ORIGINAL ARTICLE

Genetic diversity of colistin resistance Nosocomial *Acinetobacter baumannii* strains from Iran

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Background: Drug-resistant *Acinetobacter baumannii* is a global health problem since its ability to acquire new resistance mechanisms. Here, we aimed to determine the association of common types of *A. baumannii* and assess their drug resistance of *A. baumannii* and contribution of integrons (*Ints*) and oxacillinase genes in Zanjan, Iran. **Materials and Methods:** Among 68 isolated *Acinetobacters* from patients, 48 isolates were *A. baumannii* strains. Antibiotic susceptibility pattern and colistin resistance were determined by disk diffusion and broth microdilution, respectively. The presence of Int *I, II, III*, and oxacillinase genes examined using polymerase chain reaction. The clonal relationship of clinical isolates of A. baumannii determined by Pulsed Field Gel Electrophoresis method. **Results:** The results showed the highest antibiotic susceptibility (58%) for colistin. 96% of isolates were considered as multidrug resistant, and 46% as extensively drug resistant, and 16% as pandrug resistant. Frequencies of *Int I, II, III* resistance genes were 60%, 28%, and 0%, respectively, and 12% of strains had no isoform of *Ints*. Frequencies of Carbapenem resistance genes were 74%, 24%, 100%, and 4% for *blaOXA-23, blaOXA-24, blaOXA-51*, and *blaOXA-58*, respectively. The above samples were group into 26 pulsotypes. **Conclusion:** The studied *A. baumannii* strains had several resistance genes, and the colistin resistance showed an extraordinary ascending tendency that could be a severe issue in nosocomial infections, and the presence of high genetic diversity indicated a variation in *A. baumannii* strains and possibly a variety of sources of contamination or infection.

Key words: *Acinetobacter baumannii,* antibiotic resistance, molecular typing

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INTRODUCTION

Acinetobacter baumannii is of global importance due to its ability to develop resistance to several classes of antibiotics, and its unusual innate ability to survive long in all hospital environments is a factor that strengthens this resistance system leading to the spread of this bacterium in hospitals. Thus, it is necessary to determine the organism reservoir and its transmission method to control the spread of *A. baumannii* in hospitals.[1,2] Carbapenemase genes are often encoded on plasmids or mobile genetic elements such as integrons (*Ints*) that can potentially carry additional resistance genes against other classes of antibiotics.[3]

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Horizontal transfer of *Ints* is the most successful way of propagating resistance genes and the emergence of species with multiple resistance. Given that the resistance genes are located on *Ints*, they can be transferred from one strain to another, making it essential to identify these resistance genes. The importance of antibiotic resistance-related *Ints* is mainly reflected in clinical settings, especially in epidemiological surveillance, control, prevalence, and evolution worldwide.[4]

The subtyping process is epidemiologically crucial for identifying the prevalence of infection, detecting the cross-transmission of nosocomial pathogens, determining the source of infection, identifying the pathogenic species of microorganisms, and monitoring the vaccination programs.[5] In epidemiological

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surveys, to select a method or combination of methods for typing, we need to consider criteria such as the ability to type, reproducibility, power of differentiation, simplicity of the procedure, the scope of application, speed of implementation, and simplicity of method interpretation.[6]

In recent years, significant advances in genotype methods have changed current approaches to identify the *A. baumannii* species. Unfortunately, there is no absolute genotypic method. Each genotypic approach has its advantages and disadvantages. On this basis, one or more genetic methods can be used, but the selection of each method depends on the available laboratory equipment and financial support. If the disease spreads to different geographical regions, we should use more powerful techniques such as Pulsed Field Gel Electrophoresis (PFGE), as this method facilitates the comparison of results in different laboratories.[7]

PFGE is a powerful tool for monitoring bacterial genetic changes worldwide. For instance, any changes in the genes resistant to antibiotics in important bacteria of clinical and hospital infections, such as *Staphylococcus aureus, A. baumannii, Pseudomonas aeruginosa,* and *Mycobacterium tuberculosis* in regions and the world, can provide appropriate solutions to deal with the antibiotic resistance phenomenon. Regardless of the high costs of materials and equipment, and the time-consuming nature of PFGE, it is still a practical and applied typing method.[8] Using computer software, scanning, and gel analysis, we can create databases of PFGE patterns for all organisms. The reference databases can be created to detect any new strains and identify their phylogenetic relationships with other similar species.[5]

Given the increasing prevalence of multidrug resistant (MDR), extensively drug resistant (XDR), and pandrug resistant (PDR) isolates of *A. baumannii* and the prevalence of *Ints* in different regions, detecting and tracking isolates can be essential steps in treating infections and nosocomial infection control. Accordingly, different carbapenemases will be generated, such as oxacillinases. Hence, it is significantly vital to identify these types of antibiotic resistance genes to implement the infection control programs and prevent the spread of resistant strains. The present descriptive cross-sectional study aimed to determine the association of common types (CTs) of isolated *A. baumannii* samples from patients hospitalized in the intensive care units of Zanjan city with their antibiotic resistance patterns, especially with colistin resistance. Identification of the clonal relationships of MDR, XDR, and PDR strains of the isolates with the frequencies of oxacillinase gene and integrons and molecular typing of the isolates were also other aims of this study.

