Association of the genes encoding Metallo-β-Lactamase with the presence of integrons among multidrug-resistant clinical isolates of Acinetobacter baumannii

Mansour Amin1,2, Tahereh Navidifar2, Farkhonde Saleh Shooshtari2, Hamed Goodarzi2

1Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; 2Department of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: Metallo-β-Lactamases (MBL) are usually encoded on the gene cassettes harboring integrons and disseminated easily among Acinetobacter baumannii isolates. This study was aimed to investigate the association of the genes encoding MBL with the presence of class 1 and 2 integrons among multidrug-resistant (MDR) A. baumannii isolates.

Methodology: A total of 85 non-duplicated A. baumannii isolates were collected and evaluated for the amplification of blaOXA-51. The presence of genes encoding MBLs, including blaTEM, blaSHV, blaGIM, blaSIM, blaVIM, blaIMP, blaDHA, and blaNDM, as well as intI1 and intI2 was evaluated by PCR. Also, the production of MBLs was screened phenotypically by the combination of EDTA and meropenem.

Results: In this study, 77 out of 85 isolates were MDR. Also, 34 isolates had only intI1, 10 had only intI2 and 15 had both intI1 and intI2. The phenotypic detection of MBLs was found in 30 isolates, among which blaSIM was as the most common the gene encoding MBL followed by blaDHA, blaVIM, and blaNDM. The gene cassettes analysis revealed that class 1 integron is often responsible for transferring the genes harboring MBLs.

Conclusion: The production of MBLs among A. baumannii strains is one of the main mechanisms of resistance to carbapenems. Therefore, the development of inexpensive screening methods for the phenotypic detection of MBLs in clinical laboratories settings is essential. Also, our data revealed that the class 1 integron is often responsible for the dissemination of the MBL genes among A. baumannii isolates.

Keywords: acinetobacter baumannii, blaVIM, blaIMP integron, Metallo-Beta-Lactamase

Introduction
Multidrug-resistant (MDR) bacterial strains have emerged as one of the leading causes of nosocomial infections worldwide. Infections caused by A. baumannii are frequent and increasing in hospitalized patients, especially in the intensive care units (ICU).1 Nowadays, the development of antibiotic resistance among A. baumannii strains is considered as one of the major public health concerns in hospital setting.2 Moreover, A. baumannii strains have a high capacity to acquire the multiple antibiotic resistance determinants through the mobile elements, such as integrons harboring single or multiple gene cassettes.

Integrons are conserved, transposon-like DNA elements that mostly encode antibiotic resistance determinants and have a high ability for chromosomal integration in

Correspondence: Farkhonde Saleh Shooshtari
Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Golestan Street, Ahvaz, Iran
Tel +98 916 601 4404
Email farkhondeh.salehshooshtari@gmail.com
bacteria. To date, several classes of integrons have been described; among them, class 1 and 2 integrons are frequently reported from MDR A. baumannii strains.

Carbapenems have a potent activity against multidrug-resistant gram-negative bacilli and are usually the choice antibiotics against A.baumannii strains. However, the resistance rate to carbapenems in this bacterium is increasing throughout the world. The resistance to carbapenems can be led through various mechanisms, such as the production of Metallo-β-Lactamase and oxacillinase enzymes.

More specifically, the infections caused by Metallo-Beta-Lactamase (MBL)-producing organisms are associated with the high rates of morbidity and mortality. MBLs belong to class B beta-lactamas that can hydrolyze all beta-lactam classes except monobactams. MBLs are usually encoded on the gene cassettes harboring class 1 integron and disseminated in bacterial populations. To date, several MBLs were recognized such as the blaTEM, blaIMP, blaGIM, blaSPM, blaDDH, blaSAM and blaNDM which of those, the blaTEM and blaIMP allelic variants have emerged as the dominant MBLs worldwide. The high levels of resistance to carbapenems among MDR A. baumannii strains have made some demands for the reintroduction older antibiotics such as colistin and polymyxin B that had not been used for many years because of their toxicity. Moreover, recent studies have shown that gram-negative bacilli resistant to aminoglycosides, beta-lactams, and fluoroquinolones are often sensitive to polymyxin B. This study was aimed to investigate the association of the genes encoding MBLs with the presence of integrons among multidrug-resistant clinical isolates of Acinetobacter Baumannii.

Materials and methods

Bacterial isolates and identification

The present study was conducted from July 2017 to March 2018. A total of 85 A.baumannii clinical isolates were collected from different clinical samples of hospitalized patients in hospitals of Imam Khomeini and Taleghani in Ahvaz, Iran. The collected samples were as part of the routine hospital laboratory procedure and were transferred to Department of Microbiology, school of medicine, Ahvaz Jundishapur University of Medical Sciences. Then, they were cultured on Blood agar and MacConkey agar (Merck–Germany) and incubated for 24 hrs at 37°C. The gram-negative bacilli were monitored for more biochemical tests, including the sugar fermentation, motility, citrate utilization, urease, oxidative/fermentative glucose (O/F) test, catalase, oxidase and growth ability at 37°C and 42°C. In addition, the identification of A. baumannii isolates was confirmed by the amplification of blaOXA-51-like gene using the previously described primers by Turton et al.

