JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Molecular study of *blaVIM* and *blaIMP* genes in *Acinetobacter baumannii* strains isolated from burn patients in Duhok City, Iraq

Haval Mohammed Khalid[1]

[1] *Department of Biology, Faculty of Science, University of Zakho, Kurdistan Region, Iraq*

Abstract

Introduction: *Acinetobacter baumannii* (*A. baumannii*) is an opportunistic pathogenic bacterium mainly associated with hospital acquired infections and in immunocompromised individuals who stay in hospitals for a long time. In recent years, it has become increasingly resistant to many different types of antibiotics. The production of the metallo-beta-lactamase (MBL) enzyme is one of the primary causes of this resistance. This study aimed to detect the presence of MBL genes that belong to the verona integrin metallo-β-lactamase (*bla-VIM*) and imipenemase (*bla-IMP*) groups in the isolates of *Acinetobacter baumannii* from burn patients.

Methodology: One hundred and seventeen (117) isolates of *A. baumannii* were obtained from patient specimens using traditional methods followed by using the VITEK 2 (BioMérieux, Les Pennes-Mirabeau, France) identification system. Metallo β-lactamases were detected in the imipenem-resistant strains by using imipenem disks on Muller-Hinton agar. The polymerase chain reaction (PCR) technique was utilized to examine 117 isolates for the detection of MBLs encoding genes such as *bla-VIM*, and *bla-IMP*.

Results: Imipenem resistance was detected in 78.6% of the patients. The PCR assays of the isolates identified *bla-VIM-1*, *bla-VIM-2*, *bla-IMP-1* and *bla-IMP-2* genes at the rates of 17%, 40.1%, 29.9% and 4.2%, respectively.

Conclusions: The findings suggest that the majority of *A. baumannii* isolates harbour one or more of the detected genes, signifying that the production of MBLs plays a pivotal role in resistance mechanisms.

Key words: *Acinetobacter baumannii*; metallo-β-lactamases; *bla-VIM*; *bla-IMP*.

J Infect Dev Ctries 2024; 18(1):101-105. doi:10.3855/jidc.18570

(Received 19 May 2023 – Accepted 19 July 2023)

Introduction

Acinetobacter baumannii, is a member of family Moraxellaceae. It is a Gram-negative coccobacillus and does not undergo fermentation. Although most members of genus *Acinetobacter* are present in the environment and generally do not cause human disease, certain *Acinetobacter baumannii* strains have evolved to exhibit resistance to multiple drugs, leading to the emergence of respiratory, bloodstream, and skin infections in recent years. This particular microorganism poses a significant challenge in hospitals, particularly in the intensive care units (ICU) where managing several infections can be problematic [1,2].

One of the main public health concerns, particularly in developing countries, is the prevalence of infections resulting from burn injuries [3]. It is estimated that infections with *A. baumannii* account for nearly 75% of fatalities among patients with burn injuries [4]. *A. baumannii* is a common hospital-acquired infection that can result in high levels of illness and death among patients who are hospitalized, especially those in burn and intensive care units, and it is estimated that it causes nearly 75% of the fatalities among burn patients [5].

Nowadays bacterial resistance to antibiotics is a major issue worldwide, especially in the case of *A. baumannii*. Carbapenems are currently the primary medications used for the treatment of infections caused by multidrug-resistant *A. baumannii*. Nonetheless, there is growing concern as carbapenem resistance among *A. baumannii* isolates may become more widespread [6]. Resistance of *A. baumannii* to carbapenems could be caused by a variety of factors, such as the development of beta-lactamases, changes in proteins that bind to penicillin and outer membrane proteins, and an excess of efflux pumps [7]. Huang and colleagues [8] reported that the synthesis of β-lactamase enzymes is a critical factor contributing to the antibiotic resistance observed in *A. baumannii*. The genes encoding these enzymes are located on mobile genetic elements such as integrons.

Beta-lactamases are divided into four groups based on their amino acid sequence (A, B, C, and D). The class B-lactamases (MBLs) and D-type carbapenemase (OXA-type) are frequently linked to carbapenem

resistance [9]. MBLs are of greater significance than other types of β-lactamases because of their capability to break down a broad spectrum of β-lactam antibiotics, specifically carbapenems [10].

A number of MBLs genes, including verona integrin metallo-β-lactamase (*bla-VIM*) and imipenemase (*bla-IMP*), have been found in *A. baumannii* isolates. Globally, Gram-negative bacteria, including Enterobacterales and non-fermenting microorganisms such as *Acinetobacter* spp., have been identified as producing different *bla-IMP* type enzymes [11].

