

Emergence of Multidrug Resistance and Metallo-beta-lactamase Producing *Acinetobacter baumannii* Isolated from Patients in Shiraz, Iran

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Abstract

Background: Metallo-beta-lactamase (MβL) enzymes production is one of the most important resistance mechanisms against carbapenems in some bacteria including *Acinetobacter baumannii*. **Aims:** This study was aimed to determine the antimicrobial susceptibility and the prevalence of MβL among carbapenem-resistant isolates of *A. baumannii*. **Materials and Methods:** In this cross-sectional study from October 2012 to April 2013, 98 isolates were identified as *A. baumannii* using Microgen™ kits and confirmed by molecular method. These isolates were tested for antimicrobial susceptibilities by disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines. Carbapenem-resistant isolates were further detected phenotypically by MβL minimal inhibitory concentration (MIC)-test strips, and subsequently positive MβL isolates were confirmed by polymerase chain reaction (PCR). **Results:** Overall, 98% (96/98) of *A. baumannii* isolates were detected as carbapenem-resistant by MIC test. Highest sensitivity to the tested antibiotic with 42.9% (42/98) was observed to colistin. Of 96 carbapenem-resistant isolates, 43 were phenotypically positive for MβL; out of 43 isolates, 37 were confirmed for the presence of MβL genes by PCR. **Conclusion:** The frequency of drug resistance among the clinical samples of *A. baumannii* isolated in our study against most of the antibiotics was very high. Moreover, all MβL producing isolates were multidrug resistance. Therefore, systematic surveillance to detect MβL producing bacteria and rational prescription and use of carbapenems could be helpful to prevent the spread of carbapenem resistance.

Keywords: *Acinetobacter baumannii*, Antibiotic resistance, Carbapenem, Iran, Metallo-beta-lactamase

Introduction

Acinetobacter baumannii is a nonmotile, Gram-negative, nonfermentative, oxidase-negative, and aerobic *Bacilli*, which is one of the most opportunistic pathogens against human.^[1,2] The bacteria are widespread in the environment and are considerably resistant to most antibiotics, low nutrient, and arid condition.^[3] *Acinetobacter* spp. cause a

variety of nosocomial infections, but *A. baumannii* is the prevalent species with high morbidity and mortality, including pneumonia, bacteremia, urinary tract, and skin and soft tissue infections, especially in patients with severe illness.^[1,3,4] At the recent years, the continuously increasing prevalence rates

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of nosocomial infections by multidrug-resistant (MDR) *A. baumannii* in the Intensive Care Units (ICUs) have led to rise mortalities at the hospitals.^[5-7]

A. baumannii has propensity to acquire resistance; a mechanism of this resistance is being characterized by the production of a specific enzyme called metallo-beta-lactamases (MβLs).^[4,7] These enzymes belong to Ambler class B beta-lactamases based on their amino acid sequence homology and to Group 3 according to the Bush classification based on their substrate profiles (imipenem hydrolysis).^[7] These enzymes are inhibited by ethylenediaminetetraacetic acid (EDTA).^[8]

In Iran, several studies have previously been carried out on drug resistance of *A. baumannii*, revealing a high resistance rate to most of the antibiotics.^[9-11] However, a few studies have been conducted in Iran, especially in our region, aiming to find the prevalence of MβL producing genes among clinical isolates of *A. baumannii*. The aims of the present study were to investigate MDR and determine the prevalence of MβL genes among carbapenem-resistant isolates of *A. baumannii* from clinical specimens in Shiraz, Southwest of Iran.

Materials and Methods

Sampling and bacterial isolates

In this cross-sectional study from October 2012 to April 2013, 120 *Acinetobacter* isolates were recovered from clinical specimens. The specimens were randomly collected from patients in different wards of two hospitals, Nemazee and Faghihi in Shiraz, a major city in Southwest of Iran. Nemazee and Faghihi hospitals are the two major tertiary care hospitals with 1000 beds, affiliated to Shiraz University of Medical Science, Shiraz, Iran. Sample size was calculated as about 100 using the formula^[12]:

$$n = \left(\frac{z1 - \frac{a}{2}}{d} \right)^2 pq$$

The study was in accordance with declaration of Helsinki; however, because we only used laboratory clinical specimens and did not harm any of the patients, the local ethics committee waived the need for informed consent.

