

## Molecular Detection of Metallo-Beta-Lactamase genes in Clinical Isolates of *Acinetobacter baumannii*

Hossein Goudarzi<sup>1</sup>, Elnaz Sadat Mirsamadi\*<sup>1</sup>, Zohreh Ghalavand<sup>1</sup>,  
Mojdeh Hakemi Vala<sup>1</sup>, Hamed Mirjalali<sup>2, 3</sup>, Ali Hashemi<sup>1</sup> and Ehsan Ghasemi<sup>1</sup>

<sup>1</sup>Department of Microbiology, Faculty of Medicine,  
Shahid Beheshti University of Medical Sciences, Tehran, Iran.

<sup>2</sup>Foodborne and Waterborne Diseases Research Center, Research institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

<sup>3</sup>Gastroenterology and Liver Diseases Research Center, Research institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

(Received: 10 July 2015; accepted: 09 September 2015)

*Acinetobacter baumannii* is an opportunistic pathogen causing infections in hospitalized patients, particularly in intensive care units (ICUs). The treatment of these infections is one of the most important concerns of physicians due to multidrug-resistant. The  $\beta$ -lactam antimicrobial drugs are choice treatment of infections caused by *A. baumannii*. The specimens included CSF (cerebrospinal fluid), wound, sputum, urine, BAL (Broncho Alveolar Lavage), blood and so on were taken from 128 patients who were hospitalized in several wards. The antimicrobial susceptibility test was carried out using kirby-bauer disc diffusion method for Piperacillin-Tazobactam, Ceftriaxon, Cefazidime, Cefepime, Imipenem, Doripenem, Ertapenem, Cefotaxime, Ampicillin-Sulbactam antibiotics for all isolates. DNA of isolates was extracted and primers were designed and as well as amplification was carried out for *blaIMP*, *blaVIM*, *blaNDM1* genes. The resistance to all discs used in this study was seen in all isolates. The Highest and the lowest rate of resistance were seen in Piperacillin-tazobactam and Ampicillin-sulbactam, 100% and 21% isolates, respectively. The results of PCR of *blaIMP*, *blaVIM* and *blaNDM* genes were as follows: *blaIMP* 5/128 (3.9%), *blaVIM* 9/128(7.03%) and *blaNDM* 0/128(0%). The highest sensitivity to antibiotics group was seen in Ampicillin-Sulbactam groups and also *blaVIM* was the most prevalent gene of resistance among Metallo-Beta-Lactamase genes in *Acinetobacter baumannii* isolates.

**Key words:** Iran, *Acinetobacter baumannii*, Polymerase Chain Reaction (PCR), Metallo-Beta-Lactamase.

*Acinetobacter baumannii* is gram-negative, none-fermenting, nonmotile, oxidase negative, catalase positive and aerobic coccobacilli<sup>1,2</sup>. The bacterium is considered as an opportunistic pathogen in patients hospitalized particularly in intensive care units (ICUs)<sup>3,4</sup>. Wound and urinary infections, meningitis, endocarditis, septicemia and pneumonia are the severe complications resulting from *A.*

*baumannii*<sup>5</sup>. Currently, there are increasing reports of emerging strains that are resistant to various groups of antibiotics including metallo-beta-lactamases (MBL). Therefore, treatment of these infections is one of the most important concerns of physicians<sup>6,7</sup>.

Although, antibiotic therapy is controversial, but the first line drugs are  $\beta$ -lactams, Quinolones and Aminoglycosides antimicrobials. Increasing and incorrect use of these drugs resulted in widespread emerging of antibiotic resistant strains such as Multi Drug Resistant (MDR) strains.

\* To whom all correspondence should be addressed.  
Tel.: +989122839749;  
E-mail: elnaz\_mir62@yahoo.com

MDR-strains attributed to isolates, which are resistant to more than three different following classes: Carbapenems, Aminoglycosides, Ampicillin-Sulbactam, Cephalosporins and Flouroquinolones<sup>8</sup>. Carbapenems are a class of  $\beta$ -lactams that have been prescribed as choice drug of infections caused by *A. baumannii*. Unfortunately, resistance to Carbapenems in *A. baumannii* is being spread worldwide, rapidly<sup>9</sup>. The main mechanism of resistance to carbapenems is acquisition of carbapenemases genes such as MBL. MBLs genes, VIM, IMP and NDM are the most prevalent MBLs, are found on mobile genetic elements such as transposons or plasmids and can distribute throughout the communities of bacteria<sup>10</sup>.