MATERIALS AND METHODS

In designing the present study, specimens were collected from patients, who were admitted to intensive care units (ICUs) of Zanjan hospitals with being infected 72 h after hospitalization by completing the questionnaires and signing the patient consent forms. The patients selected in the hospitalization time had no obvious symptoms of infection, and the disease was not in its latent period. They showed symptoms of infection 72 h after hospitalization.

Isolation of bacterial strains and antimicrobial susceptibility testing

The clinical specimens were cultured. After the bacterial growth, the initial identification of bacteria was done based on microscopic specifications and biochemical tests (oxidase, catalase, movement, and culture in TSI medium). Then, Microgen GNA-ID system includes 12 standard biochemical mediums for lysine, carnitine, glucose, mannitol, H2S, indole, urease, topyranoside (O. N. P. G.), citrate, and tryptophan deaminase tests were used to determine the species. After that, *A. baumannii* was confirmed by amplifying the *blaOXA-51* gene, using specific primers by a polymerase chain reaction (PCR) method. Antibiotic susceptibility testing was performed using the disk diffusion method (Kirby–Bauer) on Muller–Hinton Agar medium (Merck, Germany) according to instructions of the Clinical and Laboratory Standards Institute (CLSI) in 2018. These antimicrobial disks include imipenem (IPM) 10 mg, ceftazidime (CAZ) 30 µg, gentamicin 30 µg, cefotaxime 30 µg, levofloxacin (LEV) 10 µg, co-trimoxazole 25 µg, tetracycline 30 µg, tobramycin 10 µg, amikacin (AK) 10 µg, ampicillin and sulbactam 10 µg. The standard *A. baumannii* strain ATCC 19606 was utilized as a control to assure antibiogram quality. MDR strains, XDR, and PDR were classified based on the Centers for Disease Control and Prevention. The minimum inhibitory concentration (MIC) was determined for colistin (COL)-resistant isolates by the broth microdilution method. Antibiotic susceptibility was determined based on grouping A, B, U, and O (drugs listed together in the same group are agents for which interpretive categories and clinical efficacy are similar) according to the CLSI 2018.

DNA Extraction and molecular identification of integrons and oxacillinase genes

DNAs of specimens, which were confirmed as *A. baumannii* strains, were extracted using the Qiagen Extraction Protocol and Kit. Molecular identification of *Int I, II, III,* and encoding carbapenemase genes, including *blaOXA-23, blaOXA-24, blaOXA-51*, and *blaOXA-58*, was performed by the PCR method and specific primers. Table 1 presents the specific primers for all three classes of *Ints* and oxacillinase genes, the annealing temperatures, and the PCR conditions used in this study [Table 1].

Table 1: Primer sequences and annealing temperatures were used in this study and polymerase chain reaction conditions

Target	Primer sequence	Amplicon size (bp)	Annealing temperature (°C)	References	PCR condition for the primers
Int I	CAG TGG ACA TAA GCC TGT TC CCC GAC GCA TAG ACT GTA	160	55	[9]	1 PCR tube contained 12.5 µl - master mix 1 µl - primers 1 µl - DNA 9.5 µl - nuclease-free water 25 µl - final volume 1 cycle 95°C - 4 min 30 cycle 95°C - 50 s 58°C - 60 s 72°C - 45 s 1 cycle 72°C - 8 min
Int II	TTG CGA GTA TCC ATA ACC TG TTA CCT GCA CTG GAT TAA GC	288	55	[9]	
Int III	GCC TCC GGC AGC GAC TTT CAG ACG GAT CTG CCA AAC CTG ACT	104	52	[9]	
bla_OXA 23	GATCGGATTGGAGAACCAGA ATTTCTGACCGCATTTCCAT	501	60	[3]	
bla_OsXA 24	GGTTAGTTGGCCCCCTTAAA AGTTGAGCGAAAAGGGGATT	246	60	[3]	
bla_OXA 51	TAATGCTTTGATCGGCCTTG TGGATTGCACTTCATCTTGG	324	60	[3]	
bla_OXA 58	AAGTATTGGGGCTTGTGCTG CCCCTCTGCGCTCTACATAC	599	60	[3]	

PCR=Polymerase chain reaction

Typing by Pulsed Field Gel Electrophoresis

Genotyping of *A. baumannii* isolates was performed by PFGE using the Bio-Rad CHEFF-DR III electrophoresis unit and Bio-Rad gel documentation system with a UV transilluminator. The bacterial genome was cut with Apa-1 restriction enzyme (RE) for *A. baumannii* and Xba-1 RE [the cutting sites depicted in Figure 1a] for *Salmonella braenderup H9812* as molecular standard [Figure 1]. PFGE can determine the length of DNA fragments concerning other samples and can give an estimate of the length of pieces by comparing their position in gel relative to a ladder, molecular size standard, which is Xba-1. Preparation of PFGE agarose plugs, lysis of bacterial cells in plugs, cutting DNA by RE in plugs, casting the gel and loading of plug slices, and electrophoresis run were followed as instructed by the protocol of.[5]

Data analysis

Using the online application software (inslico.hue.es), we compared and clustered the band patterns on the agarose gel. The application applied the dice method to compare the bands and UPGMA to cluster them. Dendrogram generated with dice coefficient and the UPGMA clustering method, showing the genetic similarity with the highest correlation. In the application or method, the cutoff of similarity of bands was considered to be 85%. We utilized Chi-square, the Fisher's exact test, and the Phi test as well as SPSS26 to determine the relationships of variables and investigate the association of demographic and clinical factors and strains and determine the clonal relationships of MDR, XDR, and PDR strains.