The A. baumannii ATCC19606 was used as the reference strain.

Antibiotic susceptibility testing

Antimicrobial susceptibility of A. baumannii isolates was determined by disc diffusion method according to the clinical and laboratory standards institute (CLSI) guidelines. Briefly, the bacterial suspensions were prepared in sterile normal saline to a turbidity equivalent of 0.5 McFarland standard. The used antibiotic discs were imipenem (10 μg), meropenem (10 μg), ceftazidime (30 μg), cefotaxime (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), amikacin (30 μg), tetracycline (30 μg), piperacillin (100 μg), cefepime (30 μg), piperacillin/tazobactam (100/10 μg), trimethoprim/sulphamethoxazole (1.25/23.75 μg), colistin (10 μg), ampicillin/sulbactam (10/10 μg), ceftazidime (30 μg) and polymyxin B (300U). Then, after 24 h incubation the diameters of the inhibition zones were measured in millimeters. Also, the minimum inhibitory concentrations (MICs) of colistin, meropenem and imipenem were measured using broth microdilution method and their results were interpreted according to CLSI (2018). In brief, for meropenem and imipenem, a MIC ≥8 μg/ml is considered as the breakpoint of resistant, as well as a MIC ≥4 μg/ml for colistin.

MDR Acinetobacter isolates are defined as strains that were resistant to at least three classes of antimicrobial agents, including all penicillins and cephalosporins, fluoroquinolones and aminoglycosides.

Phenotypic detection of MBL production

First, the bacterial suspensions adjusted to 0.5 McFarland were streaked on Mueller Hinton agar plates using the Dacron swab. Then, two discs of meropenem (10 μg), one with 5 μL of 0.35 M EDTA and the other without EDTA were placed on a Mueller Hinton agar plate and incubated at 37°C for 16–18 hrs. The discs containing EDTA alone served as the negative control. A strain was considered to be MBL positive, if there was an increase of ≥7 mm in the inhibition zone around the imipenem + EDTA disc as compared to imipenem disc alone.
ERIC-PCR typing and analysis

The genetic relationship of *A. baumannii* isolates was determined using the enterobacterial repetitive intergenic consensu-PCR (ERIC-PCR)\(^\text{18}\) with the primers sequences of ERIC-F (5'-ATGTAAGCTCTGGGGATTCAC-3') and ERIC-R (5'-AAATGATCCTGGGGTGA CCG-3'). The PCR reaction was performed in the final volume of 25 µL as follows: 1U Taq DNA polymerase, 1.5 mM MgCl\(_2\), 200 µM dNTPs, 0.35 µM of each primer, 10X PCR buffer, 6.5 µL of template DNA and distilled water up to a final volume of 25 µL. The amplification process was performed in an Eppendorf Thermal Cycler Gradient (Eppendorf, Hamburg, Germany) with one cycle of initial denaturation at 95°C for 5 mins; 35 cycles of denaturation at 94°C for 60 s, annealing at 57°C for 60 s, extension at 72°C for 80 s and a cycle of final extension at 72°C for 10 mins. The amplified products were visualized on agarose gel 1.5%, stained with safe stain. The data analysis was performed using the Gel Compare II software version 6.6 (Applied Math, Sint-Martens-Latem, Belgium). The similarity pattern was calculated using the Unweighted-Pair Group Method (UPGMA)/the Dice similarity coefficient with a position tolerance of 1%. Isolates with more than 90% similarity were considered as a clonal type.

Molecular method

The whole genomes of all MDR *A. baumannii* isolates were extracted using High Pure PCR Template Preparation Kit (Roche Diagnosis, Mannheim, Germany) according to manufacturer’s procedure. The Uniplex PCR reactions were performed for the presence of genes encoding *intI1*, *intI2*, *bla\_IMP*, *bla\_NDM*, *bla\_SIM*, *bla\_OXA*, *bla\_VIM*, *bla\_DIM*, *bla\_SPM* in a final volume of 25 µL, as described previously.\(^\text{19-22}\) In each PCR run, the distilled water was used as the negative control. The reaction mixture consisted of 1 U of AmpliTaq DNA polymerase, 1X PCR buffer, 1.5 mM MgCl\(_2\), 200 µM dNTPs, 3 µL of DNA and distilled water up to a final volume of 25 µL. The primer concentrations were as follows: 0.2 pmol/µL each of primers *intI1*-F, *intI1*-R, *intI2*-F and *intI2*-R; 0.45 pmol/µL each of primers *bla\_IMP*-F, *bla\_IMP*-R, *bla\_NDM*-F and *bla\_NDM*-R; 0.25 pmol/µL each of primers *bla*\_SIM*-F, *bla*\_SIM*-R, *bla*\_SPM*-F and *bla*\_SPM*-R. The amplification process was performed in a Mastercycler Nexus Thermal Cycler Gradient (Eppendorf, Hamburg, Germany) with one cycle initial denaturation at 95°C for 5 mins; 35 cycles with a denaturation temperature of 95°C for 45 s; annealing temperature of 51°C for the *intI1* and *intI2* genes, 54°C for the *bla\_IMP* and *bla\_VIM* genes, 53°C for the *bla\_OXA*-51-like gene, 52°C for the *bla\_SIM*, *bla\_NDM* and *bla\_SPM* genes, as well as 58°C for the *bla\_OXA*-51-like genes for 30 s and extension temperature of 72°C for 30 s, followed by a cycle of final extension at 72°C for 10 mins. All of the PCR products were visualized on 1% agarose gel stained with safe stain. DNA sequencing of PCR products was performed by (Bioneer, South Korea) for the determination of the MBL allelic variants.

