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Original Article

An evaluation of clonal relationship by PFGE method in *Acinetobacter baumannii* isolates, examining integron presence and antibiotic resistance

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Abstract

Introduction: This study aims to investigate the presence of class 1, 2, and 3 integrons in *Acinetobacter baumannii* isolates, evaluate the relationship between integrons and antibiotic resistance and determine the clonal relationship between isolates by PFGE method.

Methodology: A total of 188 *A. baumannii* strains between February 2020 and March 2023 were included in the study. The antibiotic susceptibility was assessed per the recommendations of "The European Committee on Antimicrobial Susceptibility Testing". Integrons have been identified by polymerase chain reaction. The clonal relationship between isolates was evaluated with PFGE.

Results: Integron carriage was observed in 45.7% of isolates. Of these, 44.1% carried class 0.5% carried class 2, and 2 (1.1%) carried both classes of integrons. Among the integron positive isolates, the rate of only class 1 integron was 96.5% (83/86), the rate of only class 2 was 1.2% (1/86), and the rate of only two integron classes together was 2.3% (2/86). In the presence of class 1 integrons, the MDR and XDR phenotypes were found to be high ($p = 0.005$, $p = 0.029$). PFGE method detected 26 clusters and 177 pulsotypes on the dendrogram. When the clusters are evaluated separately within themselves, in all of the isolates in the Y and Z clusters (100%), class 1 integron positivity was observed in cluster A at 52.2%, cluster J at 42.1%, and cluster N at 22.2%.

Conclusions: Class 1 integron positivity observed in all of the isolates in the two clusters suggests the likelihood of clonal transfer alongside the horizontal transfer.

Key words: *Acinetobacter baumannii*; integrons; antibiotic resistance; clonal relationship; PFGE.

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Introduction

Acinetobacter baumannii is one of the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa and Enterobacter spp*.) microorganisms that mostly cause hospital infection. Recent misuse of treatments and errors in treatment management have led to multiple drug resistance (MDR). Due to antibiotic resistance, *A. baumannii* is considered among the most problematic nosocomial ESKAPE pathogens. *A. baumannii* is among the priority bacteria for the study of antibiotic resistance by the World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC) [1-3]. *Acinetobacter baumannii* is an opportunistic pathogen that can cause infections of community and hospital origin. It is the agent of many infections, such as sepsis, meningitis, pneumonia, wounds, and urinary tract infection. Risk factors for infections with *A. baumannii* include catheters, mechanical ventilation, open wounds, prolonged hospitalization and poor immune system [2,4].

Acinetobacter baumannii has a wide variety of resistance mechanisms against antibiotics. Mobile genetic elements (insertion sequences, transposons and integrons/gene cassettes, plasmids and integrative conjugative elements) are thought to play a significant role in the acquisition and spread of antibiotic-resistance genes. Integrons use region-specific recombination to transmit resistance genes [4-5]. Integrons are dsDNA segments and consist of an integrase (intI gene), a promoter (Pc), and a recombination region (attI). The most common types of resistance integrons are class 1, class 2, and 3 integrons, respectively [6]. The focus of surveillance of pathogens in hospitals is antimicrobial resistance surveillance. Horizontal spread, clonal spread, and contagion differ according to the characteristics of the pathogen [7]. The clonal relationship among many pathogenic organisms can be identified with Pulsed Field Gel Electrophoresis (PFGE), which is considered the "gold standard" for the

detection of subtypes of bacteria that cause outbreaks [8].

This study aimed to investigate the presence of class 1, 2, and 3 integrons in *A. baumannii* strains obtained from various clinical samples, evaluate the relationship between integrons and antibiotic resistance, and determine the clonal relationship between strains by PFGE method.

Methodology

Identification of Bacteria Isolates and Antimicrobial Susceptibility Tests

Different sample cultures sent to Pamukkale University Health Research and Application Hospital Medical Microbiology Laboratory from intensive care, service, and policlinics between 05/02/2020 and 03/03/2023 were evaluated. *A. baumannii* strains isolated and stocked between these dates were cultured. The first isolate from the same patient was included in the study if the culture result showed growth. In the study, 188 *A. baumannii* bacterial isolates were evaluated. The identification of bacteria that reproduce in culture samples was carried out with the Phoenix™ automated system. Non-colistin antibiotic susceptibility was determined by Phoenix™ automatized system and colistin susceptibility was determined by the liquid microdilution method [9]. Antibiotic discs used included amikacin, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, and trimethoprim/sulfamethoxazol. Antibiotic susceptibility was evaluated in accordance with the recommendations of the "European Committee on Antimicrobial Susceptibility Testing" [10]. Multi-drug resistant (MDR) was defined to be resistant to three or more classes of antibiotics and resistant to at least one antibiotic in the class of antibiotics; extensively drug-resistant (XDR) to be non-susceptible to ≥ 1 agents in all categories except ≤ 2 in all listed class of antibiotics; and pan-drug-resistant (PDR) was identified as being resistant to all listed antimicrobial agents [11].