The existence of MBLs genes in *A. baumannii* was detected by PCR (Polymerase Chain Reaction) method. Existence of MBLs genes in *A. baumannii* is reported all over the world. Up to now, several studies in Iran have reported MBLs resistance in *Acinetobacter* species but the rate of resistance is different, according to the targeted populations and also the methods of study<sup>11, 12, 13</sup>. Thus, according to the above subjects, we aimed to study the existence of MBLs genes in *A. baumannii* isolated from clinical specimens such as blood, CSF, wound and also respiratory secretions using new specific primers. Furthermore we determined pattern of antimicrobial susceptibility of isolates.

## MATERIALS AND METHODS

### Patients and Specimens

Sampling was performed on 128 patients who were hospitalized in different wards including ICU, Surgery, Neurosurgery, Orthopedics, Infectious, etc., in two hospitals in Tehran. Specimens included CSF, blood, BAL (Broncho Alveolar Lavage), urine, sputum, wound and secretions of eye, vulva, mouth, throat. All isolates were identified as *Acinetobacter* spp., based on colony characteristics, Gram's staining, motility test, oxidase and alkaline reaction on TSI agar, growth at 44°C, OF-glucose and citrate utilization<sup>14</sup>. For confirmation of species, 353bp fragment of bla<sub>OXA</sub>-51-like gene was amplified in all isolates that identified as *A. baumannii* using morphological and biochemical criteria<sup>15</sup>.

### Drug Susceptibility Test

Drug susceptibility test was carried out using kirby-bauer disc diffusion method for Piperacillin-tazobactam, Ceftriaxon, Ceftazidime, Cefepime, Imipenem, Doripenem, Ertapenem, Cefotaxime, Ampicillin-Sulbactam discs (Mast Co.,UK) regarding to CLSI 2013 guidelines<sup>16</sup>. *Pseudomonase aeroginasa* ATCC 27853 used as quality control.

### DNA Extraction

All *A. baumannii* isolates were incubated overnight at 36°C in LB medium and then DNA extraction was carried out using DynaBio™ Blood/Tissue DNA Extraction Mini Kit (Takapouzist company, Iran, Cat. No: kj0015) based on manufacture instruments.

### Primer Designing and Detection of blaIMP, blaVIM, blaNDM1 genes

Primer designing was performed using an online software Primer-BLAST (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/>) on blaIMP, blaVIM, blaNDM1 genes, which were already deposited in GeneBank database with accession numbers: KF723585, GQ288396 and KF951474, respectively. The sequences of designed primers used in present study were indicated in Table 1. Specificity of primers was checked using non- MBLs resistant isolates of *A. baumannii* and *Pseudomonas* spp. that were characterized previously.

Amplification of the fragments was carried out by single PCR with following amplification conditions: initial denaturation at 95°C for 5 min, followed by 35 cycle (1min at 94°C, 1min at 50°C and 1 min at 72°C) and a final extension for 10 min at 72°C for IMP-1 gene; and initial denaturation for 5 min at 95°C, followed by 35 cycle (1min at 94°C, 1min at 51°C and 1 min at 72°C) and 10 min at 72°C for VIM gene, initial denaturation at 95°C for 5 min, followed by 35 cycle (1min at 94°C, 1min at 48°C and 1 min at 72°C) and a final extension for 10 min at 72°C for NDM1 gene.

The amplified products were electrophoresed on 1.5% agarose gel and then were visualized by ethidium bromide staining. DNA Sequencing was performed based on Sanger Dideoxynucleotides by Bioneer sequencing company. The acquired sequences results were studied by Chromas and MEGA6 softwares.

## RESULTS

Female/Male ratio of the patients that were included in this study was 53/75 (41.4%/58.6) and the mean + standard deviation (sd) of age of patients was 53.54+21.04 year.