Sequencing of integron gene cassettes

Amplification of the variable region of class 1 and 2 integrons was performed, as previously by Moura et al.\(^\text{23}\). Then, the purification of the PCR products was performed by the QIAquick Gel Extraction Kit (Qiagen, Germany) and subjected to sequencing with an ABI Prism 377 automated sequencer (Applied Biosystems, USA). The obtained sequences were assembled using MEGA 7\(^\text{24}\) and compared with those in the NCBI database using a BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the integron database INTEGRALL (http://integrall.bio.uu.pt/).

Statistical analysis

The descriptive statistics and Chi-Square test were performed in SPSS version 16.00 with a significance level of *p*<0.05.

Results

Bacterial isolates and determination of antibiotic susceptibility

In this cross-sectional study, 85 non-duplicated *A. baumannii* isolates were collected from the different clinical samples, including burn wounds 22 (25.88%), tracheal secretion 31 (36.47%), blood 16 (18.82%), bronchial lavage 12 (14.11%) and urine 4 (4.7%) isolates and the mean age of the patients was 62.1±4.75 years. According to antibiotic susceptibility testing, 77 out of 85 (90.58%) *A. baumannii* isolates were identified as MDR.

In our study, among 77 MDR *A. baumannii* isolates, resistance to amikacin, ceftazidime, ceftriaxone, cefepime, ciprofloxacin, cefotaxime, gentamicin, imipenem, meropenem, piperacillin/tazobactam, piperacillin, ampicillin/sulbactam, trimethoprim/sulfamethoxazole and tetracycline was seen in 71 (92.2%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%), 76 (98.7%), 75 (97.4%) isolates.

Infection and Drug Resistance 2019:12 submit your manuscript | www.dovepress.com Infection and Drug Resistance downloaded from https://www.dovepress.com/ by 207.241.231.108 on 16-Feb-2020
For personal use only.
isolate carried only bla\textsubscript{VIM} gene cassettes incorporated into class 1 integron and one isolate carried only bla\textsubscript{IMP} gene cassette incorporated into class 1 integron among 10 isolates and as the part of gene cassettes in class 2 integron among 2 isolates. According to these results, bla\textsubscript{VIM} allelic variants were as the part of gene cassettes incorporated into class 1 integron among 10 isolates and as the part of gene cassettes in class 2 integron among 2 isolates. On the other hand, bla\textsubscript{IMP} derivatives were as the part of gene cassettes incorporated into class 1 integron among 4 isolates and into class 2 integron among 1 isolate. Also, 2 isolates carried both bla\textsubscript{VIM} and bla\textsubscript{IMP} allelic variants in gene cassettes incorporated into class 1 integron and one isolate carried only bla\textsubscript{IMP} in gene cassette incorporated into class 1 integron. In addition, 2 isolates carrying bla\textsubscript{VIM} and 2 isolates carrying bla\textsubscript{IMP} were lack either int\textsubscript{I1} or int\textsubscript{I2}.

According to the results shown in Table 3, the isolates belonging to a same clone type had the similar gene cassette array in class 1 and 2 integron.


discussion

\textit{A. baumannii} is an important nosocomial pathogen with the high associated mortality. In the last few years, the resistance to the almost commonly prescribed antibiotics among \textit{A. baumannii} strains is increasing which will cause a treatment challenge in the future. The results of our study showed that 90.58\% of \textit{A. baumannii} isolates were MDR. In agreement with our results, the high prevalence of MDR \textit{A. baumannii} isolates was reported from other studies, ranged from 49.6\% to 100\%. The multidrug antibiotic resistance has often limited the efficacy of the common therapeutic options especially for the strains that are resistant to carbapenems.
In the current study, the resistance rates to carbapenem agents (imipenem or meropenem) were similar to a previous study by Shoja et al in the same region during 2011 to 2012 years, indicating that the prevalence of MDR A. baumannii isolates is still high in our region.