DNA isolation and integron-specific in-house polymerase chain reaction (PCR)

A. baumannii isolates were cultured in 5% sheep-blooded agar, and a single colony was taken from the growing pure colonies. The single colony was passed into 2 mL of Brain Heart Infusion growth medium and incubated at 37 ºC for 24 hours. Then, 1 ml of the suspension was centrifuged for 10 minutes at 13,000 rpm. Discarding the supernatant, the pellet was taken; then 200 µL of distilled water was added and boiled at 100 °C for 10 minutes. It was re-centrifuged for 10 minutes at 13,000 rpm, and the supernatant at the top was transferred to Eppendorf tubes to be used as template DNA for PCR [12].

Standard PCR blend was prepared to contain 5 µL PCR Buffer (10X), 1 µL forward primer (100 pmol/µL), 1 µL reverse primer (100 pmol/µL), 1 µL dNTP (10 mM), 5 µL MgCl2 (25 mM), 0.5 µL Taq DNA polymerase (5 U/µL), 5 µL DNA template and 31.5 µL sterile deionized water, totaling 50 µL. The primers used are shown in Table 1 [13].

PCR amplification conditions for IntI1 and IntI2 were applied as follows: initial denaturation at 95 °C for 5 min, followed by 30 cycles at 95 °C, 55 °C and 72 °C for 30 seconds, and a final synthesis step at 72 °C for 7 minutes. PCR amplification for intI3 was performed at 95 °C for 5 minutes (first denaturation), followed by 30 seconds (30 cycles) at 95 °C and 50 °C, 60 seconds (30 cycles) at 72 °C, and a final synthesis at 72 °C for 7 minutes [13]. The amplification products were separated in an electrophoresis device in 1.5% agarous gel for 45 minutes at a current of 120 volts and the DNA bands were imaged with a UV transluminator. After amplification, band detection of 160 bp for integron 1, 288 bp for integron 2, and 1041 bp for integron 3 was evaluated as positive [13].

PFGE (Pulsed Field Gel Electrophoresis)

The PFGE method was used to determine the clonal relationship between strains. The species-identified bacteria were cultivated in 5% sheep-blood agar and incubated for 24 hours. The growing pure colonies were collected with the extract and suspended in the cell suspension buffer. The isolates were buried in the prepared agarose. The agarose with bacteria was removed from the mold and placed in the lysis solution. After the cell lysis was performed, agarose molds were washed. Agarose with purified DNA was obtained. The DNA in agarose molds was cut by the restriction

Table 1. Integron-specific PCR test primers.

Gene	Primer	Sequence (5′ to 3′)
intl1	F	CAG TGG ACA TAA GCC TGT TC
	R	CCC GAG GCA TAG ACT GTA
intl2	F	TTG CGA GTA TCC ATA ACC TG
	R	TTA CCT GCA CTG GAT TAA GC
intl3	F	GCC TCC GGC AGC GAC TTT CAG
	R	ACG GAT CTG CCA AAC CTG ACT

enzyme Apa I (30U). The agarose gel was prepared and the DNA molds were loaded into the gel [14]. In the CHEF-DR II system (BioRad, California, USA) electrophoresis conditions were applied for 20 hours with an initial stroke time of 5 seconds and an end stroke time of 30 seconds at 6 V/cm^2 current at 14 °C. After electrophoresis, the gel was stained in pure water containing ethidium bromide. It was visualized using UV light and photographed with the Gel Logic 2200 imaging system (Kodak, USA).

Image analysis

DNA band profiles in the gel image were analyzed with Syngene Gene Directory application version 2.01.02 (UK). Using the unweighted pair group method with arithmetic mean (UPGMA) and the Dice similarity coefficient, a dendrogram was created with a tolerance of 2%, and a cluster analysis was performed [15]. In the analysis, isolates with 100% compatibility were considered indistinguishable, and isolates with at least 80% similarity were considered clusters.

