ABSTRACT

Background: Acinetobacter baumannii is one of the most important hospital pathogenic bacteria that cause infectious diseases. The present study aimed to determine the frequency of carbapenem resistance genes in association with transposable elements and molecular typing of carbapenem-resistant A. baumannii bacteria collected from patients in Shiraz, Iran.

Materials and Methods: A total of 170 carbapenem-resistant A. baumannii isolates were obtained from different clinical specimens in two hospitals. The minimum inhibitory concentrations (MIC) of imipenem were determined and the prevalence of OXA Carbapenemases, Metallo-β-lactamases genes, insertion sequences (IS) elements, and transposons were evaluated by the polymerase chain reaction (PCR) method. Finally, molecular typing of the isolates was performed by the Enterobacterial Repetitive Intergenic Consensus-PCR method.

Results: The MICs ranged from 16 to 1,024 µg/mL for imipenem-resistant A. baumannii isolates. Out of the 170 carbapenem resistant A. baumannii isolates, blaOXA-24-like (94, 55.3%) followed by blaOXA-23-like (71, 41.7%) were predominant. In addition, A. baumannii isolates carried blaVIM (71, 41.7%), blaGES (32, 18.8%), blaSPM (4, 2.3%), and blaKPC (1, 0.6%). Moreover, ISAba1 (94.2%) and Tn2009 (39.2%) were the most frequent transposable elements. Furthermore, (71, 44.0%) and (161, 94.7%) of the ISAba1 of the isolates were associated with blaOXA-23 and blaOXA-51 genes, respectively. Besides (3, 1.7%), (1, 0.6%) and (5, 2.9%) of blaOXA-23 were associated with IS18, ISAba4, and ISAba2, respectively. Considering an 80.0% cut off, clusters and four singletons were detected.

Conclusion: According to the results, transposable elements played an important role in the development of resistance genes and resistance to carbapenems. The results also indicated carbapenem-resistant A. baumannii bacteria as a public health concern.

Keywords: Acinetobacter baumannii; Carbapenem-resistant A. baumannii; OXA Carbapenemase; Insertion sequence; Transposon
INTRODUCTION

Acinetobacter baumannii is a widespread opportunistic pathogen in hospitals, which causes morbidity and mortality, especially in intensive care units (ICUs) [1, 2]. A. baumannii causes a broad range of infections including urinary tract infections, blood infections, ventilator-associated pneumonia, and meningitis [3, 4]. The last option for the treatment of A. baumannii-related infections is carbapenem antibiotics [1]. However, over the past decade, carbapenem-resistant A. baumannii (CRAB) strains have emerged as a serious public health threat [5]. The combined resistance mechanisms include penicillin-binding proteins modification, production of Metallo beta lactamases (MBL, *bla*OXA*), outer membrane impermeability, and increased expression of efflux pumps in A. baumannii, which have been attributed to carbapenem-resistance in A. baumannii [2, 6]. The ambler class A, B, C, and D β-lactamases can cause various antibiotic resistances. Carbapenem-hydrolyzing class D β-lactamases (*bla*OXA-51, *bla*OXA-23, *bla*OXA-24, and *bla*OXA-58) and Metallo-β-lactamases class B (*bla*IMP-, *bla*VIM-, *bla*SIM-1, and *bla*NDM) have been mentioned as important resistance mechanisms in A. baumannii [3]. Insertion sequences (ISs) are strong promoters that facilitate the expression of OXA genes [3]. The presence of ISaba2, ISaba3, and ISaba4 elements in the upstream of *bla*OXA-58 and *bla*OXA-23 genes in A. baumannii may increase the expression of these genes [4]. Transposons are another important genetic element responsible for the rapid spread of resistance genes worldwide. So far, the existence of transposons such as Tn2006, Tn2007, and Tn2008 in A. baumannii isolates have been described and these elements have been shown to carry the *bla*OXA-23 gene [6].

Generally, studying the molecular characteristics of different antibiotic resistance mechanisms through molecular epidemiology analysis in different regions is crucial for the development of therapeutic strategies and to control multidrug-resistant A. baumannii infections [1]. Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR) has been used as a suitable method for molecular typing of A. baumannii [1, 7]. Therefore, the present study aims at evaluation of the frequency of carbapenem resistance genes associated with transposable elements (IS and transposon) that may enhance gene expression and expansion of resistance genes, molecular typing of carbapenem-resistant A. baumannii, and determination of the related IS elements and transposons that are involved in the amplification of OXA-genes in the collected samples from two hospitals in Shiraz, Iran.

MATERIALS AND METHODS

1. Sample collection and bacterial isolates

   Bacterial isolation and identification were initiated in January 2018 and ended in May 2019. In total, 170 carbapenem resistant A. baumannii isolates were collected from two tertiary hospitals (Namazi and Faghihi). Conventional biochemical tests were used for the initial identification of Acinetobacter spp. and were evaluated for the existence of *bla*OXA-51-*bla* genes by PCR methods [8].

2. Ethics statement

   This study was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.MED.REC.1397.301). The informed consent was obtained from all the participants, and informed consent obtained was written.
3. Antimicrobial susceptibility test

The antimicrobial susceptibility test was performed using the disk diffusion method on Mueller-Hinton agar (Sigma-Aldrich, Tehran, Iran) agar according to the Clinical and Laboratory Standards Institute’s (CLSI) guidelines for imipenem. The minimum inhibitory concentrations (MICs) of imipenem were determined by the micro broth method [9]. *Pseudomonas aeruginosa* ATCC 27853 (Pasteur Institute, Tehran, Iran) was used as the control strain.