Our results showed that the antibiotic resistance rates to amikacin, ceftazidime, ceftriaxone, cefepime, ciprofloxacin, cefotaxime, gentamicin, meropenem, piperacillin/tazobactam and piperacillin among MDR A. baumannii strains were more than 90%. Similar to our work,

| Strain | Type | IRI | MEM | COL | Strain | Type | IRI | MEM | COL |
|--------|------|-----|-----|-----|--------|------|-----|-----|-----|
| SF01   | ST01 | 16  | 32  | 0.5 | SF44   | CT13 | 64  | 32  | 1   |
| SF02   | CT01 | 64  | 128 | 1   | SF45   | CT14 | 128 | 64  | 0.25|
| SF03   | CT01 | 64  | 128 | 1   | SF46   | CT14 | 128 | 64  | 0.25|
| SF04   | ST02 | 128 | 64  | 0.5 | SF47   | CT14 | 128 | 64  | 0.25|
| SF05   | ST03 | 256 | 64  | 1   | SF48   | CT14 | 128 | 64  | 0.25|
| SF06   | CT02 | 1   | 1   | 2   | SF49   | CT14 | 128 | 64  | 0.25|
| SF07   | CT02 | 1   | 1   | 2   | SF50   | ST13 | 64  | 64  | 1   |
| SF08   | CT02 | 1   | 1   | 2   | SF51   | ST14 | 32  | 64  | 8   |
| SF09   | ST04 | 32  | 64  | 0.5 | SF52   | ST15 | 128 | 512 | 0.5 |
| SF10   | CT03 | 0.5 | 0.5 | 0.5 | SF53   | CT15 | 32  | 128 | 1   |
| SF11   | CT03 | 0.5 | 0.5 | 0.5 | SF54   | CT15 | 32  | 128 | 1   |
| SF12   | ST05 | 32  | 64  | 4   | SF55   | CT15 | 32  | 128 | 1   |
| SF13   | ST06 | 128 | 64  | 1   | SF56   | CT15 | 32  | 128 | 1   |
| SF14   | CT04 | 2   | 2   | 2   | SF57   | CT16 | 16  | 64  | 0.25|
| SF15   | CT04 | 2   | 2   | 2   | SF58   | CT16 | 16  | 64  | 0.25|
| SF16   | CT05 | 64  | 256 | 0.5 | SF59   | CT16 | 16  | 64  | 0.25|
| SF17   | CT05 | 64  | 256 | 0.5 | SF60   | CT16 | 16  | 64  | 0.25|
| SF18   | CT05 | 64  | 256 | 0.5 | SF61   | CT16 | 16  | 64  | 0.25|
| SF19   | CT06 | 32  | 64  | 0.25| SF62   | CT16 | 16  | 64  | 0.25|
| SF20   | CT06 | 32  | 64  | 0.25| SF63   | CT17 | 256 | 512 | 0.5 |
| SF21   | CT06 | 32  | 64  | 0.25| SF64   | CT17 | 256 | 512 | 0.5 |
| SF22   | CT07 | 2   | 2   | 1   | SF65   | CT17 | 256 | 512 | 0.5 |
| SF23   | CT07 | 2   | 2   | 1   | SF66   | CT17 | 256 | 512 | 0.5 |
| SF24   | CT08 | 2   | 4   | 1   | SF67   | ST16 | 64  | 128 | 2   |
| SF25   | CT08 | 2   | 4   | 1   | SF68   | CT18 | 32  | 64  | 1   |
| SF26   | CT07 | 0.5 | 0.5 | 0.5 | SF69   | CT18 | 32  | 64  | 1   |
| SF27   | CT09 | 1   | 1   | 1   | SF70   | CT18 | 32  | 64  | 1   |
| SF28   | CT09 | 1   | 1   | 1   | SF71   | CT19 | 512 | 128 | 2   |
| SF29   | ST08 | 128 | 64  | 0.5 | SF72   | CT19 | 512 | 128 | 2   |
| SF30   | ST09 | 512 | 256 | 0.5 | SF73   | CT19 | 512 | 128 | 2   |
| SF31   | CT10 | 16  | 32  | 0.5 | SF74   | CT19 | 512 | 128 | 2   |
| SF32   | CT10 | 16  | 32  | 0.5 | SF75   | ST17 | 64  | 32  | 1   |
| SF33   | ST10 | 512 | 256 | 2   | SF76   | ST18 | 32  | 128 | 0.5 |
| SF34   | ST11 | 32  | 64  | 1   | SF77   | ST19 | 512 | 64  | 0.25|
| SF35   | ST12 | 128 | 64  | 1   | SF78   | ST20 | 128 | 64  | 2   |
| SF36   | CT11 | 16  | 64  | 0.5 | SF79   | CT20 | 1   | 1   | 1   |
| SF37   | CT11 | 16  | 64  | 0.5 | SF80   | CT20 | 1   | 1   | 1   |
| SF38   | CT11 | 16  | 64  | 0.5 | SF81   | CT21 | 32  | 64  | 2   |
| SF39   | CT11 | 16  | 64  | 0.5 | SF82   | CT21 | 32  | 64  | 2   |
| SF40   | CT11 | 16  | 64  | 0.5 | SF83   | ST21 | 16  | 32  | 2   |
| SF41   | CT12 | 32  | 128 | 2   | SF84   | ST22 | 32  | 64  | 0.5 |
| SF42   | CT12 | 32  | 128 | 2   | SF85   | ST23 | 128 | 512 | 0.25|
| SF43   | CT13 | 64  | 32  | 1   |

Abbreviations: CT, clone type; ST, single type; MEM, Meropenem; IRI, Imipenem; COL, Colistin.
Figure 1 Dendrogram of 85 A. baumannii clinical isolates based on ERIC-PCR types.

