Rapid detection and molecular survey of \textit{bla}VIM, \textit{bla}IMP and \textit{bla}NDM genes among clinical isolates of \textit{Acinetobacter baumannii} using new multiplex real-time PCR and melting curve analysis

Hossein Goudarzi\textsuperscript{1}, Elnaz Sadat Mirsamadi\textsuperscript{1,2*}, Zohreh Ghalavand\textsuperscript{1}, Mojdeh Hakemi Vala\textsuperscript{1}, Hamed Mirjalali\textsuperscript{3} and Ali Hashemi\textsuperscript{1}

**Abstract**

**Background:** \textit{Acinetobacter baumannii} is a cosmopolitan bacterium that is frequently reported from hospitalized patients, especially those patients who admitted in the intensive care unit. Recently, multiplex real-time PCR has been introduced for rapid detection of the resistance genes in clinical isolates of bacteria. The current study aimed to develop and evaluate multiplex real-time PCR to detect common resistance genes among clinical isolates of \textit{A. baumannii}.

**Results:** Multiplex real-time PCR based on melting curve analysis showed different T\textsubscript{m} corresponding to the amplified fragment consisted of 83.5 °C, 93.3 °C and 89.3 °C for \textit{bla}IMP, \textit{bla}VIM and \textit{bla}NDM, respectively. Results of multiplex real-time PCR showed that the prevalence of \textit{bla}IMP, \textit{bla}VIM and \textit{bla}NDM among the clinical isolates of \textit{A. baumannii} were 5/128(3.9%), 9/128(7.03%) and 0/128(0%), respectively. Multiplex real-time PCR was able to simultaneously identify the resistance genes, while showed 100% concordance with the results of conventional PCR.

**Conclusions:** The current study showed that \textit{bla}VIM, was the most prevalent MBL gene among the clinical isolates of \textit{A. baumannii} while no amplification of \textit{bla}NDM was seen. Multiplex real-time PCR can be sensitive and reliable technique for rapid detection of resistance genes in clinical isolates.

**Keywords:** \textit{Acinetobacter baumannii}, Melting curve analysis, Multiplex real-time PCR, Single tube reaction

**Background**

\textit{Acinetobacter baumannii} is known as one of the most common bacteria that is frequently found in the hospitalized patients in Intensive Care Unit (ICU) \cite{1, 2}. Several types of infections resulting from \textit{A. baumannii} have been reported, with systemic infection and pneumonia currently the most important forms \cite{3–5}. Currently, several studies have indicated the resistance of \textit{A. baumannii} to the broad spectrum of antibiotics such as beta-lactams \cite{6–8}. Meanwhile, incorrect and imprecise prescription of the drugs leads to the emergence of bacteria that are resistant to more than one class of antibiotics, knowing as multi drug resistant (MDR) and extreme drug resistant (XDR) strains. According to the guidelines, those bacteria resistant to more than three different following classes: carbapenems, aminoglycosides, ampicillin-sulbactam, cephalosporins and fluoroquinolones, are known as MDR strains \cite{9–12}. However, emergence of MDR among \textit{A. baumannii} strains, as one of the most important concerns of
physicians, decreases the number of drugs of choice, especially in the clinical practice.

Antibiotics from the carbapenems family are recommended as the most effective drugs for treatment of _A. baumannii_ infections [6, 13]. However, _A. baumannii_ is able to rapidly acquire the carbapenems resistance genes such as Metallo-Beta-Lactamases (MBLs) [14]. Several reports of MBL-producing strains has led to increased numbers of studies on the prevalence as well as designing reliable methods for detection of the most prevalent MBL genes among clinical strains of _A. baumannii_ [7, 15, 16]. MBLs-producing strains of _A. baumannii_ have been frequently reported in Iran [17–19]. Therefore, laboratory identification of the drug resistance genes is a pivotal step in the early assessment and management of hospital infections due to _A. baumannii_, particularly in ICU patients.

