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ORIGINAL RESEARCH

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

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Mansour Amin[1,2]
Tahereh Navidifar[2]
Farkhondeh Saleh Shooshtari[2]
Hamed Goodarzi[2]

[1]Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran;
[2]Department 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 bla_{OXA-51}. The presence of genes encoding MBLs, including bla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{SPM}, bla_{GIM}, bla_{DIM} and bla_{NDM}, as well as *intI 1* and *intI 2* 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 *intI 1*, 10 had only *intI 2* and 15 had both *intI 1* and *intI 2*. The phenotypic detection of MBLs was found in 30 isolates, among which bla_{VIM} was as the most common the gene encoding MBL followed by bla_{IMP}, bla_{SPM} and bla_{SIM}. 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, bla_{VIM}, bla_{IMP}, 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: Farkhondeh 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

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bacteria.[3] To date, several classes of integrons have been described; among them, class 1 and 2 integrons are frequently reported from MDR *A. baumannii* strains.[4,5]

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.[6]

More specially, the infections caused by Metallo-Beta -Lactamase (MBL)-producing organisms are associated with the high rates of morbidity and mortality.[7] MBLs belong to class B beta-lactamases that can hydrolyze all beta-lactam classes except monobactams.[8] MBLs are usually encoded on the gene cassettes harboring class 1 integron and disseminated easily in bacterial populations.[9] To date, several MBLs were recognized such as the bla_{VIM}, bla_{IMP}, bla_{GIM}, bla_{SPM}, bla_{DIM}, bla_{SIM} and bla_{NDM} which of those, the bla_{VIM} and bla_{IMP} allelic variants have emerged as the dominant MBLs worldwide.[8,10] 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.[11] Moreover, recent studies have shown that gram-negative bacilli resistant to aminoglycosides, beta-lactams, and fluoroquinolones are often sensitive to polymyxin B.[12] 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.[13]In addition, the identification of *A. baumannii* isolates was confirmed by the amplification of

$bla_{\text{OXA-51-like}}$ gene using the previously described primers by Turton et al.[14] 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.[15] 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), ceftriaxone (30 µg) and polymexin 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).[15] 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.[16]

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.[17]

ERIC-PCR typing and analysis

The genetic relationship of *A. baumannii* isolates was determined using the enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR)[18] with the primers sequences of ERIC-F (5′-ATGTAAGCTCCTGGGGATTCAC-3′) and ERIC-R (5′AAGTAAGTGACTGGGGTGA GCG-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 Mastercycler Nexus Thermal Cycler Gradient (Eppendorf, Hamburg, Germany) with one cycle of initial denaturation at 94°C for 5 mins, followed by 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_{VIM}, bla_{DIM}, bla_{GIM}, bla_{SIM}, bla_{NDM} and bla_{SPM} in a final volume of 25 µL, as described previously.[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_{VIM} -F, bla_{VIM}-R, bla_{IMP}-F and bla_{IMP} -R; 0.25 pmol/µL each of primers bla_{GIM}-F, bla_{GIM}-R, bla_{DIM}-F and bla_{DIM}-R; and 0.45 pmol/µL each of primers bla_{SIM}-F, bla_{SIM}-R, bla_{NDM}-F, bla_{NDM}-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 $bla_{\text{OXA-51-like}}$ gene, 52°C for the bla_{GIM}, bla_{SIM} and bla_{SPM} genes, as well as 58°C for the bla_{NDM} and bla_{DIM} 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[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[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.ua.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%),

75 (97.4%), 76 (98.7%), 76 (98.7%), 69 (89.6%), 73 (94.8%), 75 (97.4%), 75 (97.4%), 43 (55.8%), 74 (96.1%) and 47 (61.03%) isolates, respectively. Also, all isolates were sensitive to polymyxin B and only two isolates were resistant to colistin. The MICs of carbapenems and colistin among 85 *A.baumanni* isolates are shown in Table 1.

ERIC-PCR analysis

In our study, 85 *A.baumanni* isolates were classified into 21 clone types and 23 single type of ERIC-PCR. Figure 1 is shown the dendrogram of ERIC-PCR of these isolates. Also, Table 1 shows the distribution of MICs of imipenem, meropenem and colistin among these isolates with respect to ERIC-PCR types. According to these results, there was a significant association ($p<0.05$) between the clone types and antibiotic susceptibility to carbapenem agents and colistin.