Statistical analysis

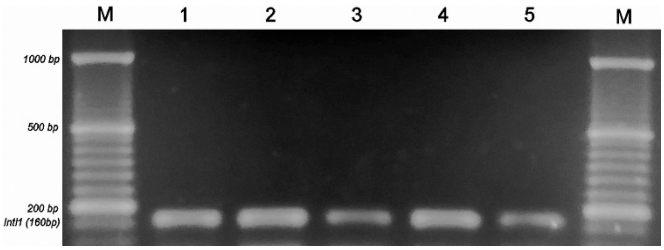
The data were analyzed with SPSS 25.0 statistical program. Continuous variables are summarized using minimum, maximum, mean, and standard deviation. Categorical variables are described using the number and percentage of observations in each category. The differences between the categorical variables were analyzed by the Chi-square analysis and Fisher's Exact test. As part of evaluation, $p < 0.05$ was taken to indicate statistical significance.

This study was approved by Pamukkale University Faculty of Medicine Ethics Committee for Non-Invasive Clinical Research (doc. date and number: 10.06.2022-E.217417).

Results

Of the *A. baumannii* isolates included in the study, 91 (48.4%) were isolated from intensive care units, 86 (45.7%) from inpatient service, and 11 (5.9%) from cultures sent from outpatient clinics. Of the isolates included in the study, 54 (28.7%) were derived from blood culture, 42 (22.3%) from sputum culture, 35 (18.3%) from wound culture and 57 (30.6%) from other cultures. Isolates were found at a higher rate in blood, a central venous catheter (CVC), and respiratory cultures in the intensive care unit (ICU), and in wound and sputum cultures in the inpatient services ($p = 0.0001$) (Table 2). In total, 78 of the isolates were female (41.5%) and 110 (58.5%) belonged to male patients. The average age of patients with isolated strains was 56.3 ± 21.75 (min: 0; max: 90).

Figure 1. PCR gel electrophoresis images of class 1 integron.



(M: 50-1000 bp DNA leader, in wells 1-5, 160 bp band of class 1 integron positive isolate).

Integron presence

Integron carriage was observed in 86 (45.7%) isolates. Of these, 83 (44.1%) carried class 1, 1 (0.5%) carried class 2, and 2 (1.1%) carried both classes of integrons, 102 (54.3%) isolates did not carry any integrons. Among the integron positive isolates, the rate of only class 1 integron was 96.5% (83/86), the rate of only class 2 was 1.2% (1/86), and the rate of only two integron classes together was 2.3% (2/86). No class 3 integron positivity was detected. An assessment of integron positivity showed class 1 integron in 52% of strains isolated from CVC cultures, 47.6% of strains isolated from sputum cultures, and 42.6% of strains isolated from blood cultures, class 2 integron was found in blood culture, and class 1 + 2 integron was found in wound culture (Table 2). Gel images of class 1 and class 2 integron positivity are shown in Figure 1 and 2. Gel images of class 1 and class 2 integron positivity are shown in Figures 1 and 2, respectively.

Table 2. Distribution of isolates according to clinics and integron positivity n (%).

Clinics-Integron /Sample	Blood, 54 (28.7)	CVC, 25 (13.3)	Sputum, 42 (22.3)	CSF, 4 (2.1)	Urine, 2 (1.0)	RTS*, 26 (13.8)	Wound, 35 (18.6)	*p*
Intensive care 91 (48,4)	32 (59.3)	22 (88)	5 (11.9)	3 (75)	0 (0)	24 (92.3)	5 (14.3)	
Service 86 (45,7)	21 (38.9)	3 (12)	37 (88.1)	1 (25)	1 (50)	2 (7.7)	21 (60)	0.0001* (cs = 112,115)
Policlinic 11 (5.9)	1 (1.9)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	9 (25.7)	
Class 1: 83 (44.1)	23 (42.6)	13 (52)	20 (47.6)	2 (50)	1 (50)	11 (42.3)	13 (37.1)	0.945 (cs = 1,703)
Class 2: 1 (0.5)	1 (1.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.868 (cs = 2,508)
Class 1 + 2: 2 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (5.7)	0.338 (cs = 6,820)

RTS*: Respiratory tract specimens *Bronchoalveolar lavage/deep tracheal aspirate/endotracheal aspirate. CVC: Central venous catheter CSF: cerebrospinal fluid *$p < 0.05$ statistically significant; cs: Pearson Chi-Square; γ: Fisher Exact test.