During the past two decades, molecular approaches have been introduced as a useful tool in order to screen antimicrobial resistance genes in different isolates of _A. baumannii_ [20–22]. Real-time PCR is a molecular tool that is able to provide more accurate and rapid results in comparison with conventional PCR techniques. Recently, multiplex real-time PCR was applied as a reliable test for immediate and coincident identification of more than one MBL gene in the clinical isolates of bacteria [23–26].

The current study aimed to apply multiplex real-time PCR assay in a single tube for simultaneous identification of three common types of MBL genes (blaIMP, blaVIM and blaNDM), in clinical isolates of _A. baumannii_ using the melting curves analysis. To our knowledge, this is the first study that used multiplex real-time PCR in a single tube, for the concurrent identification of _blaIMP_, _blaVIM_ and _blaNDM_ genes in the clinical isolates of _A. baumannii_.

**Results**

In order to perform multiplex real-time PCR, _A. baumannii_ isolates were selected from those strains that were previously phenotypically examined for piperacillin-tazobactam, ceftiraxon, ceftazidime, cefepime, imipenem, doripenem-ertapenem, cefotaxime, and ampicillin-sulbactam, using disc diffusion (Mast Co., UK) regarding to CLSI 2013 guidelines. Accordingly, antibiotic susceptibility pattern showed that all isolates were resistant to piperacillin-tazobactam, while 71.1% of isolates were sensitive to ampicillin-sulbactam [20].

In silico analysis showed that designed primers amplified all available alleles of the mentioned MBL gene families (Fig. 1). To evaluate the accuracy of the amplified fragment of each gene, multiplex SYBR green real-time PCR was separately conducted on the positive control of _blaIMP_, _blaVIM_ and _blaNDM_. Melting curve analysis of each amplicon was generated that showed the fragment Tm 83.5 °C, 93.3 °C and 89.3 °C for _blaIMP_, _blaVIM_ and _blaNDM_, respectively (Table 1). Multiplex real-time PCR was also performed for all three MBL genes in a single tube and showed discriminative melting temperatures in the melting curve analysis (Fig. 2). For more confirmation, all amplified fragments were electrophoresed on 2% agarose gel and stained with ethidium bromide that showed discriminative bands (Fig. 3). The results of the multiplex real-time PCR showed that all the PCR-positive isolates had the amplification plots and also relevant melting temperatures for the resistance genes. The results of multiplex real-time PCR showed that the prevalence of _blaIMP_, _blaVIM_ and _blaNDM_ among the clinical isolates of _A. baumannii_ were 5/128(3.9%), 9/128(7.03%) and 0/128(0%), respectively. However, the results of antibiotic susceptibility pattern and multiplex real-time PCR were compared in (Table 2).

**Discussion**

_A. baumannii_ is a major pathogen in hospitalized patients, particularly in the ICU ward. During the recent years, the increasing reports of the multidrug resistance strains of this bacterium has been highlighted _A. baumannii_ as a major public health issue, in the world [27, 28]. Multidrug resistance of _A. baumannii_ usually occurs to beta-lactams, aminoglycosides, fluoroquinolones and also carbapenems. Notably, the wide distribution of the resistance strains of this worldwide bacterium to beta-lactams drugs family has been led to increase of concerns of physicians in the clinical practices for the rapid detection of drug resistance isolates, particularly in the hospitalized patients.