Detection of genes encoding MBLs and intI1 and intI2

In our study, the frequency rates of the genes encoding bla_{IMP}, bla_{VIM}, bla_{SIM} and bla_{SPM}, among 77 MDR *A. baumannii* isolates were 10 (12.98%), 17 (22.07%), 2 (2.59%) and 4 (5.19%), respectively. In addition, none of the genes encoding bla_{GIM}, bla_{DIM} and bla_{NDM} was detected in these isolates. Also, none of the genes encoding MBLs was detected in non-MDR isolates.

Moreover, 7 isolates carried only the bla_{IMP} gene derivatives, 14 carried only the bla_{VIM} gene derivatives, 3 carried both the bla_{VIM} and bla_{IMP} genes derivatives, 4 carried only the bla_{SPM-1} gene and 2 carried only the bla_{SIM-1} gene. The distribution of allelic variants of bla_{IMP} and bla_{VIM} is shown in Table 2. According to these results, bla_{VIM-2} was the most prevalent variant of bla_{VIM} gene. In this study, the amplification of the *intI 1* and *intI2* genes was performed using PCR. Of the 77 MDR *A. baumannii* isolates, 34 had only *intI 1*, 10 had only *int 2* and 15 had both the *intI 1* and *intI 2* genes.

Association of phenotypic detection of MBL production with genes encoding MBLs

Among 73 carbapenem-resistant *A. baumannii* isolates, 30 were phenotypically as MBL-producing isolates. Moreover, of these 30 isolates, 7 carried only the bla_{IMP} gene derivatives, 14carried only the bla_{VIM} gene derivatives, 2 carried both the bla_{VIM} and bla_{IMP} gene derivatives, 4 carried the bla_{SPM-1} gene and 2 carried the bla_{SIM-1} gene. However, one strain did not carry any gene encoding MBL. Overall, 29 isolates presenting MBL phenotype carried at least one of the MBL genes, confirming the efficacy of the phenotypic detection of MBL producing strains with the PCR results.

On the other hand, the phenotypic detection of MBL was negative for one bla_{VIM} positive *A. baumannii* isolate and one bla_{IMP} positive isolates.

Association of the presence of integrons with genes encoding MBLs among MDR *A. baumannii*

Table 3 indicates the distribution of gene cassettes carrying MBLs among integron-positive *A.baumannii* isolates. Eight gene cassette arrays were detected within class 1 integron and three gene cassette arrays within class 2 integron. The most prevalent gene cassette arrays among positive class 1 integron isolates, bla_{IMP-19},*aacA31*, bla_{OXA-21},*aadA-1* and bla_{VIM-1},*qacED-1*, were detected among 10 isolates. According to these results, bla_{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_{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_{VIM} and bla_{IMP} allelic variants in gene cassettes incorporated into class 1 integron and one isolate carried only bla_{IMP} in gene cassette incorporated into class 1 integron. In addition, 2 isolates carring bla_{VIM} and 2 isolates carring bla_{IMP} were lack either *intI1* or *intI2*.

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

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 *A.baumannii* strains is increasing which will cause a treatment challenge in the future.[25]

The results of our study showed that 90.58% of *A. baumannii* isolates were MDR. In agreement with our results, the high prevalence of MDR *A. baumannii* isolates was reported from other studies, ranged from 49.6% to 100%.[26–31]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[32] 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,

Table 1 Distribution of resistance to meropenem, imipenem and colistin with regard to ERIC PCR types among 85 *A. baumannii* isolates

Strain	Type	IMI	MEM	COL	Strain	Type	IMI	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	ST07	0.5	0.5	2	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; IMI, Imipenem; COL, Colistin.