4. Detection of OXA carbapenemases and IS elements by PCR

DNA templates were extracted by a genomic DNA extraction kit (Bioneer, Daejeon, Korea) according to the manufacturer’s instructions. The primers used in this study have been listed in Table 1. The following carbapenemase-encoding genes were detected: class A β-lactamase gene: *blaKPC*, class B MBL genes: *blaIMP*, *blaVIM*, *blaSPM*, *blaSIM*, and *blaGIM*, class D oxacillinases genes: *blaOXA-23-like*, *blaOXA-24-like*, *blaOXA-51-like*, and *blaOXA-58-like*, and IS elements such as IS*Ab1*, IS*Ab2*, IS*Ab3*, IS*Ab4*, IS*J8*, IS*Ab1–blaOXA-51-like*, and IS*Ab1–blaOXA-23-like*. PCR amplification was performed in a total volume of 25 µl containing 0.5 µl of each primer (10 pmol), 12.5 µl of DNA polymerase master mix RED (Ampliqon A/S, Odense, Denmark), 1 µl of DNA, and 10.5 µl of water (DNase and RNase free water). The PCR cycle consisted of denaturation at 94°C for 5 min followed by 35 cycles at 94°C for 30 s, annealing at 51 – 59°C for 40 s, and extension at 72°C for 40 s.

5. Identification of transposons (Tn2006, Tn2007, and Tn2008)

The Tn2006, Tn2007, Tn2009 and Tn2008 genes were amplified at the final volume of 25 µl containing 12.5 µl master mix (Ampliqon A/S, Denmark), 0.2 µl of each primer at the concentration of 10 pmol/µl, 2 µl of DNA, and 10.1 µl of water (DNase and RNase free water). The PCR protocol included an initial denaturation step at 94°C for 5 min followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 59 – 61°C for 45 s, extension at 72°C for 45 s, and of a final cycle of extension at 72°C for 5 min. The PCR products were detected by electrophoresis using 1.5% agarose and were stained with SYBR DNA safe stain. Then, they were visualized under ultraviolet light.

6. Sequencing technique

Sequencing of the PCR products was performed by Bioneer Company (Korea). The nucleotide sequences were analyzed by the basic local alignment search tool (BLAST) in NCBI (https://nblast.ncbi.nlm.nih.gov/Blast.cgi).

7. Molecular typing by ERIC-PCR

ERIC-PCR was used to determine the similarity between the isolates by the clonal relation ERIC2 primer (5’-AAGTAAGTGACTGGGGTGAGCG-3’) [10]. The PCR cycle consisted of denaturation at 94°C for 5 min followed by 35 cycles at 94°C for 30 s, annealing at 52°C for 45 s, and extension at 72°C for 40 s. The obtained PCR fragments were electrophoresed in 2.0% agarose gel and the gel was analyzed using the GelJ v.1.3 software (GelJ company, San Diego, CA, USA) by considering a cutoff of 80.0% to discriminate the isolates.

8. Statistical analysis

The data were analyzed using the SPSS 22 software (SPSS Inc., Chicago, IL, USA). Chi-square test was used to determine significant differences. P ≤ 0.05 was considered statistically significant.
**RESULTS**

1. Sample collection and patients’ demographic data

A total of 170 non-duplicate *A. baumannii* isolates were mainly collected from sputum (42.3%), endotracheal tube (17.8%), and blood (11.8%) specimens from Faghihi and Namazi hospitals in Shiraz. Out of these 170 isolates, 55.8% were from male patients and 44.2% were from female ones. The patients’ ages ranged from 1 to 90 years (Mean: 51.7, standard deviation: 27.8).

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**Table 1. The primers used in this study**

| Primer name       | Primer sequence                   | Target   | Reference |
|-------------------|-----------------------------------|----------|-----------|
| OXA51-F           | TAA TGC TTT GAT CGG CCT TG        | OXA51    | [32]      |
| OXA51-R           | TGG ATT GCA CTT CAT CTT GG        |          |           |
| OXA58-F           | AAG TAT TGG GCC TTG TGC TG        | OXA58    | [32]      |
| OXA58-R           | CCC CTC TGC GCT CTA CAT AC        |          |           |
| OXA23-F           | GAT CGG ATT GGA GAA CCA           | OXA23    | [32]      |
| OXA23-R           | ATT TCT GAC CGC ATT TCC AT        |          |           |
| OXA24-F           | GGT TAG TTG GCC CCC TTA AA        | OXA24    | [32]      |
| OXA24-R           | AGT TGA GCG AAA AGG GGA TT        |          |           |
| ISAbo1-F          | AGGCCTATAAAGCGTTGTA               | ISAb1-OXA51 | [33]  |
| ISAbo1-R          | CTTCTGTTGGTTGGTC                 |          |           |
| ISAbo2-F          | AATCCGAGATAGAGCGGTTC             | ISAb2    | [34]      |
| ISAbo2-R          | TGACACATACCTAGTGCA               |          |           |
| ISAbo3-F          | CAATCAAAATGTCCAACCTG             | ISAb3    | [34]      |
| ISAbo3-R          | CGTTTACCCAAAACATAAGC             |          |           |
| ISAbo4-F          | ATTTGAACCCCATCATTGCGC            | ISAb4    | [29]      |
| ISAbo4-R          | ACTCTCATATTTTTTCTTGG             |          |           |
| IS18-F            | CACCCAACCTTCTCAAGATG             | IS18     | [34]      |
| IS18-R            | ACACCCACAAACTTCCACTCG            |          |           |
| P3                | GTCTATCGAGAACCTGGCGGC            | Tn2008   | [35]      |
| P5                | GGCTCTACACAGCAGTACAAATG          | Tn2006   | [35]      |
| P4                | GCAAGGCTTATGATGGCAAGA            | Tn2007   | [6]       |
| P6                | ACTCTCATATTTTTTCTTGG             | Tn2007   | [35]      |
| P7                | ATTTGAACCCCATCATTGTC             |          |           |
| bla_shv           | F-GGAATAGATGTTGCTTAYCTCT         | IMP      | [36]      |
| bla_shv           | R-GGTTTAAYAAACAAACACC            |          |           |
| bla_shv           | F-ATGTTAAAAGGTATTAGATG           | VIM      | [36]      |
| bla_shv           | R-CTACTCGCCGACCTGAGGAT           |          |           |
| bla_shv           | F-TCGACACACCTTCTGTGA             | GIM      | [36]      |
| bla_shv           | R-AACCTCCACACTTGCCCATG           |          |           |
| bla_shv           | F-TACAAGGGATCGGCGATCG            | SIM      | [36]      |
| bla_shv           | R-TAAGGCGTTGCTTCCATG            |          |           |
| bla_shv           | F-ATGCCTCCTACTACGCA             | GES      | [37]      |
| bla_shv           | R-CTATTGTCGTCTCAGG              |          |           |
| bla_shv           | F-AAATCCTGGATCGGCAACCG           | SPM      | [36]      |
| bla_shv           | R-ACATATCCGCTGACCCAGG           |          |           |
| bla_shv           | F-GGTGCTGGCGATCGTGGTTTC         | NDM      | [36]      |
| bla_shv           | R-CGGGACTGCTCATCAGGT            |          |           |
| bla_kpc           | F-TCTGACACACCTTCTGTGA           | KPC      |           |
| bla_kpc           | R-TGCGGCTGGACCCCATCC            |          |           |