Totally, 98 isolates were identified as *A. baumannii* isolates using Microgen™ diagnosis Kits. These 98 isolates were recovered from 35 sputum, 15 wound swab, 13 body fluids, 9 blood, 9 urine, 8 endotracheal tube, 5 cerebrospinal fluid, and 4 other samples (included 2 bronchoalveolar lavage, 1 eye swab, and 1 axillary swab). Overall, 53 samples were collected from ICUs and 45 samples from other wards (included gastroenterology, skin, surgery, and transplant) in the two above-mentioned hospitals. Confirmed isolates were kept at -70°C until for long preservation.

Antimicrobial susceptibility testing

The susceptibility of the isolates to 16 antibiotics disk (MAST, UK) was investigated using disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) recommendation.^[13] The test was performed on Mueller-Hinton agar (Merck, Germany) with followed antibiotics disks containing 10 µg imipenem, 10 µg meropenem, 30 µg amikacin, 10 µg gentamicin, 30 µg aztreonam, 30 µg ceftazidime, 5 µg ciprofloxacin, 5 µg levofloxacin, 100 µg piperacillin, 10 µg tobramycin, 25 µg colistin, 20 µg ampicillin/sulbactam, 10 µg ampicillin, and 110 µg piperacillin/tazobactam. *Pseudomonas aeruginosa* ATCC 27853 was used as the control strain in susceptibility testing.

Minimal inhibitory concentration

Minimal inhibitory concentration (MIC)-test strips (Liofilchem, Italy), containing imipenem and meropenem, were used to determine the MIC according to the instruction provided by the company on Muller-Hinton agar (Merck, Germany). Results were interpreted using CLSI criteria.

Phenotypic metallo-beta-lactamase detection

MIC-test strip (Liofilchem, Italy), containing imipenem/imipenem + EDTA, was used to determine the phenotypic MβL enzyme production. MIC-test strip was performed according to the manufacturer's instructions. A reduction in the MIC of imipenem of ≥ 3 dilutions in the presence of EDTA was interpreted as a positive test. In addition, a strain was considered MβL producer if a phantom zone or deformation of the eclipse was observed.

DNA extraction and molecular typing

The isolates which were intermediately resistant or resistant to imipenem or meropenem were considered carbapenem-resistant. Extraction of genomic DNA from *A. baumannii* isolates was performed according to the protocol as previously described.^[14] Polymerase chain reaction (PCR) assay was performed for the detection of *bla*_{OXA-51-like} gene, a 353 base-pair (bp) amplicon, as an internal gene for molecular confirmation of *A. baumannii* isolates at the species level (primer sequence: F/5'-TAA TGC TTT GAT CGG CCT TG-3', R/5'-TGG ATT GCA CTT CAT CTT GG-3'),^[15] and for amplification of MβL encoding genes, *bla*_{IMP}, *bla*_{VIM}, and *bla*_{SPM} using the following consensus primer sets, *bla*_{IMP}: F/5'-GAA GGC GTT TAT GTT CAT AC-3', *bla*_{IMP}: R/5'-GTA TGT TTC AAG AGT GAT GC-3' which amplify a 587 bp amplicon, *bla*_{VIM}: F/5'-GTT TGG TCG CAT ATC GCA AC-3', *bla*_{VIM}: R/5'-AAT GCG CAG CAC CAG GAT AG-3' which amplify a 382 bp amplicon,^[16] and *bla*_{SPM}: F/5'-AAA ATC TGG GTA CGC AAA CG-3', *bla*_{SPM}: R/5'-ACA TTA TCC GCT GGA ACA GG-3' which amplify a 271 bp amplicon.^[17] A *P. aeruginosa* harboring *bla*_{IMP}, *bla*_{VIM}, *bla*_{SPM} genes (obtained from Pasteur Institute of Iran) was used as the positive control.^[18]

Statistical analysis was performed using SPSS™ software version 19.0 (IBM Corp., Armonk, NY, USA). The results for infectious agents and antimicrobial susceptibility presented as descriptive statistics in terms of relative frequency. Chi-square or Fisher's exact test was used to analyze the results wherever they needed. $P < 0.05$ was considered as statistically significant clinical relevance.

