

Rapid identification of carbapenemases by CarbAcineto NP test and the rate of beta-lactamases among *Acinetobacter baumannii* from a teaching hospital

Morubagal Raghavendra Rao, Tejashree Anantharaj Urs, Vidyavathi B Chitharagi, Sowmya Shivappa*, Rashmi Padmanabha Mahale, Ranjitha Shankare Gowda, Kavya Shree

Department of Microbiology, JSS Medical College, JSSAHER, Mysore, India

Received: May 2021, Accepted: March 2022

ABSTRACT

Background and Objectives: *Acinetobacter baumannii* has emerged as a major organism accounting for hospital acquired infections particularly in intensive care units. Due to production of different kinds of beta lactamases these bacteria have developed drug resistance rendering the treatment of such infections very difficult and expensive. Rapid identification of *A. baumannii* producing such beta-lactamases is the need of the hour in reducing morbidity and mortality associated with *A. baumannii* infections.

Materials and Methods: *A. baumannii* was isolated from clinical samples like endotracheal aspirates, sputum, urine, exudates using standard culture techniques. Identification and drug sensitivity was done using Vitek 2 system. All the isolates were subjected to detection of ESBLs using phenotypic confirmatory test, plasmid mediated AmpC beta- lactamase by AmpC disc test, Carbapenemase production by CarbAcineto NP Test and Modified hodge method.

Results: 149 *A. baumannii* isolates were analysed for antimicrobial susceptibility and various beta-lactamase production. Results were evaluated for statistical significance using Chi-Square and P value. 81.8% of isolates were from male patients with majority of them above 50 years of age. 88.5% of samples were from ventilator associated pneumonia patients. 83.8% of isolates were sensitive to tigecycline. Only 10% to 12% of isolates were sensitive to carbapenems. 23.4% of isolates were ESBL producers and 46.9% of them were AmpC producers. Modified Hodge test method identified 63.7% of *A. baumannii* as carbapenemase producers where as CarbAcineto NP test identified 63% and exhibiting 94.74% sensitivity, 93.22% specificity when compared to Modified Hodge test.

Conclusion: Multidrug resistant *Acinetobacter* spp. is on the rise. Present study showed that high percentage of drug resistance in *A. baumannii* could be due to production of ESBLs, AmpC and carbapenemases. Among all beta lactamases carbapenemase producers are more and quickly raising in *A. baumannii*. Rapid, cost effective assay which can be adopted in all clinical laboratories is critical to prevent their further transmission particularly in hospital environment.

Keywords: AmpC; Beta-lactamase; Carbapenemase; Extended spectrum beta lactamase

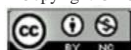
INTRODUCTION

Antimicrobial resistance among Gram negative bacteria is growing problem globally as reported by

various scientific studies. *Acinetobacter baumannii* a Gram negative non fermenter is now emerging as an important multidrug resistant pathogen causing nosocomial infections. This renders the treatment of

*Corresponding author: Sowmya Shivappa, MD, Department of Microbiology, JSS Medical College, JSSAHER, Mysore, India. Tel: +919-886686854 Fax: +918-212548345 Email: sowmyashivappa@gmail.com

Copyright © 2022 The Authors. Published by Tehran University of Medical Sciences.



This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International license (<https://creativecommons.org/licenses/by-nc/4.0/>). Noncommercial uses of the work are permitted, provided the original work is properly cited.

such hospital acquired infections very difficult and expensive.

A. baumannii infections are frequently treated with β -lactam antibiotics like cephalosporins and carbapenems or with other group of antimicrobials like aminoglycosides, polymyxins and glycolcycline. Majority of *A. baumannii* strains have developed resistant to most of these antibacterial agents due to the presence of mobile genetic elements, such as insertion sequences (ISs), plasmids, integrons and resistance islands (2). The most important mechanism of β -lactam resistance in *A. baumannii* is production of various types of beta-lactamases like extended spectrum betalactamases (ESBLs), AmpC and carbapenemases. The Ambler classification divides the β -lactamases into four major molecular classes (A-D). The extended-spectrum β -lactamases (ESBLs) belong to class A and can hydrolyze first, second and third-generation of Cephalosporins, Penicillins, and monobactams but are inhibited by clavulanate (3). AmpC β -lactamases production is mediated either by chromosomes or by plasmids and cannot be inhibited by clavulanic acid, but can be inhibited by cloxacillin. The enzymes belong to the molecular class C (4). Chromosomally encoded AmpC beta-lactamases in *A. baumannii* have a low level of expression that does not cause clinically appreciable resistance, however, the addition of a promoter insertion sequence ISAba1 next to the *ampC* gene increases beta-lactamase production, causing resistance to cephalosporins (5). Plasmid mediated AmpC and ESBLs can effortlessly move from one bacteria to another resulting in the faster spread of the resistance genes (2). Due to the emergence of ESBLs and AmpC beta-lactamase, Carbapenems like imipenem, meropenem are used extensively to treat infections in hospitalized patients. This resulted in unresponsiveness among bacteria to carbapenems. carbapenem resistance in *A. baumannii* is due to a range of mechanisms like decreased expression of outer membrane proteins and production of carbapenemases (6). Increasing proportion of *A. baumannii* producing carbapenemases has resulted in growing therapeutic concern worldwide due to very few treatment options available right now. The rapid identification of such isolates is the need of the hour to manage infections associated with such organisms and to prevent their spread (7). This study was carried out with an intention to quickly identify the alarmingly emerging carbapenemases and also to know the rate of ESBL and AmpC beta-lactamase

