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ORIGINAL ARTICLE

Comparative Study of Phenotypic Detection Methods of Metallo-beta-lactamase (MβL) Among Carbapenem Resistant Gram-Negative Bacilli (GNB) Isolated from Intensive Care Units (IUC) Patients

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ABSTRACT

Background: *Metallo-beta-lactamase (MβL) mediated resistance is an emergency threat in health care settings, and its identification is essential for treatment and infection control.* ***Objectives****: this study aimed to detect the MβL prevalence in Gram negative bacilli (GNB) and to compare its phenotypic detection methods.* ***Methodology****: Ninety six (96) isolates of GNB were isolated from different clinical specimens collected from patients admitted to intensive care units (ICU) of Ain Shams Hospitals, from February 2018 to June 2018. Isolates were screened for carbapenem resistance with imipenem 10 µg and meropenem 10 µg discs. The resistant isolates were tested for antibiotic susceptibility by disc diffusion method, and Meropenem minimum inhibitory concentration (MIC) were determinated, then the production of MβL was detected by imipenem-ethylene diamine tetra-acetic acid (EDTA) combined disc test (IPM-EDTA CDT), ceftazidime -EDTA combined disc test (CAZ-EDTA CDT) and Imipenem - EDTA double disc synergy test (IPM-EDTA DDST).* ***Results:*** *Forty three (43) isolates (44.7%) were resistant to carbapenem. Klebsiella pneumoniae (K.pneumoniae) was the most common isolated species; 29 (67.4%) isolates. Forty (40) isolates (93%) were positive for MβL by IPM-EDTA CDT method, whereas 36 (83.7%) were positive by CAZ -EDTA CDT method and 19 isolates (44.2%) were positive for MβL by IPM-EDTA DDST.* ***Conclusion****: High prevalence of MβL was detected among our isolates and IMP-EDTA CDT can be used as a phenotypic test in detection of MβL production.*

INTRODUCTION

Carbapenems possess a broad-spectrum activity and stability to hydrolysis by most beta lactamase (β-lactamase). However, the recent emerging resistance to this class of β-lactams among *Enterobacteriaceae* due to production of carbapenemases leaves the health care system vulnerable and reduces therapeutic choices[1]. Carbapenemases may be defined as beta-lactamases that significantly hydrolyze at least imipenem or meropenem. Carbapenemases are members of the molecular class A, B, and D β-lactamases. Class B or the metallo-β-lactamases (MβL) are the most significant carbapenemases[2].

The worldwide dissemination of MβL acquired from *Pseudomonas* spp to members of the *Enterobacteriaceae* family, has been emerged and become a great concern[3]

MβL are becoming a challenge as these enzymes cause hydrolysis of all β-lactam antibiotics including carbapenems[4], which often used as "last-line agents" or "antibiotics of last resort" in treatment of severe infections[5].

MβL carbapenemases are mostly encoded by genes located on integron structures that present on mobile plasmids and transposons, and thus facilitating its wide spreading and outbreaks[6].

Detection of MβL producing organisms is of utmost importance in establishing an appropriate antimicrobial therapy and in prevention of their propagation[7]. Currently, the MβL production among *Enterobacteriaceae* isolates can be detected via an initial screening test to evaluate strain susceptibility to carbapenems followed by phenotypic and/or genotypic confirmation test.

Although molecular methods as whole genome sequencing and polymerase chain reaction (PCR) are the gold standard methods and highly accurate, its availability is often limited to reference laboratories[8].

The phenotypic confirmation of carbapenemase production is based on the detection of diffusible carbapenemases or on inhibition of carbapenemase activity using MβL inhibitor like EDTA metal-chelating agent[9].

This study focuses on simple phenotypic detection methods of carbapenem resistance and aims at knowing

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the prevalence of MβL production among Carbapenem resistant Gram Negative Bacilli (CRGNB).