Results

Of 98 *A. baumannii* isolates, 54.1% (53/98) of isolates were collected from female patients' samples. Seventy isolates were obtained from Namazee and 28 from Faghihi Hospital. Most of the 98 *A. baumannii* isolates were recovered from sputum ($n = 35$), wound swab ($n = 15$), and body fluids ($n = 13$).

The highest antibiotic resistance rates were observed against aztreonam, ceftazidime, ciprofloxacin, piperacillin, and cefotaxime since all of the tested isolates showed resistance. In addition, no sensitive isolates were seen against aztreonam and cefotaxime; just a few isolates were in the intermediate level of susceptibility. The results of antibiotic susceptibility patterns for the tested isolates are displayed [Table 1].

Totally, 98% (96/98) of the isolates were detected as carbapenem-resistant by MIC-test strip. Among the carbapenem-resistant *A. baumannii* isolates, 44.8% (43/96) were found to be M β L producers by M β L MIC-test strips. Interestingly, the majority 85.7% (84/98) of the isolates showed an MIC ≥ 48 μ g/ml. MIC ranges for the tested isolates to imipenem are presented in Table 2. All phenotypically M β L producing isolates were MDR and exhibited high resistance to beta-lactams, aminoglycosides, and fluoroquinolones. Furthermore, three of the M β L producing isolates were sensitive to ampicillin-sulbactam.

Finally from M β L producing isolates, 86% (37/43) were positive for M β L genotypes, of which 53.4% (23/43) isolates carried bla_{IMP} , 32.6% (14/43) carried bla_{VIM} M β L genes, and none of the isolates carried bla_{SPM} gene. PCR results for amplification of M β L producing genes are displayed in Table 3.

From the total ICUs recovered isolates, 47.2% (25/53) carried one of the bla_{IMP} or bla_{VIM} genes; this rate for isolates from other hospital wards was 26.7% (12/45). Despite the higher proportion for bla_{IMP} and bla_{VIM} gene in ICUs compared to other wards, no significant differences were observed ($P = 0.06$). The frequencies of bla_{IMP} and bla_{VIM} genes for Namazee hospital isolates were 22.9% (16/70) and 12.9% (9/70), respectively. The similar rates for Faghihi Hospital isolates were 25% (7/28) for bla_{IMP} and 17.9% (5/28) for bla_{VIM} genes.

Discussion

One of the carbapenem resistance mechanisms is production of carbapenem-hydrolyzing β -lactamases, which is called

Table 1: Susceptibility patterns of *Acinetobacter baumannii* isolates to 16 tested antibiotics by disc diffusion method

Antibiotic disks	Total (n=98) n (%)		
	Sensitive	Intermediate	Resistance
Meropenem	3 (3.1)	0	95 (96.9)
Imipenem ^a	4 (4.1)	0	94 (95.9)
Amikacin	24 (24.5)	6 (6.1)	68 (69.4)
Gentamicin	13 (13.3)	11 (11.2)	74 (75.5)
Aztreonam	0	0	98 (100)
Cefoxitin	0	0	98 (100)
Ceftazidime	0	0	98 (100)
Ciprofloxacin	0	0	98 (100)
Levofloxacin	0	0	98 (100)
Piperacillin	0	0	98 (100)
Piperacillin-tazobactam	0	0	98 (100)
Ampicillin	0	0	98 (100)
Ampicillin-sulbactam	5 (5.1)	0	93 (94.9)
Colistin	42 (42.9)	0	56 (57.1)

^aResistance rate against imipenem using MIC was 99%, which is higher compared to 96% detected by disc diffusion method. MIC: Minimal inhibitory concentration

