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Prevalence of metallo-beta-lactamase producing *Acinetobacter baumannii* isolated from intensive care unit in tertiary care hospitals

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Key words: Acinetobacter baumannii, Intensive Care Unit (ICU), metallo-beta-lactamase
Parole chiave: Acinetobacter baumannii, unità di terapia intensiva, metallo beta-lattamasi

Abstract

Introduction. *The emergence of Metallo-beta-lactamase (MBL)-producing Acinetobacter baumannii has become a global concern in nosocomial infections. The aim of this study is to determine the prevalence of MBL producing genes among clinical isolates of A. baumannii from hospitalized patients.*
Methods. *This study was performed from October 2015 to October 2016 at three teaching hospitals located in Isfahan, Iran. Totally, 100 A-baumannii isolates were collected from clinical specimens and identified as A-baumannii using standard microbiological methods. Antimicrobial susceptibility test was determined by disc diffusion method according to the CLSI. Furthermore, the determination of bla $_{IMP-1}$, bla $_{IMP-2}$, bla $_{VIM-1}$, bla $_{VIM-2}$ and bla $_{SIM-1}$ was detected by PCR.*
Results. *Totally, Sixty-eight percent (68%) of isolates of A. baumannii were recovered from tracheal aspirate. According to the antibiotic susceptibility pattern, the highest level of resistance was against ciprofloxacin (99%), while among tested antibiotics amikacin (10%) was found to be the most effective. 21%, 4%, 7% and 6% isolates carried bla $_{IMP-1}$, bla $_{IMP-2}$, bla $_{VIM-1}$ and bla $_{VIM-2}$ genes, respectively. Also, bla $_{SIM-1}$ was not detected in any of the isolates.*
Conclusion. *The results of this study showed high rate of the MBL producing A-baumannii isolates in our region and displayed that MBLs producing A-baumannii strains are emerging threats to ICUs.*

Introduction

Acinetobacter baumannii is a non-fermenting Gram-negative *Bacilli*, nonmotile and oxidase-negative which is one of the most important opportunistic pathogens in hospitalized patient, especially in the intensive care units (ICU) (1). The first identification of *A. baumannii* in ICU goes back to the1960 (2). *A. baumannii* not only has been commonly discovered in soil and water, but also has been as a commensal bacterium on the skin of 25% of healthy people (1, 3). This organism can cause a wide range of infections, such as pneumonia, urinary tract infections, blood stream infections, meningitis, endocarditis, skin infections and surgical site infections. Some groups such as immunocompromised or burn patients and hematological patients are at higher risk of *A. baumannii* colonization and infections (4). In the recent years, extensive use of antibiotics has led to acquired resistance against a variety of antibiotics

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through various mechanisms (4). This trait is a difficult challenge to the treatment of infections caused by these types of bacteria and is a major concern worldwide. These types of bacteria can resistant to antibiotics with three mechanisms: 1. enzyme production, 2. reduce penetration of the antibiotics to the target sites, or 3. target mutations. Resistant to carbapenems has been increased due to the production of β-lactamases including enzymes of Ambler Classes A, D and B, that their genes mostly transfer via mobile genetic elements such as plasmids or transposons. Resistance to antibiotics belonging to carbapenems is a major concern for the healthcare settings throughout the world. Carbapenems-hydrolyzing metallo-β-lactamase (MBLs) belongs to the group B Ambler classification which is responsible for carbapenem resistance (4-6). This type of beta-lactamases enzymes has zinc as a cofactor in the active site that can help phenotypic detection of MBL producer organisms with ethylenediamine-tetra-acetic acid (EDTA) inhibition as a co-factor inhibitor (6). Several types of MBL genes identified in *A. baumannii*, include of imipenemase (IMP), Sno Paolo metallo (SPM), Verona integron-encoded metallo-beta-lactamases (VIM), Seoul imipenemase (SIM), Japan, Kyorin University Hospital imipenemase (KHM), German imipenemase (GIM), New-Delhi metallo-beta-lactamase (NDM-1) and Australian imipenemase (AIM) (4). IMP gene was first reported in 1980 from Japan and then widespread in around the world (7). Previous studies have reported increasing prevalence of MBL producing genes among clinical isolates of *A. baumannii* (8). However, a few studies have been conducted in our region about the prevalence of MBL producing genes among clinical isolates of *A. baumannii*, therefore, the aim of this study is to determine the prevalence of MBL producing genes among clinical isolates of *A. baumannii* from hospitalized patients.