Antimicrobial resistance and models

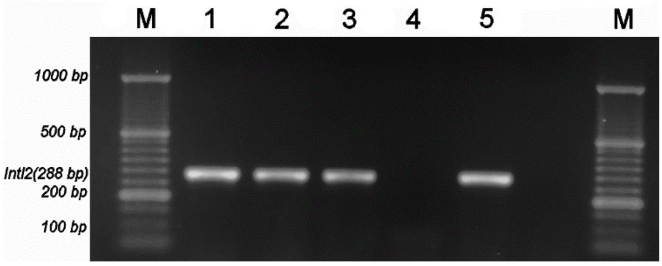
The antibiotic to which *A. baumannii* isolates were the least resistant one was colistin and the most resistant antimicrobials were identified as ciprofloxacin and levofloxacin (75%), imipenem and meropenem (73.4%), respectively (Table 3). MDR was detected in 122 (64.9%) strains and XDR in 19 (10.1%) strains. MDR was found in 72 (79.1%) of the strains isolated from intensive care samples, 44 (51.5%) of the strains isolated from samples sent from the inpatient service, and 6 (54.2%) strains isolated from outpatient clinic samples. MDR was found to be higher in intensive care isolates (p = 0.0001). XDR was found in 10 (11%) samples from the intensive care unit, 7 (8.1%) samples from the services, and 2 (18.2%) in the outpatient clinic and showed no statistically significant difference in terms of observation location (p = 0.571).

The rate of multi-drug resistance was significantly higher than that of 63 (75.9%) strains that were class 1 integron-positive compared to 59 (56.2%) strains that were negative (p = 0.005). Similarly, the incidence rate of XDR was significantly higher in the class 1 integron-positive 13 (15.7%) strains than that of negative 6 (5.7%) strains (p = 0.029). The rate of MDR showed no significant difference in 1 (100%) class 2 integron-positive strain relative to 121 (64.7%) class 2 integron-negative strains and in 1 (50%) class 1 + 2 integron-positive strain relative to 121 (65.1%) class 1 + 2 integron-negative strains (p = 1.000). Similarly, when the XDR incidence rate was assessed, class 2 and class 1 + 2 integron positivity was not observed, and negativity was detected in 19 (10.2%) samples, which presented no significant difference (p = 1.000).

Relationship between integron presence and antimicrobial resistance

Amicacin, colistin, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, and trimethoprim/sulfamethoxazole resistance was found to be significantly higher in class 1 integron-positive samples than in class 1 integron-negative samples (p = 0.011, p = 0.014, p = 0.0001 respectively) (Table 3). A total of 3 isolates with class 2 and class 1 + 2 integron positivity were found to be resistant to all antimicrobials studied, except colistin.

Figure 2. PCR gel electrophoresis images of class 2 integron.



(M: 50-1000 bp DNA leader, in wells1-3,5, 288 bp band of class 2 integron positive isolate).

The clonal relationship between isolates

Clonal analysis of 188 *A. baumannii* strains was performed by the PFGE method. Clusters are represented by letters of the alphabet and pulsotypes by roman numerals. The dendrogram identified 26 clusters and 177 pulsotypes. The clusters with a large number of isolates were N (n = 45), A (n = 23), Y (n = 21), J, Z (n = 19), M (n = 10), and D (n = 7). Five clusters included only one member (Figure 3). Cluster A was found to be of the longest duration in intensive care, inpatient service, and outpatient clinic (05.02.2023-24.02.2020). Twenty-one isolates showed 100% associated profiles in 10 pulsotypes in 6 different clusters, and the integron positivity and negativity of isolates in the same pulsotype were found to be 100% compatible. In class 1 integron-positive samples, 13 clusters and 78 pulsotypes were detected. In all of the isolates in the Y and Z clusters (100%), class 1 integron positivity was observed in cluster A at 52.2%, cluster J at 42.1%, and cluster N at 22.2%. MDR was observed at 73.7% in clusters Z and J, 76.2% in cluster Y, 60.9% in cluster A, and 60.0% in cluster N. XDR was found at 21.1% in cluster Z, 17.4% in cluster A, and 14.3% in cluster Y.

Table 3. Association between integron positivity and antibiotic resistance n (%).