RESULTS

Sixty-eight out of 250 clinical samples were identified as *Acinetobacter*, among which 48 isolates were *A. baumannii*, and 20 isolates were other *Acinetobacter* species. The isolates were respiratory (44%), wound drainage (32%), urinary tract infections (18%), and blood (6%) specimens belonging to 28 (56%) female and 22 (44%) male patients. The mean age of patients was 58.2 years, ranging from 29 to 84 years. Table 2 shows the antibiotic resistance profiles of 48 isolates. 96% of isolates were considered as MDR, and 46% as XDR, and 16.6% as PDR [Table 2]. Among clinical isolates, 100% respiratory and wound drainage, 88.8% urine specimens, and 66.6% blood samples of specimens MDR were diagnosed. The maximum and minimum amounts of XDR belonged to wound drainage (50%) and blood (33.3%), respectively; and PDR bacteria was mainly isolated from respiratory secretion (9%), urine specimens (4.4%), blood (33.3%), and wound drainage (25%).

Figure 2 also shows the susceptibility profile of *A. baumannii* isolates against eleven antimicrobial agents representing the A, B, U, and O groups of antimicrobial agents. The highest resistance rates were among Group B (80%–96%), followed by Group A (64%–94%), Group U (72%), and Group O antimicrobial agents (41.6%). Among clinical isolates, 100% respiratory and wound drainage, 88.8% urine specimens, and 66.6% blood samples of specimens MDR were diagnosed. The maximum and minimum amounts of XDR belonged to wound drainage (56.25%) and blood (33.3%), respectively [Figure 2].

Table 2 shows resistance genes profiles of isolates in the present study. 60% were with *Int I*, 28% with *Int II*, 12% with no *Ints*; 2% simultaneously carried both *Ints I* and *II*, and *Int III* had no isolate [Table 2]. The present study investigated the relationship between the presence of integron genes and microbial susceptibility to antibiotics, and there was a statistically significant relationship between the presence of *Int*

Table 2: Antimicrobial susceptibility test based on the clinical and laboratory standards institute 2018 guidelines and resistance genes profiles of *A. baumannii* isolates

	Antimicrobial-resistance testing and resistance determinants profiles																					
	TET	CAZ	TOB	IPM	CTX	AK	LEV	GM	COT	SAM	COL	Int 1		Int 2		Int 3		Int 2, 3	
R	72	94	64	86	96	80	82	74	94	72	42								
I	10	2	2	8	4	2	10	4	4	2	0								
S	18	4	34	6	0	18	8	24	2	26	58								
G												60		28		0		2	
???	bla OXA-23	bla OXA-24	bla OXA-51	bla OXA-58	bla OXA-23,24	bla OXA-23,51	bla OXA-23,58	bla OXA-24,51	bla OXA-24,58	bla OXA-51,58	bla OXA-23,24,51	bla OXA-23,24,58	bla OXA-24,51,58	No bla-OXA	No Int							
G	74	24	100	4	18	74	4	24	0	4	18	0	0	4	12							

R=Resistance rate; I=Intermediate rate; S=Susceptible rate; G=Resistance gene; TET=Tetracycline; TOB= Tobramycin; AK=Amikacin; IPM=Include imipenem; CAZ=Ceftazidime; GM=Gentamicin; CTX=Cefotaxime; LEV=Levofloxacin; COT=Co-trimoxazole; SAM=Ampicillin and sulbactam; COL=Colistin