IMP, imipenemase; VIM, verona integron-encoded metallo-beta-lactamase; GIM, German imipenemase; SIM, Seoul imipenemase; GES, Guiana extended spectrum; SPM, São Paulo metallo-beta-lactamase; NDM, New Delhi metallo-beta-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase.
27.6). The majority of the specimens were isolated from ICUs (87, 51.2%) followed by internal (39, 23.0%) and surgical (15, 8.8%) wards. The demographic characteristics of the A. baumannii isolates have been listed in Table 2.

### Table 2. Demographic and clinical characteristics of the Acinetobacter baumannii isolates

| Characteristic     | No (%): |
|--------------------|---------|
| Age range          | 5 – 82  |
| Gender             | M (95, 55.8%), F (75, 44.2%) |
| Type of specimen   |         |
| Sputum             | 72 (42.3) |
| Blood              | 20 (11.7) |
| Tracheal           | 8 (4.7)  |
| Urine              | 8 (4.7)  |
| ETT                | 30 (17.6) |
| Tip catheter       | 3 (1.7)  |
| Pleural            | 6 (3.5)  |
| CSF                | 1 (0.6)  |
| Wound              | 12 (7)   |
| Nasal              | 1 (0.6)  |
| Abscess            | 1 (0.6)  |
| Throat             | 4 (2.3)  |
| Auxiliary          | 1 (0.6)  |
| Fluid              | 2 (1.2)  |
| Abdominal          | 1 (0.6)  |
| Ward               |         |
| ICU                | 87 (51.2) |
| Internal           | 39 (22)  |
| Surgery            | 15 (8.8) |
| Emergency          | 25 (14.7)|
| Infant             | 1 (0.6)  |
| Infection          | 2 (1.2)  |
| Oncology           | 1 (0.6)  |

M, male; F, female; ETT, endotracheal tube; CSF, cerebrospinal fluid; ICU, intensive care unit.

### 2. Antimicrobial susceptibility test

According to the results, 100% resistance to imipenem was detected among all the isolates by the disk diffusion method. The MIC range for imipenem was 16 – 1,024 µg/mL, while these isolates had MICs of 16 – 64 µg/mL (n = 67, 39.4%) and 128 – 1,024 µg/mL (n = 103, 61.0%) to this antibiotic.

### 3. The prevalence of class B and D carbapenemases

All the isolates carried the blaOXA-51-like gene, which is specific for A. baumannii. Among the class D isolates, carbapenemase genes were predominant regarding blaOXA-24-like (94, 55.3%) followed by blaOXA-23-like (71, 41.7%) and blaOXA-91-like (8, 4.7%). Recognition of MBL by PCR technique showed that A. baumannii isolates carried blaVIM (71, 41.7%), blaGES (32, 18.8%), blaSPM (4, 2.3%), and class A blaKPC (1, 0.6%). However, other MBL genes were not detected. The co-existence of class D and MBL genes was identified in eight A. baumannii isolates. Co-existence of class D and IS elements was also detected, as shown in Table 3.

### 4. The prevalence of IS elements and transposons

Out of the 170 isolates, 161 (94.2%), 11 (6.4%), 8 (4.7%), and 2 (1.2%) carried the ISAb1, Is18, ISAb2, and ISAb4 elements, respectively. In total, 71 (44.0%) and 161 (94.7%) ISAb1 were associated with the blaOXA-23 and blaOXA-91 genes, respectively. Additionally, 3 (1.7%), 1

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| Tip catheter       | 3 (1.7)  |
| Pleural            | 6 (3.5)  |
| CSF                | 1 (0.6)  |
| Wound              | 12 (7)   |
| Nasal              | 1 (0.6)  |
| Abscess            | 1 (0.6)  |
| Throat             | 4 (2.3)  |
| Auxiliary          | 1 (0.6)  |
| Fluid              | 2 (1.2)  |
| Abdominal          | 1 (0.6)  |
| Ward               |         |
| ICU                | 87 (51.2) |
| Internal           | 39 (22)  |
| Surgery            | 15 (8.8) |
| Emergency          | 25 (14.7)|
| Infant             | 1 (0.6)  |
| Infection          | 2 (1.2)  |
| Oncology           | 1 (0.6)  |