Many studies have suggested molecular techniques to detect and identify carbapenemase genes among the broad spectrum of bacteria [29–31]. However, the conventional molecular methods are often time- and cost-consuming due to their need to the post-PCR analysis. Real-time PCR approaches that target resistance genes including carbapenemase have been frequently applied, so far. Martin-Pena and colleagues developed a TaqMan quantitative real-time PCR to identify resistance genes corresponding to imipenem, ciprofloxacin, colistin and amikacin in clinical isolates of _A. baumannii_ [32]. Recently, multiplex real-time PCR was employed for concurrent detection of several resistance genes from one or more than one families. In a study conducted by Mendes in Brazil, multiplex real-time PCR was exploited to detect IMP and VIM types, SPM-1, GIM-1, and SIM-1 in ATCC reference and laboratory strains of gram-negative bacteria consisted of _P. aeruginosa_, _Escherichia coli_, _Acinetobacter calcoaceticus_, _Klebsiella pneumonia_, _Neisseria meningitidis_, _Neisseria perflava_, _Escherichia coli_, _Acinetobacter calcoaceticus_, _Klebsiella pneumonia_, _Neisseria meningitidis_, _Neisseria perflava_,
Neisseria lactamica, Neisseria sicca, Salmonella serovar typhimurium, Enterobacter aerogenes [33]. In another study performed by Monteiro and colleagues SYBR green real-time PCR followed by the High Resolution Melting (HRM) curve analysis was defined to detect blaKPC, blaGES, blaIMP, blaVIM, blaOXA-48 and blaNDM-1 genes in positive strains of Enterobacteriaceae (no.21), A. baumannii (no. 1) and P. aeruginosa (no. 8) [24]. The main limitation of these studies was this fact that real-time PCR for each resistance gene was conducted in separated tubes but in a single real-time PCR run. In order to overcome this limitation, the primers of the current study were designed to identify resistance genes in a single tube using melting curve analysis based on Tm of the amplified fragments.

Zheng in 2013 introduced a duplex TaqMan real-time PCR assay for the concurrent identification of blaNDM and blaKPC genes in Enterobacteriaceae. According to this study, 7 and 10 isolates carried blaNDM and blaKPC, respectively [26]. Recently, Yang and colleagues described two sets of multiplex real-time PCR to distinguish A. baumannii and Non- baumannii Acinetobacter spp., and also detect blaNDM, blaOXA-23-like, blaOXA-40-like, blaOXA-51-like, and blaOXA-58-like genes in clinical samples using melting curve analysis [34]. In both of these studies the results of multiplex real-time PCR showed 100% concordance with the results of conventional PCR. Our findings were in line with the results of these studies and represented that all isolates that were positive for blaIMP and blaVIM by

Table 1 Designed primers and fragment Tm of the amplified fragments for multiplex real-time PCR

| Resistance genes | Primer (5’-3’) | Fragment length (bp) | Fragment melting Temperature (°C) |
|------------------|----------------|---------------------|----------------------------------|
| IMP              | F: TTGACACTCCATTACTGCTA R: TCATTGTGATTGCAGATGATA | 172 | 83.5 |
| VIM              | F: GAGTTGCTTTGATGATACAG R: TCAGAAGACCTCTATGCA | 247 | 93.3 |
| NDM              | F: AACAGGCCTGATTTTTCG R: TGATATTGTCACTGAGTG | 111 | 89.3 |

![Fig. 1](image-url) Alignment of some available subtypes of a: blaIMP, b: blaVIM and c: blaNDM using BioEdit software. The position of primers for each gene was specified using box and arrow.
conventional PCR, also showed amplification using multiplex real-time PCR.

In the current study, although 100% concordance was seen between the results of conventional PCR and multiplex real-time PCR, the results of disc diffusion showed higher range of antibiotic resistance in comparison with molecular tests. Theoretically, it was suggested that bacteria employ at least three mechanisms against beta-lactams that are including: a) destruction of the antibiotics using beta-lactam enzymes, b) applying efflux pumps to control influx and efflux of antibiotics and c) reducing the access to- and affinity of penicillin-binding protein (PBP) [35, 36]. However, it seems that A. baumannii, more likely uses several mechanisms against beta-lactams antibiotics and therefore, both phenotypic and genotypic analysis should be applied to provide more information about the drug resistance patterns.

Furthermore, in the current study, although the genes of \(\text{bla}\text{IMP} \) and \(\text{bla}\text{VIM} \) were detected in the clinical samples, there was no amplification of \(\text{bla}\text{NDM} \). Our
results were in accordance with the study performed by Milillo that showed no amplification of NDM gene in clinical isolates of *Acinetobacter*. As supported by literature, it assumed that the main reason of this observation could be related to the ability of *Acinetobacter* to harbor only one copy of NDM gene [37].