Antibiotics/ Integrons	Total Resistance	Class 1			Class 2			Class 1 + 2		
		Positive	Negative	*p*	Positive	Negative	*p*	Positive	Negative	*p*
Amikacin	124 (66)	63 (75,9)	61 (58,1)	0.011* (cs = 6,547)	1 (100)	123 (65,8)	1γ	123 (66,1)	1 (50)	1γ
Ciprofloxacin	141 (75)	77 (92,8)	64 (61)	0.0001* (cs = 25,031)	1 (100)	140 (74,9)	1γ	140 (75,3)	1 (50)	0.439γ
Gentamicin	133 (70,7)	72 (86,7)	61 (58,1)	0.0001* (cs = 18,387)	1 (100)	132 (70,6)	1γ	132 (71)	1 (50)	0.501γ
Colistin	20 (10,6)	14 (16,9)	6 (5,7)	0.014* (cs = 6,066)	0 (0)	20 (10,7)	1γ	20 (10,8)	0 (0)	1γ
Imipenem	138 (73,4)	73 (88)	65 (61,9)	0.0001* (cs = 16,11)	1 (100)	137 (73,3)	1γ	137 (73,7)	1 (50)	0.462γ
Levofloxacin	141 (75)	77 (92,8)	64 (61)	0.0001* (cs = 25,031)	1 (100)	140 (74,9)	1γ	140 (75,3)	1 (50)	0.439γ
Meropenem	138 (73,4)	74 (89,2)	64 (61)	0.0001* (cs = 18,889)	1 (100)	137 (73,3)	1γ	137 (73,7)	1 (50)	0.462γ
Trimethoprim/ Sulfamethoxazol	123 (65,4)	68 (81,9)	55 (52,4)	0.0001* (cs = 17,891)	1 (100)	122 (65,2)	1γ	122 (65,6)	1 (50)	1γ

*p < 0.05 statistically significant; cs: Pearson Chi-Square; γ: Fisher Exact test

Discussion

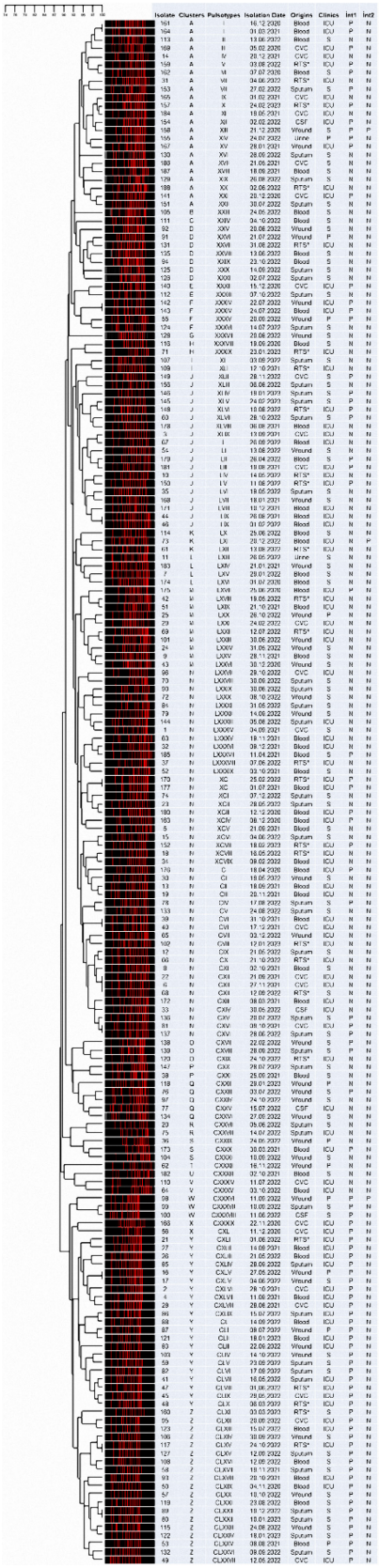
Acinetobacter baumannii is an opportunistic pathogen that can cause infections of community and hospital origin. It is the agent of many infections such as sepsis, meningitis, pneumonia, wounds, and urinary tract infection. Risk factors for infections with *A. baumannii* include catheters, mechanical ventilation, open wounds, prolonged hospitalization, and poor immune system [2,4]. In accordance with the risk factors in our study, *A. baumannii* isolates were isolated at a higher rate from bronchoalveolar lavage, deep tracheal aspirate, and endotracheal aspirate in patients with central venous catheter (CVC) in intensive care unit, and from wound cultures in inpatient services [2,4].

2020 and later saw a significant increase in the number of *Acinetobacter spp.* isolates compared to 2020. This poses a significant threat to patients and the healthcare system. The most common AMR phenotype against *Acinetobacter* species worldwide is common resistance to aminoglycosides, fluoroquinolones, and carbapenems. This also limits treatment options [16]. The carbapenem resistance rates in our study are similar to most Southern and Eastern European countries and are also lower than those in our country. Likewise, fluoroquinolone and aminoglycoside resistance were found to be lower than resistance rates in Turkey [16]. The reason for this is the importance attached to the rational drug administration in our hospital.

