RESEARCH ARTICLE

Frequency of *bla*$_{IMP}$, *bla*$_{NDM}$, *bla*$_{KPC}$, and *bla*$_{VIM}$ carbapenemase-encoding genes among gram-negative bacteria isolates from hospitalized patients in Baghdad City, Iraq

Layla Taha Yassen[1], Laith B. Alhusseini[2, *], Ban Oady Abdulsattar[3], E. Kouhsari[4, 5]

[1]Department of Medical Laboratory Techniques, Osoul Aldeen University College, Baghdad, Iraq. [2]Department of Ecology, College of Science, Kufa University, Kufa, Najaf, Iraq. [3]Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq. [4]Laboratory Sciences Research Center, [5]Department of Laboratory Sciences, Faculty of Paramedicine, Golestan University of Medical Sciences, Gorgan, Iran.

Received: June 16, 2021; **accepted:** August 6, 2021.

Carbapenemase-encoding genes have been spreading among gram-negative bacteria, which is considered the most important threats to human health. Metallo β-lactamases including IMP, VIM, and NDM are the most predominant types, which confer resistance to Carbapenem group. This study aimed to investigate the frequency of *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{VIM}$, and *bla*$_{IMP}$ genes across gram-negative bacteria isolated from different clinical specimens in Medical City Hospital in Baghdad, Iraq. Fifty-two isolates were identified phenotypically using conventional biochemical tests. Vitek 2 identification system was used for confirmation of the identification. The antimicrobial sensitivity for the isolates was performed using Kirby–Bauer disk diffusion method. The carbapenemase-encoding genes (*bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{NDM}$, and *bla*$_{KPC}$) were screened by polymerase chain reaction (PCR)- based technique. The results showed that, from a total of 52 isolates isolated from hospital in Baghdad city, 13 (25%) were *Acinetobacter baumannii* and 39 (75%) were *Enterobacteriaceae* (10 of *Serratia* spp, 17 of *E. coli*, and 12 of *Enterobacter cloacae*). According to antibiotic susceptibility results, 96% of isolates were resistance to ceftriaxone, 92.3% to ciprofloxacin, and 90.4% to cefotaxime by phenotypic testing. Within the isolates, *bla*$_{VIM}$ gene was the most prevalent gene, which was detected in 48.1% of the isolates, followed by *bla*$_{IMP}$ gene in 19.2%, *bla*$_{NDM}$ gene in 9.6%, and *bla*$_{KPC}$ gene in 5.7%. This study reveals that the dissemination rate of carbapenemase-encoding genes was not as reported to be high among isolates. The results showed higher frequency to the *bla*$_{VIM}$ gene than other encoding genes for carbapenemase-encoding genes. These results suggest a periodic screening and follow-up program to detect antibiotic resistant genes, and also the need to develop appropriate management for antimicrobial resistance.

Keywords: gram-negative bacteria; antimicrobial resistance; health; carbapenemase-encoding genes; Iraq.

[*]**Corresponding author:** Laith B. Alhusseini, Department of Ecology, College of Science, Kufa University, Kufa, Najaf, Iraq. Phone: +964 790 234 5288. Email: laithb.alhusseini@uokufa.edu.iq.

Introduction

One of the main public health threats is the antimicrobial resistance (AMR) which is increasing significantly in different hospital wards [1, 2]. A published study reported that 700,000 deaths annually were recorded due to AMR and, if an appropriate control and prevention measures are not taken, AMR would become one of the main reasons of death among hospitalized