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### 4. The prevalence of IS elements and transposons

Out of the 170 isolates, 161 (94.2%), 11 (6.4%), 8 (4.7%), and 2 (1.2%) carried the ISAb1, Is18, ISAb2, and ISAb4 elements, respectively. In total, 71 (44.0%) and 161 (94.7%) ISAb1 were associated with the blaOXA-23 and blaOXA-91 genes, respectively. Additionally, 3 (1.7%), 1
Table 3. Co-existence of OXA-type carbapenemase, MBLs genes, and IS elements among Acinetobacter baumannii isolates

| Genes                        | Number of isolates No. (%) |
|------------------------------|----------------------------|
| blaOXA23, blaOXA24           | 22 (12.9)                  |
| blaOXA23, blaOXA24, blaOXA34 | 22 (12.9)                  |
| blaOXA23, blaOXA24, blaOXA35 | 1 (0.6)                    |
| blaOXA23, blaOXA24, blaOXA34, blaOXA35 | 2 (1.17) |
| blaOXA23, blaOXA24, ISAb1     | 68 (40)                    |
| blaOXA23, ISAb2              | 5 (2.9)                    |
| blaOXA23, ISAb1              | 92 (54.1)                  |
| blaOXA23, ISAb2              | 4 (2.35)                   |
| blaOXA23, ISAb1-flaOXA3      | 51 (30)                    |
| blaOXA23, blaOXA24, ISAb1    | 16 (9.4)                   |
| blaOXA23, blaOXA24, ISAb2    | 1 (0.6)                    |
| blaOXA23, blaOXA24, ISAb1-flaOXA3 | 16 (9.4) |
| blaOXA23, ISAb1, ISAb1-flaOXA3 | 48 (28.2)  |
| blaOXA23, ISAb1, ISAb1-flaOXA3 | 18 (10.6)  |

(0.6%), and 5 (2.9%) blaOXA23 was associated with IS18, ISAb4, and ISAb2, respectively. Moreover, 8 (4.7%) ISAb1 were observed in the blaOXA24 promoter, while the blaOXA24 gene was not in the upstream insertion of ISAb2 and ISAb4. Furthermore, 92 isolates (54.1%) with ISAb1 located at the upstream of the blaOXA24 gene showed resistance to imipenem. Dissemination of the carbapenemase genes was associated with transposons. Among the identified CRAB, 67 (39.2%) were Tn2009-specific, 57 (33.3%) were Tn2008-specific, 41 (24.0%) were Tn2006-specific, and 2 (1.2%) were Tn2007-specific. The co-existence of class D and IS elements and transposons has been presented in Table 4.

5. ERIC-PCR clustering analysis

In this study, ERIC-PCR was performed on 24 isolates with a high prevalence of co-existence of blaOXA23-ikeb, blaOXA24-ikeb, and ISAb1. Considering an 80.0% cutoff, six clusters and four singletons were detected. The dendrogram showed major clusters including seven isolates, six of which were from Namazi Hospital and one was from Faghihi Hospital (Fig. 1).

DISCUSSION

A. baumannii is an important cause of nosocomial infections. Nowadays, antimicrobial resistance in A. baumannii has increased difficulties in the treatment of the related infections [11-13]. In case nosocomial A. baumannii strains become resistant to other β-lactam antibiotics, carbapenems are the best alternative for the treatment of A. baumannii infections. However, carbapenem-resistant strains of A. baumannii are increasing. Hence, it is essential to limit the use of these antibiotics [5, 14-16]. In the current study, 170 CRAB isolates were collected from two hospitals. The majority of these isolates were collected from ICUs and internal wards. Additionally, most CRAB isolates were from sputum, endotracheal tubes, and blood samples. The use of invasive instruments such as endotracheal tube, trachea, and cardiovascular catheters during the procedures and biofilm production on surfaces and devices might have played a role in the transmission of A. baumannii. Furthermore, all the isolates were resistant to imipenem with an MIC of 16 – 1,024 µg/mL. Interestingly, 105 isolates exhibited unusually high levels of resistance to imipenem, with MIC values ≥128 µg/mL. These results were
consistent with those of the previous studies conducted in Egypt, Turkey, Saudi Arabia, and China [17-20]. Increased carbapenem resistance in *A. baumannii* in different regions of the world might be associated with the extensive misuse of carbapenems and cephalosporins [17]. In the present study, however, the increased resistance to cephalosporins and carbapenemase might be attributed to the extensive prescription and use of these antibiotics in hospitals during hospitalization. The most common mechanism of carbapenem resistance in *A. baumannii* is the production of class D OXA carbapenemases and class B MBL [21]. The most prevalent carbapenemases in *A. baumannii* are class D carbapenem-hydrolysing that can be divided into four major subgroups: intrinsic *bla*OXA-23-like, acquired *bla*OXA-24-like, *bla*OXA-51-like and *bla*OXA-58-like [17]. In the current research, 55.3%, 41.7%, and 4.7% of the 170 CRAB isolates harbored the carbapenemases *bla*OXA-24, *bla*OXA-23, and *bla*OXA-51 genes, respectively. The previous studies revealed carbapenem-resistant *A. baumannii, bla*OXA-23-like, to be the most frequent type [17]. In the current study, however, *bla*OXA-24 was the most frequent type of carbapenemases.