METHODOLOGY

Bacterial isolates:

Ninety six (96) isolates of GNB were included in this study, including *Escherichia coli (E. coli), Klebsiella, Pseudomonas* and others, isolated from different clinical specimens from patients admitted to Intensive Care Units of Ain Shams Hospitals, from February 2018 to June 2018. The bacterial isolates were identified by conventional bacteriological methods according to ***Collee et al*[10].**

Antibiotics sensitivity and MβL Screening method:

Antibiotics susceptibility of all isolates was performed by the disc- diffusion method on Muller-Hinton agar plate via Kirby Baur disc diffusion method as per CLSI 2017 guidelines[11]. Susceptibilities of Amoxicillin/Clavulanic acid (AMC, 20/10 µ g), piperacillin/tazobactam (TPZ,100/10 µg), ceftriaxone (CRO,30 µg), Ceftazidime (CAZ, 30 µg), cefotaxime (CTX,30 µg),cefepime (FEP,30 µg),imipenem (IPM,10 µg), meropenem (MEM,10 µg), gentamicin (CN,10 µg), amikacin (AK,30 µg), ciprofloxacin (CIP,5 µg), levofloxacin (LEV,5 µg) and aztreonam (ATM) (30 g) were tested. *E.coli* ATCC 25922 was used as a quality control strain.

Isolates that were resistant to both imipenem (10µg), meropenem (10µg), and third generation cephalosporin by disc diffusion were considered as screening positive.

Minimum inhibitory concentration (MIC) Determination:

Carbapenem resistance were confirmed by determination of Meropenem MIC with the E test (bioMérieux, Marcy l'Etoile, France) and interpreted according to CLSI guidelines[11].

Phenotypic detection of MβL:

Combined Disc Test (CDT):

A confirmation test for MβL production was carried out using combined-disc method. A 0.5 M EDTA (Hi-Media, India) solution was prepared by dissolving 18.61 g of EDTA in 100 ml of distilled water and adjusting it pH 8.0 by using NaOH[12].

Imipenem -EDTA combined disc test (IMP-EDTA CDT):

According to Yong et al[12], 0.5 McFarland of overnight culture of test isolate was done on Muller Hinton agar. Two 10 µg imipenem discs were used, one imipenem disc alone and one with 10 µl of 0.5 M EDTA solution and placed on inoculated plates. The test was considered as positive if the zone of inhibition of imipenem + EDTA discs is >7 mm compared to imipenem alone, after 16 to 18 hours of incubation at 37◦C.

Ceftazidime-EDTA combined disc test (CAZ -EDTA CDT):

Two discs were added to an agar plate, one of them contained CAZ and the other contained CAZ in addition to EDTA solution, after an incubation period of16 to 18 hours of incubation at 37ºC the discs were observed. If the zone of inhibition around the CAZ/EDTA disc is ≥ 7mm more than that of the CAZ only disc, the test is considered positive for MβL production[12].

Imipenem - EDTA Double disc synergy test (DDST-IPM):

A blank disc containing 10µl of 0.5 M EDTA (750ug) was placed 20 mm center to center from an imipenem (10ug) disc. Plates were incubated for 16 to 18 hours at 35˚C. If there is enhancement of zone of inhibition between imipenem and EDTA disc, it was considered MβL positive[13].

Statistical analysis:

The results were analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 22.0[14]. A level of significance of 5% ($p<0.05$) was adopted for all tests.

RESULTS

Carbapenem resistant GNB (CRGNB) isolates:

Among 96 *GNB* isolates; Forty-three (43) isolates were identified as carbapenem resistant based on IPM (10 µg) and MEM (10 µg) resistance by disc diffusion method. The CRGNB isolates include; 29 *Klebsiella pneumoniae (K.pneumoniae)* (67.4%), 10 *E. coli* (23.3%), 2 *Pseudomonas aurginosa* (4.65%) and *2 Proteus* species (4.65%) (Table1).