or non-hospitalized patients in developing and developed countries [2, 3]. The treatment of bacterial infection requires a proper antibiotic usage [4]. Thus, non-prescribed antibiotics and misuse of antibiotics are among potential risk factors to the emergence of AMR pathogenic bacteria. Furthermore, AMR could restrict therapeutic options, hospitalization time, high treatment costs, and finally a high death rate [5]. The most causative agents for human's infections come from bacteria. Monitoring and effective research programs from different countries around the world should rise awareness against AMR [6]. Recently, the emergence of a great number of resistant strains in different pathogens, and multidrug-resistant (MDR) gram-negative bacteria are a becoming increasingly prevalent in the community [7, 8]. The problem of antimicrobial resistance is increasing dramatically specially when considering the very limited number of new antimicrobial agents that are in development [9]. The infections caused by MDR gram-negative bacteria are treated with carbapenems antibiotics. However, the emergence of carbapenem resistant bacteria is threatening effectiveness of these antibiotics. The resistance to carbapenems occurs mainly by carbapenemases mechanism that belongs to three of the four β-lactamase classes A, B, and D [10]. Class D carbapenemases are known as the OXA-β-lactamases [11]. On the other hand, class B carbapenemases are known as the metallo-β-lactamases (MBLs); they are mainly encoded by integrons-borne mobile gene cassettes and are transferable amongst various bacteria *via* horizontal gene transfer mechanisms mainly conjugation [12]. The B MBLs most common families of acquired class found in *Enterobacteriaceae* include the IMP and VIM groups and emerging NDM group [13]. IMP-1 was described in 1991 in Japan from a *Serratia marcescens* isolate as a cause of acquired resistance to carbapenems [14]. While the VIM (Verona integron-encoded MBL) was first time detected in 1997 in Italy from a *P. aeruginosa* strain and emerged later in *Enterobacteriaceae* [15]. Several outbreaks in Mediterranean countries including Greece, Turkey, and Italy occurred due to the dissemination of these enzymes [16]. The *bla*VIM-1 is a gene cassette, which was integrated into a class 1 integron that carries an integrase gene [15]. Then, the VIM-2 variant was identified for the first time in *P. aeruginosa* clinical isolate in France which was also integrated as a gene cassette in a class I integron and now is considered worldwide endemic [17]. While the NDM has been identified in *E. coli* and *K. pneumoniae* first time from Indian patient residing in suffered from wound infections in New Delhi before returning to Sweden in 2008 [18], and now there are nearly 24 variants of bla_{NDM} described [19]. With the ability of plasmid-mediated transfer of NDM-1 through conjugation between various gram-negative, NDM-1 can rapidly spread *via* hospital-acquired infection or community acquired infection [20], while the *Klebsiella pneumoniae* carbapenemase (KPC) family is included in class A carbapenemases which could be plasmid encoded or chromosomal [10].

The current study aimed to determine bla_{NDM}, bla_{KPC}, bla_{VIM}, and bla_{IMP} type genes frequency among gram-negative bacteria isolated from clinical specimens in Medical City Hospital, Baghdad city, Iraq.

Materials and methods

Bacterial isolates collection and species identification

Fifty-two (52) gram-negative bacterial isolates of anonymous hospitalized patients were obtained from Medical City Hospital laboratory, Baghdad, Iraq. These isolates were initially identified depending on morphological characteristics of the colonies including colonies shape, texture, edges, and hemolysis on MacConkey agar and blood agar. Then, all isolates were identified phenotypically to species level using the Vitek 2 identification gram-negative bacteria (ID-GNB) cards (bioMérieux SA, Marcy l'Etoile, France) according to the manufacturer's instructions. The Vitek 2 System (bioMérieux SA, Marcy l'Etoile, France) was used as an identification system,

Table 1. The primer sequences used for detection of carbapenemases-encoding genes and molecular size of PCR products.

Primer target	Primer sequence (5′ → 3′)	Product size (bp)
bla_{IMP}	Fw: GGAATAGAGTGGCTTAAYTCTC Rv: GGTTTAAYAAAACAACCACC	232
bla_{NDM}	Fw: GGTTTGGCGATCTGGTTTTC Rv: CGGAATGGCTCATCACGATC	621
bla_{KPC}	Fw: CGTCTAGTTCTGCTGTCTTG Rv: CTTGTCATCCTTGTTAGGCG	798
bla_{VIM}	Fw: GATGGTGTTTGGTCGCATA Rv: CGAATGCGCAGCACCAG	390

which depended on the biochemical reactions between the bacterial isolates suspended in their solutions and the media in the Vitek 2 Identification Cards. The bacterial isolates were inoculated at 37°C on MacConkey agar plates and, after overnight incubation, a single colony was taken and suspended. The turbidity measurement for bacterial suspension was fixed to match the McFarland (0.5) standard in 0.45% sodium chloride. Then, the Gram Negative Vitek 2 ID card, the bacterial suspension tubes were loaded manually into the Vitek 2 system, the software also prepared according to the manufacturer's instructions.