The percentage of positive cases isolated from origin sources was as follows: Urine (10.9%), Wound (14.1%), Blood (8.6%), Sputum (56.3%), Catheter (6.3%), Eye (0.8%), CSF (1.6%), Valve (0.8%) and Throat (0.8 %).

The Highest and lowest rate of resistance of isolates were seen in Piperacillin-tazobactam and Ampicillin-Sulbactam, 100% and 21% of isolates, respectively. Moderate resistance was seen in all used discs except in Piperacillin-tazobactam disc. Sensitivity of isolates was just seen in 4 antibiotics including: Cefepime, Imipenem, Doripenem and also Ampicillin-Sulbactam. The results of drug susceptibility test were summarized in table 2.

The expected amplified fragments 172bp, 247bp and 111 bp were attained for *blaIMP*, *blaVIM* and *blaNDM* genes, respectively (Figures 1, 2). The prevalence of these genes in our study was as follows: *blaIMP* 5/128 (3.9%), *blaVIM* 9/128(7.03%) and *blaNDM* 0/128(0%).

Primer-specificity evaluation was showed that none fragment was amplified in non- MBLs resistant isolates of *A. baumannii*. The obtained PCR products were purified and were sequenced using ABI sequencer 3130 and then all sequences were introduced to GeneBank database. Comparing the results with the sequences that were already submitted to GeneBank database previously, confirmed the specificity of the primers and accuracy of PCR products.

## DISCUSSION

One of the capabilities of *A. baumannii* is acquiring resistance to antibiotics that is being used in clinical practices. Several studies have reported worldwide increasing the resistant isolates to broad spectrum of antibiotics including penicillins, cephalosporins, aminoglycosides, quinolones and tetracyclines that have been led to limitation of drug choices<sup>8</sup>.

Resistant rate to tested antibiotics were as follows: Piperacillin-tazobactam 128 (100%), Ceftriaxone 126 (98.4%), Ceftazidime 126 (98.4%), Cefepime 114 (89.1%), Imipenem 116 (90.6%), Doripenem 124 (96.9%), Ertapenem 127 (99.2%),

**Table 1.** Oligonucleotide primers used in different PCR assays.

Designation	Name	Primer Sequence	Fragment length
IMP	IMP-F	TTGACACTCCATTTACTGCTA	172 bp
	IMP-R	TCATTTGTTAATTCAGATGCATA	
	VIM-F	GAGTTGCTTTTGATTGATACAG	
VIM	VIM-R	TCGATGAGAGTCCTTCTAGA	247 bp
	NDM-F	AACACAGCCTGACTTTCG	
NDM	NDM-R	TGATATTGTCACTGGTGTGG	111 bp

**Table 2.** Results of Antimicrobial Susceptibility Test of 128 *A.baumannii* isolates

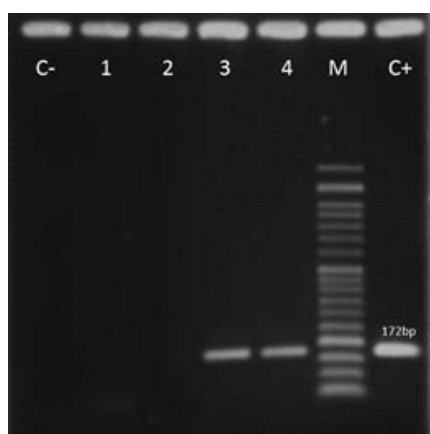
Antibiotics	Resistant, No (%)	Resistance levels Intermediate, No (%)	Sensitive, No (%)
Piperacillin-tazobactam	128 (100%)	0(0%)	0(0%)
Ceftriaxone	126 (98.4%)	2 (1.6%)	0(0%)
Ceftazidime	126 (98.4%)	2 (1.6%)	0(0%)
Cefepime	114 (89.1%)	12 (9.4%)	2 (1.6%)
Imipenem	116 (90.6%)	5 (3.9%)	7 (5.5%)
Doripenem	124 (96.9%)	3 (2.3%)	1 (0.8%)
Ertapenem	127 (99.2%)	1 (0.8%)	0(0%)
Cefotaxime	126 (96.4%)	2 (1.6%)	0(0%)
Ampicillin-sulbactam	27 (21.1%)	10 (7.8%)	91 (71.1)