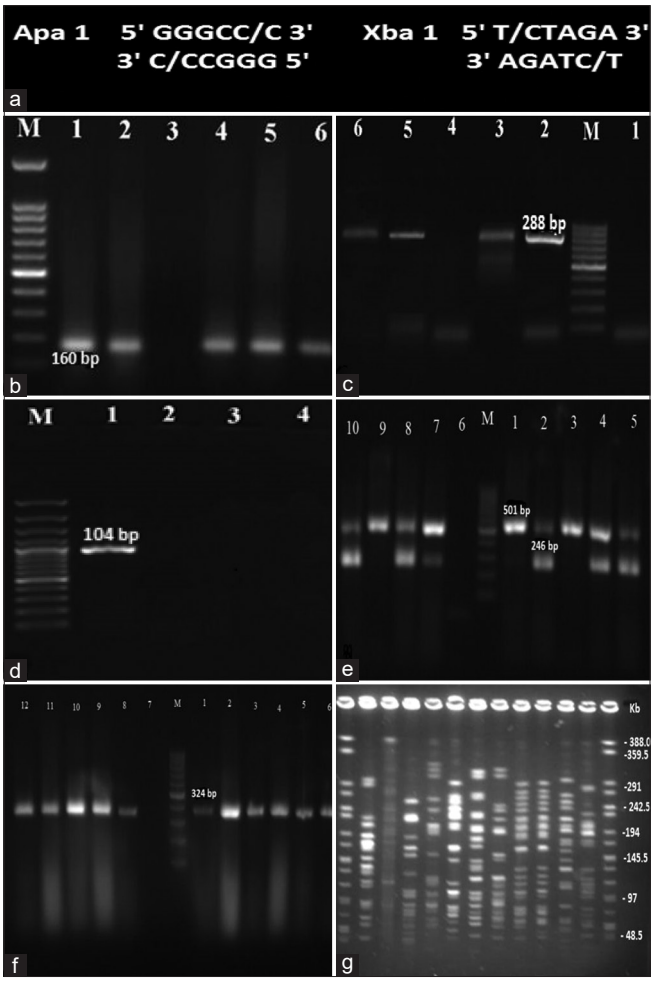

Figure 1: (a) Diagram of the location of the gene's cut sites by enzymes Apa-1 and Xba-1, gel electrophoresis of integrons, oxacillinase genes detection, and PFGE images of *A. baumannii* isolates is shown in A, B, C, D, E, F, and G parts. A. Restriction sites: Cutting sites of the Apa-1 and Xba-1. (b) PCR products of Gene Int I: M marker, 1 positive control, 3 negative control, 2, 4, 5, 6 samples positive gene Int I. (c) PCR products of Gene Int II: M marker, 1 negative control, 2 positive control, 3, 5, 6 samples positive gene Int II. (d) PCR products of Gene Int III: M marker, 1 positive control, 2 negative control, 3, 4 samples negative gene Int III. (e) PCR products of Genes bla_{OXA-23} and bla_{OXA-24}: M marker, 1 positive control Gene bla_{OXA-23}, 2 positive control Gene bla_{OXA-24}, 6 negative control. (f) PCR products of Genes bla_{OXA-51}: M marker, 1 positive control Gene bla_{OXA-51}, 7 negative control. (g) PFGE images of *A. baumannii* isolates. Markers identified by restriction enzymes cutting: The first and last well of *Salmonella braenderup H9812*. PFGE = Pulsed Field Gel Electrophoresis; PCR = Polymerase chain reaction; *A. baumannii = Acinetobacter baumannii*

I with antibiotics CAZ ،AK ،LEV ($P < 0.05$). Gel electrophoresis of *Ints* detection is shown in B, C, and D parts of Figure 1.

All isolates contained beta-lactamase *blaOXA-51*. 74% of the isolates carried the *blaOXA-23* gene, 24% *blaOXA-24*, 4% *blaOXA-58*, and 18% of the isolates carried *blaOXA-23* genes, and *blaOXA-24* simultaneously; and 4% of the isolates lacked all oxacillinase genes. 20% of IPM-resistant strains lacked *blaOXA-23* and *blaOXA-24* genes, and no isolate had all four oxacillinase genes simultaneously [Table 2]. Gel electrophoresis of oxacillinase genes detection is shown in E, and F parts of Figure 1.