from *A. baumannii*.

MATERIALS AND METHODS

The present study was carried out in a tertiary care teaching hospital for a period of one year. A total of 149 *A. baumannii* isolates were collected from clinical samples like endotracheal aspirates, sputum, urine, exudates etc from patients admitted during study period. Isolation of *A. baumannii* was done by standard culture techniques by inoculating clinical samples on to blood agar and MacConkey agar and incubating aerobically at 37°C.

Identification and drug sensitivity of *A. baumannii* was done using Vitek 2 system according to the manufacturer's guidelines. All the isolates were subjected to phenotypic detection of ESBL, AmpC and Carbapenemase.

ESBL detection. A lawn culture of *A. baumannii* was done on quality control passed and sterility checked Mueller Hinton agar plate. A sterile disc of ceftazidime (30 μ g) and ceftazidime and clavulanic acid (30 μ g/10 μ g) (Himedia) was applied at a distance of 20 mm from centre to centre on Mueller Hinton agar plate, and incubated at 37°C for 18 to 24 hours. A difference in the zone of more than or equal to 5 mm between the two discs was considered as ESBL producer (1).

AmpC disc test. Using 0.5 McFarland's matched broth of ATCC *E. coli* 252922 a lawn culture was done with sterile cotton swab. A sterile 30 μ g cefoxitin disc (Himedia) was kept on agar surface and a blank sterile disc was moistened with sterile saline and was inoculated with few colonies of *A. baumannii* isolate and placed touching the cefoxitin disc. The plates were incubated at 37°C for 18 to 24 hours. The organism was considered as AmpC producer if flattening or indentation of inhibition zone of cefoxitin disc in the vicinity of the disc with *A. baumannii* (8).

Carbapenemase production: modified Hodge method. Using 0.5 McFarland's matched broth of ATCC *E. coli* 252922 a lawn culture was done with sterile cotton swab. 10 μ g meropenem disc (Himedia) was placed at the centre of the agar plate and *A. baumannii* isolate was streaked from the disc's edge to the periphery of the plate. The plate was then incubat-

ed at 37°C for 18 to 24 hours. Appearance of a clover leaf shaped zone of inhibition along the growth of *A. baumannii* indicates carbapenemase production (9).

CarbAcineto NP. From overnight growth of *A. baumannii* on Mueller-Hinton agar (MHA) two to three colonies were placed in two 1.5-ml Eppendorf tubes (A and B) containing 100 µl of 5 M NaCl and mixed using a vortexer for 5 s. Both the tubes were mixed with 100 µl of a pH adjusted 0.05% phenol red containing 0.1 mmol/liter ZnSO₄. Then to the tube B 12 mg/ml Imipenem-cilastatin injectable drug was added. Both the tubes were incubated at 37°C for 2 hours. The carbapenemase produced by the bacterial strains could hydrolyze the imipenem into a carboxylic derivative and result in decrease in pH. This reduction in pH is indicated by color change of the phenol red solution. Any change in color from red to orange/yellow in the antibiotic containing tube (tube B) indicates carbapenemase activity (9).

RESULTS

During study period, 149 *A. baumannii* non repetitive clinical isolates were analysed for antimicrobial susceptibility and various beta-lactamase production. Results were evaluated for statistical significance using Chi-Square and P value.

Out of 149 isolates, 122 (81.8%) were from male patients and 28 (18.7%) were from female patients. Maximum number of isolates studied were from patients in the age group of 41-50 (20%) and 51-60 years (18.6%). Age wise and gender wise distribution of patients from whom these clinical isolates grown is shown in Table 1.