### Table 4. Distribution of class D lactamase genes-insertion sequences and transposons in *Acinetobacter baumannii* isolates

| Class D and its insertion sequences | Transposons | Number of isolates (%) | P-value |
|-----------------------------------|-------------|------------------------|---------|
| **IS1**-OXA51                     | TN2008      | 5 (3)                  | 0.1     |
| **IS1**-OXA58                     | TN2006      | 40 (23.5)              | 0.1     |
| **IS1**-OXA51                     | TN2007      | 2 (1.2)                | 0.1     |
| **IS1**-OXA51                     | TN2009      | 63 (37)                | 0.1     |
| **IS1**-OXA23                     | TN2008      | 39 (23)                | 0.0001  |
| **IS1**-OXA23                     | TN2006      | 36 (21.1)              | 0.0001  |
| **IS1**-OXA23                     | TN2007      | 2 (1.2)                | 0.1     |
| **IS1**-OXA23                     | TN2009      | 57 (33.5)              | 0.0001  |
| **IS1**-OXA24                     | TN2008      | 21 (12.3)              | 0.005   |
| **IS1**-OXA24                     | TN2006      | 17 (10)                | 0.02    |
| **IS1**-OXA24                     | TN2009      | 23 (13.5)              | 0.0001  |
| **IS1**-OXA58                     | TN2008      | 4 (2.35)               | 0.2     |
| **IS1**-OXA58                     | TN2006      | 3 (2)                  | 0.3     |
| **IS1**-OXA58                     | TN2009      | 5 (3)                  | 0.1     |
| **IS2**-OXA51                     | TN2008      | 3 (2)                  | 0.3     |
| **IS2**-OXA51                     | TN2006      | 3 (2)                  | 0.3     |
| **IS2**-OXA51                     | TN2009      | 5 (3)                  | 0.1     |
| **IS2**-OXA23                     | TN2008      | 3 (2)                  | 0.1     |
| **IS2**-OXA23                     | TN2009      | 3 (2)                  | 0.1     |
| **IS2**-OXA24                     | TN2006      | 5 (3)                  | 0.01    |
| **IS2**-OXA24                     | TN2008      | 1 (0.6)                | 0.5     |
| **IS2**-OXA24                     | TN2009      | 1 (0.6)                | 0.5     |
| **IS2**-OXA58                     | TN2008      | 3 (2)                  | 0.2     |
| **IS2**-OXA58                     | TN2006      | 3 (2)                  | 0.2     |
| **IS2**-OXA58                     | TN2009      | 5 (3)                  | 0.1     |
| **IS4**-OXA51                     | TN2008      | 1 (0.6)                | 0.1     |
| **IS4**-OXA51                     | TN2009      | 1 (0.6)                | 0.1     |
| **IS4**-OXA23                     | TN2009      | 1 (0.6)                | 0.5     |
| **IS4**-OXA24                     | TN2008      | 1 (0.6)                | 0.5     |
| **IS4**-OXA58                     | TN2008      | 1 (0.6)                | 0.2     |
| **IS4**-OXA58                     | TN2009      | 1 (0.6)                | 0.1     |
| **IS8**-OXA51                     | TN2008      | 2 (1.2)                | 0.1     |
| **IS8**-OXA51                     | TN2006      | 2 (1.2)                | 0.1     |
| **IS8**-OXA51                     | TN2009      | 3 (2)                  | 0.1     |
| **IS8**-OXA23                     | TN2008      | 2 (1.2)                | 0.05    |
| **IS8**-OXA23                     | TN2006      | 2 (1.2)                | 0.05    |
| **IS8**-OXA23                     | TN2009      | 3 (2)                  | 0.006   |
| **IS8**-OXA24                     | TN2008      | 1 (0.6)                | 0.5     |
| **IS8**-OXA58                     | TN2009      | 1 (0.6)                | 0.5     |
Spread of $\text{IS}$ element and $\text{Tn}$ in $A$. baumannii

| G  | Hospital | Ward       | Specimen | bla-genes                          | IS-element | Transposon |
|----|----------|------------|----------|-----------------------------------|------------|------------|
| M  | N        | Internal   | Wound    | VIM, OXA23, 24                    | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2009 |
| M  | N        | Internal   | ETT      | VIM, SPM, OXA23, 24               | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2009, 2008 |
| M  | F        | ICU        | Sputum   | OXA23, 24                         | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2009, 2008 |
| M  | N        | Surgery    | Sputum   | GES, VIM, OXA23, 24               | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2009, 2008 |
| F  | F        | Surgery    | Blood    | GES, OXA23, 24, 34, 38             | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2008 |
| M  | N        | ICU        | Sputum   | OXA23, 24                         | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2009, 2008 |
| M  | N        | ICU        | Sputum   | OXA23, 24                         | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2009, 2008 |
| M  | N        | ICU        | Sputum   | OXA23, 24                         | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2009, 2008 |
| F  | F        | ICU        | Sputum   | VIM, OXA23, 24                    | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2009, 2008 |
| M  | N        | ICU        | Sputum   | OXA23, 24                         | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2009, 2008 |
| M  | N        | ICU        | Sputum   | OXA23, 24                         | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2009, 2008 |
| F  | N        | ICU        | Blood    | GES, VIM, OXA23, 24               | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2009, 2008 |
| F  | N        | ICU        | Sputum   | OXA23, 24                         | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2009, 2008 |
| M  | N        | ICU        | Sputum   | OXA23, 24                         | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2009, 2008 |
| M  | N        | ICU        | Sputum   | OXA23, 24                         | ISAb1, ISAb1-doxa23, ISAb1-blaoxa       | Tn2006, 2009, 2008 |