Table 2: Distribution of resistance rate of the studied *Acinetobacter baumannii* isolates to imipenem by E-test based on source of isolation

MIC (μ g/mL) sample	Sensitive ≤ 4 μ g/mL	Resistance ≥ 16 μ g/mL							Total number
		2	24	32	48	64	98	128	
Sputum	0	1	2	2	10	9	11	35	
Wound	1	0	0	2	3	4	5	15	
Body fluids	0	1	0	2	1	7	2	13	
Urine	0	1	2	1	2	3	0	9	
Blood	0	0	1	2	2	4	0	9	
ETT ^a	0	2	1	2	2	1	0	8	
CSF ^b	1	0	1	2	0	1	0	5	
Other	0	0	0	2	1	0	1	4	
Total number	2	5	7	15	21	29	19	98	

^aETT: Endotracheal tube, ^bCSF: Cerebrospinal fluid. MIC: Minimal inhibitory concentration

carbapenemase; one of them, M β L is more important in drug resistance against carbapenems.^[7,19]

In the recent years, there have been numerous reports on MDR *A. baumannii* from hospital settings in Iran.^[10-11] In most of these studies, only characterized isolates were obtained from ICUs while this study attempted to determine the resistance among isolates obtained from various medical wards as well. In particular, 45 clinical samples were isolated from wards outside ICUs. Antibiotic susceptibility testing showed that the majority of the isolates were resistant to three or more antibiotics [Table 1] while all the isolates were resistant to imipenem, cefotaxime, and ciprofloxacin, and 95% and 96% of the isolates were resistant to meropenem and amikacin, respectively.

Carbapenems are the best choice for nosocomial infections of *Acinetobacter* in Iran. However, in the recent years, it has been reported that there is reduced susceptibility to imipenem.^[10,11,20]

Table 3: Distribution of metallo-beta-lactamase genes among the studied *Acinetobacter baumannii* isolates

MβL gene	Sample	Place of isolation	Total number
IMP-1	Sputum	ICU	6
IMP-1	Sputum	Gastroenterology	2
IMP-1	Body fluids	ICU	2
IMP-1	Wound	ICU	2
IMP-1	Wound	NICU	1
IMP-1	Wound	Transplant	1
IMP-1	Blood	ICU	2
IMP-1	Blood	Transplant	1
IMP-1	ETT ^a	NICU	2
IMP-1	ETT	ICU	1
IMP-1	Urine	ICU	2
IMP-1	CSF ^b	ICU	1
VIM-2	Sputum	ICU	2
VIM-2	Sputum	Gastroenterology	1
VIM-2	Wound	ICU	2
VIM-2	Wound	NICU	1
VIM-2	Urine	ICU	2
VIM-2	Blood	ICU	1
VIM-2	Blood	NICU	1
VIM-2	Body fluids	ICU	1
VIM-2	CSF	ICU	1
VIM-2	ETT	Gastroenterology	1
VIM-2	Other	NICU	1

^aETT: Endotracheal tube, ^bCSF: Cerebrospinal fluid. MβL: Metallo-beta-lactamase, ICU: Intensive Care Unit, NICU: Neonatal Intensive Care Unit

Further, resistance to colistin, polymyxin, and tigecycline which are the usual choices for nosocomial infections of carbapenem-resistance *Acinetobacter* is increased around the world, especially in Iran.^[10,11,21] Our results suggest colistin as the best choice for *A. baumannii* isolates *in-vitro*; this is in accordance with the finding of Japoni-Nejad *et al.* from Arak, Central area of Iran, that reported colistin and tigecycline as the most effective antibiotic agents against *A. baumannii* isolates.^[10]

In a relatively similar study, Feizabadi *et al.* reported the prevalence of susceptibility of *A. baumannii* to imipenem, meropenem, piperacillin-tazobactam, and amikacin rate of 50.7%, 50%, 42.1%, and 38.2%, respectively.^[22] These rates indicated higher sensitive isolates compared to our rates with 4.1%, 3.1%, 0%, and 24.5%, respectively. Our results showed a higher resistance rate of *A. baumannii* isolates against most of the studied antibiotics which were almost similar compared to some studies carried out throughout Iran and other countries.^[23-25]