Materials and methods

Bacterial isolates

In this cross-sectional study, one hundred non-replicate *A. baumannii* were isolated from October 2015 to October 2016 (a one-year period) at three teaching hospitals affiliated to Isfahan University of Medical Sciences Isfahan, Iran. The isolates were collected from different clinical samples such as endotracheal aspirates, sputum, blood, urine, intravenous catheters, aspirates, wounds, tissues and cerebrospinal fluid (CSF) from ICUs inpatients. Primary identification was done by conventional biochemical methods and were also confirmed by the PCR amplification for $bla_{\text{OXA-51}}$ gene as previously described (9). The bacterial isolates were stored in brain heart infusion broth medium containing 20% glycerol at -20°C.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested using the standard Kirby-Bauer disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) guidelines against imipenem (10 μg), meropenem (10 μg), cefepime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), piperacillin-tazobactam (100/10 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), and tetracycline (30 μg) disks (MAST, Merseyside, UK). *Escherichia coli* ATCC 25922 was used as the control strain (10).

Phenotypic detection of MBLs

Combined disk diffusion Test (CDDT) and Double Disk Synergy Test (DDST) were used to determine the MBLs.

Combined Disk Diffusion Test (CDDT)

CDDT was performed by imipenem and meropenem (Mast Group, Merseyside, UK) alone and in combination with EDTA. The increased ≥7 mm inhibition zones of the imipenem-EDTA in comparing with

imipenem disks were considered as MBL positive (11).

Double Disk Synergy Test (DDST)

DDST was performed using disks of IPM and disks of EDTA according to a study done by Lee et al. (12). Any clear extension of the inhibition zone around the carbapenem disk to the EDTA disk wa s interpreted as a positive result (12).

DNA extraction and PCR assay

Total DNAs of the *A. baumannii* isolates were performed according to the protocol as described previously. All carbapenem resistant *A. baumannii* isolates were screened for the presence of $bla_{IMP\text{-}1}$, $bla_{IMP\text{-}2}$, $bla_{VIM\text{-}1}$, $bla_{VIM\text{-}2}$ and $bla_{SIM\text{-}1}$ genes by the previously described methods (9). All used primers in the current study are listed in Table 1.

Statistical analysis

Data were analyzed using SPSS™ software (IBM corp., USA) version 21.0. The results are shown as descriptive statistics in terms of relative frequency. Chi–square exact tests were used to estimate any statistical association. Statistical significance was regarded as P values < 0.05.

Results

During the 12-month period of study one hundred confirmed *A. baumannii* were isolated from ICU inpatients of the different hospitals. Overall, 70 (70%) isolates were obtained from males and 30 (30%) from females. Sixty-eight (68%) isolates of *A. baumannii* were recovered from tracheal aspirate, followed by 10 (10%) from CSF, 9 (9%) from wounds, 3 (3%) from sputum, 3 (3%) from blood, 2 (2%) from catheters and 5 (5%) from other samples.

According to the antibiotic susceptibility pattern, the highest level of resistance was against ciprofloxacin and imipenem, (99% each), while the most effective antibiotics were amikacin (10%), gentamycin (6%) and tetracycline (6%). The total results of the antimicrobial susceptibility pattern of *A. baumannii* were shown in Table 2. Overall, 21 (21%) of isolates were found to be positive for MBL production based on DDST method, while 36 (36%) of isolates showed MBL-positive according to the CDT method.

All strains were also evaluated for the presence of the MBLs gene, and it was shown that 32 (32.0%) were positive for

Table 1 - List of used primers in the present study

Gene	Product size	Sequence	Reference
$bla_{IMP\text{-}1}$	587 (bp)	F (5-ACC GCA GCA GAG TCT TTG CC-3) R (5-ACA ACC AGT TTT GCC TTA CC-3)	9
$bla_{IMP\text{-}2}$	678 (bp)	F (5-GTT TTA TGT GTA TGC TTC C-3) R (5-AGC CTG TTC CCA TGT AC-3)	9
$bla_{VIM\text{-}1}$	261 (bp)	F (5-AGT GGT GAG TAT CCG ACA G-3) R (5-ATG AAA GTG CGT GGA GAC-3)	9
$bla_{VIM\text{-}2}$	801 (bp)	F (5-ATG TTC AAA CTT TTG AGT AAG-3) R (5-CTA CTC AAC GAC TGA GCG-3)	9
$bla_{SIM\text{-}1}$	570 (bp)	F (5-TAC AAG GGA TTC GGC ATC G-3-) R(5- TAA TGG CTT GGT CCC ATG TG-3-)	9
$bla_{OXA\text{-}51\text{-}like}$	353 (bp)	F (5′-TAA TGC TTT GAT CGG CCT TG-3′) R (5′-TGG ATT GCA CTT CAT CTT GG-3′)	9