Figure 1. Dendrogram of 24 Acinetobacter baumannii isolates with $\text{blaoxa23}$, $\text{blaoxa24}$, and $\text{ISAb1}$ genes based on ERIC-PCR patterns.
ERIC-PCR, enterobacterial repetitive intergenic consensus-polymerase chain reaction; G, gender; M, male; F, female; N, Namazi hospital; F, Faghihi hospital; ICU, Intensive care unit; ETT, endotracheal tube specimens; VIM, Verona integron-encoded; OXA, oxacillin; GES, Guiana extended spectrum; SPM, São Paulo metallo-beta-lactamase.
This finding was in agreement with the findings obtained by Alcántar-Curiel M D et al. [22]. The blaOXA-51-like gene was also detected in all the CRAB isolates, confirming that blaOXA-51-like is an intrinsic oxacillinase gene in A. baumannii [6]. In the present investigation, A. baumannii isolates were tested for ambler class A and B carbapenemases. The prevalence of the detected carbapenemases was as follows: Verona integron-encoded metallo-beta-lactamase (VIM): 71, 41.7%; Guiana extended spectrum (GES): 32, 18.8%; São Paulo metallo-beta-lactamase (SPM): 4, 23.5%; and Klebsiella pneumoniae carbapenemase (KPC): 1, 0.6%. Nevertheless, contradictory results have been reported from different countries and regions [23-27]. The present study findings revealed a considerable increase in the prevalence of carbapenem resistance genes in Shiraz, Iran in 2019 compared to 2015. This resistance originated from the extensive use of antimicrobials. The study results also showed the co-existence of OXA genes and MBL genes in the isolates (Table 3). These results indicated an increase in carbapenem-resistance. Generally, the presence of the IS elements upstream of β-lactamase genes provides promoters that increase gene expression and lead to higher levels of resistance to carbapenems [7]. In the current study, ISAb1 was detected in all the isolates that were positive for the blaOXA-23 (n = 71) and blaOXA-24 (n = 94) genes. This suggested that ISAb1 might be involved in the acquisition of carbapenem resistance. Besides, the presence of ISAb1 might promote the blaOXA-51-like gene expression, eventually leading to resistance. In the same line, various studies have demonstrated ISAb1 as a promoter for the expression of the blaOXA-51-like gene [7]. This finding supports the hypothesis that the presence of the ISAb1 upstream of the blaOXA-51-like gene reduces the ability to hydrolyze carbapenemases in A. baumannii isolates without other blaOXA genes. In a study by Al-Agamy et al., ISAb1 was found to play a role in the over-expression of blaOXA-51 and blaOXA-23, while this element was not found in the upstream of blaOXA-24 and blaOXA-51 [17]. Similarly, ISAb1 and ISAb2 could participate in the expression of OXA carbapenemases. In the current study, the prevalence of ISAb2 was 8 (4.7%), which was different from the results obtained by Owrang et al. in Tehran [28]. IS18 (n = 11, 6.4%) and ISAb4 (2, 1.2%) were also detected in this research. Hence, these IS elements could describe the enhancement of promoters related to resistance genes. IS interchange among various bacterial species, which is because of the extensive use of the third generation of cephalosporins along with carbapenemases and has been considered a threat to the expression of resistance genes. Increased resistance to carbapenem suggests that clinical isolates may have one or more transposons. The presence of transposons in Actinetobacter isolates showed that transposons were the preferred mechanism of the spread of the blaOXA genes [29]. The acquisition and dissemination of the carbapenem genes were mediated by transposons Tn2008, Tn2006, Tn2007, and Tn2009 and Mobile Genetic Elements (MGEs) [4, 6]. The current study defined MGEs as transposable elements, namely ISAb1, ISAb2, ISAb4, IS18, Tn2008, Tn2006, Tn2007, and Tn2009. The results of a previous study indicated that Tn2009 was the most widely detected transposon related to the OXA genes [30]. In the current investigation, the blaOXA-23 genes were embedded in transposons Tn2006 (n = 41), Tn2007 (n = 2), Tn2008 (n = 57), and Tn2009 (n = 67) in the clinical isolates of A. baumannii. One of the most tangible factors for the increased resistance is “antibiotic pressure” due to the great use of imipenem and the third generation of cephalosporins as well as the transmission of resistance genes through plasmids and chromosomes in this region. Moreover, the transmission of CRAB strains among hospitals can be associated with the transfer of resistant pathogens through infected patients, hospital staff, and medical equipment such as ventilators.

In general, clonal relationship analysis among pathogens is important for a better understanding of their molecular epidemiology [1]. In the present study, ERIC-PCR was performed for molecular typing of the A. baumannii isolates with blaOXA-23-like, blaOXA-24-like, and ISAb1 genes.
Considering an 80.0% cutoff, six clusters and four singletons were detected. The rate of carriers in hospitals has been reported as 60.0 – 70.0%. Besides, nosocomial infections have been found to transmit through contaminated healthcare personnel's skin, environment, contaminated water and food, and contact with shared items and surfaces [31]. Given the similarity of these isolates, the possibility of transfer between patients, wards, and hospitals increases [e.g., in the ICU and internal wards (Fig. 1)]. These results confirmed the spread of \textit{A. baumannii} clones (\textit{bla}_{OXA} and MBL) as well as similarities among CRAB isolates through ERIC-PCR typing methods.

In conclusion, due to the increase in antibiotic resistance in \textit{A. baumannii}, this pathogen has been considered a general concern, especially in hospitalized patients. Since IS element and transposons play an important role in the development of resistance to antibiotics, the aim of this study was to investigate the simultaneous presence and association of IS element and transposons with carbapenem resistance genes. The current study revealed the promotion of carbapenem-resistant \textit{A. baumannii} genes as the major cause of carbapenem resistance in \textit{A. baumannii}. Moreover, ISAba1 and transposons Tn2009, Tn2006, and Tn2008 were found to play an important role in the overexpression of \textit{bla}_{OXA-23} and \textit{bla}_{OXA-24}. Yet, further studies are needed to investigate the association between IS and the genes carrying antibiotic resistance.