Table 1. Age and sex wise distribution of the cases

Age group	Male	Female	Percentage
0 - 1	1	0	0.6
1-10 years	8	1	6
11-20 years	6	1	4.6
21-30 years	25	1	17.4
31-40 years	16	8	16
41-50 years	25	5	20
51-60 years	20	8	18.6
61-70 years	10	4	9.3
>71 years	10	0	6.6

Maximum number of clinical isolates were from endotracheal aspirates (88.5%) of patients with suspected ventilator associated pneumonia (VAP) followed by sputum from patients diagnosed with nosocomial pneumonia and exudates samples (4.6%) two each from diabetic wound and burns wound. Distribution of isolates according to sample source is shown in Table 2.

Out of 132 patients with suspected VAP 84 were admitted in neuro ICU, 37 were admitted in RICU and 11 patients were in MICU. Piperacillin-tazobactam, meropenem or imipenem were predominant antibiotics started in these patients. Later most of these patients were started with tigecycline or colistin based on antimicrobial susceptibility which was performed using N-281 card of Vitek 2 system. Majority of the isolates were resistance to commonly used cephalosporins like ceftazidime, cefipime. Only 10% to 12% of isolates were sensitive to carbapenems like imipenem, meropenem and doripenem. The most susceptible drug was found to be the tigecycline (83.8%) followed by minocycline (61.7%). Antimicrobial resistance pattern of these isolates is depicted in Table 3.

Out of 149 *A. baumannii* only 23.4% of isolates were ESBL producers where as 46.9% of them were AmpC producers. Distribution of these beta-lactamases is shown in Table 4.

Modified Hodge test method identified 63.7% of *A. baumannii* as carbapenemase producers where as CarbAcineto NP test identified 63%. Both Modified Hodge test method (Chi-Square=10.667; P=.001) and CarbAcineto NP test (Chi-Square=9.627; P=.002) showed statistically significant result. Number of carbapenemase producers identified by both methods is shown in Table 5. when both the methods are compared 90 *A. baumannii* were carbapenemase producers by both the methods, 5 isolates only by Modified Hodge test 4 isolates only by CarbAcineto NP test. When Modified Hodge test is considered as gold standard CarbAcineto NP test has sensitivity of 94.74% and specificity of 93.22%. Positive predictive value is 95.74% and negative predictive value is 91.66%.

DISCUSSION

A. baumannii belongs to the *Moraxellaceae* family commonly associated with infections like hospital-acquired and ventilator-associated pneumonia, urinary tract infections and pyogenic infections. *A.*

Table 2. Sample wise distribution of the clinical isolates

SL.NO	SAMPLE	SAMPLE SIZE	TOTAL %
1	ENDOTRACHEAL ASPIRATE	132	88.5
2	EXUDATE	7	4.6
3	SPUTUM	7	4.6
4	URINE	1	0.6
5	BILE	1	0.6
6	CENTRAL VENOUS CATHETER TIP	1	0.6

Table 3. Antibiotic sensitivity pattern of clinical isolates

Antibiotics	Sensitive	Resistance	Intermediate
Ceftazidime	7 (4.6%)	128 (85.9%)	14 (9.3%)
Piperacillin-tazobactam	17 (11.4%)	132 (88.5%)	-
Cefepime	9 (6%)	135 (90.6%)	5 (3.3)
Aztreonam	4 (2.6%)	145 (97.3%)	-
Doripenem	15 (10%)	132 (88.5%)	2 (1.3%)
Imipenem	17 (11.3%)	132 (88.5%)	-
Meropenem	17 (11.3%)	132 (88.5%)	-
Gentamycin	27 (18.1%)	118 (79.1%)	4 (2.6%)
Ciprofloxacin	16 (10.7%)	130 (87.2%)	3 (2%)
Levofloxacin	18 (12%)	115 (77.1%)	16 (10.7%)
Minocycline	92 (61.7%)	41 (27.5%)	16 (10.7%)
Tigecycline	125 (83.8%)	6 (4%)	18 (12%)
Colistin	-	1 (0.6%)	148 (99.3%)
Trimethoprim/sulfamethoxazole	19 (12.6%)	130 (87.2%)	-

Table 4. Distribution of ESBLs and AmpC beta-lactamases

Test	Positive		Negative	
	No.	%	No.	%
ESBL	35	23.4	114	76.5
AmpC disc Test	70	46.9	79	53

baumannii is one of the ESKAPE organisms that pose a global threat to human health (5).