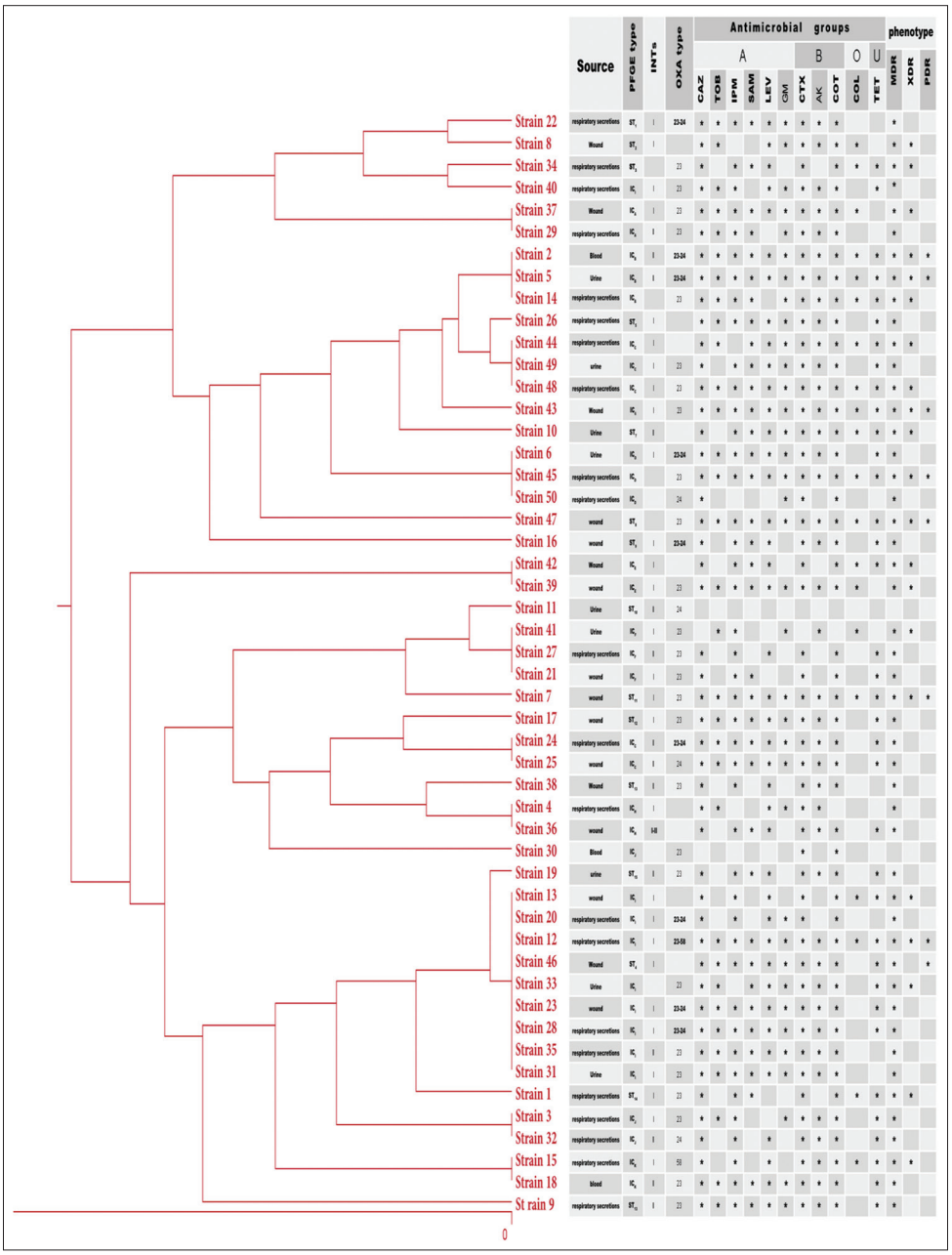

Figure 2: Comparison of PFGE genotype diversity of 48 *A. baumannii* isolates (dendrogram) with their virulence gene expression, as well as resistance to CLSI antimicrobial groups, and international clonal lineage. Figure 2 completely shows the source of infection for each isolation and the associated IC or ST, resistance phenotype, resistance transmission genes, and virulence factors. We presented the full information on resistance and susceptibility to different types of antibiotics for each type. PFGE = Pulsed Field Gel Electrophoresis; *A. baumannii* = *Acinetobacter baumannii*; CLSI = Clinical and Laboratory Standards Institute; ST = Single type

According to Table 3, there is a direct correlation between the *Int I* and *blaOXA-23* genes with phenotype MDR ($P < 0.05$; $\Phi = 0.250$). Furthermore, there is a correlation between the *Int II* with phenotype XDR ($P < 0.05$, $\Phi = 0.307$) [Table 3]. Furthermore, the present study examined the association between integron and oxacillinase genes in *A. baumannii* isolates and showed that there is a direct correlation between the *Int I* and *blaOXA-23* ($P < 0.05$, $\Phi = 0.213$) [Table 4].

Among 48 *A. baumannii* isolates, we found that 20 (41.6%) isolates were to COL according to the broth microdilution method. In this study, a statistically significant relationship was found between antibiotic COL with *Int II* and *blaOXA-24* genes ($P < 0.05$). According to Table 3, there is a direct correlation between the resistances to COL with XDR phenotype in this study (P value < 0.05, $\Phi = 0.312$) [Table 5].

Based on the analysis of the PFGE results, Figure 2 shows a comparison of PFGE genotype diversity of 48 *A. baumannii* isolates (dendrogram) with their virulence gene expression, as well as resistance to CLSI antimicrobial groups, and international clonal lineage, among 48 isolates

Table 3: Frequencies and statistical relationship between multidrug-resistant and extensively drug-resistant strains with resistance genes *Ints* and OXa-types *Acinetobacter baumannii* isolates

n	MDR		*P*	Phi	XDR		*P*	Phi
	+48, *n* (%)	−2, *n* (%)			+23, *n* (%)	−27, *n* (%)		
Int 1 (+)	30 (62.5)	0	0.077	0.250	15 (62.5)	15 (55.6)	0.487	0.098
Int 2 (+)	13 (27.1)	1 (50)	0.479	−0.100	3 (13)	11 (40.7)	0.030	0.307
Int 3 (+)	0	0	-	-	0	0	-	-
bla_OXA 23 (+)	36 (75)	1 (50)	0.03	0.212	16 (69.6)	21 (77.8)	0.509	−0.093
bla_OXA 24 (+)	10 (20.8)	1 (50)	0.329	−0.138	5 (21.7)	6 (22.2)	0.967	−0.006
bla_OXA 51 (+)	48 (100)	2 (100)	-	-	23 (100)	27 (100)	-	-
bla_OXA 58 (+)	2 (4.2)	0	0.76	0.042	0	2 (7.4)	0.183	-0.188

MDR=Multidrug resistant; XDR=Extensively drug resistant

Table 4: Correlation between Int genes and Oxa-type genes among *Acinetobacter baumannii* isolates

Oxa-type genes	Int I		*P*	Phi	Int II		*P*	*Phi*
	Positive	Negative			Positive	Negative		
bla_OXA 23								
Positive	21 (56.8)	16 (43.2)	0.043	0.21	9 (24.3)	28 (75.7)	0.329	−0.13
Negative	9 (69.2)	4 (30.8)			5 (38.5)	8 (61.5)		
bla_OXA 24								
Positive	5 (45.5)	6 (54.4)	0.256	−0.15	5 (45.5)	6 (54.5)	0.144	0.20
Negative	25 (64.1)	14 (35.9)			9 (23.1)	30 (76.9)		
bla_OXA 51								
Positive	30 (60)	20 (40)	-	-	14 (28)	36 (72)	-	-
Negative	0	0			0	0		
bla_OXA 58								
Positive	2 (100)	0	0.239	0.16	0	2 (100)	0.368	−0.12
Negative	28 (58.3)	20 (41.7)			14 (29.2)	34 (70.8)		