Antibiotic susceptibility test

The pattern of antimicrobial sensitivity was performed on all pure isolates *in vitro* by the Kirby-Bauer method on Muller-Hinton agar and interpreted based on the Clinical Laboratory Standard Institute guidelines [21]. The antibiotic discs used in this study were as follow, Amoxicillin–Clavulanate (AMG, 20 µg), Cefotaxime (CTX, 30 µg), Ceftriaxone (CRO, 30 µg), Ciprofloxacin (CIP, 5 µg), Tetracycline (TE, 5 µg), Levofloxacin (LEV, 5 µg), Trimethoprim/ Sulphamethaxazole (SXT, 5 µg), Tobramycin (TOB, 5 µg), Imipenem (IMP, 5 µg), and Piperacillin (PI, 30 µg) provided by Bioanalyse Company, Ankara, Turkey.

A fresh 18 hours' pure culture for each isolate was emulsified with normal saline to achieve inoculum density equal to 0.5 McFarland turbidity standards. Each bacterial isolate was spread on fresh Mueller-Hinton agar using sterile cotton swab and left for 10 min at room temperature to dry. Antibiotic discs were placed on the top of Mueller-Hinton agar and the plates were incubated at 37°C for 24 hrs. The test was performed in duplicate. The strain *Escherichia coli* ATCC 25922 was used for quality control, which is obtained from the teaching laboratories of Biology Department, College of Science, Mustansiriyah University, Baghdad, Iraq.

DNA extraction and gene amplification

The bacterial DNA was extracted from all samples using the DNA Extraction Kit (Promega, Madison, Wisconsin, USA) according to the manufacturer's instruction provided with the kit. The presence of carbapenemases-encoding genes was screened by using polymerase chain reaction (PCR) with the paired primers (Table 1). The best amplification conditions for the PCR were: initial denaturation at 95°C for 5 min followed by 36 cycles of 95°C for 30 seconds, 59°C for 1min, and 72°C for 1min, and a final extension at 72°C for 10 min [22]. The verification of the PCR products was resolved in 1% agarose gel electrophoresis and then visualized under UV transilluminator. All positive samples were sent to Macrogen DNA sequencing Company (Seoul, Korea) for sequencing.

Results

The samples in this study were obtained from anonymous hospitalized patients during 2019