Integron positivity was found to be 45.7% (n = 86) and the presence of class 1 integrons was seen to be dominant. Integron positivity rates and distributions vary in different countries, and rates can change even in different geographies of the same country. Of *A. baumannii* strains, 33% were class 1 positive integrons in Turkey, 70% were class 1 and 21% were class 2 positive in northwest Iran; a Chinese study based on carbapenemase-resistant isolates showed class 1 positive integrons in 10.42%, whereas another Chinese study reported class 1 positive integrons in 72% and class 2 positive integrons in 4.2% [17-20].

In the presence of class 1 integron, there was a high resistance to all antibiotics subject to evaluation. There are a wide variety of methods to detect antibiotic resistance mechanisms of non-fermentative bacteria such as *A. baumannii* and *Pseudomonas aeruginosa* [21,22]. *A. baumannii* antibiotic resistance mechanisms include horizontal transfer of resistance genes, alteration of target sites, overexpression of flow pumps, β-lactamases, modifying enzymes, and permeability defects [4,23]. Integrons play an important role in the spread of resistance genes due to gene cassettes.

Figure 3. Pulsed-field gel electrophoresis dendrogram image.



The gene cassettes are placed in the integron so that the resistance gene is expressed inside them, and more than one cassette can be contained within an integron. Gene cassettes may carry those genes encoding lactamases, aminoglycoside-modifying enzymes, and carbapenemas [5]. Among integron cassettes, DfrA is a dihydrofolate reductase and plays a role in resistance to trimethoprim, while AadA2 is an aminoglycoside adenyltransferase that plays a role in aminoglycoside resistance [20]. On the other hand, aacA4/aac (6')-Ib gene cassette variants are active in resistance to fluoroquinolones [5,18]. Several studies and our study found a significant relationship between the presence of class 1 integrons and resistance to antibiotics [20,24,25].

In our study, MDR and XDR phenotypes were found high in the presence of class 1 integrons. In A. *baumannii*, in the development of multiple drug resistance phenotype, horizontal passage of resistance genes plays an important role. Generally, MDR occurs when the genes encoding resistance are close to each other on mobile genetic elements (integrons, plasmids, transposons) [26]. Based on the relationship between class 1 integron positivity and MDR, some researchers have concluded that the presence of class 1 integron can be used as a biomarker to predict the MDR phenotype [27,28].

In our study, the number of clusters and pulsotypes of *A. baumannii* isolates varies greatly. Likewise, a multicentric study in Turkey reported high diversity among strains isolated from various clinical samples [29]. However, a study evaluating multi-drug-resistant *A. baumannii* isolates in intensive care units in the same country showed low diversity [30]. The high diversity in our study may have been due to high sample diversity and the low number of some samples. Isolates have long existed in the same cluster and sometimes in the same pulsotype across various units of the hospital. Our results were similar to those reported by Gundeslioglu *et al* [31]. Cluster A has been identified as the cluster that has been in existence for three years (05.02.2020-24.02.2023) in intensive care, inpatient service and outpatient clinic. As a result of the deterioration or improvement in the clinical condition of the patients in our hospital, the places of hospitalization can change and there are patient transfers from intensive care to inpatient service or from inpatient service to intensive care, which is effective in the spread between clinics and continuity. Ceylan *et al.* also reported that patient transfer was effective among the causes that increased clonal spread [32].

All of the isolates in the clusters Y and Z presented class 1 integron presence, and about half of the class 1 integron-positive isolates concentrated in two clusters, namely Y and Z. These findings suggested that clonal transfer could be likely along with the horizontal transition. Similarly, Sung *et al*. reported that class 1 integrons tend to be transferred horizontally and vertically between *A. baumannii* or other Acinetobacter isolates [33].

Limitations

The limitations of this study include the non-evaluation of integron gene cassettes that encode resistance to the antibiotics. Another important limitation is a single center study.

Conclusions

In conclusion, in the presence of class 1 integron, there was a high resistance to many antibiotics. There was also a significant correlation between MDR and XDR phenotypes and class 1 integron positivity. When the clonal relationship between PFGE and isolates was evaluated, the number of clusters and pulsotypes varied greatly. Class 1 integron positivity observed in all of the isolates in the two clusters suggests the likelihood of clonal transfer alongside the horizontal transition.

