Original Article

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An Outbreak of Carbapenem-Resistant *Acinetobacter baumannii* in Patients Hospitalized in Intensive Care Units of a Hospital in Western Iran

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ABSTRACT

Background & Objective: Dissemination of multiple drug-resistant *Acinetobacter baumannii* in hospitals, particularly in intensive care units (ICUs), may lead to serious outcomes. We aimed to investigate clonal relatedness and the presence of carbapenemase genes in clinical *A. baumannii* isolated during a hospital outbreak.

Materials & Methods: From October to December 2020, 41 *A. baumannii* were isolated from patients hospitalized in the ICUs of a university hospital in Kermanshah, western Iran. The sensitivity of isolates to antimicrobial agents was evaluated. The polymerase chain reaction determined the presence of OXA carbapenemases and metallo beta-lactamases. The genetic relationships of isolates were determined using the Enterobacterial repetitive intergenic consensus (ERIC) sequences analysis followed by the Pasteur multilocus sequence typing (MLST) scheme.

Results: All 41 isolates showed resistance to carbapenem meropenem. The *bla*$_{VIM}$ gene was found in all isolates. Of the 41 isolates, 85.4% carried *bla*$_{OXA-24}$ and 48.8% *bla*$_{OXA-23}$. No isolate harbored *bla*$_{OXA-58}$. Twenty-three isolates (56.1%) had the IS*Aba1* upstream of the *bla*$_{OXA-51-like}$ gene. All isolates showed multidrug resistance. ERIC typing revealed the genetic relatedness of isolates. MLST showed that some isolates belonged to sequence type (ST) 415.

Conclusion: The results showed a high level of resistance and clonal spread of *A. baumannii* in our hospital's ICUs. ST415 might be an emerging *A. baumannii* lineage carrying carbapenem resistance determinants, and creating awareness regarding preventing this ST may be necessary. Characterization of antimicrobial resistance and determination of genotypes will help track the recurrent episodes of infections with the described *A. baumannii* isolates.

Keywords: Drug resistance; beta-Lactamases; Molecular typing, OXA carbapenemase, Clonal spread

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Introduction

Acinetobacter baumannii is a non-fermenting oxidase-negative coccobacillus that mainly causes hospital-acquired infections including blood, wound, and urinary infections, pneumonia, meningitis, endocarditis, and peritonitis (1). This microorganism has become an increasingly important cause of outbreaks in intensive care units (ICUs). The majority of outbreak strains are markedly resistant to most antibiotics, including carbapenems, which are widely considered the last-resort drugs for the treatment of severe infections caused by this microorganism (2). The emergence of resistance to carbapenems in *Acinetobacter* spp. is therefore a serious concern, leaving few therapeutic possibilities remaining (3). The major mechanism responsible for carbapenem resistance is the expression of beta-lactamases,

including class D OXAs and class B metallo-beta-lactamases (MBLs) (4). The OXA carbapenemases of *Acinetobacter* spp. are categorized into four main groups: OXA-24-like; OXA-51-like; OXA-23-like; and OXA-58-like. The $bla_{\text{OXA-51-like}}$ gene is located on chromosome in *A. baumannii* and is very variably expressed, contingent on whether or not its expression is activated by upstream insertion sequences (ISs) (5). IS*Aba1*, which belongs to the IS*4* family, is a strong promoter for the expression of $bla_{\text{OXA-51-like}}$ in *A. baumannii* strains (6).

Outbreaks in healthcare settings and the dissemination of resistant strains of *A. baumannii* have been increasing in recent years (7). Determination of the relationship between the strains may guide to implementation of infection control strategies and to control of the outbreak. Enterobacterial repetitive intergenic consensus (ERIC) sequences analysis is a PCR-based typing technique that relies on the analysis of the 124–127-base pair (bp) imperfect palindromes found in the genomes of bacteria (8). This method is simple, fast, and cost-effective and provides sufficient discrimination between isolates. It requires only basic equipment, yields reproducible results over the years, and allows inter-laboratory comparisons of results (9, 10). Multi-locus sequence typing (MLST) has also been used in determining the genetic relationships of *Acinetobacter* spp. isolates (11). MLST analyzes the nucleotide polymorphisms in the sequences of the genes necessary for the cell (housekeeping genes) (12). Both approaches (ERIC-PCR and MLST) help to identify clonal lineages and genetic diversity of the *A. baumannii* isolates (10).

Drug-resistant *A. baumannii* is a serious threat in healthcare settings (13). According to the World Health Organization (WHO), this microorganism has a critical priority level in the list of drug-resistant bacteria (14). The WHO and the Centers for Disease Control and Prevention (CDC) have prioritized the development of new antibiotics to combat the global threat of drug-resistant *A. baumannii* (14, 15). In our hospital, we encountered an outbreak of pathogenic *A. baumannii* in patients hospitalized in the ICUs. In this study, we aimed to analyze the antibiotic resistance and clonal relatedness of strains isolated during this outbreak.