Cefotaxime 126 (96.4%), Ampicillin-sulbactam 27(21.1%). None of isolates were sensitive to Piperacillin-tazobactam, Ceftriaxone, Ceftazidime, Ertapenem, Cefotaxime. In a study that was performed by Noori and her colleagues the susceptibility test results showed resistant rate of *Acinetobacter* spp., to ceftazidim, Cefotaxime, imipenem, cefepime, piperacillin-tazobactam, Ampicillin-sulbactam were 95.4%, 100%, 91.7%, 95.7%, 95.4%, 98.1%, respectively<sup>12</sup>. In another study, Peymani stated that resistance to ceftazidim, imipenem, cefepime, Piperacillin-tazobactam, Ampicillin-sulbactam, Ceftriaxone were 92%, 54% 88%, 89%, 59%, 94%, respectively<sup>17</sup>. The results of mentioned study were in agreement with our findings that showed high resistance of *Acinetobacter* strains to conventional beta-lactam disks in Iran. The main difference between our susceptibility test results with mentioned studies is the susceptibility rate of *Acinetobacter* isolates to Ampicillin-sulbactam. Our study was also in accordance with Shanthi Amudhan study that showed 100% resistance to ceftazidime, piperacillin-tazobactam and imipenem disks in India<sup>18</sup>. These findings state that *Acinetobacter* have high capability to acquire resistance gene of conventional beta-lactams that are prescribed, routinely. Although several studies have performed on drug susceptibility test using conventional beta lactam disks but there are few reports that were conducted on resistance of *Acinetobacter* spp.,

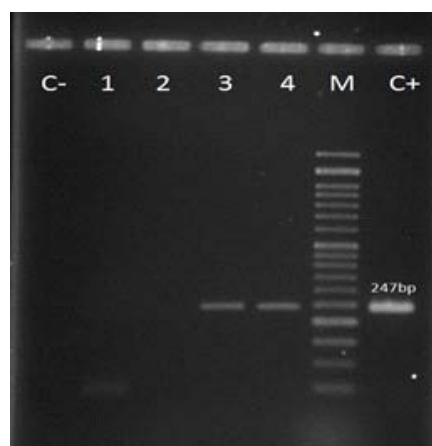
to new carbapenem disks such as Ertapenem and Doripenem. However, in our study, resistant to ertapenem and Doripenem, the new carbapenem disks, was higher than 96%. This finding demonstrates widespread and fast distribution of resistance genes to new drugs throughout *Acinetobacter* strains and increases concerns among clinicians for prescribing more suitable antibiotics in patients, particularly those patients that are hospitalized in intensive care units.

Theoretically, there are four major mechanisms, which may lead to resistance to  $\beta$ -lactam antibiotics: 1. Destruction of the antibiotic by  $\beta$ -lactamases, 2. Modification of the target molecule (i.e. penicillin binding protein), 3. Reduction of antibiotic uptake due to losing porins and 4. Increasing efflux of the drug through the outer membrane<sup>19</sup>. Since one of the most important mechanisms of resistance to betalactam drugs is production of  $\beta$ -lactamases, we investigated prevalence of the most common metallo-beta-lactamases; IMP, VIM, NDM1, using single PCR and new specific primers.

As the first cases of *bla*IMP-1 and *bla*VIM metallo-beta-lactamase genes were seen in Japan and Italy, respectively, the reports of MBLs genes among Gram-negative bacilli have increased, steadily in almost all over the world<sup>20</sup>. In present study, 7.03% of isolates harbored *bla*VIM gene and 3.9% *bla*IMP gene but none isolates harbored *bla*NDM1 gene.