**Abbreviations:** CT, clone type; ST, single type; W, wound; T, tracheal secretion; B, blood; BL, bronchial lavage; U, urine; Hospital A, Imam Khomeini; B, Taleghani Hospital.
In our study, all isolates were susceptible to polymyxin B which was in concordance with the studies conducted by Najar Peerayeh et al\textsuperscript{35} and Shoja et al\textsuperscript{32} in Iran. However, in contrast to our results, the higher resistance rates to polymyxin B were reported in other regions of Iran, including 14\% in Tehran,\textsuperscript{36} 16\% in Tabriz,\textsuperscript{37} and 11\% in Kermanshah.\textsuperscript{38} It seems that this growing resistance could be due to the excessive usage of this antibiotic in the treatment of severe infections. Surprisingly, the resistance level to polymyxin B in Brazil\textsuperscript{39} was much high (81.5\%). This high resistance might be due to the prolonged use of this antibiotic agent in treatment of carbapenem-resistant \textit{A. baumannii} infections in this country.\textsuperscript{39}

Our results showed that the majority of \textit{A. baumannii} isolates were susceptible to colistin which is in agreement with a previous study\textsuperscript{32} in our region, suggesting polymyxin B and colistin are still the most effective antibiotic agents against MDR \textit{A. baumannii} strains.

In our study, the \textit{bla}\textsubscript{IMP} and \textit{bla}\textsubscript{VIM} allelic variants were recognized as the most common genes encoding MBLs in the majority of isolate with the positive results in the phenotypic detection of MBL. However, in the one isolate that was phenotypically positive for MBL production, any gene encoding MBL was not detected using PCR. It seems that MBL phenotype in this isolate was

### Table 2 Pattern of allelic variants of \textit{bla}\textsubscript{IMP} and \textit{bla}\textsubscript{VIM}

| \textit{bla}\textsubscript{IMP} | \textit{bla}\textsubscript{VIM} |
|-----------------|-----------------|
| (5 strains)     | (5 strains)     |
| (9 strains)     | (9 strains)     |
| (3 strains)     | (3 strains)     |
| (5 strains)     | (5 strains)     |
| (5 strains)     | (5 strains)     |

### Table 3 Distribution of gene cassettes carrying MBLs among integron-positive \textit{A. baumannii} isolates

| Strain No. | Type | IntI1 and gene cassette | IntI2 and gene cassette |
|------------|------|-------------------------|-------------------------|
| SF45       | CT14 | \textit{bla}\textsubscript{VIM-1}, qacED-1 | –                       |
| SF46       | CT14 | \textit{bla}\textsubscript{VIM-1}, qacED-1 | –                       |
| SF47       | CT14 | \textit{bla}\textsubscript{VIM-1}, qacED-1 | –                       |
| SF48       | CT14 | \textit{bla}\textsubscript{VIM-1}, qacED-1 | –                       |
| SF49       | CT14 | \textit{bla}\textsubscript{VIM-1}, qacED-1 | –                       |
| SF02       | CT01 | GES-11, \textit{bla}\textsubscript{IMP-4}, \textit{bla}\textsubscript{VIM-2} | –                       |
| SF03       | CT01 | GES-11, \textit{bla}\textsubscript{IMP-4}, \textit{bla}\textsubscript{VIM-2} | –                       |
| SF12       | ST05 | \textit{bla}\textsubscript{IMP-19}, aacA31, \textit{bla}\textsubscript{OXA-21}, aadA-1 | –                       |
| SF71       | CT19 | \textit{bla}\textsubscript{IMP-19}, aacA31, \textit{bla}\textsubscript{OXA-21}, aadA-1 | –                       |
| SF72       | CT19 | \textit{bla}\textsubscript{IMP-19}, aacA31, \textit{bla}\textsubscript{OXA-21}, aadA-1 | –                       |
| SF73       | CT19 | \textit{bla}\textsubscript{IMP-19}, aacA31, \textit{bla}\textsubscript{OXA-21}, aadA-1 | –                       |
| SF74       | CT19 | \textit{bla}\textsubscript{IMP-19}, aacA31, \textit{bla}\textsubscript{OXA-21}, aadA-1 | –                       |
| SF41       | CT12 | \textit{bla}\textsubscript{VIM-25}, GES-24, qacED-1 | –                       |
| SF42       | CT12 | \textit{bla}\textsubscript{VIM-25}, GES-24, qacED-1 | –                       |
| SF68       | CT18 | \textit{bla}\textsubscript{VIM-25}, aacA7, aadA-1, qacED-1 | DfrA-1, SAT-2, aadA-1   |
| SF69       | CT18 | \textit{bla}\textsubscript{VIM-25}, aacA7, aadA-1, qacED-1 | DfrA-1, SAT-2, aadA-1   |
| SF70       | CT18 | \textit{bla}\textsubscript{VIM-25}, aacA7, aadA-1, qacED-1 | DfrA-1, SAT-2, aadA-1   |
| SF34       | ST11 | \textit{bla}\textsubscript{VIM-25}, GES-24, qacED-1 | –                       |
| SF81       | CT21 | arr-2, cmlA-7, sul-1, qacED-1 | \textit{bla}\textsubscript{VIM-2}, \textit{bla}\textsubscript{WEB}, aacA4 |
| SF82       | CT21 | arr-2, cmlA-7, sul-1, qacED-1 | \textit{bla}\textsubscript{VIM-2}, \textit{bla}\textsubscript{WEB}, aacA4 |
| SF43       | CT13 | \textit{bla}\textsubscript{OXA-21}, arr-3, aadA-1, qacED-1, sul-1 | –                       |
| SF44       | CT13 | \textit{bla}\textsubscript{OXA-21}, arr-3, aadA-1, qacED-1, sul-1 | –                       |
| SF53       | CT15 | \textit{bla}\textsubscript{OXA-21}, aacA-2, aadA-1 | –                       |
| SF54       | CT15 | \textit{bla}\textsubscript{OXA-21}, aacA-2, aadA-1 | –                       |
| SF55       | CT15 | \textit{bla}\textsubscript{OXA-21}, aacA-2, aadA-1 | –                       |
| SF56       | CT15 | \textit{bla}\textsubscript{OXA-21}, aacA-2, aadA-1 | –                       |
| SF85       | ST23 | –                       | \textit{bla}\textsubscript{IMP-4} |