**Limitations**

The Ambler class B-metallo-beta-lactamases (MBL) and carbapenem-hydrolysing class D beta-lactamases (CHDLs) are two common mechanisms for the carbapenem-resistant in *A. baumannii*. However, since in the current study, only three common types of MBL genes (blaIMP, blaVIM, and blaNDM) were investigated, it seems that high negative rate in multiplex real-time PCR results may not reflect the actual rate of resistant genes, particularly CHDL genes in *A. baumannii*.

**Conclusion**

Multiplex real-time PCR is a sensitive, rapid and precise method that is able to simultaneously detect the MBL resistance genes among clinical isolates of *A. baumannii*. Furthermore, the current study represented that there was 100% concordance between the result of conventional PCR and multiplex real-time PCR; thus, the later method could be suggested as a rapid and sensitive technique for multiplex detection of the resistance genes among the clinical isolates of bacteria, particularly in emergency cases who are hospitalized in ICU.

**Methods**

**Patients and specimens**

This study had received ethical approval from the Ethics Committee of the Shahid Beheshti University of Medical Science (SBMU), Tehran, Iran. The current study was performed on samples, which were previously collected by Goudarzi and colleagues [20]. The samples were collected from 128 patients who had been hospitalized in different wards including ICU, Surgery, Neurosurgery, Orthopedics, Infectious, etc. of two hospitals in Tehran.

**DNA extraction**

In order to achieve enough *A. baumannii* colonies for DNA extraction, the obtained isolates were incubated overnight at 36°C in LB medium. Then, each sample was centrifuged at 8000 rpm for 3 min, supernatant was discarded and the pellet was introduced to DynaBioTM Blood/Tissue DNA Extraction Mini Kit (Takapouzist Company, Iran). Purified DNA was kept out in −20°C until molecular investigation.

**Primer designing and detection**

Primers were designed using the online software Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) according to available blaIMP, blaVIM, blaNDM genes in GenBank database with accession numbers: KF723585, GQ288396 and KF951474, respectively. All the primers were checked in silico to amplify all available alleles of the mentioned MBL gene families. The primers amplified fragments with different lengths and melting temperatures to provide enough resolution in the conventional PCR and multiplex real-time PCR, respectively (Table 1). In the case of IMP and VIM, the positive isolates that were sequenced and submitted in GenBank database with accession numbers: KU685506 to KU685508 were used as positive controls. In addition, the positive case of NDM-1 was kindly provided by Dr. Shahcheraghi from Institute Pasteur of Iran. In order to determine specificity of the designed primers, clinical isolates of *A. baumannii* and *Pseudomonas* spp., which were phenotypically and molecularly non-MBLs resistant isolates, were tested using the designed primers. In addition, the isolates were screened for carrying blaIMP, blaVIM and blaNDM genes using conventional PCR [20]. It is necessary to mention that conventional PCR was performed using the current designed primers.

**Table 2** The comparison results of antibiotic susceptibility test and multiplex real-time PCR

| Antibiotics                  | Antibiotic susceptibility test | Multiplex real-time PCR | blaIMP-positive No (%) | blaVIM-positive No (%) |
|------------------------------|--------------------------------|-------------------------|------------------------|------------------------|
| Piperacillin-tazobactam      | 128 (100)                      | 5/128 (3.9)             | 9/128 (7.03)           |
| Ceftriaxone                  | 128 (100)                      | 5/128 (3.9)             | 9/128 (7.03)           |
| Ceftazidime                  | 128 (100)                      | 5/128 (3.9)             | 9/128 (7.03)           |
| Cefepime                     | 126 (98.4)                     | 5/126 (3.97)            | 9/126 (7.14)           |
| Imipenem                     | 121 (94.53)                    | 5/121 (4.13)            | 9/121 (7.44)           |
| Doripenem                    | 127 (99.22)                    | 5/127 (3.94)            | 9/127 (7.09)           |
| Ertapenem                    | 128 (100)                      | 5/128 (3.9)             | 9/128 (7.03)           |
| Cefotaxime                   | 128 (100)                      | 5/128 (3.9)             | 9/128 (7.03)           |
| Ampicillin-sulbactam         | 37 (28.9)                      | 5/37 (13.519)           | 9/37 (24.32)           |