Table 2 - The antibiotic susceptibility testing results of *A. baumannii* isolates

Antibiotic	Susceptible, n (%)	Intermediate, n (%)	Resistant, n (%)
Imipenem	1 (1.0%)	0 (0.0%)	99 (99.0%)
Meropenem	1 (1.0%)	1 (1%)	98 (98.0%)
Cefepime	2 (2.0%)	0 (0.0%)	98 (98.0%)
Ceftriaxone	0 (0.0%)	3 (3.0%)	97 (97.0%)
Ceftazidime	3 (3.0%)	1 (1.0%)	96 (96.0%)
Piperacillin/tazobactam	1 (1.0%)	1 (1.0%)	98 (98.0%)
Tetracycline	6 (6.0%)	17 (17.0%)	77 (77.0%)
Ciprofloxacin	1 (1.0%)	0 (0.0%)	99 (99.0%)
Amikacin	10 (10.0%)	9 (9.0%)	81 (81.0%)
Gentamycin	6 (6.0%)	2 (2.0%)	92 (92.0%)

MBLs gene; that $bla_{\text{IMP-1}}$ was positive in 21 (21%) and $bla_{\text{IMP-2}}$, $bla_{\text{VIM-1}}$ and $bla_{\text{VIM-2}}$ were detected in 4 (4%), 7 (7%) and 6 (6%), respectively. In addition, *sim*-1 was not detected in any MBL production isolates. According to Table 3, the isolates that have more MBLs genes among clinical samples are those recovered from tracheal aspirate, showing the prevalence of $bla_{\text{IMP-1}}$ higher than other MBLs genes, while isolates coming from blood samples only show *bla*-vim genes.

Discussion and Conclusions

A. baumannii is one of the most important agents in nosocomial infections and is causing a wide range of diseases, especially in the ICUs (13). In the present study, all of the samples were collected from the ICUs during one year, the high rate of isolation shows that *A. baumannii* is a common nosocomial pathogen in ICUs, and high rates of antibiotic resistance, specially carbapenems resistance, demonstrate that we need alternative strategies to treat this type of infection.

Based on our results, more than half of the isolates were recovered from patients with respiratory infections (68.0%) and the smallest recovery was from urine samples (1.0%).

Noori et al. (4) showed that 52.8% among 108 *A. baumannii* isolates were from respiratory tract specimens. In another study, Carvalho et al. (14) detected 56.3% *A. baumannii* isolates from tracheal secretion.

Table 3 - The rate of resistance genes among type of samples

Type of sample	Total number (n=100)	Male (n=70)	Female (n=30)	$bla_{\text{IMP-1}}$ (n=21)	$bla_{\text{IMP-2}}$ (n=4)	$bla_{\text{VIM-1}}$ (n=7)	$bla_{\text{VIM-2}}$ (n=6)	$bla_{\text{SIM-1}}$ (n=0)
Tracheal	68	47	21	16	2	3	4	0
CSF	10	6	4	1	1	2	1	0
Wound	9	7	2	1	1	0	0	0
sputum	3	3	0	1	0	0	0	0
Blood	3	1	2	0	0	2	1	0
catheter	2	2	0	0	0	0	0	0
others	5	5	0	2	0	0	0	0

These results were in accordance with our studies which report that the respiratory tract was the commonest site of infection.

The antibacterial susceptibility assay demonstrates that the highest rate of resistance was against imipenem and ciprofloxacin (99.0%) and the most effective antibiotic was amikacin with 10% sensitivity. Our results are in accordance with previous studies in Iran, which have shown that the emergence of resistant *A. baumannii* strains is increasing throughout our Country (15). Therefore, amikacin and colistin were used as the first choice drug for the treatment of infections caused by *A. baumannii* isolates.