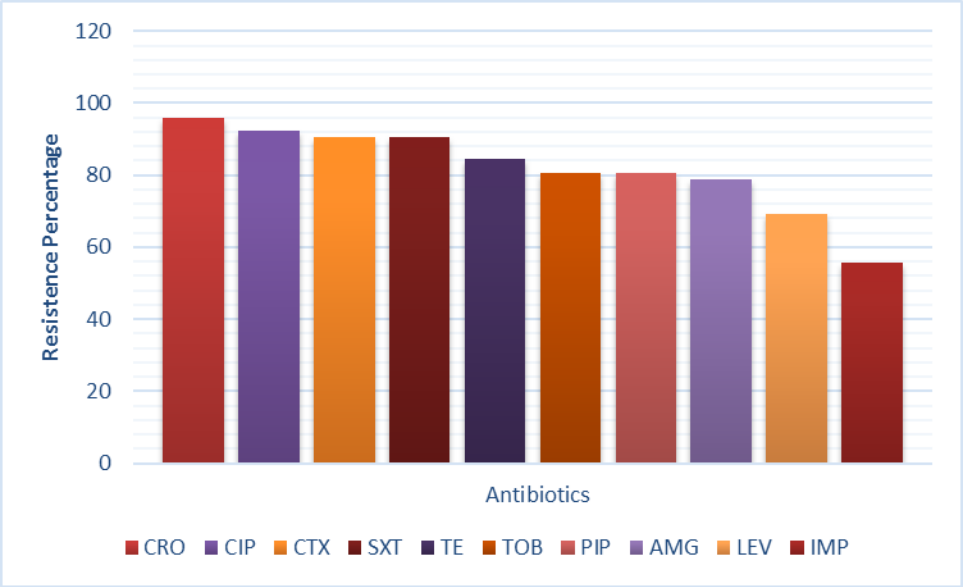

Figure 1. The percentage of antibiotics resistance among tested isolates.

and the rates of isolation sources were as follows: 12 isolates (23.07%) from blood, 19 isolates (36.54%) from urine, 12 isolates (23.07%) from burns, 3 isolates (5.77 %) from sputum, and 6 isolates (11.54%) from CSF. According to microscopic examination, culture growth, and biochemical tests, four species were identified and later were confirmed by Vitek 2 system. From 52 isolates, 13 (25%) were *Acinetobacter baumannii* and 39 (75%) were *Enterobacteriaceae* with 10 of *Serratia* spp, 17 of *E. coli*, and 12 of *Enterobacter cloacae*.

The profile of the antimicrobial resistance showed highest resistant percentage of 96% to ceftriaxone followed by 92.3% to ciprofloxacin, 90.4% to cefotaxime and trimethoprim/sulfamethoxazole, 84.6% to tetracycline, 80.7% to tobramycin and piperacillin, 78.8% to amoxicillin/clavulanate, 69.2% to levofloxacin, and 55.7% to Imipenem (Figure 1).

Uniplex PCR-based methods were conducted to detect bla_{NDM}, bla_{KPC}, bla_{VIM}, and bla_{IMP} genes. Among the 52 isolates, the bla_{VIM} gene was the most prevalent in 25 isolates (48.1%) followed by bla_{IMP} gene in 10 isolates (19.2%), bla_{NDM} gene in 5 isolates (9.6%), and bla_{KPC} gene in 3 isolates (5.7%). The results showed the presence of bla_{VIM} gene in 8 of *E. cloacae* isolates (15.4%), 7 of *A. baumannii* isolates (13.5%), 6 of *Serratia* isolates (11.5%), and 4 of *E. coli* isolates (7.7%); bla_{IMP} gene in 1 of *A. baumannii* isolates (1.9%), 1 of Serratia isolates (1.9%), 3 of *E. cloacae* isolates (5.7%), and 5 of *E. coli* isolates (9.6%); bla_{NDM} gene was presence in 2 of *A. baumannii* (3.8%), 1 isolate of each *Serratia*, *E. cloacae*, and *E. coli*. Interestingly, 3 isolates (5.8%) harbored a combination of bla_{NDM}, bla_{VIM}, and bla_{IMP} (Figure 2, Table 2).

Discussion

The gram-negative bacteria are responsible and often associate with infections in hospitals. High rates of mortality and morbidity are recorded by this bacterium [23]. Monitoring antibiotic resistance by routine surveillance studies is necessary, especially in low-income countries. The increased dependence on carbapenem as a last choice for treatment of multidrug-resistant bacteria emerged carbapenemase-producing bacteria globally [24]. Moreover, carbapenemases represent one of the most