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References

1. Kyriakidis I, Vasileiou E, Pana ZD, Tragiannidis A (2021) *Acinetobacter baumannii* antibiotic resistance mechanisms. Pathogens 10: 373. doi: 10.3390/pathogens10030373
2. Whiteway C, Breine A, Philippe C, Van der Henst C (2022) *Acinetobacter baumannii*. Trends Microbiol 30: 199-200. doi: 10.1016/j.tim.2021.11.008
3. Valcek A, Philippe C, Whiteway C, Robino E, Nesporova K, Bové M, Coenye T, De Pooter T, De Coster W, Strazisar M, Van der Henst C (2023) Phenotypic characterization and heterogeneity among modern clinical isolates of *Acinetobacter baumannii*. Microbiol Spectr 11: e0306122. doi: 10.1128/spectrum.03061-22
4. Cain AK, Hamidian M (2023) Portrait of a killer: uncovering resistance mechanisms and global spread of *Acinetobacter baumannii*. PLoS Pathog 19: e1011520. doi: 10.1371/journal.ppat.1011520
5. Partridge SR, Kwong SM, Firth N, Jensen SO (2018) Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev 31: e00088-17. doi: 10.1128/CMR.00088-17

6. Akrami F, Rajabnia M, Pournajaf A (2019) Resistance integrons; a mini review. Caspian J Intern Med 10: 370-376. doi: 10.22088/cjim.10.4.370
7. Werner G, Couto N, Feil EJ, Novais A, Hegstad K, Howden BP, Friedrich AW, Reuter S (2023) Taking hospital pathogen surveillance to the next level. Microb Genom 9: mgen001008. doi: 10.1099/mgen.0.001008
8. Lopez-Canovas L, Martinez Benitez MB, Herrera Isidron JA, Flores Soto E (2019) Pulsed field gel electrophoresis: past, present, and future. Anal Biochem 573: 17-29. doi: 10.1016/j.ab.2019.02.020.
9. European Committee on Antimicrobial Susceptibility Testing (2016) Recommendations_ for_MIC_determination_of_colistin_March_2016 Available: https: //www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/ General_documents/Recommendations_for_MIC_determinati on_of_colistin_March_2016.pdf. Accessed: 14.01.2024.
10. European Committee on Antimicrobial Susceptibility Testing (2023) Breakpoint tables for interpretation of MICs and zone diameters Version 13.0, valid from 2023-01-01. Available: https: //www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/ Breakpoint_tables/v_13.0_Breakpoint_Tables.pdf. Accessed: 14.01.2024.
11. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18: 268-281. doi: 10.1111/j.1469-0691.2011.03570.x.
12. Ripon MH, Hasan M, Ahasan MM, Alam MW, Kabir S (2011) Comparison of three different methods of genomic DNA extraction from Gram positive and Gram negative bacteria. J Expt Biosci 2: 55-60.
13. Chen J, Li H, Yang J, Zhan R, Chen A, Yan Y (2015) Prevalence and characterization of integrons in multidrug resistant *Acinetobacter baumannii* in Eastern China: a multiple-hospital study. Int J Environ Res Public Health 12: 10093-10105. doi: 10.3390/ijerph120810093.
14. 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.
15. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33: 2233-2239. doi: 10.1128/jcm.33.9.2233-2239.1995
16. Antimicrobial resistance surveillance in Europe 2023 - 2021 data (2023) Stockholm: European Centre for Disease Prevention and Control and World Health Organization; 2023. Available: http://apps.who.int/iris. Accessed: 16.01.2024.
17. Mengeloğlu FZ, Copur Çiçek A, Koçoğlu E, Sandallı C, Budak EE, Ozgümüş OB (2014) Carriage of class 1 and 2 integrons in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolated from clinical specimens and a novel gene cassette array: blaOXA-11-cmlA7. Mikrobiyol Bul 48: 48-58.
18. Ashouri P, Mohammadshahi J, Nikbin VS, Peeridogaheh H, Mohammadi-Ghalehbin B, Refahi S, Teimourpour A, Esmaelizad M, Mirzaneghad H, Teimourpour R (2022) Antimicrobial resistance, integron carriage, and fluoroquinolone resistance genes in *Acinetobacter baumannii* isolates. Arch Clin Infect Dis 17: e120590. doi: 10.5812/archcid-120590.