A. baumannii has developed resistance to many groups of antibiotics due to indiscriminate use of antimicrobials (10). One of the major concerns is development of resistance to beta-lactam group of antibiotics as this result in restricted treatment options for treating *A. baumannii* infections and can contribute to increased morbidity and mortality in ICU patients (11). The overall prevalence of multi-drug-resistant strains in patients with *A. baumannii*

Table 5. Carbapenemase producers identified by Modified Hodge test and CarbaacinetNP test

Test	Positive		Negative	
	No.	%	No.	%
Modified Hodge test	95	63.7	54	36.2
CarbaacinetNP test	94	63	55	36.9

hospital-acquired and ventilator-associated pneumonia is estimated to be 79.9%, ranging from 56.5% in Argentina and 61.8% in Taiwan to 100% in Central America, Pakistan, Lebanon, Qatar, and Croatia, while its overall mortality can be as high as 56.2%. Carbapenems were the treatment of choice for multi-drug resistant *A. baumannii* infections. Their extensive use has led to increased incidence of carbapenem resistance in recent years (5). One of the frequent ways of developing resistance to carbapenems in *A. baumannii* is production of carbapenemase especial-

ly Ambler class D β -lactamases and to a lesser extent class B β -lactamases [metallo- β -lactamases (MBLs)] which includes four types like IMP, VIM, NDM, and SIM. Most of the class D β -lactamases are not inhibited by β -lactamase inhibitors (12). Other types of beta-lactamases in *A. baumannii* includes Class A beta-lactamases like ESBLs, KPCs and Class C beta-lactamases, which are chromosomally encoded cephalosporinases (*Acinetobacter* derived cephalosporinase, ADC. Carbapenem resistant *A. baumannii* was ranked in 2018 by WHO as number one priority for antibiotic research and development (5). So need of the hour is rapid identification of *A. baumannii* producing such carbapenemase. In the present study around 149 *A. baumannii* were screened for rapid identification of carbapenemase and also other beta lactamases like ESBLs and AmpC.

In the present study 122 (81.8%) clinical isolates included were from male patients with highest number of isolates from patients above 50 years of age. In accordance to present study TrangDinhVan et al. also observed male predominance (72.7%) and aged above 50 years (13). These findings suggest that male elderly were more vulnerable for infections in both the studies. 88% of *A. baumannii* during study period were from endotracheal aspirates followed by exudate samples. In a study done by Uzma Rehman et al. also reported similar findings (8). These findings highlights that *A. baumannii* is the predominant organism causing ventilator associated pneumonia.

In this study 83.8% of *A. baumannii* were sensitive to tigecycline. Sensitivity to carbapenems ranged between 10% to 12%. Michelle Lowe et al. from South Africa also, reported carbapenem sensitivity in the range of 11% to 13% and 90% of the isolates were resistant to tigecycline (12). In a study from Lebanon, Micheline Hajjar Soudeihia et al. reported carbapenem sensitivity between 16% to 23% and only one isolate was reported as resistant to colistin (14). These findings suggest that antimicrobial susceptibility patterns vary from place to place. Since present study was done in a tertiary care hospital where the patients are referred who are terminally ill and treated with broad spectrum antibiotics elsewhere could be the reason behind increased resistance to higher antibiotics.

ESBL identification in *Acinetobacter* spp. is not easy as standard protocols are not available unlike in *Enterobacteraceae* (15). 23.4% of *A. baumannii* were ESBL producers in the present study and results

are almost in agreement with another Indian study by Amandeep Kaur et al. (1) who reported 27.5% and a study from Malaysia by Khan M et al. who reported 22% (16). However study by Rehab M. Abd El-Baky et al. (17) from Egypt reported 35%, Abdar et al. (2) from Iran reported 59%. In the present study 46.9% of isolates were AmpC producers. Similar rate of AmpC producers were also reported by Yadav SK et al. (18) (38.5%) and Richa Hans et al. (19) (56%). However Batra et al. (15) has reported only 12% AmpC producers. Based on these observations it is clear that percentage of ESBLs and AmpC produced by *A. baumannii* varied from study to study in different geographical areas.