Materials and Methods

Bacterial isolates

During three months (October to December 2020), 41 consecutive, non-duplicate pathogenic *A. baumannii* were isolated from patients hospitalized in the adult ICUs of the largest university hospital in Kermanshah city (the capital of Kermanshah province), western Iran. Only the first *A. baumannii* isolate per patient was included in this study.

We received the isolates from the hospital laboratory and did not have access to the ICUs; therefore, sampling from the environment of the ICUs was excluded. The strains were isolated from different samples including urine, blood, sputum, and tracheal secretions (a cutoff of $\geq 10^5$ colony-forming units /mL was applied for cultures of airway specimens) (16) and identified using standard tests such as Gram staining, oxidase, catalase, urease test, citrate utilization, and oxidation/fermentation (O/F) reaction (17). Isolates were further confirmed by a VITEK 2 compact mass spectrometry microbial identification system (bioMerieux, USA). Molecular detection was performed by amplification of *gyrB* and $bla_{\text{OXA-51-like}}$ beta-lactamase according to the previous studies (18, 19). The isolates were stored in Brain-Heart Infusion (BHI) broth (Merck, Germany), containing 20% (v/v) glycerol at -70°C for further investigations.

Antibiotic sensitivity assay

The disc diffusion assay was carried out to determine the antibiotic sensitivity pattern according to the 2020 Clinical and Laboratory Standards Institute (CLSI) guidelines (20). Antibiotic discs (Mast, UK) belonging to the five antimicrobial classes were included: beta-lactam [cefotaxime (30 µg), cefepime (30 µg), and meropenem (10 µg)]; aminoglycoside [gentamicin (10 µg)]; fluoroquinolone [ciprofloxacin (5µg)], tetracycline (30 µg), and trimethoprim/sulfamethoxazole. Briefly, Mueller Hinton agar (Merck, Germany) plates were inoculated with a 0.5 McFarland standard inoculum from overnight cultures. Antibiotic discs were placed at a standard distance from each other and from the edge of the plate and incubated at 35 °C for 20 to 24 hours. The zone of inhibition around each disk was measured and interpreted. *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as the quality control strains. Resistance to meropenem was verified by determination of minimum inhibitory concentration (MIC) using broth microdilution according to the CLSI 2020 criteria (≤2 µg/mL: susceptible, 4 µg/mL: intermediate, and ≥8 µg/mL: resistant).

DNA extraction and identification of carbapenemase genes

Boiling method was used to extract genomic DNA. Briefly, overnight culture of each isolate in BHI agar (Gibco, Scotland) was washed and suspended in sterile deionized water. The suspensions were boiled at 100 °C for 10 min and immediately incubated on ice for 5 min. After centrifugation, the supernatants containing DNA were transferred to fresh tubes and stored at -20°C. The quality and quantity of DNA extracts were determined by agarose gel electrophoresis and by measuring the absorbance at 260 nm, respectively. The ratio of absorbance at 260 and 280 nm was obtained for each DNA sample to assess DNA purity, and the average value between 1.6 and 2.0 was considered optimal.

DNA stocks were used as the template for polymerase chain reaction (PCR). All PCR reactions in the present study were performed in an Analytik Jena (Germany) thermal cycler. A positive control (from our

laboratory's collection) and a negative control (DNase-free water) were included in PCR reactions.

Amplification of MBL genes (bla_{SPM}, bla_{VIM}, bla_{SIM}, bla_{IMP}, bla_{GIM}, and bla_{NDM}) was performed using primers (4) under the following thermal cycling conditions: initial denaturation for 5 min at 94°C; followed by 30 cycles of 94°C denaturation temperature for 1 min, annealing at different temperatures for 1 min (Table 1)], and 72°C elongation temperature for 1 min; and 5 min at 72°C for the final elongation.

Detection of the three groups of OXA carbapenemase genes ($bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}23\text{-}like}$, and $bla_{OXA\text{-}58\text{-}like}$) was performed under the following conditions: an initial denaturation step at 94 °C for 5 min, then 30 cycles of 94 °C denaturation temperature for 1 min, primer annealing at different temperatures for 1 min, and 72 °C extension temperature for 1 min and final elongation at 72 °C for 5 min**.** The primers used are shown in (Table 1).