**Abbreviations:** CT, clone type; MBL, Metallo-\beta-Lactamase; ST, single type.
caused by other mechanisms rather than the presence of genes encoding MBLs that unfortunately were not considered in our study.

In consistent with our work, Lee et al. in Seoul found the bla_{IMP} and bla_{VIM} genes allelic variants in most A. baumannii isolates, whereas the bla_{SIM-1} gene was recognized only in a few isolates. However, in contrast to our results, Shahcheraghi et al. in Iran did not find either bla_{IMP} or bla_{VIM} genes, instead the bla_{SPM} gene was recognized in the A. baumannii isolates.

In our study, the phenotypic detection of MBL was negative in one bla_{VIM} -positive isolate and one bla_{IMP} -positive isolate. Similar to our study, Ikonomidis et al. also, reported two A. baumannii isolates harboring bla_{VIM-1} gene which were phenotypically negative for MBL production. Moreover, to find the reason of this phenomenon, the researchers evaluated the bla_{VIM-1} expression in these two isolates, indicating that one of these isolates had a weak P1 promoter, and both these isolates had the inactivated P2 promoters. Hence, the bla_{VIM-1} expression level was reduced significantly and these isolates showed a negative phenotype in MBL test.

The integrons as the mobile genetic elements play an important role in the dissemination of antibiotic resistance determinants among A. baumannii isolates. In recent years, the frequency rates of integrons are increasing, so that they have caused a serious threat for the spread of antibiotic resistance elements. In our study, the prevalence of the intI1 gene was more than the intI2 gene that is in agreement with the results obtained from studies of Huang et al. in China, Japoni et al. and Taherikalani et al. in Iran. However, unlike our study, Mirnejad et al. in Tehran and Ramirez et al. in Buenos Aires found higher frequency of the intI2 gene than the intI1 gene. The difference in data is often dependent on the integron classes of clones which are widely disseminated in the community and nosocomial settings.

Our results showed that class 1 integron is often responsible for transferring the gene cassettes harboring MBLs, especially the bla_{VIM} and bla_{IMP} allelic variants. In consistent with our results, Tsakris et al. and Mendes et al. associated the presence of class 1 integron with gene cassettes encoding bla_{VIM} and bla_{IMP} allelic variants. Moreover, Mendes et al. indicated the presence of the bla_{IMP-1} gene in the gene cassette of bla_{IMP-1, aac(6’)-31, aadA1} which was plasmid located in five of the seven isolates. Also, Goudarzi et al. showed the presence of gene cassettes encoding bla_{VIM} and bla_{IMP} allelic within both class 1 and 2 integrons, suggesting the class 1 integron has the important role in the horizontal transfer of gene cassettes encoding MBLs.46

In our study, the most prevalent gene cassette arrays among positive class 1 integron and MBLs isolates were bla_{IMP-19, aacA31, bla_{OXA-21, aadA1}} and bla_{VIM-1, qacED-1}.

In consistent with our results, Goudarzi et al. showed seven different gene cassettes in 89 class 1 integron-carrying isolates and three gene cassettes in 15 class 2 integron-harboring A. baumannii isolates that among them, five different gene cassettes harbored gene encoding MBLs (VIM-25-GES-24-qacF, IMP-4, VIM-2-VEB-aacA4 and GES-11-IMP-4-VIM-2).

In our study, the majority of gene cassettes encoding MBL genes harbored genes encoding resistance to aminoglycosides as shown in a previous study by Farshadzadeh et al. Moreover, they indicated that gene cassettes encoding resistance to aminoglycosides were present in the majority of MDR A. baumannii isolates, suggesting the high-level resistance rates to aminoglycoside agents among A. baumannii isolates.

Also, according to the results obtained from ERIC-PCR analysis, the isolates belonging to a same clone type had the similar gene cassette array in class 1 and 2 integrons, indicating the importance of molecular typing methods in epidemiological studies for finding the distribution of clonal types disseminated in a hospital or a geographical region.