Carbapenemase producing *A. baumannii* strains are on rise due to indiscriminate use of carbapenems in hospitalized patients. This has resulted in increase morbidity and mortality globally due to treatment failure. One of the approaches in preventing treatment failures is quicker identification of carbapenemase producing *A. baumannii*. In the present study CarbAcineto NP test method for rapid identification of carbapenemase was adapted which gives results in two hours. By this method 63% of *A. baumannii* were carbapenemase producers where as by Modified Hodge method 63.7% of isolates were carbapenemase producers. Alaa Abouelfetouh et al. (9) has reported 78.4% of carbapenemases and 71.2% by Uzma Rehman et al. (8) in Modified Hodge method almost similar to present study. Moulana et al. (20) reported 84% carbapenemase by Modified Hodge method. Where as in the Alaa Abouelfetouh et al. (9) study 95.94% of *A. baumannii* were positive for carbapenemase by CarbAcineto NP test similarly Jiang L et al. (21) also reported 93.4% carbapenemases. These reports of carbapenemases producing *A. baumannii* from different parts of world clearly suggest that carbapenem resistance in *A. baumannii* is increasing globally and is a threat to mankind.

When two methods for carbapenemase detection was compared in the current study, it showed that 90 *A. baumannii* were positive for carbapenemase by both Modified Hodge method and CarbAcineto NP test. Whereas CarbAcineto NP test alone identified 4 carbapenemase producers and Modified Hodge method 5 isolates. CarbAcineto NP test has sensitivity of 94.74% and specificity of 93.22%. This findings show that both the methods are reliable to detect the carbapenemase even though some marginal difference was observed. However when compared

to turnaround time, CarbAcineto NP test is rapid, results are available in 2 hours where as Modified Hodge method takes 18-24 hours.

Sensitivity and specificity of different carbapenemase detection methods varies greatly as reported by different studies like Abouelfetouh et al. (9), Lee YT et al. (22), Labeeb A (23). Phenotypic detection of carbapenemases has the advantages of low cost, ease of procedure and the absence of complicated or expensive equipment; however, it suffers from poor specificity and sensitivity unlike PCR. So it is better to use more than one method for not missing out carbapenemase producers however it may not be possible to adapt all the different methods in routine laboratory practice. It is better to use more rapid, reliable, cost effective methods like CarbAcineto NP or modified carbapenem inactivation method (mCIM) methods. One of the limitations of the present study is not evaluating sensitivity and specificity of phenotypic methods by genotypic detection methods due to cost constraints.

CONCLUSION

Acinetobacter are “superbugs” causing infections in the intensive care units. Multidrug resistant *Acinetobacter* spp. is on the rise making the treatment more difficult. The present study showed that high percentage of drug resistance in *A. baumannii* and also prevalence of ESBLs, AmpC and carbapenemase among these isolates is high. Rapid, cost effective, assay which can be adopted in all clinical laboratories is critical to prevent their further transmission particularly in hospital environment.