PCR experiment to detect the presence of the $bla_{OXA\text{-}51\text{-}like}$ gene with an upstream IS*Aba1* (IS*Aba1*- $bla_{OXA\text{-}51\text{-}like}$ gene) was carried out using a combination of the IS*Aba1* forward (F) primer and the OXA-51-like reverse (R) primer (21) under the same conditions, but with a primer annealing temperature of 55°C. The amplified PCR fragments obtained with the ISAba1F/OXA51-likeR primers were sequenced in both directions using an automated DNA sequencer (ABI 3730XL sequencer; Macrogen, Korea).The sequencing results were analyzed and compared to a sequence database via the BLAST algorithm at the NCBI website (http://www.ncbi.nlm.nih.gov).

Each PCR reaction was performed in a final volume of 20 µL, containing DNA template, forward and reverse primers, and Taq PCR Master Mix (0.1 U/µl Taq polymerase in reaction buffer, 1.5 mM $MgCl_2$, and 0.2 mM of each dNTP; SinaClon, Iran). Electrophoresis was performed using 0.5X Tris-Borate Ethylene diamine tetraacetic acid (TBE) buffer on a 1.5% agarose gel stained with Safe Gel Stain (SinaClon) to visualize DNA under ultraviolet (UV) light. A 100 bp Plus DNA ladder (Parstous, Iran) was used as a molecular weight marker.

Table 1. Genes, sequences and annealing temperatures of the primers, and product sizes for PCR amplification

Gene	Primer sequence (5′-3′)	Annealing temperature (°C)	Product size (bp)	Reference
	Metallo-beta-lactamases			
***bla*SIM**	TACAAGGGATTCGGCATCG/ TAATGGCCTGTTCCCATGTG	64	570	(4)
***bla*NDM**	GGTTTGGCGATCTGGTTTTC/ CGGAATGGCTCATCACGATC	64	621	
***bla*SPM**	AAAATCTGGGTACGCAAACG/ ACATTATCCGCTGGAACAGG	61	271	
***bla*GIM**	TCGACACACCTTGGTCTGAA/ AACTTCCAACTTTGCCATGC	61	477	
***bla*VIM**	GATGGTGTTTGGTCGCATA/ CGAATGCGCAGCACCAG	59	390	
***bla*IMP**	GGAATAGAGTGGCTTAAYTCTC/ GGTTTAAYAAAACAACCACC	54	232	
	OXA carbapenemases			
***bla*OXA23-like**	GATCGGATTGGAGAACCAGA/ ATTTCTGACCGCATTTCCAT	56	501	(5)
***bla*OXA24-like**	GGTTAGTTGGCCCCCTTAAA/ AGTTGAGCGAAAAGGGGATT	56	246	
***bla*OXA58-like**	AAGTATTGGGGCTTGTGCTG/ CCCCTCTGCGCTCTACATAC	59	599	
IS*Aba1*- *bla*OXA51-like	CACGAATGCAGAAGTTG/ TGGATTGCACTTCATCTTGG	55	Variable	(21)

Molecular typing

ERIC fingerprinting

The genetic relatedness of isolates was evaluated using the ERIC-PCR with the primers ERIC1: 5′ATGTAAGCTCCTGGGGATTCAC3′ and ERIC2: 5′AAGTAAGTGACTGGGGTGAGCG3′ as described previously (22). All reactions were done in a final PCR volume of 20 µL containing genomic DNA template, forward and reverse primers, and PCR Master Mix (0.1 U/µl Taq polymerase in reaction buffer, 1.5 mM $MgCl_2$, and 0.2 mM of each dNTP; SinaClon). *A. baumannii* ATCC 17978 was used as a control isolate. Electrophoresis was performed using 0.5X TBE buffer on a 1.5% agarose gel stained with Safe DNA Gel Stain for visualization of DNA by UV transilluminator. A 100 bp DNA ladder (SinaClon) was used as a molecular weight marker.

Multi-locus sequence typing

Isolates were also subjected to MLST based on the Pasteur scheme (11). Seven housekeeping genes (*cpn60, fusA*, *gltA*, *pyrG*, *recA*, *rplB*, and *rpoB*) were amplified using the PCR conditions described by the MLST database for *A. baumannii* (https://pubmlst.org/primers-used-mlst-acinetobacter-baumannii-complex-pasteur-scheme) and purified PCR products were then sequenced in both directions with the ABI 3730XL automated DNA sequencer (Macrogen, Korea).

Data analyses

Experiments were performed in triplicate. Statistical analysis was performed using the SPSS version 21.0 software (SPSS, USA). Isolates were considered multidrug-resistant (MDR) if they showed non-susceptibility to at least one antimicrobial agent in three or more antimicrobial classes (23). In MLST, sequence types (STs) and DNA sequence variations were analyzed using the Acinetobacter MLST database (http://pubmlst.org/abaumannii/). In ERIC typing, we used a stringent definition for ERIC types with no band differences between isolates in the same type and isolates that differed by one band were considered different types (24).