Carbapenem-resistant *A. baumannii* can significantly prolong hospitalization, cause a high mortality rate and increase the medical costs; therefore, early investigation and detection of molecular characteristics of carbapenem-resistant *A. baumannii* can provide an effective health policy to the control of carbapenem-resistant *A. baumannii* (16-21).

DDST and CDT methods are recommended for phenotypic identification MBL enzymes by CLSI (22). According to our results, 21% and 36% of the 99 (99%) carbapenem-resistant *A. baumannii,* were found to be MBL producers by DDST and CDT test, respectively. In a similar study conducted by Pandya et al. (23) it was shown that 96.3% of isolates were MBL positive using CDT and 81.4% were positive using DDST. Peymani et al. (24) described that among 63 carbapenems non-susceptible *A. baumannii* isolates, 31 (49%) were found to be MBL producers, which is relatively similar to our results. In contrast with our study, Noori et al (4) reported that 86.86% of isolates were MBL positive, and Irfan et al. (25) showed a higher rate of positive results by CDT method. In the current study, from a total of 36 MBL producing isolates, 29% were positive for MBL genotypes. The highest frequency of them belonged to bla_{IMP} (21%), followed by bla_{IMP-2}, bla_{VIM-1}, bla_{VIM-2} (4.0%, (7.0% and 6.0%, respectively). Based on the results of this study, the phenotypic and the genotypic results of the detection of MBL producing isolates were different, which can be due to the presence of other interfering genes and other resistance mechanisms for MBL production. In a study whose results are similar to our findings, from a total of 63 carbapenem-resistant *A. baumannii*, 31 (49%) were found to be MBL producers by MIC-test strips, of which 19 isolates carried bla_{IMP} and 9 carried bla_{VIM} genes (24). In addition, in a study conducted in Shiraz, Moghadam et al. (26) reported 43 MBL producing isolates, 53.4% and 32.6% of the isolates carried bla_{IMP} and bla_{VIM} genes, respectively.

In a previous study done in northern Iran, Davoodi et al. (27) revealed that 6.7%, 41.7%, 50% and 1.7% of *A. baumannii* isolates were positive for bla_{VIM-1}, bla_{VIM-2}, bla_{IMP-1} and bla_{IMP-2} genes, respectively. Similar to other studies in Iran, our results showed that bla_{SIM-1} was not detected in any of *A. baumannii* isolates, probably also there may be specific genotypic characters against antimicrobial agents in *A. baumannii* isolates that could lack bla_{SIM-1} (21). Based on previous studies in the world, there was a variation in the frequencies of these genes, which are due to several factors, such as geographical areas, infection control programs and inappropriate use of antibiotics, especially in ICUs (28-31).

There are some limitations related to the present study. First, we have not assessed all mechanism of the carbapenem-resistance, since some of carbapenem-resistant isolates lack any MBLs genes. Second, because we didn't use any typing method, the association of clinical isolates was not clarified. In conclusion, beside some limitations possibly associated with the characteristics of our study, our results showed a high rate of the carbapenem-resistant *A. baumannii* isolates in our region. In addition, these results display that MBLs producing *A. baumannii* strains are an emerging threat to ICUs: therefore, early investigation is suggested

for preventing the emergence of more resistance and the spread of this pathogen in our region.

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Conflict of interest
None declared.

Riassunto

Prevalenza di *Acinetobacter baumannii* produttore di metallo beta-lattamasi in unità di terapia intensiva in grandi ospedali per acuti

Introduzione. L'emergenza legata agli *Acinetobacter baumannii* produttori di metallo-beta-lattamasi (MBL) è divenuta una preoccupazione generale nell'ambito delle infezioni nosocomiali. L'obiettivo di questo studio è di determinare la prevalenza di geni produttori di MBL tra gli isolati per *A. baumannii* nei pazienti ospedalizzati.

Metodi. Questo studio è stato condotto tra ottobre 2015 ed ottobre 2016 in due ospedali universitari collocati in Isfahan, Iran. In totale, 100 *A. baumannii* isolati sono stati raccolti da campioni clinici ed identificati come *A. baumannii* utilizzando comuni metodiche microbiologiche. Il test di suscettibilità antimicrobica è stato determinato mediante il metodo per diffusione su piastra in accordo con CLSI. Inoltre, la determinazione di $bla_{\text{IMP-1}}$, $bla_{\text{IMP-2}}$, $bla_{\text{VIM-1}}$, $bla_{\text{VIM-2}}$ e $bla_{\text{SIM-1}}$ è stata indagata mediante PCR.