of *A. baumannii* isolated from patients admitted to the ICUs of Zanjan hospitals, 26 different pulsotypes were detected based on 80% similarity [Figure 2]. Among the 26 types, 11 types were similar or CT as CTA to CTK, and 15 types were as single type (ST) from ST1 to ST15, as shown in Figure 1. Among the CTs in types A, E, G, H, J, and K, two isolates or 25% of isolates were in two-member clusters. In types B, C, D, and F, three isolates or 25% of the isolates were in three-member clusters; and in type I, nine isolates, or 18.75%, were in a 9-member cluster that was the most significant CT among the isolates of *A. baumannii*. Among the isolates in the cluster, 88.9% were isolated from female patients, and 44.4%, 33.3%, and 22.2% of the isolates were isolated from clinical respiratory samples, wound discharge, and urinary sediment, respectively. In terms of resistance phenotype, 33.3% were from XDR phenotype and 88.9% from MDR. 77.8% of type I isolates had *Int I* gene, and 22.2% carried the *Int II* gene. PFGE images of *A. baumannii* isolates are shown in G part of Figure 1.

DISCUSSION

Members of *A. baumannii* have a high tendency to develop resistance to antibiotics and are inherently resistant to some antibiotics. These bacilli can acquire new resistance mechanisms and rapidly transmit their resistance patterns.[9,10]

In the present study, the percentages of isolates with phenotypes MDR, XDR, and PDR were 96%, 46%, and 16.6%, respectively. The rate of MDR was 92.9% in a study by Shaheli *et al.*,[8] 100% by Shirmohammadlou *et al.*,[3] 100% by Simo Tchuinte *et al.*,[11] which were consistent with the present study, indicating high rates of MDR in different geographical areas. Saeidi *et al.*[4] reported 15.7% phenotype XDR, and Golafshan *et al.*[12] reported 22.5%, which were lower than phenotype XDR, in the present study. The available evidence and the emergence and spread of different antibiotic resistance suggest a very high possibility of increasing XDR strains.

In the present study, colistin antibiotic showed better activity than other antibiotics, despite its high resistance (41.6%) [Table 5]. The higher prevalence of colistin resistance has been reported in isolates of this bacterium worldwide, but the reports widely vary in different geographical regions. The level of antibiotic resistance in each region is directly related to the pattern of antibiotic use in that region. Colistin is a critical therapeutic option for carbapenem-resistant *A. baumannii* that limits treatment options against this pathogen as the emergence of colistin resistance increases.[13] The attention to colistin resistance in studies and their geographical locations indicates the rapid release of resistance and its upward trend. The highest rates

Table 5: Frequencies and statistical relationship between antibiotic colistin and variables used in this study

Variables	COL (susceptibility), *n* (%)	COL (resistance), *n* (%)	*P*
Int I			
+	16 (53.3)	14 (46.7)	>0.05
−	13 (65)	7 (35)	
Int II			
+	11 (78.6)	3 (21.4)	<0.05
−	18 (50)	18 (50)	
Int III			
+	0	0	-
−	29 (58)	21 (42)	
bla_OXA 23			
+	23 (62.2)	14 (37.8)	>0.05
−	6 (46.2)	7 (53.80)	
bla_OXA 24			
+	8 (72.8)	3 (27.3)	<0.05
−	21 (53.8)	18 (46.2)	
bla_OXA 51			
+	29 (58)	21 (42)	-
−	0	0	
bla_OXA 58			
+	2 (100)	0	>0.05
−	27 (56.3)	21 (43.8)	
MDR			
+	27 (56.3)	21 (43.8)	>0.05
−	2 (100)	0	
XDR			
+	3 (13)	20 (87)	<0.05
−	26 (96.3)	1 (3.7)	

COL=Colistin; MDR=Multidrug resistant; XDR=Extensively drug resistant

of resistance in Iran are 11.6% by Vakili *et al*. and 14.2% by Bahador *et al*. Compared to the present study (42%), it indicates a rapid growth rate of this antibiotic.[14,15] In 2011, the colistin resistance of 12%, 16%, and 18.7% was reported for Kuwait, India, and Argentina, respectively. In 2012, it was reported at 10.4% for Taiwan.[16-19] The resistance rate was 34.6% in Italy in 2014 and 35% in Brazil in 2016. Referring to this amount of resistance in different regions of the world indicates strong evidence that there is an increasing movement toward colistin resistance in MDR specimens, especially carbapenem-resistant strains.[13,20]

The significant relationship was between the presence of *Int I* and resistance to antibiotics CAZ ،AK ،LEV in the present study ($P < 0.05$), and results of studies by Peymani *et al*. ($P < 0.05$) and Japoni-Nejad *et al*. ($P < 0.05$) indicated the uninterrupted progress of this resistance mechanism; hence, it is essential to find preventive solutions.[21,22] Class 1 *Ints* can carry 40 types of resistance genes such as aminoglycosides, fluoroquinolones, and beta-lactams, therefore, this indicates that our research area represents a lack of proper infection control in the region.[3] The frequency of resistance gene *Int II* varies in studies on different geographic regions so that it was 66% in research by Golafshani *et al*. and 12.5% by Goudarzi *et al*.[12,23] However, a more detailed study indicated an upward trend of *Int I* and *Int II* in Iran. In cases without any significant relationship between the presence of *Ints* and antibiotic resistance, the resulting resistance can be obtained by various means, such as defective autolytic enzymes in the cell wall or under plasmid control or chromosome-controlled resistance.