Research conducted recently showed increase in the incidence of MBLs producing *A. baumannii* isolates globally, though there are variable prevalences in different geographical regions [11].

The goal of the current investigation was to identify MBL-encoding genes in the clinical isolates of *A. baumannii* from burn patients.

Methodology

Specimens' collection and identification

This cross-sectional study was performed on patients with infected burn wounds who were referred to a private medical health centre in Duhok city, Iraq. A total of 117 sterilised swab samples of *A. baumannii* had been isolated from burn patients between January 2019 and December 2021. These isolates were identified using, Gram staining and MacConkey agar growth. Additionally, standard recommended tests such as catalase and oxidase tests were carried out. Following the manufacturer's instructions, a VITEK 2 system (BioMérieux, Les Pennes-Mirabeau, France) that uses identification of Gram-negative bacilli card (ID-GNB) to identify bacterial species was used to further confirm the presence of *Acinetobacter baumannii*. All swabs were taken from patients who were not administered antibiotics for the last 3 days, and those who were taking antibiotics were excluded from the study.

Imipenem sensitivity tests

The susceptibility patterns to imipenem were determined using imipenem disks on Muller-Hinton agar and the disk diffusion technique. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI).

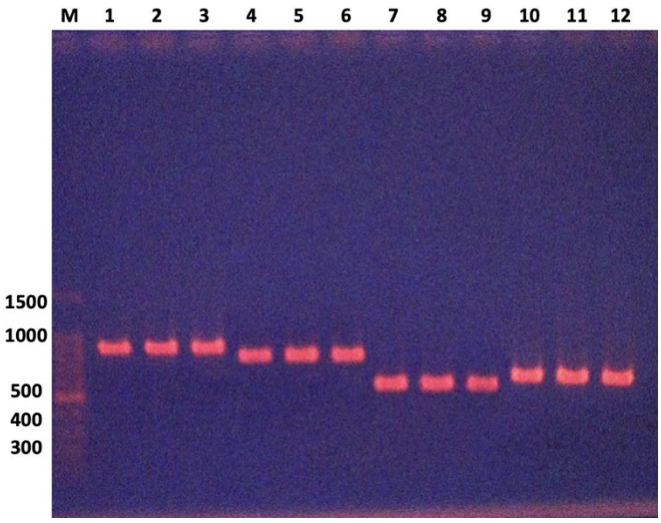
Molecular methods

The genomic DNA was extracted using the PrimePrep™ Genomic Extraction Kit for DNA (GeNetBio, Daejeon, South Korea). DNA was measured for determining its concentration and purity with the use of a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and used for polymerase chain reaction (PCR) amplification. Four different primer pairs were used: bla-VIN1, bla-VIN2, bla-IMP1, and bla-IMP2 (Table 1, Figure 1).

PCR amplification and gel electrophoresis

A total of 20 µL reaction mixture were used for the PCR amplification reaction. The reaction mix comprised of 5 µL of the master mix, 2 µL each of the

Figure 1. PCR amplification of the four MBL-genes.



Lane M: DNA ladder (1500-100 pb), lane 1-3: bla-VIM1(924 bp), lane 4-6: bla-VIM2 (801 bp), lane 7-9: bla-IMP1 (587), lane 10-12: bla-IMP2 (678 bp).

Table 1. Primer sequences utilized in this research.

Primer	Primer sequence	Amplicon size (bp)	Ref.
bla-VIM1	F: 5'-TATATGTGAGACAGCATACGCATGAT-3' R: 5'-ATAAAGGTACCCGC-TCACAATCGA-3	924	[25]
Bla-VIM2	F: 5'-ACTGTATCATACTTTCTGAGAT-AAG-3' R: 5'- CTACATCATACGATCTGAGAGG-3	801	
bla-IMP1	F: 5'-ATCCGACAGCCAGAACTGCTTGTGCAC-3 R: 5'- ACTAACTCACGTTTATGCTC-TTGACC-3'	587	
bla-IMP2	F: 5'-GATTTCTATGCTGCTA-TGACTTAC-C-3' R: 5'AGACCTTGTATCCCGATGATAC-3'	678	

Table 2. Amplification cycle conditions.