**Goudarzi et al. BMC Microbiology (2019) 19:122 Page 5 of 7**
Multiplex real-time PCR
Each reaction was accomplished in final volume of 25 μl containing 2X Real-time PCR Mastermix (Bioneer, Korea), 5 pmol of each primer, 1 μl of ROX, 6.5 μl of DEPC water and 2 μl of template using Rotor Gene 6000 (Corbett, Australia) instrument. The reaction mixture was subjected to 95°C, 10 min, 40 Cycles including 95°C, 10 s, 55°C, 37 s, 72°C, hold 20 s ramping from 70°C to 99°C at 0.02°C s⁻¹. Furthermore, melting curve analysis was determined using Rotor Gene 6000 software.

Abbreviations
ICU: Intensive Care Unit; MBLs: Metallo-Beta-Lactamases; MDR: Multi drug resistant

Acknowledgments
The authors would like to appreciate all colleagues of department of microbiology for their laboratory cooperation.

Authors’ contributions
Conceived and designed the experiments: ESM HG. Performed the experiments: ESM. Analyzed the data: HM ESM AH. Contributed reagents/materials/analysis tools/positive samples: ZGH MHV. Wrote the paper: ESM HM. All authors read and approved the final version of the manuscript.

Funding
This Project was financially supported by Shahid Beheshti University of Medical Sciences, Tehran. The funding body had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Availability of data and materials
The data associated with this manuscript consisted of normalized melting curves are included in the article.

Ethics approval and consent to participate
All procedures performed in this study were in accordance with the ethical standards released by Ethical Review Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran. Informed oral and written consent was obtained from all patients included in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Department of Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. 2Department of Microbiology, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran. 3Foodborne and Waterborne Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Received: 13 May 2018 Accepted: 3 June 2019
Published online: 10 June 2019