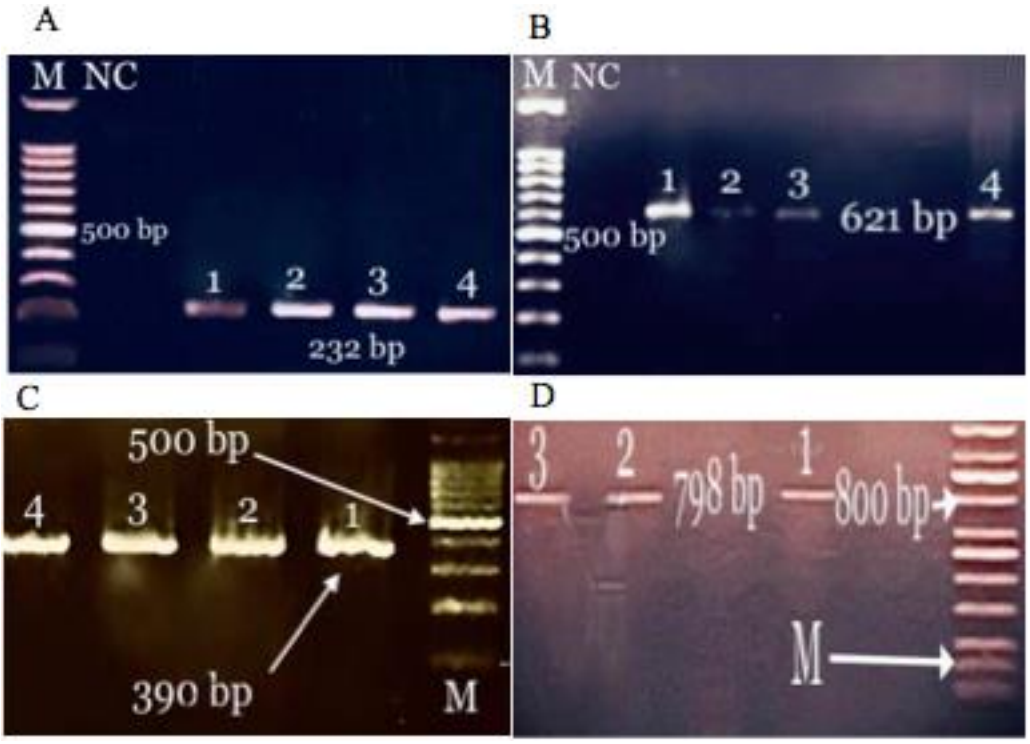

Figure 2. Agarose gel electrophoresis (1% agarose, 5-10 V/cm for 50 min). **A.** *bla*$_{IMP}$, **B.** *bla*$_{NDM}$, **C.** *bla*$_{VIM}$, **D.** *bla*$_{KPC}$. Lane M: DNA Ladder (1,000 bp), Lane NC: negative control, Lanes 1-4: represent of isolates bands.

Table 2. Distribution of *bla*$_{IMP}$, *bla*$_{NDM}$, *bla*$_{KPC}$, and *bla*$_{VIM}$ genes among isolates.

Bacterial isolate	Number of producer's isolates (%)			
	***bla*$_{VIM}$**	***bla*$_{IMP}$**	***bla*$_{NDM}$**	***bla*$_{KPC}$**
Enterobacter cloacae	8 (15.4%)	3 (5.7%)	1 (1.9%)	0 (0%)
Escherichia coli	4 (7.7%)	5 (9.6%)	1 (1.9%)	1 (1.9%)
Acinetobacter baumannii	7 (13.5%)	1 (1.9%)	2 (3.8%)	1 (1.9%)
***Serratia* spp**	6 (11.5%)	1 (1.9%)	1 (1.9%)	1 (1.9%)
Total number	25 (48.1%)	10 (19.2%)	5 (9.6%)	3 (5.7%)

challenging issues in the last decades on human health [16].

The most globally common type of MBL appeared to be *bla*$_{IMP}$-type MBLs. Among the isolates in this study, the results showed that the existence rate of *bla*$_{IMP}$ gene among isolates was 19.2% (Table 2). The *bla*$_{KPC}$ gene is frequently encoded in a transposon structure on a transferable plasmid so enabling it to spread to other gram-negative bacteria, predominantly in *Enterobacteriaceae*. However, the less prevalent MBL gene was *bla*$_{KPC}$, and the prevalence rate of *bla*$_{KPC}$ in isolates was 5.7% (Table 2). The evolution, maintenance, and dissemination of MBL resistance genes among gram-negative bacteria population in geographic regions is a complex and dynamic field that needs to be studied in detail. Careful monitoring for the presence of MBL-positive isolates among hospitalized patients is recommended to prevent wide dissemination of antibiotics resistance and to limit the indiscriminative use of cephalosporins and carbapenems antibiotic in the hospitals [25].