**Fig. 1.** a 172bp fragment of *bla* IMP gene amplified using specific primers. M: marker 50bp; C-: Negative Control; 1, 2, 3 and 4: clinical samples; C+: positive Control



**Fig. 2.** a 247bp fragment of *bla* VIM gene amplified using specific primers. M: marker 50bp; C-: Negative Control; 1, 2, 3 and 4: clinical samples; C+: positive Control

Up to now, several studies had shown various frequencies of metallo-beta lactamase resistant strains belonging to different bacteria. Johann and colleagues showed that the high frequency of *blaVIM* gene in clinical isolates of *Pseudomonas aeruginosa*. In this study the frequency of *blaIMP* was 4%<sup>21</sup>. In a study that was conducted by Amudha and colleagues illustrated that 45.68% and 0.86% of 116 isolates of *A. baumannii* had *blaVIM* and *blaIMP* genes, respectively<sup>22</sup>. In other study that was performed by Ikonmidis, 2 of 87 *A. baumannii* isolates carried *blaVIM* gene<sup>23</sup>. As mentioned above studies and also most of other studies, among MBLs genes *blaVIM* have higher frequency in *A. baumannii* isolates. These studies are in agreement with our finding that demonstrated higher frequency of *blaVIM* gene among *A. baumannii* isolates.

In Iran, *blaVIM* gene is also more prevalent comparing with *blaIMP* gene. Fallah and colleagues showed that *blaVIM* and *blaIMP* genes had a frequency 13.88% and 2.7% of 108 *A. baumannii* isolates, respectively<sup>24</sup>. In another study, Shahcheraghi reported that there were not seen any *blaVIM* and *blaIMP* among *A. baumannii* isolated from hospitalized patients<sup>25</sup>. It is necessary to mention that, the primers that were used in the present study produced shorter products in comparison with other study's primers that this feature increases the chance of detection of MBLs genes, even when the copies of MBLs genes are few or the quantity of DNA is insufficient.

As our finding showed, in accordance to the most of studies that were conducted in Iran, *blaVIM* gene had more prevalent frequency comparing with *blaIMP*, except one study that declared more frequency of *blaIMP* among clinical isolates<sup>17</sup>.

*blaNDM* was another MBLs gene that was surveyed in our study. Since the first case of *blaNDM* gene was reported from a Swedish patient admitted to a hospital in New Delhi, the importance of *Acinetobacter* strains that carry this gene, has being increased, steadily<sup>26</sup>. The bacteria containing *blaNDM1* gene are resistant to almost all antibiotic groups. The *blaNDM1* gene had been reported from *Klebsiella pneumoniae*, *E. coli*, *Enterobacter cloacae*, *Proteus* spp., *Citrobacter freundii*, *k. oxytoca*, *Morganella morganii*, *Providencia* spp., and *A. baumannii*. The ability

of plasmids containing *blaNDM1* gene to transferring among bacterial populations has led to detecting and identifying this gene in clinical<sup>27</sup>. In 2011 Chen reported four isolates of *A. baumannii* that carried *blaNDM1* gene<sup>28</sup>. Decousser and colleagues in 2013 reported an outbreak of carbapenem-resistant NDM-1-producing *A. baumannii* in Europe in a french intensive-care unit<sup>29</sup>. In Iran the first case of multi-drug resistant bacterium containing *blaNDM1* gene was reported by Shahcheraghi in 2012 from a clinical isolate of *K. pneumoniae*<sup>30</sup>. In other studies, there are not proved evidences of presence of *blaNDM1* gene in several bacterial groups. Our findings also showed that there was not any *blaNDM1* gene in *A. baumannii* isolates. Although, there are not proved reports of *blaNDM1* gene in studies that were conducted after Shahcheraghi finding, but increasing rate of immigration and travel between countries increases the chance of emerging resistance to several groups of antibiotics.

However, the high frequency of resistant strains of opportunistic bacteria, particularly *A. baumannii* that are responsible for most of hospitalized infections, increases concerns on selecting an appropriate antibiotic.

## CONCLUSION

The clinical *A. baumannii* isolates showed highest sensitivity to Ampicillin-Sulbactam in drug susceptibility test and on the other hand, *blaVIM* and *blaNDM1* have most prevalent and less prevalent MBLs-resistant genes, respectively.

## ACKNOWLEDGMENT

We would like to thank the Microbiology Department of Shaheed Beheshti University for providing facilities and support this research.