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REFERENCES

1. Jiang L, Liang Y, Yao W, Ai J, Wang X, Zhao Z. Molecular epidemiology and genetic characterisation of carbapenem-resistant \textit{Acinetobacter baumannii} isolates from Guangdong Province, South China. J Glob Antimicrob Resist 2019;17:84-9.

2. Hashemi B, Afkhami H, Khaledi M, Kiani M, Bialvaei AZ, Fathi J, Sarley H, Divsalar S, Ahanjan M. Frequency of metalo beta lactamase genes, \textit{bla} IMP1, \textit{INT} 1 in \textit{Acinetobacter baumannii} isolated from burn patients North of Iran. Gene Reports 2020;21:100800.

3. Simo Tchuinte PL, Rabenandrassana MAN, Kowalewicz C, Andrianoelina VH, Rakotondrasoa A, Andrianirina ZZ, Enouf V, Ratsima EH, Randriantirina F, Collard JM. Phenotypic and molecular characterisations of carbapenem-resistant \textit{Acinetobacter baumannii} strains isolated in Madagascar. Antimicrob Resist Infect Control 2019;8:31.

4. Pagano M, Martins AF, Barth AL. Mobile genetic elements related to carbapenem resistance in \textit{Acinetobacter baumannii}. Braz J Microbiol 2016;47:785-92.

5. Mugnier PD, Poirel L, Naas T, Nordmann P. Worldwide dissemination of the \textit{bla}_{OXA-23} carbapenemase gene of \textit{Acinetobacter baumannii}. Emerg Infect Dis 2010;16:35-40.

6. Chen Y, Gao J, Zhang H, Ying C. Spread of the \textit{bla}_{OXA-23} Containing Tn2008 in Carbapenem-Resistant \textit{Acinetobacter baumannii} Isolates Grouped in CC92 from China. Front Microbiol 2017;8:163.

7. Zhao Y, Hu K, Zhang J, Guo Y, Fan X, Wang Y, Mensah SD, Zhang X. Outbreak of carbapenem-resistant \textit{Acinetobacter baumannii} carrying the carbapenemase OXA-23 in ICU of the eastern Heilongjiang Province, China. BMC Infect Dis 2019;19:452.
8. Mahon CR, Lehman DC. Manuselis. Textbook of diagnostic microbiology: e-book. 6th ed. Amsterdam: Elsevier Health Sciences 2018.

9. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; Twenty-first informational supplement. CLSI document M100-S21. Wayne, PA: CLSI; 2011.

10. Stehling EG, Leite DS, Silveira WD. Molecular typing and biological characteristics of Pseudomonas aeruginosa isolated from cystic fibrosis patients in Brazil. Braz J Infect Dis 2010;14:462-7. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)

11. Tavakol M, Motmaz H, Mohajeri P, Shokoohizadeh L, Tajbakhe E. Genotyping and distribution of putative virulence factors and antibiotic resistance genes of Acinetobacter baumannii strains isolated from raw meat. Antimicrob Resist Infect Control 2018;7:120. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)

12. Nazari M, Youzbashi Z, Khaledi M, Fathi J, Afkhami H. Detection of carbapenem resistance and virulence genes among Acinetobacter baumannii isolated from hospital environments in center of Iran. J Curr Biomed Rep 2021;2:14. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)

13. Farshadzadeh Z, Taheri B, Rahimi S, Shoja S, Pourhajibagher M, Haghighi MA, Bahador A. Growth rate and biofilm formation ability of clinical and laboratory-evolved colistin-resistant strains of Acinetobacter baumannii. Front Microbiol 2018;9:153. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)

14. Xie R, Zhang XD, Zhao Q, Peng B, Zheng J. Analysis of global prevalence of antibiotic resistance in Acinetobacter baumannii infections disclosed a faster increase in OECD countries. Emerg Microbes Infect 2018;7:31. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)

15. Coelho JM, Turton JF, Kaufmann ME, Glover J, Woodford N, Warner M, Palepou MF, Pike R, Pitt TL, Patel BC, Livermore DM. Occurrence of carbapenem-resistant Acinetobacter baumannii clones at multiple hospitals in London and Southeast England. J Clin Microbiol 2006;44:3623-7. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)

16. Rahimi S, Farshadzadeh Z, Taheri B, Mohammadi M, Haghighi MA, Bahador A. The relationship between antibiotic resistance phenotypes and biofilm formation capacity in clinical isolates of Acinetobacter baumannii. Jundishapur J Microbiol 2018;11:e74315. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)

17. Al-Agamy MH, Khalaf NG, Tawfick MM, Shibli AM, El Kholy A. Molecular characterization of carbapenem-insensitive Acinetobacter baumannii in Egypt. Int J Infect Dis 2014;22:49-54. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)

18. Elabd FM, Al-Ayed MS, Asaad AM, Alsareii SA, Qureshi MA, Musa HA. Molecular characterization of oxacillinases among carbapenem-resistant Acinetobacter baumannii nosocomial isolates in a Saudi hospital. J Infect Public Health 2015;8:242-7. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)

19. Cicek AC, Saral A, Iraz M, Ceylan A, Duzgun AO, Peleg AY, Sandalli C. OXA- and GES-type β-lactamases predominate in extensively drug-resistant Acinetobacter baumannii isolates from a Turkish University Hospital. Clin Microbiol Infect 2014;20:410-5. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)