The *bla*$_{VIM}$ gene showed high prevalence rate. Al-Jubori *et al*. (2016) showed a prevalence rate of *bla*$_{VIM}$ gene as 25% in *A. baumannii* [26], while another study reported that all *E. coli* isolates did not carry *bla*$_{VIM}$ gene [27]. Among class B carbapenemases, the VIM types are the most frequent to be detected in all continents [28]. The first VIM enzymes were reported in *P. aeruginosa* isolates and emerged later in other *Enterobacteriaceae* as well. As for regional countries, A study in Saudi Arabia described that *P. aeruginosa* strain harborined the *bla*$_{VIM}$-2 gene [29]. Another study carried out in Iran by Rajabnia *et al*. (2015) indicated that the *bla*$_{VIM}$-1 gene was presence in 30% of *K. pneumoniae* isolates [30]. In Romania, a study showed that the *bla*$_{VIM}$-2 gene was presence 48% of *P. aeruginosa* isolates [31]. Furthermore, Touati *et al*. (2013) showed that the percentage rate was 82% of the studied isolates [32].

The NDM-1 was firstly reported in India and, since this detection, NDM-1 carrying organisms were disseminated globally. The first report for the existence of NDM-1 was *Enterobacteriaceae*, but recent reports indicated their spread in *Acinetobacter* spp. and *Pseudomonas* spp. as well [33]. The NDM-1 enzyme produced by bacterial isolates might have the ability to express numerous other unrelated resistance genes, like OXA-48 type and VIM type which encode other carbapenemases, AmpC, extended-spectrum beta-lactamases, and other classes of antimicrobials [34]. Different countries including the Gulf Corporation Council (GCC) investigated prevalence of NDM-1 producing isolates in a total of 200 isolates collected from 16 hospitals in Saudi Arabia, Kuwait, Oman, and the United Arab Emirates. The result showed that NDM-1 was the most common encountered carbapenemase gene (46.5%), while 47.6% in Egypt [24, 35], 29.5% in Turkey [36], and 7.8% in Tunisia [37].

There are different reasons for various ratio of the carbapenemase producing isolates such as, by geographic region, type of infection, specimen source, and selective pressure due to antibiotics. Moreover, the different patients studied, and the different rates of antibiotic used in different hospitals may also affect this ratio variation [38]. In our country, the wars, antimicrobial misuses, and high numbers of workers, especially from endemic region might play a significant role for the different variants of carbapenemase dissemination. There is an urgent need to find guidelines and active system to control infections among patients. Antimicrobial resistance gene surveillance in hospitals will help efforts to better implement appropriate measures and more studies are required to investigate prevalence of carbapenemase-encoding genes to control the spread of multidrug-resistant bacteria.