Primer	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Cycles
bla-VIM1	95 ºC, 4 min	95 ºC, 1 min	55 ºC, 30 sec	72 ºC, 45 sec	72 ºC, 7 min	35
bla-VIM2	95 ºC ,4 min	95 ºC, 1 min	53 ºC, 1 min	72 ºC, 1.5 min.	72 ºC, 7 min	35
bla-IMP1	95 ºC ,4 min	95 ºC, 1 min	53 ºC, 1 min	72 ºC, 1.5 min.	72 ºC, 7 min	35
bla-IMP2	95 ºC ,4 min	95 ºC, 1 min	53 ºC, 1 min	72 ºC, 1.5 in.	72 ºC, 7 min	35

primers (with a concentration of 10 pmol/µL), 2 µL of DNA (with a concentration of 25-50 ng/µL), and 9 µL of PCR deionized distilled water. Table 2 presents the conditions under which the amplification was carried out. Following amplification, electrophoresis was used to separate the PCR products using a 1.2% (w/v) agarose solution prepared in 1x Tris-Boric EDTA (TBE) buffer. The molecular weight of the DNA bands was determined using a 50-1000 bp ladder, and the resulting DNA bands on the agarose gel were observed under a UV transilluminator.

Results

A total of 117 *A. baumannii* isolates were recovered from patients referred to a private medical health centre in Duhok city during three years, of which 73 (62.3%) and 44 (37.6%) were isolated from burn wounds of male and female patients respectively. Out of these isolates, 25 (21.3%) were sensitive to imipenem, while 92 (78.6%) were resistant (Table 3).

The PCR findings showed that 20 (17.1%), 47 (40.1%), 35 (29.9%), and 5 (4.2%) of the isolates carried *VIM-1*, *VIM-2*, *IMP-1*, and *IMP-2* genes, respectively. Out of the 117 isolates analyzed, there were 9 (7.6%) that harboured both *bla-VIM1* and *bla-VIM2* genes, 18 (15.3%) that carried both *bla-IMP1* and *bla-VIM2* genes, 3 (2.5%) that had both *bla-IMP2* and *bla-VIM1* genes, and 31 (26.4%) that lacked all of these genes (Table 4).

Discussion

Acinetobacter baumannii, is a Gram-negative bacterium, frequently isolated from ICU patients with burn wounds. It poses a significant risk due to its potential for opportunistic infections, high mortality rates, and its ability to develop resistance to multiple drugs. In recent years, the clinical relevance of *A. baumannii* has grown considerably, largely because of its capacity to acquire resistance quickly, which further exacerbates the challenge of treating patients infected with this bacterium. β-lactamases production is the most significant contributor to the development of resistance against β-lactam antibiotic in this bacterium [12].

Acinetobacter baumannii has been found to be resistant to the majority of lactamases, making imipenem a relatively ineffective antibiotic against this pathogen. A hindrance to this mechanism, however, comes in the form of a group of enzymes called MBLs, which have been identified in only a limited number of bacterial species such as *P. aeruginosa* and *A. baumannii*.

Gordon and Wareham noted that resistance to imipenem is becoming increasingly common in *Acinetobacter*, and one of the ways in which this bacterium becomes resistant is by acquiring genes that encode MBLs [1]. These genes include *VIM-1*, *VIM-2*, *IMP-1*, and *IMP-2*.

The present study findings indicate that the frequency of resistance to imipenem among *A. baumannii* strains at a private clinical health centre was 78.6%. This high prevalence of resistance against imipenem is in accordance to the results of numerous other studies such as, Smail and AL-otrachi in Erbil [13] who stated that 100% of their isolates were resistant to imipenem and meropenem. Another study performed by Abd El-Hady and Abdelhadi [14] in Egypt demonstrated that 82.8% of the patient's exhibited resistance to imipenem. Similarly, Das *et al*.

Table 3. Distribution among genders and imipenem susceptibility of *Acinetobacter baumannii* isolates.

Characteristic	n (%)
Gender	
Male	73 (62.3)
Female	44 (37.6)
Total	117 (100)
Imipenem susceptibility	
Sensitive	25 (21.3)
Resistant	92 (78.6)
Total	117 (100)

Table 4. Distribution patterns of bla-VIM-1, bla-VIM-2, bla-IMP-1 and bla-IMP-2 genes among *Acinetobacter baumannii* isolates.