Risultati. In totale, il 68% degli *A. baumannii* isolati sono stati ottenuti da aspirato tracheale. In accordo con il modello di suscettibilità antibiotica, i più alti livelli di resistenza erano nei confrontio della ciprofloxacina (99%), mentre tra gli antibiotici testati l'amikacina (10%) è risultata la più efficace con 21%, 4%, 7% e 6% degli isolati trasmessi dai geni $bla_{\text{IMP-1}}$, $bla_{\text{IMP-2}}$, $bla_{\text{VIM-1}}$ e $bla_{\text{VIM-2}}$, rispettivamente. Inoltre, $bla_{\text{SIM-1}}$ non è stato rilevato in alcun isolato.

Conclusioni. I risultati di questo studio hanno dimostrato un elevato tasso di *A. baumannii* produttori di MBL nelle nostre regioni ed hanno dimostrato che i ceppi di *A. baumannii* produttori di MBL costituiscono una minaccia emergente per le unità di terapia intensiva.

References

1. Borgmann S, Wolz C, Gröbner S, et al. Metallo-beta-lactamase expressing multi-resistant *Acinetobacter baumannii* transmitted in the operation area. J Hosp Infect 2004; **57**: 308-15.
2. Stirland RM, Hillier VF, Steyger MG. Analysis of hospital bacteriological data. J Clin Pathol 1969; **3**: 82-6.
3. Barrie A, Gorman M. *Acinetobacter baumannii*—The New MRSA? Eplasty 2016; **16**: ic10.
4. Noori M, Karimi A, Fallah F, et al. High prevalence of metallo-beta-lactamase producing *Acinetobacter baumannii* isolated from two hospitals of Tehran, Iran. Arch Pediatr Infect Dis 2014; **2**: e15439.
5. Eliopoulos GM, Maragakis LL, Perl TM. *Acinetobacter baumannii*: Epidemiology, Antimicrobial Resistance, and Treatment Options. Clin Infect Dis 2008; **46**: 1254-63.
6. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-β-Lactamases: the Quiet before the Storm? Clin Microbiol Rev 2005; **18**: 306-25.
7. Fallah F, Taherpour A, Vala MH, Hashemi A. Global spread of New Delhi metallo-beta-lactamase-1-(NDM-1). Arch Clin Infect Dis 2011; **7**: 171-7.
8. Zahedi Bialvaei A, Samadi Kafil H, Ebrahimzadeh Leylabadlo H, Asgharzadeh M, Aghazadeh M. Dissemination of carbapenemases producing Gram negative bacteria in the Middle East. Iran J Microbiol 2015; **7**: 226-46.
9. 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**: 2974-6.
10. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; 25th Informational Supplement. M100-S25. Wayne, PA: CLSI, 2015.
11. Aktaş Z, Kayacan ÇB. Investigation of metallo-beta-lactamase producing strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* by E-test, disk synergy and PCR. Scand J Infect Dis 2008; **40**: 320-25.
12. Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge and EDTA-disk synergy tests to screen metallo-beta-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. Clin Microbiol Infect 2001; **7**: 88-91.
13. Shahcheraghi F, Abbasalipour M, Feizabadi M, Ebrahimipour G, Akbari N. Isolation and genetic characterization of metallo-beta-lactamase and carbapenamase producing strains of *Acinetobacter baumannii* from patients at Tehran hospitals. Iran J Microbiol 2011; **3**: 68-74.