Another aim of the present study was to investigate the prevalence of oxacillinase genes as the main mechanism of carbapenem resistance in *A. baumannii* isolates. In the present study, the prevalence of *bla-OXA23*, *bla-OXA24*, *bla-OXA51*, and *bla-OXA58* was 74%, 24%, 100%, and 4%, respectively, and the result was consistent with results of studies by Golafshan *et al*.,[12] and Shirmohammadlou *et al*.,[3] but exceedingly inconsistent with studies by Goudarzi *et al*.,[23] Kooti *et al*.[24] probably due to different geographical conditions. Based on the present study and other studies in Iran and the world, three genes, namely *bla-OXA58*, *bla-OXA24*, and *bla-OXA23* alone or in combination, caused resistance to carbapenems. In the present study, 18% of IPM-resistant samples lacked *bla-OXA24* and *bla-OXA23* genes. The resistance might be due to the lower membrane permeability, changes in penicillin-binding protein, production of other carbapenem hydrolyzing enzymes, or expression of efflux pumps.

The isolates, which were analyzed by PFGE in the present study, indicated 26 specific genetic patterns. The number of clusters was higher than studies by Farahani *et al*.,[2] Raka *et al*.,[25] and Gholipour *et al*.,[26] who found seven isolated genetic patterns, but it was lower than a study by Anvarinejad *et al*.,[27] who examined 47 genetic patterns. The differences may be related to different geographical regions, sources of infection, and the genetic diversity of strains in the case study. The presence of genes *blaOXA-23* and *blaOXA-24* in members of clusters B, C, D, F, and G, as results obtained from the present study, was consistent with a study by Ranjbar *et al*.[28] Based on the results, there was no statistical correlation between genetic patterns and the presence of resistance genes of *Int I* and *II*, and it was consistent with studies by Eghbalimoghadam *et al*.[29]

CONCLUSION

Resistant strains of *A. baumannii* isolated in the intensive care units of Zanjan hospitals had several resistance genes, indicated resistance to a wide range of antibiotics, high genetic diversity, and probably various sources of infection. Furthermore, and to control them, we need basic measures in terms of infection control. The examination of previous results and comparison with the present study indicated