Genes	n (%)
bla-VIM1	20 (17.1)
bla-VIM2	47 (40.1)
bla-IMP1	35 (29.9)
bla-IMP2	(4.2)
bla-VIM1+ bla-VIM2	9 (7.6)
bla-VIM2 + bla-IMP1	18 (15.3)
bla-VIM1+ bla-IMP2	3 (2.5)
None of them	31 (26.4)

[15] recorded that 71.21% of *A. baumannii* isolates collected were resistant to imipenem. In another study, Hoang *et al*. [16], found that more than 80% of *A. baumannii* isolates were resistant to carbapenem. Furthermore, a cross-sectional study performed by Nikibakhsh *et al*. [17] in Iran showed that 100 % of the *A. baumannii* isolates examined exhibited resistance to imipenem. Moreover, the current results contradict with those of Scott *et al*. [18] as they found that 10% of *A. baumannii* strains isolated from American service members who had been hurt in Iraq in 2007 were carbapenem-resistant. Furthermore, Hawley *et al.* [19] discovered that 37% of the 142 strains of *A. baumannii* isolated from American service members wounded in Iraq during 2007 were resistant to imipenem. In addition, a thorough investigation conducted by Unal and Garcia-Rodriguez in Latin America between 2002 and 2004 concluded that meropenem or imipenem resistance was evident in 29 % of *Acinetobacter* isolates [20].

The findings of this study demonstrated a different level of imipenem resistance in *A. baumannii* compared to previous studies. These variations could be attributed to geographical differences, variations in medical facilities, and the use of diverse antibiotic regimens.

In the current study, the percentages of *VIM-1*, *IMP-2*, *IMP-1*, and *IMP-2* genes in *A. baumannii* were 17.1%, 40.1%, 29.9%, and 4.2%, respectively. However, in a study conducted by Abd El-Hady and Abdelhadi in Egypt, *bla-VIM* gene expression was found to be higher than other genes, while *bla-IMP* gene expression was not found [14]. Variable rates of *VIM-I* and *IMP-I* genes were reported in a number of studies in Iran, as 17.44 and 3.48%, 18.18% and 5.3% and 96.2% and 58.5% [4,11,17], respectively.

In contrast, Ruiz *et al*. examined 83 *A. baumannii* isolates in Spain and found that none of them had *VIM* and *IMP* genes [21]. In addition, Shahcheraghi *et al*. examined 100 *A. baumannii* strains from more than one hospital in Tehran, Iran, and did not detect any *bla-VIM* or *bla-IMP* genes MBL-encoding genes [22]. Furthermore, a study conducted by Ikonomidis *et al*. in Greece, identified only 2 (2.3%) isolates carrying *bla-VIM1* gene among the 87 isolates of *A. baumannii* examined [23]. On the other hand, Azim *et al.* conducted a study in India, by investigating 38 *Acinetobacter baumannii* isolates, and reported that 20 (52.6%) of the isolates carried *bla-IMP* genes and 14 (36.8%) had *bla-VIM* genes [23].

It is evident that significant variations are present in the frequency of *bla-VIM* and *bla-IMP* type MBL encoding genes across different areas and years. These differences may be attributed to several factors including geographical locations, antibiotic therapies, and the number of analyzed isolates. In order to address this discrepancy and to obtain an accurate statistic on the occurrence of MBL expression genes, an overall study is suggested. This study should encompass samples from multiple countries, collected at a specific time interval, and with complete patient and specimen data including the location of collection, hospitalization ward, and antibiotic action protocol. A consistent and well-defined methodology should be employed for data collection and analysis.

In this study, it was found that 91.3% of the strains had at least one of the four mentioned genes, representing that MBL production is one of the primary methods of resistance to imipenem by *A. baumannii* in the hospitals. However, 8.7% of the isolates in this study did not have these genes, and it is possible that other mechanisms of resistance were responsible for their imipenem resistance. It is also possible that other MBL enzymes, which were not studied in this research, may have caused resistance in the isolates lacking these four genes.

Conclusions

In order to prevent the dissemination of imipenem-resistant bacteria in hospitals it is crucial to reduce the use of key antibiotics like imipenem, implement accurate health protocols for preventing transmission through personnel or hospital equipment, and regularly estimate the incidence of MBL-expression genes and resistance to imipenem among *A. baumannii* strains.

Additionally, the high prevalence of MBL-coding genes and the potential for resistance transfer between different bacteria and strains underscores the importance of taking proactive measures to combat antibiotic resistance.

Acknowledgements

The author expresses gratitude to the biology department, College of Science, Zakho University, Duhok Province, Iraq, for providing research facilities for this work, and the private clinical center for providing the samples.