14. Carvalho RM, Marques SG, Goncalves LH, Abreu AG, Monteiro SG, Goncalves AG. Phenotypic detection of metallo-beta-lactamases in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from hospitalized patients in Sao Luis, State of Maranhao, Brazil. Rev Soc Bras Med Trop 2013; **46**: 506-9.
15. Jasemi S, Douraghi M, Adibhesami H, et al. Trend of extensively drug-resistant *Acinetobacter baumannii* and the remaining therapeutic options: a multicenter study in Tehran, Iran over a 3-year period. Lett Appl Microbiol 2016; **63**: 466-72.
16. Al Atrouni A, Hamze M, Jisr T, et al. Wide spread of OXA-23-producing carbapenem-resistant *Acinetobacter baumannii* belonging to clonal complex II in different hospitals in Lebanon. Int J Infect Dis 2016; **30**: 29-36.
17. Pagano M, Rocha L, Sampaio JL, Martins AF, Barth AL. Emergence of OXA-72-producing *Acinetobacter baumannii* Belonging to High-Risk Clones (CC15 and CC79) in Different Brazilian States. Infect Control Hosp Epidemiol 2017; **38**(2): 252-4.
18. Zanganeh Z, Eftekhar F. Correlation of Oxacillinase Gene Carriage With the Genetic Fingerprints of Imipenem-Resistant Clinical Isolates of *Acinetobacter baumannii*. Jundishapur J Microbiol 2015; **8**: e26545.
19. Farshadzadeh Z, Hashemi FB , Rahimi S, et al. Wide distribution of carbapenem resistant *Acinetobacter baumannii* in burns patients in Iran. Front Microbiol Oct 20, 2015.
20. Salimizand H, Noori N, Meshkat Z, Ghazvini K, Amel SJ. Prevalence of *Acinetobacter baumannii* harboring ISAba1/bla OXA-23-like family in a burn center. Burns 2015; **41**: 1100-6.
21. Safari M, Mozaffari Nejad AS, Bahador A, Jafari R, Alikhani MY. Prevalence of ESBL and MBL encoding genes in *Acinetobacter baumannii* strains isolated from patients of intensive care units (ICU). Saudi J Biol Sci 2015; **22**: 424-9.
22. Owlia P, Azimi L, Gholami A, Asghari B, Lari AR. ESBL- and MBL-mediated resistance in *Acinetobacter baumannii*: a global threat to burn patients. Infezioni in Medicina 2012; 182e187.
23. Pandya NP, Prajapati SB, Mehta SJ, Kikani KM, Joshi PJ. Evaluation of various methods for detection of metallo β-lactamase (mbl) production in gram negative bacilli. Int J Biol Med Res 2011; **2**: 775-7.
24 Peymani A, Nahaei MR, Farajnia S, et al. High prevalence of metallo-beta-lactamase-producing *acinetobacter baumannii* in a teaching hospital in Tabriz, Iran. Jpn J Infect Dis 2011; **6**: 469-71.
25. Irfan S, Zafar A, Guhar D, Ahsan T, Hasan R. Metallo-beta-lactamase-producing clinical isolates of *Acinetobacter* species and *Pseudomonas aeruginosa* from intensive care unit patients of a tertiary care hospital. Indian J Med Microbiol 2008; **26**: 243-5.
26. Moghadam MN, Motamedifar M, Sarvari J, Sedigh ES, Mousavi SM, Moghadam FN. Emergence of Multidrug Resistance and Metallo-beta-lactamase Producing *Acinetobacter baumannii* Isolated from Patients in Shiraz, Iran. Ann Med Health Sci Res 2016; **6**: 162-7.
27. Davoodi S, Boroumand MA, Sepehriseresht S, Pourgholi L. Detection of VIM-and IMP-type Metallo-Beta-Lactamase Genes in *Acinetobacter baumannii* Isolates from Patients in Two Hospitals in Tehran. Iran J Biotech 2015; **13**: 63-7.
28. Lee K, Ha GY, Shin B-M, et al. Metallo-β-lactamase-producing Gram-negative bacilli in Korean Nationwide Surveillance of Antimicrobial Resistance group hospitals in 2003: continued prevalence of VIM-producing *Pseudomonas* spp. and increase of IMP-producing *Acinetobacter* spp. Diagn Microbiol Infect Dis 2004; **50**: 51-8.
29. Sader HS, Castanheira M, Mendes RE, Toleman M, Walsh TR, Jones RN. Dissemination and diversity of metallo-beta-lactamases in Latin America: report from the SENTRY Antimicrobial Surveillance Program. Int J Antimicrob Agents 2005; **25**: 57-61.
30. Fazeli H, Bafghi MF, Faghri J, Akbari R. Molecular study of PER and VEB genes is multidrug resistant *Pseudomonas aeroginosa* isolated. J. Kerman Univ. Med. Sci 2012; 11: **19**: 345-53.
31. Sedighi M, Hasanzadeh A, Safiri S, et al. Detection of blaSPM-1 Metallo-β-Lactamase Gene in Imipenem-Resistant *Pseudomonas aeruginosa* Strains Isolated From Hospitalized Patients in Isfahan Hospitals, J Arch Mil Med 2015; **3**: e26977

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