## REFERENCES

1. Cornaglia, G., Giamarellou, H., Rossolini G. M. Metallo-beta-lactamases: a last frontier for beta-lactams? *Lancet Infect Dis.*, 2011; **11**: 381-393.
2. Kaur, A., Gupta, V., Chhina, D. Prevalence of metallo-  $\beta$ -lactamase-producing (MBL) *Acinetobacter* species in a tertiary care hospital. *IRAN. J. Microbiol.*, 2014; **6**(1): 22-25.

3. Dijkshoorn, L., Nemec, A., Seifert, H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol.*, 2007; **5**: 939-951.
4. Abbott, I., Cerqueira, G., Bhuiyan, S., Peleg, A. Carbapenem Resistance in *Acinetobacter baumannii*. *Expert Rev Anti Infect Ther.*, 2013; **11**(4):395-409.
5. Bergogne-Bérézin, E., Towner, K.J. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin. Microbiol. Rev.*, 1996; **9**: 148-65.
6. Hawkey, P. M (a).Molecular epidemiology of clinically significant antibiotic resistance genes. *Brit J Pharmacol.*, 2008; **153**: 406-413.
7. Hawkey, P. M. (b).The growing burden of antimicrobial resistance. *J. Antimicrob. Chemother.*, 2008; **62**: 1-9.
8. Valencia,R., Arroyo, L.A., Conde, M., Aldana, J. M., Torres, M. J., Fernández-Cuenca, F., Garnacho-Montero, J., Cisneros, J. M., Ortíz ,C., Pachón, J., Aznar ,J. Nosocomial epidemic of infection with pan-drug resistant *Acinetobacte baumannii* in a tertiary care university hospital. *Infect. Contr. Hosp. Ep.*, 2009; **30**: 257-263.
9. Peleg, A. Y., Seifert, H., Paterson, D. L. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin. Microbiol. Rev.*, 2008; **21**: 538-582.
10. Walsh, T. R. Emerging carbapenemases: a global perspective. *Int. J. Antimicrob. Agents.*, 2010; **36**: 8-14.
11. Noori, N., Vandyosefi, J., Sabet, F., Ashrafi, S., Ghazvini, K. Frequency of IMP -1 and VIM Genes among Metallo-beta-Lactamase Producing *Acinetobacter* spp. Isolated from Health Care Associated Infections in Northeast of Iran. *J. Med. Bacteriol.* , 2013; **3**(2): 11-16.
12. Noori, M., Karimi ,A., Fallah, F., Hashemi, A., Alimehr, Sh., Goudarzi, H., Aghamohammad , Sh. High Prevalence of Metallo-beta-lactamase Producing *Acinetobacter baumannii* Isolated From Two Hospitals of Tehran, Iran. *Arch. Pediatr. Infect. Dis.*, 2014; **2**(3): e15439.
13. Safari, M., Alikhani, M. Y., Arabestani, M. R., Kamali Kakhki, R., Jafari, R. Prevalence of Metallo-â-lactamases Encoding Genes Among *Pseudomonas aeruginosa* Strains Isolated From the Bedridden Patients in the Intensive Care Units. *Avicenna J. Clin. Microb. Infect.*, 2014; **1**(1): e19216.
14. Winn, W.J., Allen, S., Janda, W., Koneman, E., Procop, G., Schreckenberger, P., Woods, G. (ed): Nonfermenting Gram negative bacilli. Koneman's Color Atlas and textbook of Diagnostic Microbiology, 6nd edn. USA: Lippincott Williams and Wilkins Company, 2006; pp. 305-391.
15. Merkier A. K., Centron D. blaOXA-51 type â-lactamase genes are ubiquitous and vary within a strain in *Acinetobacter baumannii*. *Int. J. Antimicrob. Agent*, 2006; **28**: 110-113.
16. Clinical Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing: Twenty-Third informational supplement* Wayne, PA: CLSI; 2013.
17. Peymani, A., Nahaei, M. R., Farajnia, S., Hasani, A., Mirsalehian, A., Sohrabi, N., Abbasi, L. high prevalence of metallo-beta-lactamase-producing *Acinetobacter baumannii* in a teaching hospital in Tabriz, Iran. *Jpn. J. Infect. Dis.*, 2011; **64**:69-71.
18. Amudhan, M., Sekar, U., Kamalanathan, A., Balaraman, S. blaIMP and blaVIM mediated carbapenem resistance in *Pseudomonas* and *Acinetobacter* species in India. *J. Infect. Dev. Ctries.*, 2012; **6**(11):757-762.
19. Vila, J., Pachon, J. Therapeutic options for *Acinetobacter baumannii* infections. *Expert Opin. Pharmacother.*, 2008; **9**: 587-599.
20. Lee, K., Yim Ha., G., Shin, B., Kimd, J., Kange, J., Jangf S.J., Yong D., Chong, Y., Metallo-beta-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. *Diag. Microb. Infect. Dis.*, 2004; **50**:51-58.
21. Johann, D., Pitout, D., Daniel, B., Poirrel, L., McClure, J., Le, Ph., Church, D. Detection of *Pseudomonas aeruginosa* Producing Metallo - beta-Lactamasesin a Large Centralized Laboratory. *J. Clin. Microbiol.*, 2005; **43**: 3129-35.
22. Amudhan, MS., Sekar, U., Kamalanathan, A., Balaraman, S. blaIMP and blaVIM mediated carbapenem resistance in *Pseudomonas* and *Acinetobacter* species in India. *J.Infect. Dev. Ctries.*, 2012; **6**: 757-62.
23. Ikonomidis, A., Ntokou, E., Maniatis, A. N., Tsakris, A., Pournaras, S. Hidden VIM-1 Metallo-beta-Lactamase Phenotypes among *Acinetobacter baumannii* Clinical isolates. *J. Clin. Microbiol.*, 2008; **46**: 346-9.
24. Fallah, F., Noori, M., Hashemi, A., Goudarzi, H., Karimi, A., Erfanimanesh, S., Alimehr, Sh. Prevalence of blaNDM, blaPER, blaVEB, blaIMP, and blaVIM Genes among *Acinetobacter baumannii* Isolated from Two