Results

A total of 41 pathogenic *A. baumannii* was isolated during three months from patients hospitalized in the adult ICUs of the largest hospital in western Iran. Out of the 41 isolates, 18 (43.9%) were isolated from tracheal secretions, 14 (34.1%) from sputum, 7 (17.1%) from urine, and 2 (4.9%) were isolated from blood. Twenty-five (61%) patients were male and therefore, 16 patients (39%) were female. More than half of the patients (56.1%) were 65 years old or over. The oldest patient was a 97-year-old male. One patient was between 17 to 24 years old, 1 patient was between 25 to 34, 3 patients were between 35 to 44, 4 patients were between 45 to 54, 9 patients were between 55 to 64, and 23 patients were 65 or over. We did not have access to more data about patients such as their outcomes or the type of treatment at the time of sampling.

Antimicrobial resistance rate

All 41 isolates showed resistance to meropenem, cefotaxime, and ciprofloxacin (no inhibition zone (0 mm)). Against tetracycline, 39 (95.1%) isolates were resistant and one isolate showed intermediate resistance. Forty isolates (97.6%) showed resistance to gentamicin and 37 (90.2%) isolates were resistant to trimethoprim/sulfamethoxazole. Against cefepime, 35 (85.4%) and 6 isolates showed resistance and intermediate resistance, respectively. The MIC of meropenem was ≥ 8 µg/mL (resistant category) for all isolates.

Seven patterns of antimicrobial resistance were identified among the isolates (Table 2). All isolates were resistant to at least five antibiotics. The most common pattern was resistance to all 7 agents, which was seen in 30 of 41 isolates (73.2%), followed by the pattern of resistance to 6 agents, which was found in 9 isolates (21.9%). All isolates exhibited a multidrug-resistant phenotype.

Table 2. Antimicrobial resistance patterns of 41 pathogenic *Acinetobacter baumannii* isolated during an outbreak in intensive care units

Number of agents	MEN	TE	SXT	CTX	GM	CP	FEP	Number of isolates (%)
7	R	R	R	R	R	R	R	30 (73.2)
	R	R	R	R	R	R	I	5 (12.2)
	R	I	R	R	R	R	R	1 (2.4)
6	R	R	S	R	R	R	R	2 (4.9)
	R	R	R	R	S	R	R	1 (2.4)
	R	R	S	R	R	R	I	1 (2.4)
5	R	S	S	R	R	R	R	1 (2.4)

***Abbreviation: MEN meropenem, TE tetracycline, SXT trimethoprim/sulfamethoxazole, CTX cefotaxime, GM gentamicin, CP ciprofloxacin, and FEP cefepime. R: resistant, I: intermediate, S: susceptible.**

Detection of carbapenemase genes

Among the MBLs, *bla*$_{VIM}$ was present in all isolates (100%) (Figure 1), while the other genes (*bla*$_{SIM}$, *bla*$_{GIM}$, *bla*$_{NDM}$, *bla*$_{IMP}$, and *bla*$_{SPM}$) were not found.

PCR for detection of OXA carbapenemases showed that out of the 41 isolates, 35 isolates (85.4%) carried *bla*$_{OXA-24}$ and 20 isolates (48.8%) *bla*$_{OXA-23}$ (Figure 1). No isolate harbored *bla*$_{OXA-58}$. Twenty-three isolates (56.1%) carried the *bla*$_{OXA-51-like}$ gene with an upstream IS*Aba1*.