Ethics approval

All specimens involving participants were approved by the Ethics Committee of the University of Zakho (Approval no.: BSCUoz/03/01/2023).

References

1. Gordon NC, Wareham DW (2010) Multidrug-resistant *Acinetobacter baumannii*: mechanisms of virulence and resistance. Int J Antimicrob Agents 35: 219-226. doi: 10.1016/j.ijantimicag.2009.10.024.

2. Eze EC, Chenia HY, El Zowalaty ME (2018) *Acinetobacter baumannii* biofilms: effects of physicochemical factors, virulence, antibiotic resistance determinants, gene regulation, and future antimicrobial treatments. Infect Drug Resist 11: 2277-2299. doi: 10.2147/IDR.S169894.
3. Church D, Elsayed S, Reid O, Winston B, Lindsay R (2006) Burn wound infections. Clin Microbiol Rev 19: 403-434. doi: 10.1128/CMR.19.2.403-434.2006.
4. Tarashi S, Goudarzi H, Erfanimanesh S, Pormohammad A, Hashemi A (2016) Phenotypic and molecular detection of metallo-beta-lactamase genes among imipenem resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains isolated from patients with burn injuries. Arch Clin Infect Dis 11: e39036. doi: 10.5812/archcid.39036.
5. Akrami F, Ebrahimzadeh NA (2019) *Acinetobacter baumannii* as nosocomial pathogenic bacteria. Mol Genet Microbiol Virol 34: 84-96. doi: 10.3103/S0891416819020046.
6. Pournajaf A, Rajabnia R, Razavi S, Solgi S, Ardebili A, Yaghoubi S, Khodabandeh M, Yahyapour Y, Emadi B, Irajian G (2018) Molecular characterization of carbapenem-resistant *Acinetobacter baumannii* isolated from pediatric burns patients in an Iranian hospital. Trop J Pharm Res 17: 135-141. doi: 10.4314/tjpr.v17i1.19.
7. Naas T, Oueslati S, Bonnin RA, Dabos ML, Zavala A, Dortet L, Retailleau P, Iorga BI (2017) Beta-lactamase database (BLDB) - structure and function. J Enzyme Inhib Med Chem 32: 917-919. doi: 10.1080/14756366.2017.1344235.
8. Huang C, Long Q, Qian K, Fu T, Zhang Z, Liao P, Xie J (2015) Resistance and integron characterization of *Acinetobacter baumannii* in a teaching hospital in Chongqing, China. New Microbes New Infect 8: 103-108. doi: 10.1016/j.nmni.2015.09.015.
9. Ibrahim ME (2019) Prevalence of *Acinetobacter baumannii* in Saudi Arabia: risk factors, antimicrobial resistance patterns and mechanisms of carbapenem resistance. Ann Clin Microbiol Antimicrob 18: 1. doi: 10.1186/s12941-018-0301-x.
10. Firoozeh F, Mahluji Z, Shams E, Khorshidi A, Zibaei M (2017) New Delhi metallo-β-lactamase-1-producing *Klebsiella pneumoniae* isolates in hospitalized patients in Kashan, Iran. Iran J Microbiol 9: 283-287.
11. Fallah F, Noori M, Hashemi A, Goudarzi H, Karimi A, Erfanimanesh S, Alimehr S (2014) Prevalence of *blaNDM*, *blaPER*, *blaVEB*, *blaIMP*, and *blaVIM* genes among *Acinetobacter baumannii* isolated from two hospitals of Tehran, Iran. Scientifica 2014: 245162. doi: 10.1155/2014/245162.
12. Thomson JM, Bonomo RA (2005) The threat of antibiotic resistance in Gram-negative pathogenic bacteria: beta-lactams in peril! Curr Opin Microbiol 8: 518-524. doi: 10.1016/j.mib.2005.08.014.
13. Smail SB, AL-Otrachi KI (2020) Phenotypic characterization of extended-spectrum beta-lactamases and metallo-beta-lactamase of multi drug resistant *Acinetobacter baumannii* causing nosocomial infections in Erbil city. Al-Mustansiriyah Journal of Science 30: 51-56. doi: 10.23851/mjs.v30i4.671.
14. Abd El-Hady H, Abdelhadi A (2021) Expression of metallo-β-lactamase genes in carbapenem resistant *Acinetobacter baumannii* isolates from intensive care unit patients. Microbes and Infectious Diseases 2: 797-806. doi: 10.21608/mid.2021.97430.1196.