an increase in resistance to different classes of antibiotics, especially colistin resistance, therefore suggesting that local antibiotic prescription policies should be frequently reviewed, and to be sure of the drug effect before its prescription using the MIC test.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Wang X, Qin LJ. A review on *Acinetobacter baumannii*. J Acute Dis 2019;8:1-16.
2. Farahani N, Mirnejad R, Ahmadi Z, Amirmozafari N, Masjedian F. Molecular typing of *Acinetobacter baumannii* clinical strains in Tehran by pulsed-field gel electrophoresis. J Fasa Univ Med Sci 2013;2:259-65.
3. Shirmohammadlou N, Zeighami H, Haghi F, Kashefieh M. Resistance pattern and distribution of carbapenemase and antiseptic resistance genes among multidrug-resistant *Acinetobacter baumannii* isolated from intensive care unit patients. J Med Microbiol 2018;67:1467-73.
4. Saeedi S, Abdolsalehi MR, Khodabandeh M, Alvandimanesh A, Pournajaf A, Rajabnia R. Survey of integron types and carbapenem resistance encoding genes in *Acinetobacter baumannii* isolated from burn wound samples. Alborz Univ Med J 2018;7:323-32.
5. Goering RV. Pulsed field gel electrophoresis: A review of application and interpretation in the molecular epidemiology of infectious disease. Infect Genet Evol 2010;10:866-75.
6. Parizad EG, Parizad EG, Valizadeh A. The application of pulsed field gel electrophoresis in clinical studies. J Clin Diagn Res 2016;10:E01-4.
7. Ahmed SS, Alp E. Genotyping methods for monitoring the epidemic evolution of *A. baumannii* strains. J Infect Dev Ctries 2015;9:347-54.
8. Shaheli M, Baseri Salehi M, Bahador N. Antibiotic resistance dependent on efflux pump in the isolates of clinical and environmental *Acinetobacter baumannii*. Journal of Microbial World 2019;12: 15-26.
9. Moammadi F, Arabestani MR, Safari M, Roshanaii G, Alikhani MY. Prevalence of class1, 2 and 3 integrons among extensive drug resistance *Acinetobacter baumanii* strains isolated from intensive care units in Hamadan, West Province, Iran. Iran J Med Microbiol 2014;8:8-14.
10. Ardeshiri N, Nasrollahi M, Goudarzi H, Goudarzi M, Ghalavand Z, Dadashi M. The prevalence of integron 1, 2 and 3 classes in *Acinetobacter baumanii* clinical isolates from Sari Hospitals, Iran. pajouhesh Dar Pezeshki 2017;41:217-25.
11. Simo Tchuinte PL, Rabenandrasana MA, Kowalewicz C, Andrianoelina VH, Rakotondrasoa A, Andrianirina ZZ, *et al*. Phenotypic and molecular characterisations of carbapenem-resistant *Acinetobacter baumannii* strains isolated in Madagascar. Antimicrob Resist Infect Control 2019;8:31.
12. Golafshani FB, Kaboosi H, Armaki MT, Ghadikolaii FP, Fattahi E. Molecular investigation of integron types and imipenem-resistance encoded genes in *Acinetobacter baumannii* strains isolated from burns patients in Iran. Gene Rep 2019;17:100486.
13. Leite GC, Oliveira MS, Perdigão-Neto LV, Rocha CK, Guimarães T, Rizek C, *et al.* Antimicrobial combinations against pan-resistant *Acinetobacter baumannii* isolates with different resistance mechanisms. PLoS One 2016;11:e0151270.
14. Vakili B, Fazeli H, Shoaei P, Yaran M, Ataei B, Khorvash F, *et al.* Detection of colistin sensitivity in clinical isolates of *Acinetobacter baumannii* in Iran. J Res Med Sci 2014;19:S67-70.
15. Bahador A, Taheri M, Pourakbari B, Hashemizadeh Z, Rostami H, Mansoori N, *et al.* Emergence of rifampicin, tigecycline, and colistin-resistant *Acinetobacter baumannii* in Iran; spreading of MDR strains of novel International Clone variants. Microb Drug Resist 2013;19:397-406.
16. Al-Sweih NA, Al-Hubail MA, Rotimi VO. Emergence of tigecycline and colistin resistance in Acinetobacter species isolated from patients in Kuwait hospitals. J Chemother 2011;23:13-6.
17. Taneja N, Singh G, Singh M, Sharma M. Emergence of tigecycline and colistin resistant *Acinetobacter baumanii* in patients with complicated urinary tract infections in north India. Indian J Med Res 2011;133:681-4.
18. Herrera ME, Mobilia LN, Posse GR. Comparative evaluation of the sensitivity of Acinetobacter to colistin, using the prediffusion and minimum inhibitory concentration methods: Detection of heteroresistant isolates. Rev Argent Microbiol 2011;43:115-9.
19. Chang KC, Lin MF, Lin NT, Wu WJ, Kuo HY, Lin TY, *et al.* Clonal spread of multidrug-resistant *Acinetobacter baumannii* in eastern Taiwan. J Microbiol Immunol Infect 2012;45:37-42.
20. Agodi A, Voulgari E, Barchitta M, Quattrocchi A, Bellocchi P, Poulou A, *et al.* Spread of a carbapenem- and colistin-resistant *Acinetobacter baumannii* ST2 clonal strain causing outbreaks in two Sicilian hospitals. J Hosp Infect 2014;86:260-6.
21. Peymani A, Farajnia S, Nahaei MR, Sohrabi N, Abbasi L, Ansarin K, *et al.* Prevalence of class 1 integron among multidrug-resistant *Acinetobacter baumannii* in Tabriz, northwest of Iran. Pol J Microbiol 2012;61:57-60.
22. Japoni-Nejad A, Farshad S, van Belkum A, Ghaznavi-Rad E. Novel cassette array in a class 1 integron in clinical isolates of *Acinetobacter baumannii* from central Iran. Int J Med Microbiol 2013;303:645-50.
23. Goudarzi H, Azad M, Seyedjavadi SS, Azimi H, Chirani AS, Omrani VF. Characterization of integrons and associated gene cassettes in *Acinetobacter baumannii* strains isolated from intensive care unit in Tehran, Iran. J Acute Dis 2016;5:386-92.
24. Kooti S, Motamedifar M, Sarvari J. Antibiotic Resistance Profile and Distribution of Oxacillinase Genes Among Clinical Isolates of *Acinetobacter baumannii* in Shiraz Teaching Hospitals, 2012 - 2013. Jundishapur J Microbiol 2015;8:e20215. doi: 10.5812/jjm.20215v2. PMID: 26464764; PMCID: PMC4600599.
25. Raka L, Kalenć S, Bosnjak Z, Budimir A, Katić S, Sijak D, *et al.* Molecular epidemiology of *Acinetobacter baumannii* in central intensive care unit in Kosova Teaching Hospital. Braz J Infect Dis 2009;13:408-13.
26. Gholipur A, Ansari N, Damavandi MS, Rabiei M, Mirnezhad R. Genotyping and antibiotic resistance of *Acinetobacter baumannii* strains isolated from patients hospitalized in teaching hospitals of Shahrekord by Pulsed- Field Gel Electrophorsis. J Shahid Sadoughi Univ Med Sci 2017;25:241-51.
27. Anvarinejad M, Japoni A, Davarpanah MA, Mahmudi H, Mammina C, Vazin A. Phenotypic and Molecular Epidemiology of *Acinetobacter calcoaceticus* baumannii Complex Strains Spread at Nemazee Hospital of Shiraz, Iran. Jundishapur journal of microbiology 2015;8:e19180. https://doi.org/10.5812/

jjm.8(5)2015.19180.
28. Ranjbar R, Farahani A. Study of genetic diversity, biofilm formation, and detection of Carbapenemase, MBL, ESBL, and tetracycline resistance genes in multidrug-resistant *Acinetobacter baumannii* isolated from burn wound infections in Iran. Antimicrob Resist Infect Control 2019;8:172.
29. Eghbalimoghadam M, Farahani A, Akbar FN, Mohajeri P. Frequency of class 1 integron and genetic diversity of *Acinetobacter baumannii* isolated from medical centers in Kermanshah. J Nat Sci Biol Med 2017;8:193.