldo Cruz, 2019; 25; 114: e190232.

33. Salgado-Camargo AD, Castro-Jaimes S, Gutierrez-Rios RM, et al. Structure and Evolution of Acinetobacter baumannii Plasmids. Front Microbiol, 2020; 11: 1283.

34. Lima WG, Alves MC, Cruz WS, Paiva MC. Chromosomally encoded and plasmid-mediated polymyxins resistance in Acinetobacter baumannii: a huge public health threat. Eur J Clin Microbiol Infect Dis, 2018; 37 (6): 1009-19.

35. Thummeepak R, Kongthai P, Leungtongkam U, Sitthisak S. Distribution of virulence genes involved in biofilm formation in multi-drug resistant Acinetobacter baumannii clinical isolates. Int Microbiol, 2016; 19 (2): 121-9.

36. Qi L, Li H, Zhang C, Liang B, Li J, Wang L, et al. Relationship between antibiotic resistance, biofilm formation, and biofilm-specific resistance in Acinetobacter baumannii. Front Microbiol, 2016; 7: 483.

37. Li Z, Ding Z, Liu Y, Jin X, Xie J, Li T, et al. Phenotypic and Genotypic Characteristics of Biofilm Formation in Clinical Isolates of Acinetobacter baumannii. Infect Drug Resist, 2021; 14: 2613-24.