Conclusion
We demonstrated a high prevalence of resistance to carbapenems, as well as the genes encoding MBLs among MDR A. baumannii isolates. Hence, the results of our study showed that MBLs have an important role in the resistance to carbapenem among MDR A. baumannii isolates. Therefore, the development of simple and inexpensive screening methods for detecting MBL production in microbiology laboratories is essential. In this study, we indicated polymyxins as the only option of effective antibiotic in vitro against MDR A. baumannii isolates. Also, our data revealed that the class I integron had a significant role in the dissemination of bla_{VIM} gene among clinical isolates of A. baumannii in Ahvaz, Iran.

Acknowledgments
This work is a part of M. Sc. thesis of Farkhondeh Saleh Shooshtari which has been approved in the Department of Microbiology of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. The authors thank the Health Research Institute, Infectious and Tropical Diseases...
Research Center, Jundishapur University of Medical Sciences, Ahvaz, Iran for financial support. This study was supported by funds from Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Author contributions
All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure
The authors report no conflicts of interest in this work.

References
1. Ghabavand H, Esfahani BN, Havaei SA, Moghim S, Fazeli H. Molecular identification of Acinetobacter baumannii isolated from intensive care units and their antimicrobial resistance patterns. Adv Biomed Res. 2015;4:110.
2. Hanlon GW. The emergence of multidrug resistant Acinetobacter species: a major concern in the hospital setting. Lett Appl Microbiol. 2005;41(5):375–378. doi:10.1111/j.1472-765X.2005.00791.x
3. Gillings MR. Integrons: past, present, and future. Microbiol Mol Biol Rev. 2014;78(2):257–277. doi:10.1128/MMBR.00056-13
4. Martins N, Picão RC, Adams-Sapper S, Riley LW, Bm M. Association of class 1 and 2 integrons with multidrug-resistant Acinetobacter baumannii international clones and Acinetobacter nosocomialis isolates. Antimicrob Agents Chemother. 2015;59(1):698–701. doi:10.1128/AAC.02415-14
5. Huang C, Long Q, Qian K, et al. Resistance and integrin characterization of Acinetobacter baumannii in a teaching hospital in Chongqing, China. New Microbes New Infect. 2015;8:103–108. doi:10.1016/j.nmn.2015.09.015
6. Quale J, Bratu S, Landman D, Heddarshetti R. Molecular epidemiology of Acinetobacter baumannii endemic in New York City. Clin Infect Dis. 2003;37(2):214–220. doi:10.1086/375875
7. Tasris A, Poulou A, Kristo I, et al. Large dissemination of VIM-2-metallo-β-lactamase-producing pseudomonas aeruginosa strains causing health care-associated community-onset infections. J Clin Microbiol. 2009;47(11):3524–3529. doi:10.1128/JCM.01099-09
8. Bebrone C. Metallo-β-lactamases (classification, activity, genetic organization, zinc structure, co-factor requirement) and their superfamily. Biochem Pharmacol. 2007;74(2):1686–1701. doi:10.1016/j.bcp.2007.05.021
9. Khosravi Y, Tay ST, Vadiuvel J. Analysis of integrons and associated gene cassettes of metallo-β-lactamase-positive Pseudomonas aeruginosa in Malaysia. J Med Microbiol. 2011;60(Pt7):988–994. doi:10.1099/jmm.0.029868-0
10. Anoor KA, Ali FA, Omer SA. Detection of metallo [β]-lactamase enzyme in somegram negative bacteria isolated from burn patients in Sulaimani city, Iraq. Eur Sci J. 2014;10(3):485–496.
11. Zavasci AP, Goldani LZ, Li J, Nation RL. Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. J Antimicrob Chemother. 2007;60(2):1206–1215. doi:10.1093/jac/dkm357
12. Evans ME, Feola DJ, Rapp RP. Polymyxin B sulfate and colistin: old antibiotics for emerging multiresistant gram-negative bacteria. Ann Pharmacother. 1999;33(9):960–967. doi:10.1345/aph.18426
13. Hall GS. Non-fermenting and miscellaneous gram-negative bacilli. In: Mahon CR, Lehman DC, Manuels G, Heights M, editors. Textbook of Diagnostic Microbiology: 4th ed. Mo: Saunders/Elsevier; 2011:482–501.
14. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of Acinetobacter baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. J Clin Microbiol. 2006;44:2974–2976. doi:10.1128/JCM.01021-06
15. CLSI. M100-S28. Performance standards for antimicrobial susceptibility testing; Twenty-eight informational supplement; 2018.
16. Manchanda V, Sanchaita S, Singh N. Multidrug resistant acinetobacter. J Glob Infect Dis. 2010;2(3):291–304. doi:10.4103/0974-777X.68538
17. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Impenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol. 2002;40(10):3798–3801.
18. Tavakol M, Montaz M, Mohajeri P, Shokoolizadeh L, Tajbakhsh 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. doi:10.1186/s13756-018-0405-2
19. Koellemann JGM, Stooft J, Van Der Bijl MW, Cmje V-G, Savelkoul PHM. Identification of epidemic strains of Acinetobacter baumannii by integrase gene PCR. J Clin Microbiol. 2001;39(1):8–13. doi:10.1128/JCM.39.1.8-13.2001
20. Fallah F, Noori M, Hashemi A, et al. Prevalence of blaNDM, blaPER, blaVEB, blalMP, and blalVM Genes among Acinetobacter baumannii isolated from Two Hospitals of Tehran, Iran. Scientifica (Cairo). 2014;2015.
21. Aksoy MD, Çavuşlu Ş, Tuğrul HM. Investigation of Metallo Beta lactamases and oxacilinases in carbapenem resistant Acinetobacter baumannii strains isolated from inpatients. Balkan Med J. 2015;32(1):79–83. doi:10.5152/balkanmedj.2015.15302
22. Mlynarcik P, Roderova M, Kolar M. Primer evaluation for PCR and its application for detection of carbapenemases in enterobacteriaceae. Jundishapur J Microbiol. 2016;9(1):e29314. doi:10.5812/jjm
23. Moura A, Henriques I, Ribeiro R, Correia A. Prevalence and characterization of integrons from bacteria isolated from a slaughterhouse wastewater treatment plant. J Antimicrob Chemother. 2007;60(6):1243–1250. doi:10.1093/jac/dkm340
24. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870–1874. doi:10.1093/molev/msw054
25. Gaynes R, Edwards JR. National nosocomial infections surveillance system. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis. 2005;41(6):848–854. doi:10.1086/444499
26. Banerjee T, Mishra A, Das A, Sharma S, Banman H, Yadav G. High prevalence and endemcity of multidrug resistant Acinetobacter spp. in intensive care unit of a tertiary care hospital, Varanasi, India. J Pathog. 2018;2018:9129083. doi:10.1155/2018/9129083
27. Begum S, Hasan F, Hussain S, Ali Shah A. Prevalence of multidrug resistant Acinetobacter baumannii in the clinical samples from Tertiary care hospital in Islamabad, Pakistan. Pak J Med Sci. 2013;29(5):1253–1258.
28. De Francesco MA, Ravizzola G, Peroni L, Bonfanti C, Manca N. Prevalence of multidrug-resistant Acinetobacter baumannii and Pseudomonas aeruginosa in an Italian hospital. J Infect Public Health. 2013;6(3):179–185. doi:10.1016/j.jiph.2012.11.006
39. Gentilucci GL, Gomes DB, Souza MJ, Carvalho KR, Villas-Bôas MH. Emergence of polymyxin B-resistant Acinetobacter baumannii in hospitals in Rio de Janeiro. J Bras Patol Med Lab. 2016;52(2):91–95.