In this study, of the 96 (98%) carbapenem-resistant *A. baumannii*, 44.8% were found to be MβL producers by MIC-test strips. Such a high resistance rate to imipenem is not uncommon since two separate studies by Safari *et al.* and Noori *et al.* have previously shown 99% resistance among their tested isolated by MIC-test strips and disk diffusion methods, respectively.^[11,21] Abdalhamid *et al.* from Saudi Arabia, in contrast with Iranian studies, reported lower carbapenem-resistant *A. baumannii* (46/141; 32.6%);

however, MβL was much higher than that of Iranian report since 43 of 46 carbapenem-resistant *A. baumannii* isolates were MβL positive.^[26]

All MβL producing isolates in our study were MDR and exhibited high resistance to beta-lactams, aminoglycosides, and fluoroquinolones. This high rate of MDR in our study was in agreement with a study from our neighboring country, Pakistan, which reported the prevalence of MDR 100% among *A. baumannii* isolates.^[27] Three of our MβL-producing isolates were sensitive to ampicillin-sulbactam.

In the present study, from a total of 43 MβL producing isolates, 86% were positive for MβL genotypes and subsequently 53.4% and 32.6% of the isolates carried bla_{IMP} and bla_{VIM} genes, respectively.

As it is seen in our results, phenotypic and genotypic results of the detection of MβL producing isolates were not the same. Possible reason of higher positive rates for phenotypic MβL production could be the presence of MβL genes other than those we have screened.^[28,29]

In a previous Iranian study conducted in Tabriz, from a total of 63 carbapenem-resistant *A. baumannii*, 31 (49%) were found to be MβL producers by MIC-test strips, of which 19 isolates carried bla_{IMP} and 9 carried bla_{VIM} genes; these rates were remarkably close to our findings.^[20] In addition, Noori *et al.* from the capital of Iran reported a bla_{IMP-1} gene prevalence of 3 of 86 (3.48%) among MβL *A. baumannii* isolates.^[21]

In the current study, no bla_{SPM} gene was detected among *A. baumannii* isolates; previously, two similar attempts for detection of bla_{SPM} gene by Shahcheraghi *et al.* (2009–2010) and Noori *et al.* (2012–2013) were made in Tehran, Iran; only, Shahcheraghi *et al.* was able to detect this gene in 6 out of 100 tested *A. baumannii* isolates.^[18,21] Reports about the prevalence of these genes among *A. baumannii* isolates from other parts of the world have shown different patterns. Abdalhamid *et al.* from Saudi Arabia and Al-Agamy *et al.* from Egypt reported negative PCR results for bla_{IMP} , bla_{VIM} , and bla_{SPM} genes among all MβL producing isolates.^[26,30] However, previously, bla_{IMP} and bla_{VIM} prevalences have been reported from Greece, China, Korea, and India.^[31-34] The present study has some limitations. First, lack of DNA sequencing for knowing the IMP and VIM variants must be mentioned as a limitation of our study. Moreover, employed a typing method could clear the connection between isolates and source of infections in studied hospitals.

Conclusion

The prevalence of drug resistance among the clinical samples of *A. baumannii* isolated in our study against most of the antibiotics is very high. Hence, early detection and optimization of infection control practices are the best defenses

against these organisms. Therefore, systematic surveillance to detect M β L producing bacteria and rational prescription and use of carbapenems could be helpful to prevent the spread of carbapenem resistance.

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Conflicts of interest

There are no conflicts of interest.