19. Xu L, Deng S, Wen W, Tang Y, Chen L, Li Y, Zhong G, Li J, Ting WJ, Fu B (2020) Molecular typing, and integron and associated gene cassette analyses in *Acinetobacter baumannii* strains isolated from clinical samples. Exp Ther Med 20: 1943-1952. doi: 10.3892/etm.2020.8911.
20. Huang C, Long Q, Qian K, Fu T, Zhang Z, Liao P, Xie J (2015) Resistance and integron characterization of *Acinetobacter baumannii* in a teaching hospital in Chongqing, China. New Microbes New Infect 8: 103-108. doi: 10.1016/j.nmni.2015.09.015.
21. Uddin F, McHugh TD, Roulston K, Platt G, Khan TA, Sohail M (2018) Detection of carbapenemases, AmpC and ESBL genes in *Acinetobacter* isolates from ICUs by DNA microarray. J Microbiol Methods 155: 19-23. doi: 10.1016/j.mimet.2018.11.004.
22. Uddin F, Sohail M, Shaikh QH, Ahmed S, Khan S, Roulston K, McHugh TD (2021) PCR and microarray analysis of AmpC and ESBLs producing *Pseudomonas aeruginosa* isolates from intensive care units. Gene Reports 23: 101178. doi: 10.1016/j.genrep.2021.101178.
23. Abdi SN, Ghotaslou R, Ganbarov K, Mobed A, Tanomand A, Yousefi M, Asgharzadeh M, Kafil HS (2020) *Acinetobacter baumannii* efflux pumps and antibiotic resistance. Infect Drug Resist 13: 423-434. doi: 10.2147/IDR.S228089.
24. Eghbalimoghadam M, Farahani A, Akbar FN, Mohajeri P (2017) Frequency of Class 1 integron and genetic diversity of *Acinetobacter baumannii* isolated from medical centers in Kermanshah. J Nat Sci Biol Med 8: 193-198. doi: 10.4103/0976-9668.210007.
25. Seyyedi M, Shapouri R, Zeighami H, Shokoohizadeh L (2021) Genetic diversity of colistin resistance nosocomial *Acinetobacter baumannii* strains from Iran. J Res Med Sci 26: 125. doi: 10.4103/jrms.JRMS_1023_20.
26. Monem S, Furmanek-Blaszk B, Łupkowska A, Kuczyńska-Wiśnik D, Stojowska-Swędrzyńska K, Laskowska E (2020) Mechanisms protecting *Acinetobacter baumannii* against multiple stresses triggered by the host immune response, antibiotics and outside-host environment. Int J Mol Sci 21: 5498. doi: 10.3390/ijms21155498.
27. Azizi O, Fereshteh S, Nasiri O, Ghorbani M, Barzi SM, Badmasti F (2021) The occurrence and characterization of class I, II, and III integrons among carbapenemase-producing clinical strains of *Acinetobacter baumannii* in Tehran, Iran. Jundishapur J Microbiol 14: e117766. doi: 10.5812/jjm.117766.
28. Alabdali YAJ (2022) Antibiotic resistance and carriage class I integron in clinical isolates of *Acinetobacter baumannii* from Al Muthanna, Iraq. J Antibiot (Tokyo) 75: 691-697. doi: 10.1038/s41429-022-00569-9.
29. Tunç AK, Nagiyev T, Kandemir T, Öcal MM, Köksal F (2023) Multicenter research of the molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* infections. Kafkas J Med Sci 13: 197–205. doi: 10.5505/kjms.2023.10692.
30. Boral B, Unaldi Ö, Ergin A, Durmaz R, Eser ÖK, Acinetobacter Study Group A (2019) Prospective multicenter study on the evaluation of antimicrobial resistance and

molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* infections in intensive care units with clinical and environmental features. Ann Clin Microbiol Antimicrob 18: 19. doi: 10.1186/s12941-019-0319-8.
31. Gündeşlioğlu ÖÖ, Gökmen TG, Horoz ÖÖ, Aksaray N, Köksal F, Yaman A, Yıldızdaş RD, Alhan E, Kocabaş E, Alabaz D (2014) Molecular epidemiology and antibiotic susceptibility pattern of *Acinetobacter baumannii* isolated from children in a Turkish university hospital. Turk J Pediatr 56: 360-367.
32. Ceylan MR, Karahoca M, Karagöz A, Çıkman A, Durmaz R (2020) The investigation of clonal relationship among multiple drug resistant *Acinetobacter baumannii* isolates with pulsed-field gel electrophoresis. J Harran Unıv Med Fac 17: 297-305. doi: 10.35440/hutfd.757137.
33. Sung JY, Koo SH, Kim S, Kwon KC (2014) Epidemiological characterizations of class 1 integrons from multidrug-resistant *Acinetobacter* isolates in Daejeon, Korea. Ann Lab Med 34: 293-299. doi: 10.3343/alm.2014.34.4.293.

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