20. Li S, Duan X, Peng Y, Rui Y. Molecular characteristics of carbapenem-resistant Acinetobacter spp. from clinical infection samples and fecal survey samples in Southern China. BMC Infect Dis 2019;19:900. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)

21. Vijayakumar S, Gopi R, Gunasekaran P, Bharathy M, Walia K, Anandan S, Veeraraghavan B. Molecular characterization of invasive carbapenem-resistant Acinetobacter baumannii from a tertiary care hospital in South India. Infect Dis Ther 2016;5:379-87. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)

22. Alcántar-Curiel MD, Rosales-Reyes R, Jarillo-Quijada MD, Gayoso-Vázquez C, Fernández-Vázquez JL, Toledano-Tableros JE, Giono-Cerezo S, Garza-Villafuerte P, López-Huerta A, Vences-Vences D, Morfin-Otero R, Rodríguez-Noriega E, López-Álvarez MDR, Espinosa-Sotero MDC, Santos-Preciado JI. Carbapenem-resistant Acinetobacter baumannii in three tertiary care hospitals in Mexico: virulence profiles, innate immune response and clonal dissemination. Front Microbiol 2019;10:2116. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)

23. Pragasam AK, Vijayakumar S, Balthavatchalam YD, Kapil A, Das BK, Roy P, Gautam V, Sistla S, Parija SC, Walia K, Ohri VC, Anandan S, Veeraraghavan B. Molecular characterisation of antimicrobial resistance in Pseudomonas aeruginosa and Acinetobacter baumannii during 2014 and 2015 collected across India. Indian J Med Microbiol 2016;34:433-41. [PUBMED | CROSSREF](https://doi.org/10.3947/ic.2022.0022)
24. Alkasaby NM, El Sayed Zaki M. Molecular Study of Acinetobacter baumannii isolates for metallo-β-lactamases and extended-spectrum-β-lactamases genes in intensive care unit, Mansoura University Hospital, Egypt. Int J Microbiol 2017;2017:3925868. 

25. Noori M, Karimi A, Fallah F, Hashemi A, Alimehr S, Goudarzi H, Aghamohammad S. High prevalence of metallo-beta-lactamase producing Acinetobacter baumannii isolated from two hospitals of Tehran, Iran. Arch Pediatr Infect Dis 2014;2:e15439. 

26. Peymani A, Nahezi MR, Farajnia S, Hasani A, Mirsaleheian A, Sohrabi N, Abbasi L. High prevalence of metallo-beta-lactamase-producing Acinetobacter baumannii in a teaching hospital in Tabriz, Iran. Jpn J Infect Dis 2011;64:69-71. 

27. Abouelfetouh A, Torky AS, Aboulmagd E. Phenotypic and genotypic characterization of carbapenem-resistant Acinetobacter baumannii isolated from Egypt. Antimicrob Resist Infect Control 2019;8:185. 

28. Owrang M, Fallah F, Irani S, Rahbar M, Eslami G. Identification and isolation of insertion sequences, in carbapenem resistant clinical isolates of Acinetobacter baumannii from Tehran hospitals. Jundishapur J Microbiol 2018;11:e58251. 

29. Wang D, Yan D, Hou W, Zeng X, Qi Y, Chen J. Characterization of bla(OXA-23) gene regions in isolates of Acinetobacter baumannii. J Microbiol Immunol Infect 2015;48:284-90. 

30. Lee HY, Chang RC, Su LH, Liu SY, Wu SR, Chuang CH, Chen CL, Chiu CH. Wide spread of Tn2006 in an AbaR4-type resistance island among carbapenem-resistant Acinetobacter baumannii clinical isolates in Taiwan. Int J Antimicrob Agents 2012;40:163-7. 

31. Solgi H, Badmasti F, Aminzadeh Z, Giske CG, Pourahmad M, Vaziri F, Havaei SA, Shahcheraghi F. Molecular characterization of intestinal carriage of carbapenem-resistant Enterobacteriaceae among inpatients at two Iranian university hospitals: first report of co-production of blaOXA-23 and blaOXA-48. Eur J Clin Microbiol Infect Dis 2017;36:2127-35. 

32. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, Pitt TL. The role of ISAba1 in expression of OXA carbapenemase genes in Acinetobacter baumannii. FEMS Microbiol Lett 2006;258:72-7. 

33. Zhou H, Pi BR, Yang Q, Yu YS, Chen YG, Li LJ, Zheng SS. Dissemination of imipenem-resistant Acinetobacter baumannii strains carrying the ISAba1 blaOXA-23 genes in a Chinese hospital. J Med Microbiol 2007;56:1076-80. 

34. Merkier AK, Catalano M, Ramirez MS, Quiroga C, Orman B, Ratier L, Famiglietti A, Vay C, Di Martino A, Kaufman S, Centron D. Polyclonal spread of bla(OXA-23) and bla(OXA-58) in Acinetobacter baumannii isolates from Argentina. J Infect Dev Ctries 2008;2:235-40. 

35. Lee MH, Chen TL, Lee YT, Huang L, Kuo SC, Yu KW, Hsueh PR, Dou HY, Su IJ, Fung CP. Dissemination of multidrug-resistant Acinetobacter baumannii carrying BlaOXA-23 from hospitals in central Taiwan. J Microbiol Immunol Infect 2013;46:19-24. 

36. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70:119-23. 

37. Labuschagne Cde J, Weldhagen GF, Ehlers MM, Dove MG. Emergence of class 1 integron-associated GES-5 and GES-5-like extended-spectrum beta-lactamases in clinical isolates of Pseudomonas aeruginosa in South Africa. Int J Antimicrob Agents 2008;31:527-30.