Table (1): Types of CRGNB isolated organisms:

Types of isolated organisms	CRGNB N (%) N=43
E .coli	10 (23.3%)
Klebsiella pneumoniae	29 (67.4%)
Proteus species	2 (4.65%)
Pseudomonas aurginosa	2 (4.65%)

Most of the CRGNB isolates were collected from Urine ,18 isolates (41.9%), 12 isolates (27.9%) were isolated from tracheal aspirate (TA), 7 isolates (16.2%) were isolated from sputum and 6 isolates (14 %) were isolated from blood (Table 2)

Table 2: Types of collected specimens and its percentages

Types of specimens	CRGNB N(%) N=43
Urine	18 (41.9%)
Blood	6 (14%)
Tracheal aspirate	12 (27.9%)
Sputum	7 (16.2%)

Antibiotics susceptibility testing of CRGNB isolates:

All the CRGNB isolates (100%) were resistant to all beta-lactam/beta-lactamase inhibitor combinations, 3rd generation cephalosporine, cefepime, ciprofloxacin and levofloxacin. 34 isolates (79.1%) were resistant to gentamicin, and 41 isolates (95.3%) were resistant to amikacin (Table 3).

Table 3:Antibiotics sensitivity of the CRGNB isolates

Antibiotics	Sensitive	Resistant
Amoxicillin-Clavulenic acid	0	43 (100%)
Pipracillin –Tazobactam	0	43 (100%)
Cefoxitin	0	43 (100%)
Ceftriaxone	0	43 (100%)
Ceftazidime	0	43 (100%)
Cefitaxime	0	43 (100%)
Cefepime	0	43 (100%)
Amikacin	2 (4.7%)	41(95.3%)
Gentamicin	9 (20.9%)	34(79.1%)
Ciprofloxacin	0	43 (100%)
Levofloxacin	0	43 (100%)
Azteronam	19 (44.2%)	24 (55.8%)
Imipenem	0	43 (100%)
Meropenem	0	43 (100%)

MIC of Meropenem of CRGNB isolates:

The MIC of Meropenem was ranged 12-32μg/ml. Out of 43 CRGNB, one isolate (2.3%) was resistant to meropenem with MIC (12μg/mL) and 42 isolates (97.7%) were resistant to meropenem with MIC >32μg/mL as shown in figure 1 and table 4.

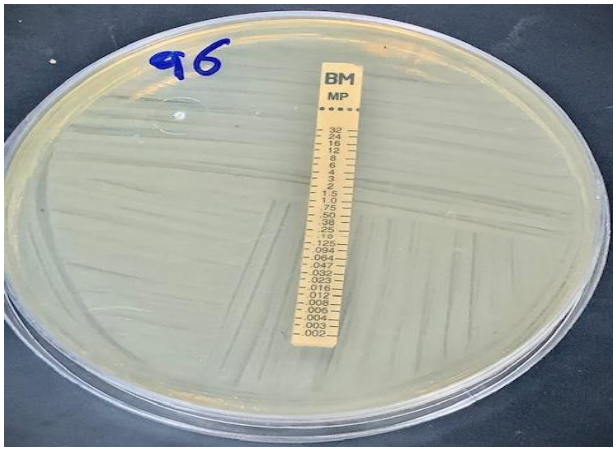

Fig. 1: MIC of meropenem by E test: MIC >32μg/mL

Table 4: Meropenem MIC by E test:

Meropenem MIC results	No of isolates (%)
MIC >32μg/mL	42 (97.7%)
MIC =12μg/mL	1 (2.3%)

Phenotypic detection of MβL by IMP-EDTA CDT and CAZ -EDTA CDT:

Out of 43 CRGNB isolates, 40 isolates (93%) were positive for MβL by IPM-EDTA CDT method, whereas 36 (83.7%) were positive by CAZ -EDTA CDT method (Figure 2 and table 5).