40. Lee K, Yum JH, Yong D, et al. Novel acquired metallobeta-lactamase gene, bla(SIM-1), in a class 1 integron from Acinetobacter baumannii clinical isolates from Korea. Antimicrob Agents Chemother. 2005;49(11):4485–4491. doi:10.1128/AAC.49.11.4485-4491.2005

41. Shahcheraghi F, Abbasalipour M, Feizabadi M, Ebrahimipour G, Akbari N. Isolation and genetic characterization of metallo-β-lactamase and carbapenemase producing strains of Acinetobacter baumannii from patients at Tehran hospitals. Iran J Microbiol. 2011;3(2):68–74.

42. Ikonomidis A, Ntokou E, Maniatis AN, Tsakis A, Pourmaras S. Hidden VIM-1 metallobeta-lactamase phenotypes among Acinetobacter baumannii clinical isolates. J Clin Microbiol. 2008;46(1):346–349. doi:10.1128/JCM.01670-07

43. Ramírez MS, Stietz MS, Vilacoba E, et al. Increasing frequency of class 1 and 2 integrons in multidrug-resistant clones of Acinetobacter baumannii reveals the need for continuous molecular surveillance. Int J Antimicrob Agents. 2011;37(2):175–177. doi:10.1016/j.ijantimicag.2010.10.006

44. Japoni S, Japoni A, Farshad S, Ali AA, Jamalidoust M. Association between existence of integrons and multi-drug resistance in Acinetobacter isolated from patients in southern Iran. Pol J Microbiol. 2011;60(2):163–168.

45. Tsakis A, Ikonomidis A, Pourmaras S, et al. VIM-1 metallo-beta-lactamase in Acinetobacter baumannii. Emerg Infect Dis. 2006;12(6):981–983.

46. Mendes RE, Castanheira M, Toleman MA, Sader HS, Jones RN, Walsh TR. Characterization of an integron carrying blaIMP-1 and a new aminoglycoside resistance gene, aac(6')-31, and its dissemination among genetically unrelated clinical isolates in a Brazilian hospital. Antimicrob Agents Chemother. 2007;51(7):2611–2614. doi:10.1128/AAC.00838-06

47. Goudarzi H, Azad M, Seyejavadi SS, et al. Characterization of integrons and associated gene cassettes in Acinetobacter baumannii strains isolated from intensive care unit in Tehran, Iran. J Acute Dis. 2016;5(5):386–392. doi:10.1016/j.joad.2016.08.004

48. Farshadzadeh Z, Hashemi FB, Rahimi S, et al. Wide distribution of carbapenem resistant Acinetobacter baumannii in burns patients in Iran. Front Microbiol. 2015;6:1146. doi:10.3389/fmicb.2015.01146