References

1. Pormohammad A, Nasiri MJ, Azimi T. 2019. Prevalence of antibiotic resistance in *Escherichia coli* strains simultaneously isolated from humans, animals, food, and the environment: a systematic review and meta-analysis. Infect Drug Resist. 12: 1181–1197.
2. Azimi T, Maham S, Fallah F, Azimi L, Gholinejad Z. 2019. Evaluating the antimicrobial resistance patterns among major bacterial pathogens isolated from clinical specimens taken from patients in Mofid Children's Hospital, Tehran, Iran: 2013–2018. Infect Drug Resist. 12: 2089–2102.
3. De Kraker MEA, Stewardson AJ, Harbarth S. 2016. Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050? PLoS Med. 13(11): e1002184.
4. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, *et al*. 2018. Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist. 11:1645–1658.
5. Llor C, Bjerrum L. 2014. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. Ther Adv Drug Saf. 5(6):229–241.
6. Organization WH. Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2017-2018. http://wwwwhoint/drugresistance/surveillance/GLASSmeeting/en/.
7. Ventola CL. 2015. The antibiotic resistance crisis: part 1: causes and threats. Pharm Ther. 40(4):277.
8. Alhusseini LB, Maleki A, Kouhsari E, Ghafourian S, Mahmoudi M, Al Marjani MF. 2019. Evaluation of type II toxin-antitoxin systems, antibiotic resistance, and biofilm production in clinical MDR *Pseudomonas aeruginosa* isolates in Iraq. Gene Rep. 17: 100546.
9. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, *et al*. 2009. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 48(1):1-12.
10. Manenzhe RI, Zar HJ, Nicol MP, Kaba M. 2015. The spread of carbapenemase-producing bacteria in Africa: a systematic review. J Antimicrob Chemother. 70(1):23–40.
11. Antunes NT, Lamoureaux TL, Toth M, Stewart NK, Frase H, Vakulenko SB. 2014. Class D β-lactamases: are they all carbapenemases? Antimicrob Agents Chemother. 58(4):2119–2125.