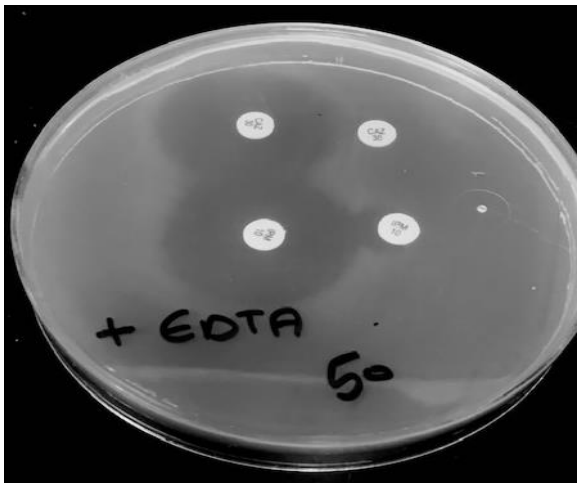

Fig. 2: Phenotypic detection of MβL by IMP-EDTA CDT and CAZ -EDTA CDT

Phenotypic detection of MβL by DDST-IPM:

Out of 43 CRGNB isolates, 19 isolates (44.2%) were positive for MβL and 24 isolates (55.8%) were negative for MβL production (Figure3 and table 5).

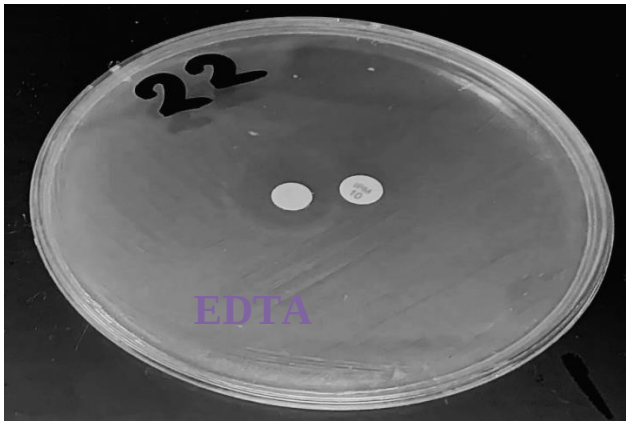

Fig. 3: Positive MβL by DDST-IPM

Table 5: Prevalence of MβL by different phenotypic detection methods:

Phenotypic test	MβL Positive	MβL Negative
IMP-EDTA CDT	40 (93%)	3 (7%)
CAZ-EDTA CDT	36 (83.7)	7(16.3%)
DDST-IPM	19 (44.2%)	24(55.8%)

DISCUSSION

The presence of a MβL -positive isolate in a hospital setting is a serious therapeutic problem. The accurate identification of such organisms is of great use in infection control and prevention of spread of this multidrug resistant bacteria[15].Although different phenotypic methods had been described, CLSI[16] does not include standardized recommendations for MβL screening. These Phenotypic tests include: Modified Hodge test (MHT), DDST, CDT and MβL E test[17].

Among these different methods, MβL E test is considered as the most widely accepted standardized MβL screening test[18].However, most of clinical microbiology laboratories use other screening methods, like DDST and CDT because the E test strips are expensive and unavailable.

In the current study we used EDTA as chelating agent with imipenem and ceftazidime. The DDST and the CDT were simple to perform, and the materials were not expensive, nontoxic and easily accessible, which makes them highly applicable for detection of MβL[18]. However, these methods may lack high sensitivity or specificity especially when compared to other methods such as whole genome sequencing and PCR which are the gold standard despite their limited availability[12].

In present study, the prevalence of MβL production among the collected 96 isolates was 44.8% (43/96). Lower prevalence rates were reported by Pandya et al[1] and Panchal et al[19]who found prevalence of MβL production in GNB was 6% (27/450) and 19.62% (21/107) respectively. In the present study, IMP-EDTA CDT was found to be the most sensitive confirmation test, it was positive in 40/43 (93%) of the isolates. This result was in agreement with Hebat-allah et al[20] who found MEM-EDTA CDT sensitivity 94% in detection of MβL among their *E.coli* isolates. Also Meawed and Gad[21], found 18(41.8%) of 43 *Pseudomonas* isolates were MβLs by IPM–EDTA CDT with the best sensitivity 100% and specificity of 93.1%.