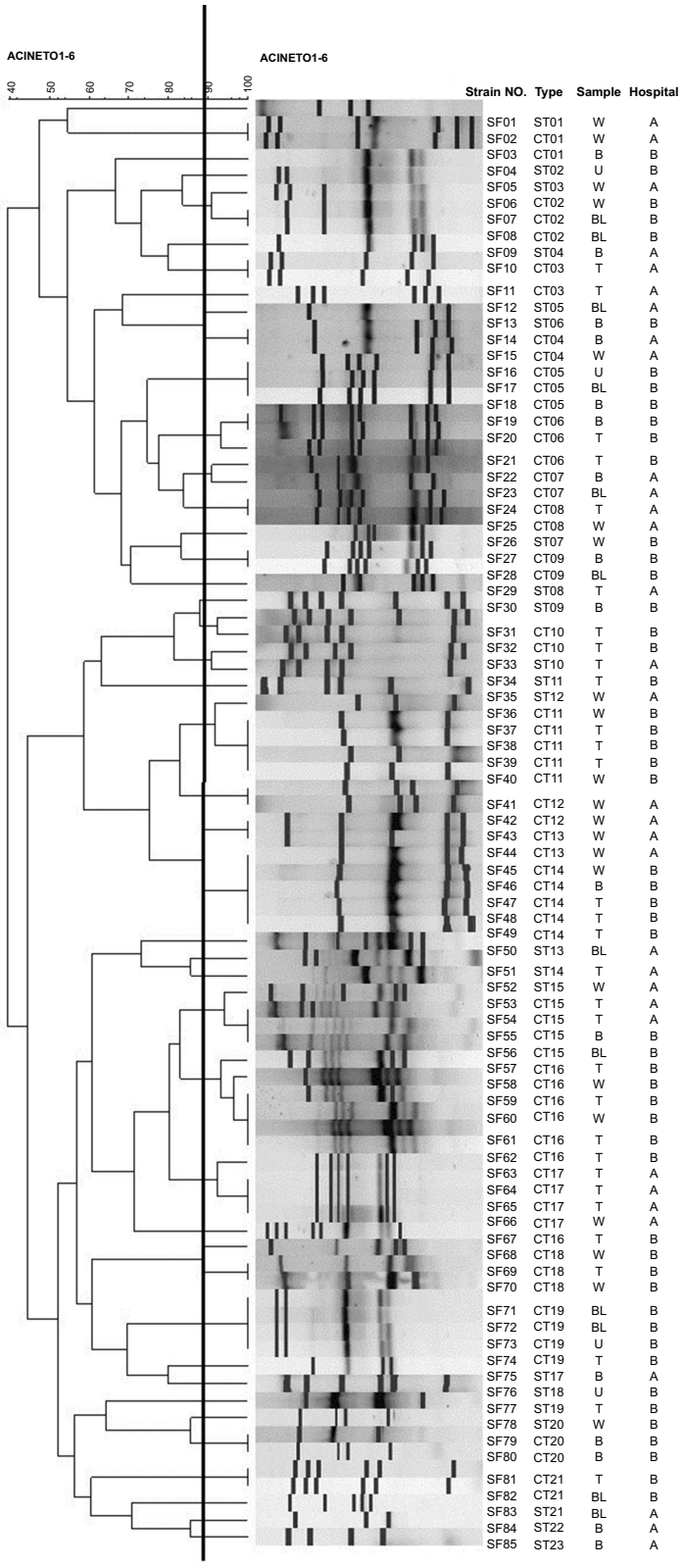

Figure 1 Dendrogram of 85 *A. baumannii* clinical isolates based on ERIC-PCR types.
Abbreviations: CT, clone type; ST, single type; W, burn wound; T, tracheal secretion; B, blood; BL, bronchial lavage; U, urine; Hospital A, Imam Khomeini; B, Taleghani Hospital.

Table 2 Pattern of allelic variants of *blaIMP* and bla_{VIM}

bla_{VIM}	bla_{VIM-1} (5 strains) bla_{VIM-2}(9 strains) bla_{VIM-25}(3 strains)
bla_{IMP}	bla_{IMP-4} (5 strains) bla_{IMP-19}(5 strains)

Mirnejad et al[33], Huang et al[5] and Taherikalani et al[34] also reported the high percentages of the antibiotic resistance among *A. baumannii* isolates.

As mentioned earlier, polymyxins are recommended as the antibiotic choices for MDR *A. baumannii* infections. In our study, all isolates were susceptible to polymyxin B which was in concordance with the studies conducted by Najar Peerayeh et al[35] and Shoja et al[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,[36] 16% in Tabriz[37] and 11% in Kermanshah.[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[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 *A. baumannii* infections in this country.[39] Our results showed that the majority of *A. baumannii* isolates were susceptible to colistin which is in agreement with a previous study[32] in our region, suggesting polymyxin B and colistin are still the most effective antibiotic agents against MDR *A. baumannii* strains.