REFERENCES

1. Kaur A, Singh S. Prevalence of extended spectrum betalactamase (ESBL) and metallobeta lactamase (MBL) producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from various clinical samples. *J Pathog* 2018;2018: 6845985.
2. Abdar MH, Taheri-Kalani M, Taheri K, Emadi B, Hasanzadeh A, Sedighi A, et al. Prevalence of extended-spectrum beta-lactamase genes in *Acinetobacter baumannii* strains isolated from nosocomial infections in Tehran, Iran. *GMS Hyg Infect Control* 2019;14: Doc02.
3. Zarabadi-Pour M, Peymani A, Habibollah-Pourzereshki N, Sarookhani MR, Karami AA, Javadi A. Detection of extended-spectrum β -Lactamases among *Acinetobacter baumannii* isolated from hospitals of Qazvin, Iran. *Ethiop J Health Sci* 2021;31:229-236.
4. Liu Y, Liu X. Detection of AmpC β -lactamases in *Acinetobacter baumannii* in the Xuzhou region and analysis of drug resistance. *Exp Ther Med* 2015;10:933-936.
5. Kyriakidis I, Vasileiou E, Pana ZD, Tragiannidis A. *Acinetobacter baumannii* antibiotic resistance mechanisms. *Pathogens* 2021;10:373.
6. Gladstone P, Rajendran P, Brahmadathan KN. Incidence of carbapenem resistant nonfermenting gram negative bacilli from patients with respiratory infections in the intensive care units. *Indian J Med Microbiol* 2005;23:189-191.
7. Hodiwala A, Dhoke R, Urhekar AD. Incidence of metallo-beta lactamase producing pseudomonas, acinetobacter & enterobacterial isolates in hospitalised patients. *Int J Pharma Bio Sci* 2013;3:79-83.
8. Rehman U, Dolma KG, Singh TS. Comparative analysis of various phenotypic methods for the detection of beta-lactamase in *Acinetobacter baumannii* isolates from a referral hospital in Sikkim, India. *IJBAMR* 2017;6:226-233.
9. Abouelfetouh A, Torkey AS, Aboulmagd E. Phenotypic and genotypic characterization of carbapenem-resistant *Acinetobacter baumannii* isolates from Egypt. *Antimicrob Resist Infect Control* 2019;8:185.
10. Lee K, Yong D, Jeong SH, Chong Y. Multidrug-resistant *Acinetobacter* spp.: increasingly problematic nosocomial pathogens. *Yonsei Med J* 2011;52:879-891.
11. Richet HM, Mohammed J, McDonald LC, Jarvis WR. Building communication networks: international network for the study and prevention of emerging antimicrobial resistance. *Emerg Infect Dis* 2001;7:319-322.
12. Lowe M, Ehlers MM, Ismail F, Peirano G, Becker PJ, Pitout JDD, et al. *Acinetobacter baumannii*: epidemiological and beta-lactamase data from two tertiary academic hospitals in Tshwane, South Africa. *Front Microbiol* 2018;9:1280.
13. Van TD, Dinh QD, Vu PD, Nguyen TV, Pham CV, Dao TT, et al. Antibiotic susceptibility and molecular epidemiology of *Acinetobacter calcoaceticus*-*baumannii* complex strains isolated from a referral hospital in northern Vietnam. *J Glob Antimicrob Resist* 2014;2:318-321.
14. Soudeihia MH, Dahdouh E, Daoud Z, Sarkis DK. Phenotypic and genotypic detection of β -lactamases in *Acinetobacter* spp. isolates recovered from Lebanese patients over a 1-year period. *J Glob Antimicrob Resist* 2018;12:107-112.
15. Batra P, Khurana S, Govindaswamy A, Aravinda A,

- Bajpai V, Ayyanar M, et al. Antibiotic resistance profile and co-production of extended spectrum beta lactamases and AmpC in *Acinetobacter* spp. in a level 1 trauma center from India. *J Lab Physicians* 2019;11:128-132.
16. Khan MFK, Rashid SS, Ramli ANM, Ishmael UC, Maziz MNH. Detection of ESBL and MBL in *Acinetobacter* spp. and their plasmid profile analysis. *Jordan J Biol Sci* 2019;12:373-378.
 17. Abd El-Baky RM, Farhan SM, Ibrahim RA, Mahran KM, Hetta HF. Antimicrobial resistance pattern and molecular epidemiology of ESBL and MBL producing *Acinetobacter baumannii* isolated from hospitals in Minia, Egypt. *Alexandria J Med* 2020;56:4-13.
 18. Yadav SK, Bhujel R, Hamal P, Mishra SK, Sharma S, Sherchand JB. Burden of multidrug-resistant *Acinetobacter baumannii* infection in hospitalized patients in a tertiary care hospital of Nepal. *Infect Drug Resist* 2020;13:725-732.
 19. Hans R, Bisht D, Agarwal R, Irfan M. Phenotypic detection of MBL, Ampc beta-lactamase and carbapenemases in multi drug resistant isolates of *Acinetobacter baumannii*. *Int J Med Res Heal Sci* 2015;4:311-316.
 20. Moulana Z, Babazadeh A, Eslamdost Z, Shokri M, Ebrahimipour S. Phenotypic and genotypic detection of metallo-beta-lactamases in Carbapenem resistant *Acinetobacter baumannii*. *Caspian J Intern Med* 2020;11:171-176.
 21. Jiang L, Liang Y, Yao W, Ai J, Wang X, Zhao Z. Molecular epidemiology and genetic characterisation of carbapenem-resistant *Acinetobacter baumannii* isolates from Guangdong province, South China. *J Glob Antimicrob Resist* 2019;17:84-89
 22. Lee YT, Huang TW, Liu IF, Kuo SC, Yang YS, Lin PY, et al. The prediction values of carbapenemase detection methods and carbapenem susceptibility testing for clinical outcomes of patients with *Acinetobacter* bacteremia under carbapenem treatment. *J Microbiol Immunol Infect* 2022; 55:257-265.
 23. Labeeb AZ, Elbrolosy AM. Comparative study for detection of carbapenemase producers among non-fermenting gram negative uropathogens with special reference to risk factors and fosfomycin susceptibility. *J Clin Diagn Res* 2019; 13:DC14-DC21.