Sachdeva et al[22]also demonstrated that the CDT is the best method for screening of MβL production in *P. aeruginosa*. it was positive in 144 isolates out of 147 isolates (97.9%). Another study was conducted by Makled et al[23], who used Ertapenem/EDTA combined disk (ETP/EDTA-CD) test to detect MβL production among ESBL producing E.coli isolates. The test showed sensitivity of 91%, specificity of 94% and accuracy of 93% .it was able to detect 20/22 (90.9%) of *bla*VIM-positive isolates. In the present study we used CAZ-EDTACDT as the using a CAZ disc instead of IPM disc for CDT/DDST was recommended by Arakawa et al[24]**.**The test was positive in 36 isolates out of 43 isolates but MβL producing organisms may have CAZ resistance mechanisms. With such strains, CDT/DDST using CAZ will not show MβL production and, therefore, IPM disc must be used for detection of MβL[25].As regarding IMP -DDST it was positive in 19 isolate out of 43 isolates (54.8%). However, higher percentage was reported by Mishra et al[26]who found 89 (93.68%) out of 95 imipenem-resistant *Pseudomonas* isolates were positive by IPM DDST method. Also, Ranjan et al[27]demonstrated that DDST was more specific in detecting MβL in comparison to the CDT. However, Biradar and Roopa[28]**,** reported that CDT surpasses DDST as a screening test. They used both tests to detect MβL production among 200 *P. aeruginosa* isolates and they found, 49(24.5%) to be MβL producers by CDT while DDST were positive in 32(16%) isolates only. Also, Mehta and Prabhu [29]found 12(60%) isolates out of 20 imipenem resistant *P. aeurginosa* were positive for production of MβL by CDT, while only 8(40%) were detected by DDST, showing that CDT is superior and these results were in accordance of our results. This discrepancy in findings may be due to differences in population structure of MβL genes between different geographical area. False-positive MβL producers were detected by all phenotypic assays. These false-positive cases might actually be producing an unknown and weaker β-lactamases, which should be further investigated[27].

In the present study *K.pneumoniae* 29 (67.4%) was the most common isolated species, followed by *E. coli* 10 (23.), then *Pseudomonas and Proteus*, 2 isolates for each (4.65%). This results were in agreement with El-Rehewy et al[30] who found Klebsiella spp as the most common isolated spp, 34% of CRGNB isolates, followed by Pseudomonas spp 23.71%, 18.56% were E.coli and 8.25% were Proteus spp. this results were against Naim et al [31]**,** as *Citrobacter* species (25.2%) were the predominant CRGNB, followed by *E. coli* (24.2%), and *P. aeruginosa* (18.2%) followed by *Serratia* species (13.1%) and Klebsiella species (8.1%) in their study. Also, another study conducted by Wankhede et al[32] reported that *P. aeruginosa* (23.7%), *Acinetobacter spp*. (18.4%), *K. pneumoniae* (8.3%), and *E. coli* (5%) were the most common MβL producers. In this study all the MβL producing isolates were resistant to all beta-lactam antibiotics, ciprofloxacin and levofloxacin. This result was in accordance with Behera et al[25] and Ranjan et al[27]who reported that MβL –positive isolates are usually resistant to all β-lactam antibiotics, aminoglycosides, tetracycline, and fluoroquinolones.

Azteronam was the most sensitive drug.it was sensitive in 19 isolates (44.2%) and this results were against Naim et al[31] who observed high resistance of aztreonam in their study. Most of CRGNB isolates were obtained from urine (41.%) followed by tracheal aspirate (27.9%), sputum (16.2%) and blood (14%).this distribution was in agreement with El-Rehewy et al[28] who found the highest numbers of samples collected were the endotracheal aspirate (24.5%), followed by sputum samples (20%), urine samples (17.75%), blood

samples (16.06%), wound swabs (15.77%), and then throat swabs (5.92%) in their study.