References
1. Murray CK, Hospenthal DR. Acinetobacter infection in the ICU. Crit Care Clin. 2008;24(2):237–48 viii.
2. Nitou NB, Badri M, Khalefly H, Whitelaw A, Oliver S, Piercy J, Raine R, Loubert I, Dheda K. ICU-associated Acinetobacter baumannii colonisation/infection in a high HIV-prevalence resource-poor setting. PLoS One. 2012;7(12):e52452.
3. Munoz-Price LS, Weinstein RA. Acinetobacter infection. N Engl J Med. 2008;358(12):1271–81.
4. Dexter C, Murray GL, Paulsen IT, Peleg AT. Community-acquired Acinetobacter baumannii: clinical characteristics, epidemiology and pathogenesis. Expert Rev Anti-Infect Ther. 2015;13(5):567–73.
5. Wong D, Nielsen TB, Bonombo RA, Pantapalangkoor P, Luna B, Spellberg B. Clinical and pathophysiological overview of Acinetobacter infections: a century of challenges. Clin Microbiol Rev. 2017;30(1):409–47.
6. Mathlouthi N, Ben Lamine Y, Somar R, Boughalla-Beibes S, Bakour S, Rolain JM, Chouchani C. Incidence of OXA-23 and OXA-58 Carbapenemases Coexpressed in clinical isolates of Acinetobacter baumannii in Tunisia. Microb Drug Resist (Larchmont, NY). 2017.
7. Mathlouthi N, El Salabi AA, Ben Jomaa-Jermil M, Bakour S, Al-Baysari C, Zorgani AA, Klaeria A, Elharrer O, Okdah L, Rolain JM, et al. Early detection of metallo-beta-lactamase NDM-1- and OXA-23 carbapenemase-producing Acinetobacter baumannii in Libyan hospitals. Int J Antimicrob Agents. 2016;48(1):46–50.
8. Khurana S, Mathur P, Kapil A, Vaisan C, Behera B. Molecular epidemiology of beta-lactamase producing nosocomial gram-negative pathogens from North and South Indian hospitals. J Med Microbiol. 2017;66(7):999–1004.
9. Karaiskos I, Giamarellou H. Multidrug-resistant and extensively drug-resistant gram-negative pathogens: current and emerging therapeutic approaches. Expert Opin Pharmacother. 2014;15(10):1351–70.
10. Seruga Music M, Hrenovic J, Goic-Barisic I, Hunkaj B, Skotic D, Ivanovic T. Emission of extensively-drug-resistant Acinetobacter baumannii from hospital settings to the natural environment. J Hosp Infect. 2017;96(3):233–7.
11. Teo J, Lim TP, Hsu LY, Tan TY, Sasakal S, Hon PY, Kwa AL, Apsamthanarak A. Extensively drug-resistant Acinetobacter baumannii in a Thai hospital: a molecular epidemiologic analysis and identification of bacterial Polymyxin B-based combinations. Antimicrob Resist Infect Control. 2015;4(1):2.
12. Medina E, Pieper DH. Tackling threats and future problems of multidrug-resistant Bacteria. Curr Top Microbiol Immunol. 2016;398:33–3.
13. Poirel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin Microbiol Infect. 2006;12(9):826–36.
14. Alksasy NM, El Sayed Zak Z, Molar study of Acinetobacter baumannii isolates for Metallo-beta-lactamases and extended-Spectrum-beta-lactamases genes in intensive care unit, Mansoura University Hospital, Egypt. Int J Microbiol. 2017;2017:3925868.
15. Kimura Y, Miymamoto T, Aoki K, Ishii Y, Harada K, Watarai M, Hatoya S. Analysis of IMP-1 metallo-beta-lactamase-producing Acinetobacter radioresistens isolated from companion animals. J Infect Chemother. 2017;23(9):655–7.
16. Gomaa FAM, Helal ZH, Khan MI. High prevalence of blaNDM-1, blaVIM, qacE, and qacEDelta1 genes and their association with decreased susceptibility to antibiotics and common hospital biocides in clinical isolates of Acinetobacter baumannii. Microorganisms. 2017;5(2):18.
17. Aghamiri S, Amirmozaftani N, Fallah Mehrabadi J, Foulahtan B, Hanafi Abidar M. Antibiotic resistance patterns and a survey of Metallo-beta-lactamase genes including bla-IMP and bla-IMV types in Acinetobacter baumannii isolated from hospital patients in Tehran, Chemotherapy. 2016;61(5):275–80.
18. Fallah F, Noori M, Hashemi A, Goudarzi H, Karimi A, Erfanianmehr S, Alimehr S. Prevalence of bla NDM, bla PER, bla VEB, bla IMP, and bla VM genes among Acinetobacter baumannii isolated from two hospitals of Tehran, Iran. Scientificia. 2014;2014:245162.
19. Safari M, Moazzafar Nejad AS, Bahador A, Jafari R, Alkhanly MY. Prevalence of ESBL and MBL encoding genes in Acinetobacter baumannii strains isolated from patients of intensive care units (ICU). Saudi J Biol Sci. 2012;22(4):424–9.
20. Goudarzi H, Mirsamadi ES, Ghavam Z, Hakami Vala M, Mirjali H, Hashemi A, Ghasemi E. Molecular detection of Metallo-beta-lactamase genes in clinical isolates of Acinetobacter baumannii. J Pure App Microbiol. 2015;9(Spl. Edn 2):145–51.
21. Mehmkt Z, Salimzard H, Arminy V, Khaskhoor M, Mansouri D, Farsiani H, Ghazvini K, Najafi A. Molecular characterization and genetic relatedness of clinically Acinetobacter baumannii isolates conferring increased resistance to the first and second generations of tetracyclines in Iran. Ann Clin Microbiol Antimicrob. 2017;16(1):51.
carbapenemase genes in gram-negative clinical isolates. J Microbiol Methods. 2015;113:4–9.