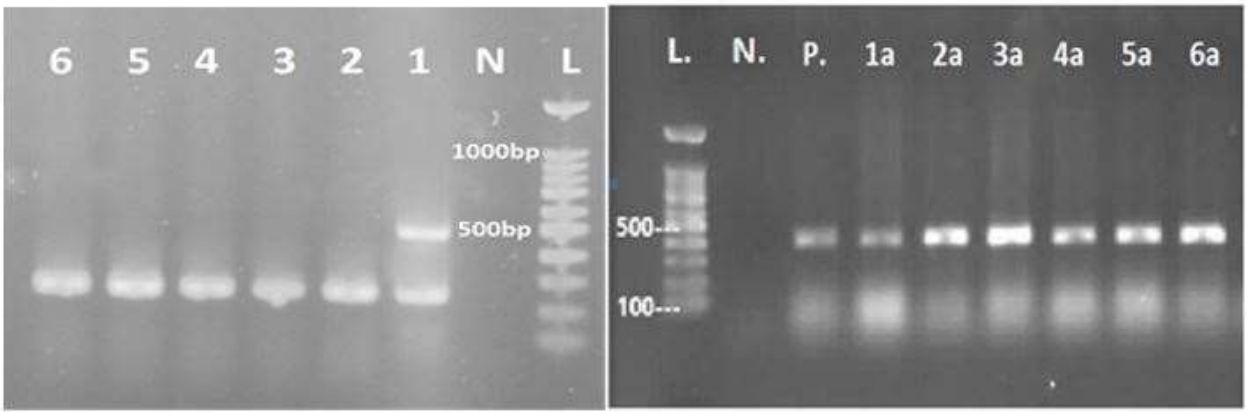


Figure 1. Gel image of *bla*$_{VIM}$, *bla*$_{OXA-24}$, and *bla*$_{OXA-23}$ genes in pathogenic *Acinetobacter baumannii* isolated from patients hospitalized in intensive care units. Right: gel image of *bla*$_{VIM}$ gene. L.: 100 bp plus ladder; N.: negative control; P.: positive control; other wells: *Acinetobacter baumannii* strains (fragments with an amplicon size of 390 bp are seen). Left: gel image of *bla*$_{OXA-24}$ and *bla*$_{OXA-23}$ genes with amplicon sizes of 246 and 501 bp, respectively. L: 100 bp plus ladder; N: negative control; 1: positive control; other wells: isolates of *Acinetobacter baumannii*. Well 1: *bla*$_{OXA-24}$ and *bla*$_{OXA-23}$ positive, wells 2-6: *bla*$_{OXA-24}$ positive.

In general, out of 10 resistance determinants investigated (6 MBLs, 3 OXAs, and IS*Aba1*-*bla*$_{OXA-51}$), the isolates were negative for 6 determinants. Considering the 4 determinants together, which for them at least one isolate was positive; the isolates exhibited 6 profiles (Table 3). Of these 6 combinations, the most common pattern was characterized by the presence of IS*Aba1*-*bla*$_{OXA-51}$, *bla*$_{OXA-24}$, and *bla*$_{VIM}$ (19 isolates) followed by the *bla*$_{OXA-23}$, *bla*$_{OXA-24}$, and *bla*$_{VIM}$ profile which was found in 11 isolates. The least distributed profile was detected in only one isolate and characterized by the simultaneous presence of IS*Aba1*-*bla*$_{OXA51}$, *bla*$_{OXA-23}$, and *bla*$_{VIM}$. Other profiles detected were: the profile characterized by the presence of IS*Aba1*-*bla*$_{OXA51}$, *bla*$_{OXA-24}$, *bla*$_{OXA-23}$, and *bla*$_{VIM}$ in 3 isolates; the profile characterized by the presence of *bla*$_{VIM}$ and *bla*$_{OXA-23}$ in 5 isolates, and the profile characterized by the simultaneous presence of *bla*$_{OXA-24}$ and *bla*$_{VIM}$ in 2 isolates. The *bla*$_{OXA-58}$, *bla*$_{SIM}$, *bla*$_{GIM}$, *bla*$_{NDM}$, *bla*$_{IMP}$, and *bla*$_{SPM}$ were not found in any isolates and therefore were not included. Table 3 shows the characteristics of *A. baumannii* strains isolated during the outbreak including sample, patient age, phenotypic non-susceptibility pattern, and resistance genotypes. As shown in this table, the most prevalent pattern (non-susceptibility to all tested antibiotics) exhibited 6 resistance genotypes which ranged from two-gene patterns to a five-gene pattern.

Table 3. Characteristics of 41 pathogenic *Acinetobacter baumannii* isolated during an outbreak in intensive care unit patients

Isolate	Gender	Age (years)	Sample	*Resistance genotype	**Phenotypic non-susceptibility pattern
38Ab	M	73	Urine	IS*Aba1*-*bla*$_{OXA-51}$,*bla*$_{OXA-23}$, *bla*$_{OXA-24}$, *bla*$_{VIM}$	MEN, TE, SXT, CTX, GM, CP, FEP
32Ab	M	52	Blood		
5Ab	M	32	Urine	IS*Aba1*-*bla*$_{OXA-51}$, *bla*$_{OXA-24}$, *bla*$_{VIM}$	
39Ab	F	65	Urine		
3Ab	M	78	Sputum		
6Ab	M	75	Trachea.		
7Ab	F	80	Trachea.		
8Ab	M	67	Sputum		