CONCLUSION

The prevalence of MβL -producing *GNB* isolates in the ICU were high, with treatment failure due to high resistance to the commonly used antibiotics. IMP-EDTA CDT can be used as a routine for detection of MβL -producing strains to identify MβL production before antibiotic use.

Conflicts of interest:
The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

Limitation of the study

Unfortunately, there was inability to use MβL E test or molecular methods in our study due to lack of financial support.

REFERENCES

1. Pandya NP, Prajapati SB, Mehta SJ, Kikani KM, and Joshi PJ. Evaluation of various methods for detection of metallo-β-lactamase (MBL) production in gram-negative bacilli. Int J Biol Med Res 2011; 2:775-7.
2. Walsh T R, Toleman MA, Poirel L, and Nordmann P. Metallo-β-lactamases: the quiet before the storm? Clin. Microbiol. Rev 2005; 18:306-325.
3. Gupta V. Metallo beta lactamases in Pseudomonas aeruginosa and Acinetobacter species. Expert Opin. Investig. Drug 2008; 17:131–143.
4. Galani I, Rekatsina PD, Hatzaki D, Plachouras D, Souli M, and Giamarellou H. Evaluation of different laboratory tests for the detection of metallo-ßlactamase production in Enterobacteriaceae. J Antimicrob Chemother 2008:61;548-53.
5. Torres J A, Villegas MV, and Quinn JP. Current concepts in antibiotic-resistant gram-negative bacteria. Expert Rev. Anti Infect. Ther 2007; 5.
6. Maltezou HC, Giakkoupi P, Maragos A, Bolikas M, Raftopoulos V, Papahatzaki H, et al. Outbreak of infec- tions due to KPC-2-producing Klebsiella pneumoniae in a hospital in Crete (Greece). J Infect 2009; 58:213- 219.
7. Schwaber MJ and Carmeli Y. An ongoing national intervention to contain the spread of carbapenem-resistant Enterobacteriaceae. Clin Infect Dis 2014; 58:697–703.
8. Franklin C, Liolios L, and Peleg AY. Phenotypic detection of carbapenem-susceptible metallo-beta-lactamase-producing gram-negative bacilli in the clinical laboratory. J Clin Microbiol 2006; 44:3139-44.
9. Bartolini A, Frasson, I, Cavallaro A, Richter SN, and Palù G. Comparison of phenotypic methods for the detection of carbapenem non-susceptible *Enterobacteriaceae*. Gut Pathog 2014*;* 6:13.
10. Colle JG, Miles RS and Wan B. Tests for the identification of bacteria. In: Colle JG, Fraser AG, Marimon BP, Simmons A, editors. Mackei and McCartney Practical Medical Microbiology. 14th ed. Edinburg: Churchill Livingstone; 1996. p. 131-50.
11. CLSI. 2017. Performance Standards for Antimicrobial Susceptibility Testing. M100-S27. Clinical and Laboratory Standards Institute, Wayne, PA
12. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, and Chong Y. Imipenem–EDTA disc method for differentiation of metallo-ß-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol 2002; 40:3798-801.
13. Lee K, Lim YS, Yong D, Yum JH, and Chong Y. Evaluation of the Hodge test and the imipenem–EDTA double-disc synergy test for differentiating metallo-ßlactamase-producing isolates of Pseudomonas spp. and Acinetobacter spp.J Clin Microbiol 2003; 41:4623-9.
14. IBM Corp. IBM SPSS statistics for Windows, Version 22.0. Armonk, NY: IBM Corp 2013.
15. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT and Daikos G L. Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin. Microbiol. Rev.2012; 25, 682–707.
16. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 22nd Informational Supplement. CLSI document M100-S22. Wayne. PA: USA.2012.
17. Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, and Miriagou V. "Identification and screening of carbapenemase-producing

Enterobacteriaceae," Clinical Microbiology and Infection 2012;18(5): 432–438.