24. Monteiro J, Widen RH, Pignatari AC, Kubasek C, Silbert S. Rapid detection of carbapenemase genes by multiplex real-time PCR. J Antimicrob Chemother. 2012;67(4):906–9.

25. Roscosínski N, Fischer J, Guerra B, Roessler U. Development of a multiplex real-time PCR for the rapid detection of the predominant beta-lactamase genes CTX-M, SHV, TEM and CIT-type AmpCs in Enterobacteriaceae. PLoS One. 2014;9(7):e100956.

26. Zheng F, Sun J, Cheng C, Rui Y. The establishment of a duplex real-time PCR assay for rapid and simultaneous detection of blaNDM and blaKPC genes in bacteria. Ann Clin Microbiol Antimicrob. 2013;12:90.

27. Gootz TD, Marra A. Acinetobacter baumannii: an emerging multidrug-resistant threat. Expert Rev Anti-Infect Ther. 2008;6(3):309–25.

28. Nwadike VU, Ojide CK, Kalu EI. Multidrug resistant acinetobacter infection and their antimicrobial susceptibility pattern in a nigerian tertiary hospital ICU. Afr J Infect Dis. 2014;8(1):14–8.

29. Bocanegra-Barias P, Garza-Gonzalez E, Morfin-Otero R, Barrios H, Villareal-Trevino L, Rodriguez-Noriega E, Garza-Ramos U, Petersen-Morfin S, Silva-Sanchez J. Molecular and microbiological report of a hospital outbreak of NDM-1-carrying Enterobacteriaceae in Mexico. PLoS One. 2017;12(8):e0179651.

30. Mansour W, Haenni M, Saras E, Grami R, Mani Y, Ben Haj Khalifa A, El Atroous S, Kheder M, Fekih Hassen M, Boujaafar N, et al. Outbreak of colistin-resistant carbapenemase-producing Klebsiella pneumoniae in Tunisia. J Glob Antimicrob Resist. 2017;10:88–94.

31. Marinimuthu K, Venkatachalam I, Khong WX, Koh TH, Cheng BPZ, Van La M, De PP, Krishnan PU, Tan TY, Choon RFF, et al. Clinical and molecular epidemiology of Carbapenem-resistant Enterobacteriaceae among adult inpatients in Singapore. Clin Infect Dis. 2017;64(suppl_2):S1–S5.

32. Martin-Peña R, Domínguez-Herrera J, Pachón J, McConnell MJ. Rapid detection of antibiotic resistance in Acinetobacter baumannii using qualitative real-time PCR. J Antimicrob Chemother. 2013;68(7):1572–5.

33. Mendes RE, Kyota KA, Monteiro J, Castanheira M, Andrade SS, Gales AC, Pignatari AC, Tuflik S. Rapid detection and identification of metallo-beta-lactamase-encoding genes by multiplex real-time PCR assay and melt curve analysis. J Clin Microbiol. 2007;45(2):544–7.

34. Yang Q, Rui Y. Two multiplex real-time PCR assays to detect and differentiate Acinetobacter baumannii and non-baumannii Acinetobacter spp. carrying blaNDM, blaOXA-23-like, blaOXA-40-like, blaOXA-51-like, and blaOXA-58-like genes. PLoS One. 2016;11(7):e0158958.

35. Petchiappan A, Chatterji D. Antibiotic resistance: current perspectives. ACS Omega. 2017;2(10):7400–9.

36. Rice LB. Mechanisms of resistance and clinical relevance of resistance to beta-lactams, glycopeptides, and fluoroquinolones. Mayo Clin Proc. 2012;87(2):198–208.

37. Millño M, Kwak Y, Snesrud E, Waterman PE, Lesho E, McGann P. Rapid and simultaneous detection of blaKPC and blaNDM by use of multiplex real-time PCR. J Clin Microbiol. 2013;51(4):1247–9.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.