15. Das NK, Grover N, Sriram R, Kumar M, Dudhat VL, Prasanna S (2016) Prevalence of carbapenem resistance and comparison between different phenotypic methods for detection of metallo-β-lactamases in Gram negative non-fermentative bacteria in the acute wards of a tertiary care centre. Int J Curr Microbiol App Sci 5: 109-119. doi: 10.20546/ijcmas.2016.505.012.
16. Hoang Quoc C, Nguyen Thi Phuong T, Nguyen Duc H, Tran Le T, Tran Thi Thu H, Nguyen Tuan S, Phan Trong L (2019) Carbapenemase genes and multidrug resistance of *Acinetobacter baumannii*: a cross sectional study of patients with pneumonia in southern Vietnam. Antibiotics 8: 148. doi: 10.3390/antibiotics8030148.
17. Nikibakhsh M, Firoozeh F, Badmasti F, Kabir K, Zibaei M (2021) Molecular study of metallo-β-lactamases and integrons in *Acinetobacter baumannii* isolates from burn patients. BMC Infect Dis 9: 782. doi: 10.1186/s12879-021-06513-w.
18. Scott P, Deye G, Srinivasan A, Murray C, Moran K, Hulten E, Fishbain J, Craft D, Riddell S, Lindler L, Mancuso J, Milstrey E, Bautista CT, Patel J, Ewell A, Hamilton T, Gaddy C, Tenney M, Christopher G, Petersen K, Endy T, Petruccelli B (2007) An outbreak of multidrug-resistant *Acinetobacter baumannii-calcoaceticus* complex infection in the US military health care system associated with military operations in Iraq. Clin Infect Dis 15: 1577-1584. doi: 10.1086/518170.
19. Hawley JS, Murray CK, Griffith ME, McElmeel ML, Fulcher LC, Hospenthal DR, Jorgensen JH (2007) Susceptibility of *Acinetobacter* strains isolated from deployed U.S. military personnel. Antimicrob Agents Chemother 51: 376-378. doi: 10.1128/AAC.00858-06.
20. Unal S, Garcia-Rodriguez JA (2005) Activity of meropenem and comparators against *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolated in the MYSTIC Program, 2002-2004. Diagn Microbiol Infect Dis 53: 265-271. doi: 10.1016/j.diagmicrobio.2005.10.002.
21. Ruiz M, Marti S, Fernandez-Cuenca F, Pascual A, Vila J (2007) High prevalence of carbapenem-hydrolysing oxacillinases in epidemiologically related and unrelated *Acinetobacter baumannii* clinical isolates in Spain. Clin Microbiol Infect 13: 1192-1198. doi: 10.1111/j.1469-0691.2007.01825.x.
22. Shahcheraghi F, Abbasalipour M, Feizabadi M, Ebrahimipour G, Akbari N (2011) Isolation and genetic characterization of metallo-β-lactamase and carbapenamase producing strains of *Acinetobacter baumannii* from patients at Tehran hospitals. Iran J Microbiol 3: 68-74.
23. Ikonomidis A, Ntokou E, Maniatis AN, Tsakris A, Pournaras S (2008) Hidden *VIM-1* metallo-beta-lactamase phenotypes among *Acinetobacter baumannii* clinical isolates. J Clin Microbiol 46: 346-349. doi: 10.1128/JCM.01670-07.
24. Azim A, Dwivedi M, Rao PB, Baronia AK, Singh RK, Prasad KN, Poddar B, Mishra A, Gurjar M, Dhole TN (2010) Epidemiology of bacterial colonization at intensive care unit admission with emphasis on extended-spectrum beta-lactamase- and metallo-beta-lactamase-producing Gram-negative bacteria - an Indian experience. J Med Microbiol 59: 955-960. doi: 10.1099/jmm.0.018085-0.
25. Shibata N, Doi Y, Yamane K, Yagi T, Kurokawa H, Shibayama K, Kato H, Kai K, Arakawa Y (2003) PCR typing of genetic determinants for metallo-beta-lactamases and integrases carried by Gram-negative bacteria isolated in Japan, with focus on the class 3 integron. J Clin Microbiol 41: 5407-5413. doi: 10.1128/JCM.41.12.5407-5413.2003.

Corresponding author

Haval Mohammed Khalid, PhD.
Department of Biology, Faculty of Science, University of Zakho, Zakho International Road, Zakho 42002, Kurdistan Region, Iraq
Tel: 00964 7504508093
Email: Haval.khalid@uoz.edu.krd

Conflict of interests: No conflict of interests is declared.