9Ab	F	48	Sputum		
10Ab	F	80	Trachea.		
11Ab	M	55	Trachea.		
12Ab	F	83	Trachea.		
14Ab	M	79	Sputum		
16Ab	M	61	Trachea.		
17Ab	M	81	Sputum		
18Ab	M	76	Trachea.		
20Ab	M	90	Trachea.		
21Ab	M	74	Trachea.		
22Ab	M	38	Urine		
25Ab	M	60	Trachea.		
30Ab	M	85	Trachea.	*bla*$_{\text{OXA-24}}$, *bla*$_{\text{VIM}}$	
24Ab	M	97	Sputum	*bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24}}$, *bla*$_{\text{VIM}}$	
66Ab	F	84	Trachea.		
45Ab	M	62	Sputum		
27Ab	F	84	Trachea.		
28Ab	F	60	Sputum		
33Ab	F	48	Urine		
31Ab	M	69	Sputum		
34Ab	M	45	Urine		
35Ab	F	60	Urine		
37Ab	F	18	Trachea		
40Ab	F	43	Trachea.	IS*Aba1-bla*$_{\text{OXA-51}}$, *bla*$_{\text{OXA-23}}$, *bla*$_{\text{VIM}}$	
75Ab	M	57	Blood	*bla*$_{\text{OXA-23}}$, *bla*$_{\text{VIM}}$	
29Ab	F	84	Trachea.		
51Ab	F	61	Sputum		
103Ab	M	71	Sputum		
13Ab	M	68	Sputum	*bla*$_{\text{OXA-24}}$, *bla*$_{\text{VIM}}$	MEN, TE, SXT, CTX, CP, FEP
26Ab	M	57	Sputum	*bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24}}$, *bla*$_{\text{VIM}}$	MEN, TE, CTX, GM, CP, FEP
36Ab	F	37	Sputum	IS*Aba1-bla*$_{\text{OXA-51}}$, *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24}}$, *bla*$_{\text{VIM}}$	
69Ab	F	72	Trachea.	*bla*$_{\text{OXA-23}}$, *bla*$_{\text{VIM}}$	
19Ab	M	71	Trachea.	IS*Aba1-bla*$_{\text{OXA-51}}$, *bla*$_{\text{OXA-24}}$, *bla*$_{\text{VIM}}$	MEN, CTX, GM, CP, FEP

M: Male; F: Female; Trachea: Tracheal secretions; MEN meropenem, TE tetracycline, SXT trimethoprim/sulfamethoxazole, CTX cefotaxime, GM gentamicin, CP ciprofloxacin, and FEP cefepime.

*** The *bla*$_{\text{OXA-58}}$, *bla*$_{\text{SIM}}$, *bla*$_{\text{GIM}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{IMP}}$, and *bla*$_{\text{SPM}}$ were not found in any isolates and therefore were not included.**

**** Non-susceptibility (intermediate resistance+ resistance)**

Molecular typing

ERIC typing was performed in triplicate on each isolate. The isolates showed three bands, ranging in size from approximately 250 to 600 bp (Figure 2). According to the banding pattern, the isolates were assigned to the two types (A and B), indicating a genetic relatedness.

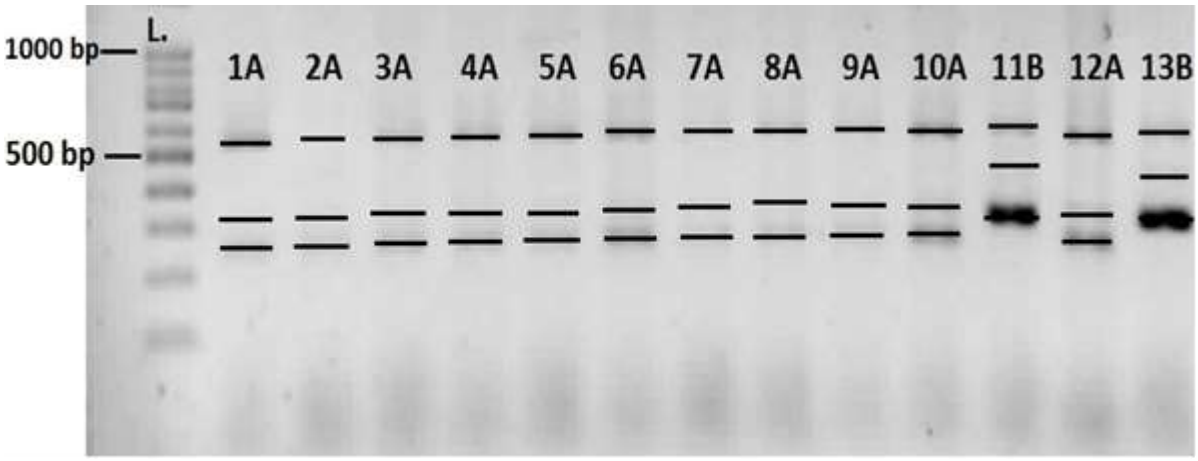


Figure 2. Gel image of ERIC fingerprinting. L: 100 bp DNA ladder, 1-13: pathogenic *Acinetobacter baumannii* isolated during an outbreak in intensive care units. Isolates were assigned to two types A and B. Fragments ranging in size from approximately 250 to 600 bp are seen.