References

- Karageorgopoulos DE, Kelesidis T, Kelesidis I, Falagas ME. Tigecycline for the treatment of multidrug-resistant (including carbapenem-resistant) *Acinetobacter* infections: A review of the scientific evidence. *J Antimicrob Chemother* 2008;62:45-55.
- Karah N, Sundsfjord A, Towner K, Samuelsen Ø. Insights into the global molecular epidemiology of carbapenem non-susceptible clones of *Acinetobacter baumannii*. *Drug Resist Updat* 2012;15:237-47.
- Cisneros JM, Reyes MJ, Pachón J, Becerril B, Caballero FJ, García-Garmendía JL, et al. Bacteremia due to *Acinetobacter baumannii*: Epidemiology, clinical findings, and prognostic features. *Clin Infect Dis* 1996;22:1026-32.
- Kuo SC, Chang SC, Wang HY, Lai JF, Chen PC, Shiau YR, et al. Emergence of extensively drug-resistant *Acinetobacter baumannii* complex over 10 years: Nationwide data from the Taiwan surveillance of antimicrobial resistance (TSAR) program. *BMC Infect Dis* 2012;12:200.
- Kooti S, Motamedifar M, Sarvari J. Antibiotic resistance profile and distribution of oxacillinase genes among clinical isolates of *Acinetobacter baumannii* in Shiraz teaching hospitals, 2012-2013. *Jundishapur J Microbiol* 2015;8:e20215.
- Zheng W, Yuan S, Li L. Analysis of hospital departmental distribution and antibiotic susceptibility of *Acinetobacter* isolated from sputum samples. *Am J Infect Control* 2013;41:e73-6.
- Maltezou HC. Metallo-beta-lactamases in Gram-negative bacteria: Introducing the era of pan-resistance? *Int J Antimicrob Agents* 2009;33:405.e1-7.
- Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2002;40:3798-801.
- HakemiVala M, Hallajzadeh M, Fallah F, Hashemi A, Goudarzi H. Characterization of the extended-spectrum beta-lactamase producers among non-fermenting Gram-negative bacteria isolated from burnt patients. *Arch Hyg Sci* 2013;2:1-6.
- Japoni-Nejad A, Sofian M, Belkum AV, Ghaznavi-Rad E. Nosocomial outbreak of extensively and pan drug-resistant *Acinetobacter baumannii* in tertiary hospital in central part of Iran. *Jundishapur J Microbiol*. 2013;6:e9892.
- Safari M, Saidijam M, Bahador A, Jafari R, Alikhani MY. High prevalence of multidrug resistance and metallo-beta-lactamase (M β L) producing *Acinetobacter baumannii* isolated from patients in ICU wards, Hamadan, Iran. *J Res Health Sci* 2013;13:162-7.
- Oladeinde B, Ekejindu I, Omoregie R, Aguh O. Awareness and knowledge of ergonomics among Medical Laboratory Scientists in Nigeria. *Ann Med Health Sci Res*. 2015;5:423-7.
- CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 24th Informational Supplement. M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
- Sambrook J, Russell DW. Purification of nucleic acids by extraction with phenol: Chloroform. *CSH Protoc* 2006;2006.pii: pdb.prot4455.
- 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.
- Khosravi AD, Mihani F. Detection of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* strains isolated from burn patients in Ahwaz, Iran. *Diagn Microbiol Infect Dis* 2008;60:125-8.
- Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* 2011;70:119-23.
- 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;3:68-74.
- Ben RJ, Yang MC, Hsueh JC, Shiang JC, Chien ST. Molecular characterisation of multiple drug-resistant *Acinetobacter baumannii* isolates in southern Taiwan. *Int J Antimicrob Agents* 2011;38:403-8.
- Peymani A, Nahaei MR, Farajnia S, Hasani A, Mirsalehian A, Sohrabi N, et al. High prevalence of metallo-beta-lactamase-producing *Acinetobacter baumannii* in a teaching hospital in Tabriz, Iran. *Jpn J Infect Dis* 2011;64:69-71.
- Noori M, Karimi A, Fallah F, Hashemi A, Alimehr S, Goudarzi H, 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.
- Feizabadi MM, Fathollahzadeh B, Taherikalani M, Rasoolinejad M, Sadeghifard N, Aligholi M, et al. Antimicrobial susceptibility patterns and distribution of blaOXA genes among *Acinetobacter* spp. Isolated from patients at Tehran hospitals. *Jpn J Infect Dis* 2008;61:274-8.
- Rahbar M, Mehrgan H, Aliakbari NH. Prevalence of antibiotic-resistant *Acinetobacter baumannii* in a 1000-bed tertiary care hospital in Tehran, Iran. *Indian J Pathol Microbiol* 2010;53:290-3.
- Akbari M, Niakan M, Taherikalani M, Feizabadi MM, Azadi NA, Soroush S, et al. Rapid identification of Iranian *Acinetobacter baumannii* strains by single PCR assay using BLA oxa-51-like carbapenemase and evaluation of the antimicrobial resistance profiles of the isolates. *Acta Microbiol Immunol Hung* 2010;57:87-94.