18. Qu TT, Zhang JL, Wang J, Tao J, Yu YS, Chen YG, et al. Evaluation of phenotypic tests for detection of metallo-beta-lactamase-producing Pseudomonas aeruginosa strains in China. J Clin Microbiol 2009; 47(4):1136–1142.
19. Panchal CA, Oza SS, and Mehta SJ. Comparison of four phenotypic methods for detection of metallo-β-lactamase-producing Gram-negative bacteria in rural teaching hospital. J Lab Physicians 2017 ;9(2):81–83.
20. Hebat-allah GR, Ahmed AO, and Moawad AK. Different Phenotypic Methods for Detection of Beta Lactam. Different Phenotypic Methods for Detection of Beta Lactam Resistance in Escherichia coli. Egyptian Journal of Medical Microbiology 2019;28(2): 19-26.
21. Meawed TE, and Gad DM. Clinical and Microbiological Characteristics of Metallo β-lactamase producing Pseudomonas aueroginosa in a sample of Egyptian Patients with Bronchiectasis. The Egyptian Journal of Medical Microbiology. 2017 Jul;38(5793):1-6.
22. Sachdeva R, Sharma B, and Sharma R. Evaluation of different phenotypic tests for detection of metallo-β-lactamases in imipenem-resistant Pseudomonas aeruginosa. J Lab Physicians 2017; 9:249-53.
23. Makled AF, Elbrolosy AM, Salem EH, and Awad ET. Prevalence of Carbapenem-Resistance among Extended Spectrum Beta Lactamase-Producing E. coli at Menoufia University Hospitals: Comparison of Phenotypic and Molecular Characterization Methods. The Egyptian Journal of Medical Microbiology. 2017 Jan;38(5782):1-9.
24. Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, and Goto M. Convenient Test for Screening Metallo-b-Lactamase-Producing Gram-Negative Bacteria by Using Thiol Compounds. Journal of Clinical Microbiology 2000; 38 (1): 40-43.
25. Behera B, Mathur P, Das A, Kapil A, and Sharma V. An evaluation of four different phenotypic techniques for detection of metallo-beta-lactamase producing *Pseudomonas aeruginosa*. Indian J Med Microbiol 2008 ;26:233–7.
26. Mishra SN, Biswal SR, Behera BK, Pattnaik D, et al. Detection of prevalence of metallo-beta lactamases in clinical isolates of imipenem resistant *Pseudomonas aeruginosa* from neonatal septicaemia cases in a tertiary hospital in Odisha, India. International Journal of Contemporary Pediatrics. Int J Contemp Pediatr 2018Jan;5(1):61-66.
27. Ranjan S, Banashankari G, and Babu PS. Comparison of epidemiological and antibiotic susceptibility pattern of metallo-Beta-lactamase-positive and metallo-Beta-lactamase-negative strains of *Pseudomonas aeruginosa*. J Lab Physicians. 2014;6(2):109–113.
28. Biradar S and Roopa C. Prevalence of Metallo-Beta-Lactamase Producing Pseudomonas aeruginosa and its antibiogram in a tertiary care center. International Journal of Current Microbiology and Applied Sciences 2015;4(9):952-956
29. Mehta A and Prabhu T. Detection and characterization of metallo-β-lactamases producing *Pseudomonas aeruginosa* clinical isolates at a tertiary care hospital of Bhopal, India. Int J Res Med Sci 2016;4 :4084-8.
30. El-Rehewy MS, Saboor EA, Afifi NA,and Ibrahim MA, Qayed SS. Detection and Characterization of Nosocomial Carbapenem-Resistant Gram Negative Bacilli from Assuit University Hospitals. The Egyptian Journal of Medical Microbiology. 2016 Jul;38(79):1-9.
31. Naim H, Rizvi M, Gupta R, et al. Drug Resistance and Molecular Epidemiology of Carbapenem Resistant Gram-negative Bacilli Isolates. *J Glob Infect Dis*.2018;10(3):133–139.
32. Wankhede SV, Iyer VS, and Bharadwaj RS. The study of MBL producers in Gram negative isolates from ICUs and wards. Indian J Basic Appl Med Res. 2011; 1:38.