In our study, the bla_{IMP} and bla_{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 3 Distribution of gene cassettes carrying MBLs among integron-positive *A.baumannii* isolates

Strain No.	Type	IntI1 and gene cassette	IntI2 and gene cassette
SF45	CT14	bla_{VIM-1},*qacED-1*	–
SF46	CT14	bla_{VIM-1},*qacED-1*	–
SF47	CT14	bla_{VIM-1},*qacED-1*	–
SF48	CT14	bla_{VIM-1},*qacED-1*	–
SF49	CT14	bla_{VIM-1},*qacED-1*	–
SF02	CT01	*GES-11*, bla_{IMP-4}, bla_{VIM-2}	–
SF03	CT01	*GES-11*, bla_{IMP-4}, bla_{VIM-2}	–
SF12	ST05	bla_{IMP-19},*aacA31*, bla_{OXA-21},*aadA-1*	–
SF71	CT19	bla_{IMP-19},*aacA31*, bla_{OXA-21},*aadA-1*	–
SF72	CT19	bla_{IMP-19},*aacA31*, bla_{OXA-21},*aadA-1*	–
SF73	CT19	bla_{IMP-19},*aacA31*, bla_{OXA-21},*aadA-1*	–
SF74	CT19	bla_{IMP-19},*aacA-31*, bla_{OXA-21},*aadA-1*	–
SF41	CT12	bla_{VIM-25}, *GES-24*, *qacED-1*	–
SF42	CT12	bla_{VIM-25}, *GES-24*, *qacED-1*	–
SF68	CT18	bla_{VIM-2}, *aacA-7*, *aadA-1*, *qacED-1*	*DfrA-1*, *SAT-2*, *aadA-1*
SF69	CT18	bla_{VIM-2}, *aacA-7*, *aadA-1*, *qacED-1*	*DfrA-1*, *SAT-2*, *aadA-1*
SF70	CT18	bla_{VIM-2}, *aacA-7*, *aadA-1*, *qacED-1*	*DfrA-1*, *SAT-2*, *aadA-1*
SF34	ST11	bla_{VIM-25}, *GES-24*, *qacED-1*	
SF81	CT21	*arr-2*, *cmlA-7*, *sul-1*, *qacED-1*	bla_{VIM-2}, bla_{VEB}, *aacA4*
SF82	CT21	*arr-2*, *cmlA-7*, *sul-1*, *qacED-1*	bla_{VIM-2}, bla_{VEB}, *aacA4*
SF43	CT13	bla_{SIM-1},*Arr-3*, *aadA-1*, *qacED-1*, *sul-1*	–
SF44	CT13	bla_{SIM-1},*Arr-3*, *aadA-1*, *qacED-1*, *sul-1*	–
SF53	CT15	bla_{SPM-1}, *aacA-2*, *aadA-1*	–
SF54	CT15	bla_{SPM-1}, *aacA-2*, *aadA-1*	–
SF55	CT15	bla_{SPM-1}, *aacA-2*, *aadA-1*	–
SF56	CT15	bla_{SPM-1}, *aacA-2*, *aadA-1*	–
SF85	ST23	–	bla_{IMP-4}

Abbreviations: CT, clone type; MBL, Metallo-β-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[40] in Seoul found the bla_{IMP} and bla_{VIM} genes allelic variants in most *A.baumannii* isolates, whereas the $bla_{SIM\text{-}1}$ gene was recognized only in a few isolates. However, in contrast to our results, Shahcheraghi et al[41] 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[42] also, reported two *A. baumannii* isolates harboring $bla_{VIM\text{-}1}$ gene which were phenotypically negative for MBL production. Moreover, to find the reason of this phenomenon, the researchers evaluated the $bla_{VIM\text{-}1}$ 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 $bla_{VIM\text{-}1}$ 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.[43]

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[5] in China, Japoni et al[44] and Taherikalani et al[34] in Iran. However, unlike our study, Mirnejad et al[33] in Tehran and Ramírez et al[43] 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[45] and Mendes et al[46] 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 $bla_{\text{IMP-1}}$ gene in the gene cassette of bla_{IMP1}_ *aac(6')-31*_ *aadA1* which was plasmid located in five of the seven isolates. Also, Goudarzi et al[47] 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 $bla_{IMP\text{-}19}$_*aacA31*_$bla_{OXA\text{-}21}$_*aadA-1* and $bla_{VIM\text{-}1}$_*qacED-1*.

In consistent with our results, Goudarzi et al[47] 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.[48] 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.

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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.

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