- Hospitals of Tehran, Iran. *Scientiûca.*, 2014; **2014**:1-6.
25. Shahcheraghi, F., Abbasalipour, M., Feizabadi, M. M., Ebrahimipour, GH., 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** (2): 68-74
  26. Yong, D., Toleman, M. A., Giske ,C. G., Cho, H. S., Sundman, K., Lee, K, Walsh, T. R. Characterization of a new metallo-beta-lactamase gene, *bla*(NDM-1), and a novel erythromycinesterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob. Agents. Chemother.*, 2009; **53**: 5046–5054
  27. Kumarasamy, K., Toleman, M. A., Walsh, T. R., Bagaria, J., Butt, F. A., Balakrishnan, R., Chaudhary, U., Doumith, M., Giske, C. G., Irfan, S., Krishnan, P., Kumar, A. V., Maharjan, S., Mushtaq,S., Noorie T., Paterson, D. L., Pearson, A., Perry, C., Pike, R., Rao, B., Ray ,U., Sarma , J. B., Sharma, M., Sheridan, E., Thirunarayan, M. A., Turton, J., Upadhyay, S., Warner, M., Welfare, W., Livermore ,D. M., Woodford N. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet. Infect. Dis.*, 2010; **10**: 597–602
  28. Chen, Y., Zhou, Z., Jiang, Y., Yu, Y. Emergence of NDM-1-producing *Acinetobacter baumannii* in China. *J. Antimicrob. Chemother.*, 2011; **66**: 1255–1259
  29. Decousser, J. W., Jansen, C., Nordmann, P., Emirian, A., Bonnin, R. A., Anais, L., Merle, J. C., Poirrel, L. Outbreak of NDM-1-producing *Acinetobacter baumannii* in France. *Euro. Surveill.*, 2013; **18**(31).
  30. Shahcheraghi, F., Nobari, S., Rahmati Ghezelgeh, F., Nasiri, S., Owlia, P., Nikbin, VS., Fooladi, I. First report of New Delhi metallo-beta-lactamase-1-producing *Klebsiella pneumoniae* in Iran. *Microb. Drug. Resist.*, 2013; **19**(1):30-6.