We used a stringent definition for ERIC types with no band differences between isolates in the same type (24). Representative isolates from each ERIC type, each non-susceptibility pattern, and each resistance genotype (25-27) were subjected to MLST (Pasteur scheme) and STs were determined. MLST showed that the representative isolates belonged to ST415. Therefore, our ST415 isolates carried the bla_{VIM} gene and showed non-susceptibility (intermediate resistance + resistance) to meropenem, ciprofloxacin, cefotaxime, and cefepime.

Discussion

Infections with drug-resistant *A. baumannii* pose a significant risk in hospitals, particularly in the ICUs. *A. baumannii* strains are spreading throughout many different countries, and susceptible isolates are acquiring resistance genes (2, 28).

In this study, we reported an outbreak of *A. baumannii* in Kermanshah, western Iran. The outbreak involved 41 cases of infection by MDR *A. baumannii* isolates during three months. The isolates encountered in this outbreak were resistant to most of the tested antibiotics; including carbapenems. Though some isolates were sensitive to trimethoprim/sulfamethoxazole in vitro, the emergence of resistance during treatment may have influenced the therapy results. Treatment options for MDR *A. baumannii* infections are limited in most cases to carbapenems (3); however, the contribution of OXAs and MBLs to carbapenem resistance at our hospital was observed. All isolates harbored the bla_{VIM} gene, while the other tested MBLs were not detected. In addition, IS*Aba1* was detected upstream of bla_{OXA-51} in more than half of the isolates. The prevalence of bla_{OXA-24} and bla_{OXA-23} in our isolates was high (48.8% and 85.4%, respectively); while no isolate harbored the bla_{OXA-58}. Similar to our outbreak, the majority of *A. baumannii* hospital outbreaks reported in recent years were caused by carbapenem-resistant strains. In northwest Iran, 112 clinical *A. baumannii* were isolated from hospitalized patients. All isolates were resistant to carbapenems: 82.1% of isolates were positive for $bla_{OXA23\text{-}like}$, 36.6% harbored $bla_{OXA24\text{-}like,}$ bla_{NDM} was found in 6.2% of isolates, and bla_{IMP} in 4.4% of isolates. None of the isolates carried $bla_{OXA\text{-}58\text{-}like}$, bla_{GIM}, bla_{VIM}, bla_{SIM}, or bla_{SPM} (29). In China, a study reported an outbreak of OXA-23 -producing carbapenem-resistant *A. baumannii* in the ICU of a university hospital. Isolates were also positive for bla_{OXA-24}; however, bla_{OXA-58}, bla_{NDM}, bla_{VIM}, and bla_{IMP} were not detected (10). In North Lebanon, 28 strains were isolated from patients hospitalized in the ICUs of three major hospitals. All isolates were resistant to carbapenems: the bla_{OXA-23} was found in 96.4% of the isolates, while only one isolate carried the bla_{NDM}. The bla_{VIM}, bla_{OXA-24}, and bla_{OXA-58} were not detected (30).

During an outbreak, it is important to determine the genetic relatedness of isolates. Determination of the relationship between isolates may guide to implementation of infection prevention and control practices and to restrict the outbreak. PCR-based methods such as ERIC typing need simple equipment and provide sufficient discrimination between isolates (9, 10). Clustering of our isolates by ERIC-PCR revealed two ERIC profiles, suggesting a clonal distribution in the ICUs of our hospital. With the Pasteur MLST scheme, one ST (ST415) could be identified in the representative isolates. MLST on all isolates could identify the ST of the remaining strains isolated during this outbreak. Previous studies showed that ST2, the main ST in the MLST clonal complex 2 (Pasteur), is the predominant *A. baumannii* clone circulating in clinical settings in Iran (31, 32). The ST2 clone has also been the most common ST in Mediterranean countries including Greece, Italy,

Lebanon, and Algeria (30, 33-35). Moreover, data have shown that the majority of outbreaks worldwide are associated with clonal complex 2 (32). Both ST2 and ST415 belong to clonal complex 2 and ST415 differs only by a single locus from ST2 (30). At present, to our knowledge, few if any reports of outbreaks caused by bla_{VIM}-positive *A. baumannii* ST415 have been published in Iran and other countries; although sporadic cases of ST415 have previously been reported among clinical isolates in Lebanon and Iran (30, 31). In Lebanon, one *A. baumannii* ST415 strain was isolated from the sputum of a patient. The isolate carried bla_{OXA-23}; however, bla_{NDM-1}, bla_{VIM}, bla_{OXA-24}, bla_{OXA-48}, and bla_{OXA-58} were not found. It was susceptible to colistin only and showed extensive resistance to beta-lactams [ticarcillin, piperacillin, ceftazidime, cefotaxime, cefepime, imipenem, and meropenem], beta-lactam/beta-lactamase inhibitors [ticarcillin/clavulanate and piperacillin/tazobactam], aminoglycosides [tobramycin, gentamicin, netilmicin, and amikacin], fluoroquinolones [levofloxacin and ciprofloxacin], tetracyclines [tetracycline and doxycycline], and also to fosfomycin, and trimethoprim/sulfamethoxazole (30). In Iran, a study found one *A. baumannii* ST415 isolate. The antibiotic susceptibility test revealed that this isolate was colistin-resistant and it also showed resistance to other tested antibiotics (31). Our ST415 isolates were not susceptible to meropenem, ciprofloxacin, cefotaxime, and cefepime and carried the bla_{VIM}, an MBL that confers a high level of resistance to carbapenems (36). It is worthwhile to detect resistance genes to antimicrobials other than carbapenems in our isolates. The presence of various resistance genes suggests that horizontal gene transfer occurred among some isolates. DNA transfer experiments and determination of the location and possible mobilization of the genes are of value to address this phenomenon.