25. Hamzeh AR, Al Najjar M, Mahfoud M. Prevalence of antibiotic resistance among *Acinetobacter baumannii* isolates from Aleppo, Syria. *Am J Infect Control* 2012;40:776-7.
26. Abdalhamid B, Hassan H, Itbaileh A, Shorman M. Characterization of carbapenem-resistant *Acinetobacter baumannii* clinical isolates in a tertiary care hospital in Saudi Arabia. *New Microbiol* 2014;37:65-73.
27. Begum S, Hasan F, Hussain S, Ali Shah A. Prevalence of multi drug resistant *Acinetobacter baumannii* in the clinical samples from tertiary care hospital in Islamabad, Pakistan. *Pak J Med Sci* 2013;29:1253-8.
28. Meletis G, Exindari M, Vavatsi N, Sofianou D, Diza E. Mechanisms responsible for the emergence of carbapenem resistance in *Pseudomonas aeruginosa*. *Hippokratia* 2012;16:303-7.
29. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: Mechanisms and epidemiology. *Clin Microbiol Infect* 2006;12:826-36.
30. Al-Agamy MH, Khalaf NG, Tawfick MM, Shibl AM, El Kholy A. Molecular characterization of carbapenem-insensitive *Acinetobacter baumannii* in Egypt. *Int J Infect Dis* 2014;22:49-54.
31. Tsakris A, Ikonomidis A, Pournaras S, Tzouveleki LS, Sofianou D, Legakis NJ, *et al.* VIM-1 metallo-beta-lactamase in *Acinetobacter baumannii*. *Emerg Infect Dis* 2006;12:981-3.
32. Chen Z, Qiu S, Wang Y, Wang Y, Liu S, Wang Z, *et al.* Coexistence of blaNDM-1 with the prevalent blaOXA23 and blaIMP in pan-drug resistant *Acinetobacter baumannii* isolates in China. *Clin Infect Dis* 2011;52:692-3.
33. Lee K, Lee WG, Uh Y, Ha GY, Cho J, Chong Y; Korean Nationwide Surveillance of Antimicrobial Resistance Group. VIM- and IMP-type metallo-beta-lactamase-producing *Pseudomonas* spp. and *Acinetobacter* spp. in Korean hospitals. *Emerg Infect Dis* 2003;9:868-71.
34. Amudhan MS, Sekar U, Kamalanathan A, Balaraman S. bla (IMP) and bla (VIM) mediated carbapenem resistance in *Pseudomonas* and *Acinetobacter* species in India. *J Infect Dev Ctries* 2012;6:757-62.

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Sheahan P, O'leary G, Lee G, Fitzgibbon J. Cystic cervical metastases: Incidence and diagnosis using fine needle aspiration biopsy. *Otolaryngol Head Neck Surg* 2002;127:294-8.
- Only the references from journals indexed in PubMed will be checked.
- Enter each reference in new line, without a serial number.
- Add up to a maximum of 15 references at a time.
- If the reference is correct for its bibliographic elements and punctuations, it will be shown as CORRECT and a link to the correct article in PubMed will be given.
- If any of the bibliographic elements are missing, incorrect or extra (such as issue number), it will be shown as INCORRECT and link to possible articles in PubMed will be given.