12. Amudhan MS, Sekar U, Kamalanathan A, Balaraman S. 2012. *bla*$_{IMP}$ and *bla*$_{VIM}$ mediated carbapenem resistance in *Pseudomonas* and *Acinetobacter* species in India. J Infect Dev Ctries. 6(11):757–762.
13. Jeon JH, Lee JH, Lee JJ, Park KS, Karim AM, Lee C-R, *et al*. 2015. Structural basis for carbapenem-hydrolyzing mechanisms of carbapenemases conferring antibiotic resistance. Int J Mol Sci. 16(5):9654–9692.
14. Osano E, Arakawa Y, Wacharotayankun R, Ohta M, Horii T, Ito H, *et al*. 1994. Molecular characterization of an enterobacterial metallo beta-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. Antimicrob Agents Chemother. 38(1):71–78.
15. Goudarzi H, Taherpour A, Fallah F, Pourkaveh B, Erfanimanesh S, Hashemi A. 2016. Laboratory detection of carbapenemases in gram-negative bacteria. Arch Clin Infect Dis. 11(2):e32816.
16. Leylabadlo HE, Asgharzadeh M, Aghazadeh M, Zahedi Bialvaei A, Samadi Kafil H, Ebrahimzadeh Leylabadlo H, *et al*. 2015. Dissemination of carbapenemases producing Gram negative bacteria in the Middle East. Iran J Microbiol. 7(5):226–246.
17. Diene SM, Rolain JM. 2014. Carbapenemase genes and genetic platforms in Gram-negative bacilli: *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* species. Clin Microbiol Infect. 20(9):831–838.
18. Rolain JM, Parola P, Cornaglia G. 2010. New Delhi metallo-beta-lactamase (NDM-1): towards a new pandemia? Clin Microbiol Infect. 16(12):1699–1701.
19. Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z. 2019. NDM metallo-β-lactamases and their bacterial producers in health care settings. Clin Microbiol Rev. 32(2):e00115–e00118.
20. Velasco JM, Valderama MT, Peacock T, Warawadee N, Nograd K, Navarro FC, *et al*. 2017. Carbapenemase-producing *Enterobacteriaceae* and nonfermentative bacteria, the Philippines, 2013–2016. Emerg Infect Dis. 23(9):1597–1598.
21. The Clinical and Laboratory Standards Institute. 2019. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.
22. Cotar AI, Chifiriuc MC, Dinu S, Pelinescu D, Banu O, Lazăr V. 2010. Quantitative real-time PCR study of the influence of probiotic culture soluble fraction on the expression of *Pseudomonas aeruginosa* quorum sensing genes. Roum Arch Microbiol Immunol. 69(4):213–223.
23. Fournier PE, Richet H, Weinstein RA. 2006. The epidemiology and control of *Acinetobacter baumannii* in health care facilities. Clin Infect Dis. 42(5):692–699.
24. Khalifa HO, Soliman AM, Ahmed AM, Shimamoto T, Hara T, Ikeda M, *et al*. 2017. High Carbapenem Resistance in Clinical Gram-Negative Pathogens Isolated in Egypt. Microb Drug Resist Larchmt N. 23(7):838–844.
25. Meletis G. 2016. Carbapenem resistance: overview of the problem and future perspectives. Ther Adv Infect Dis. 3(1):15–21.
26. AL_Jubori SS, AL_kadmy IMS, JassimAl_Ani Z. 2016. Emergence of multidrug resistance (MDR) *Acinetobacter baumannii* isolated from Iraqi hospitals. Adv Environ Biol. 10(5):265–276.
27. Hamed SL, Hasoon NA. 2019. Molecular Characterization of Carbapenemase-Producing Gram-negative Bacteria Isolated from Clinical Specimens in Baghdad, Iraq. J Pure Appl Microbiol. 13(2):1031–1040.
28. Djahmi N, Dunyach-Remy C, Pantel A, Dekhil M, Sotto A, Lavigne JP. 2014. Epidemiology of carbapenemase-producing *Enterobacteriaceae* and *Acinetobacter baumannii* in Mediterranean countries. BioMed Res Int. 2014:305784.
29. 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):1.
30. Rajabnia R, Asgharpour F, Shahandashti EF, Moulana Z. 2015. Nosocomial emerging of (VIM1) carbapenemase-producing isolates of *Klebsiella pneumoniae* in North of Iran. Iran J Microbiol. 7(2):88-93.
31. Hammami S, Boubaker IB-B, Ghozzi R, Saidani M, Amine S, Redjeb S Ben. 2011. Nosocomial outbreak of imipenem-resistant *Pseudomonas aeruginosa* producing VIM-2 metallo-β-lactamase in a kidney transplantation unit. Diagn Pathol. 6(1):106.
32. Touati M, Diene SM, Dekhil M, Djahoudi A, Racherache A, Rolain J-M. 2013. Dissemination of a class I integron carrying VIM-2 carbapenemase in *Pseudomonas aeruginosa* clinical isolates from a hospital intensive care unit in Annaba, Algeria. Antimicrob Agents Chemother. 57(5):2426–2427.
33. Kazi M, Drego L, Nikam C, Ajbani K, Soman R, Shetty A, *et al*. 2015. Molecular characterization of carbapenem-resistant *Enterobacteriaceae* at a tertiary care laboratory in Mumbai. Eur J Clin Microbiol Infect Dis. 34(3):467–472.
34. Nordmann P, Dortet L, Poirel L. 2012. Carbapenem resistance in *Enterobacteriaceae*: here is the storm! Trends Mol Med. 18(5):263–272.
35. Sonnevend Á, Ghazawi AA, Hashmey R, Jamal W, Rotimi VO, Shibl AM, *et al*. 2015. Characterization of carbapenem-resistant *Enterobacteriaceae* with high rate of autochthonous transmission in the Arabian Peninsula. PLoS One. 10(6): e0131372.
36. Ozgumus OB, Akkoyunlu Y, Doymaz MZ, Iraz M, Cicek AC, Sandalli C, *et al*. 2015. Distribution of Beta-Lactamase Genes Among Carbapenem-Resistant *Klebsiella pneumoniae* Strains Isolated from Patients in Turkey. Ann Lab Med. 35(6):595–601.
37. Ben Helal R, Dziri R, Chedly M, Klibi N, Barguellil F, El Asli MS, *et al*. 2018. Occurrence and characterization of carbapenemase-producing *Enterobacteriaceae* in a Tunisian Hospital. Microb Drug Resist. 24(9):1361–1367.
38. Lin KY, Lauderdale TL, Wang JT, Chang SC. 2016. Carbapenem-resistant *Pseudomonas aeruginosa* in Taiwan: Prevalence, risk factors, and impact on outcome of infections. J Microbiol Immunol Infect. 49(1):52–59.