The $bla_{OXA-51-like}$ gene is located on chromosome and it is also detectable in carbapenem-susceptible isolates. However, the expression of bla_{OXA-51} is up-regulated by upstream promoters such as IS*Aba1* (6). Studies showed that the $bla_{OXA-51-like}$ and its upstream IS*Aba1* can be co-transmitted between isolates (10). In addition, *A. baumannii* strains can carry the $bla_{OXA-24-like}$ and $bla_{OXA-23-like}$ genes on the structures associated with DNA mobilization and transfer these genes horizontally to other strains (37, 38). This phenomenon may further complicate managing *A. baumannii* infections.

The extensive resistance of the organism and involvement of a large number of patients makes this outbreak significant, though the severity of the patient outcomes remained unknown. Multiple factors likely contributed to the development of this outbreak, including long hospital stays, broad-spectrum antibiotic exposure (mostly carbapenems or third-generation cephalosporins), invasive medical device usage, severity of clinical conditions, immunosuppression due to diseases or treatments, or a combination of these (28, 39). Implementation of infection control measures such as (1) contact precautions for all *A. baumannii*-infected patients (2) enforcement of standard precautions by providing ongoing education and training of staff such as hand hygiene awareness, (3) antimicrobial stewardship, and (4) environmental screening and disinfection are effective in preventing the further spread of *A. baumannii* (2, 28, 37). In our hospital, the infection control team intensified interventions. Despite all the efforts, *A. baumannii* cases persisted and the outbreak could not be controlled successfully. We have to assume that serious breaches in infection control may have resulted in the transmission of this microorganism. Similarly, other authors reported the spread of carbapenem-resistant *A. baumannii* among patients despite adequate precautions (2, 40). The epidemiology of *A. baumannii* is complex and outbreaks caused by this organism are often characterized by rapid spread among patients, especially in ICUs (2, 10). The prolonged closure of units has been used as a means of controlling outbreaks caused by carbapenem-resistant *A. baumannii* with considerable extra cost (40); however, closing the ICUs during the outbreak in our hospital was not possible which also introduced challenges for infection control. Carbapenem resistance in *A. baumannii* has forced clinicians to use polymyxins as the last-resort treatment of infections caused by extensively drug-resistant *A. baumannii*. However, the emergence of polymyxin-resistant strains of *A. baumannii* has forced researchers to investigate novel combination therapy regimens such as ampicillin/sulbactam or ceftazidime/avibactam regimens with a carbapenem and polymyxin (41, 42). Susceptibility testing to polymyxins or beta-lactam combination agents could better illustrate the level of drug resistance in our strains isolated during the outbreak.

Conclusion

In conclusion, the findings revealed that the presence of bla_{VIM}, bla_{OXA-23}, bla_{OXA-24}, and IS*Aba1*- bla_{OXA-51} could be responsible for carbapenem resistance in clinical *A. baumannii* isolated during the outbreak in our hospital. This outbreak stresses the need for strict measures for successful and rapid control of the outbreaks. ST415 might be an emerging *A. baumannii* lineage carrying carbapenem resistance determinants and creating awareness regarding the detection and prevention of this ST may be necessary. Characterization of drug resistance and determination of genotypes will allow us to track the recurrent episodes of infections with the described *A. baumannii* isolates and to rapidly detect changes in genotype and resistance patterns of new emerging clones.

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Conflict of interest
The authors have nothing to declare.

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Ethics approval and consent to participate
Ethical approval was obtained from the Research Ethics Committee (REC), Faculty of Medicine, Kurdistan University of Medical Sciences (Ethical code: IR.MUK.REC.1400.108). All methods were carried out in accordance with relevant guidelines and regulations.

Authors' Contribution
KF performed the experiments and prepared the manuscript. AH performed the experiments. SD designed the study, analyzed the data, and prepared the manuscript. All authors reviewed the manuscript.

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