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ORIGINAL ARTICLE

Phenotypic Methods and Molecular Metallo-β-lactamases Genes Detection in Different Clinical Carbapenems Resistant *Acinetobacters* Isolates from Tanta University Hospitals

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ABSTRACT

Background: *Acinetobacter species are very common in hospital acquired infections; Carbapenems resistant species become common in hospital and represent a major health problem.* ***Methodology:*** *Our study was carried on 150 patients from ICU; we compared different phenotypic methods and PCR for detection of carbapenems resistance in Acinetobacters.* ***Results:*** *88.9% of resistant Acinetobacters were detected by Kirby Bauer Disk diffusion method, 54.2% by Modified Hodge test, 70.8 % by EDTA disc synergy, 85.4% by Amp C disk test, 33.3% by PCR bla-IMP and 12.5% by PCR bla-VIM.* ***Conclusion:*** *We recommended for detection of carbapenems resistance in Acinetobacters the phenotypic methods as they are rapid, accurate methods and less costly than PCR.*

Note: bla-IMP gene (NCBI GenBank Nucleotide accession # LC103138.1);
bla-VIM gene (NCBI GenBank Nucleotide accession # MT185944.1
GenBank accession number S71932

INTRODUCTION

Acinetobacter species (spp) are widely distributed in different environments including soil, water, wastewater, and vegetables [1]. Also, *Acinetobacter* spp are present in healthy individuals in many sites [2]. They can remain alive for long time on inanimate objects in hospital environment as they are resistant to the commonly used antibiotics and some disinfectants [3].The most common species in hospital acquired infections are *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex (ABC), which is comprised of 4 spp; *A. baumannii*, *Acinetobacter noso-comialis*, and *Acinetobacter pittii* that cause clinical infections in humans, whereas *A. calcoaceticus* is an environmental organism of negligible clinical importance [4]. The most common species in hospital acquired infections is *Acinetobacter baumannii.* [4-7]

Moreover, it was included in published World Health Organization list of antibiotic resistant "priority pathogens" to be prevented, controlled and to focus on developing new antibiotics against them [8]. Multi-drug resistance (MDRO) that lead to resistance to almost all used antibiotics are very common in *Acinetobacters* , thus causes many nosocomial infections as ventilator-associated pneumonia and bloodstream infections with mortality rates up to 35.5% [9-11].

Carbapenems have been the mainstay of treatment of *Acinetobacters.* However, carbapenem resistance is increasingly recognized as a threat to the effective treatment of these infections by production of carbapenamases, which hydrolyse carbapenems [9, 10]. Carbapenamases are β-lactamases, which include serine-β-lactamases and metallo-β-lactamases (MBLs). Metallo-β-lactamases (MBLs) need for their activity metal ion zinc, metal chelators like EDTA and thiol-based compounds inhibit these ions but not by sulbactam, tazobactam and/or clavulanic acid. MBLs production is usually associated with resistance to other classes of antibiotics such as aminoglycosides and fluoroquinolones, there are seven types of MBL genes, bla-IMP and bla-VIM are the most common types. [11, 12] The genes coding for MBL production can be acquired chromosomal or plasmid mediated and can be transferred to other Gram-negative bacteria.[13] Many phenotypic methods for MBL producer's detection using different chelators have been describe[14]. Also, the genotypic methods as, polymerase chain reaction (PCR) and iso-electric focusing (IEF) have been evaluated in different settings by various workers globally [11, 12]. From Egypt only few studies have evaluated PCR for the

detection of gene encoding MBLs of *A. baumannii.* Our study aimed to evaluate many phenotypic methods and PCR for detection of carbapenems resistance in different clinical carbapenems resistant *Acinetobacters* isolates from Tanta University Hospitals.

METHODOLOGY

The present study was carried out in Medical Microbiology and Immunology Department, Biochemistry Department, Clinical Pathology Department, Department of Anesthesia, Intensive Care and Pain Medicine, Faculty of Medicine, Tanta University. It was carried on 150 patients who were admitted during the period of research (one year) to the Department of Anesthesia, Intensive Care and Pain Medicine, Faculty of Medicine, Tanta University. Written informed consents were obtained from all participants in this research. The patients were selected according to specific criteria including length of hospital stay (more than 3 days), receiving antibiotics for more than 10 days without improvement and any exposure to invasive techniques. An exclusive criterion was who were admitted to hospital for less than 48 hours. All patients were subjected to proper history taking with precise clinical examination. Approval of the ethics committee was obtained from the Faculty of Medicine, Tanta University

Bacterial isolates & phenotypic identification

Specimens were collected from 150 patients under complete aseptic precautions. The samples included endotracheal aspirates and urine samples as well as wound and burn swabs. All samples were inoculated on appropriate culture media i.e. Blood agar and MacConky agar plate, incubated at 37°C. The isolated microorganisms were identified according to Gram staining, colony morphology and biochemical tests. [15] Gram negative non-lactose fermenter bacilli were subjected to testing by API 20NE (Biomerieux) for identification of *Acinetobacter baumannii.* Only *Acinetobacter baumannii* isolates were included in the study.

The antimicrobial resistant pattern

It was performed by KirbyBauer disk diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI) 2016[16, 17]. The isolates were tested for Piperacillin (Pi, 100 µg), Piperacillin-tazobactam (PIT, 100/10 µg), Cefotaxime (CTX, 30 µg), Ceftraxone (CTR, 30 µg), Ceftazidime (CAZ, 30 µg), Ceftriaxone (CPM, 30 µg), Amikacin (AK, 30 µg), Gentamicin (GEN, 30 µg), Ciprofloxacin (CIP, 5 µg), Imipenem (IMP, 10 µg), Meropenem (MRP, 10 µg), Polymyxin B (PB, 300 unit) and Tigecycline (TGC, 15 µg).

Screening tests for carbapenemases detection in the clinical isolates

It was done by Modified Hodge test as CLSI recommendations [18]. The test was done by adjusting overnight incubated suspension of *Escherichia coli* ATCC 25922 to 0.5 McFarland standards, then using sterile cotton swab to inoculate it on a Mueller-Hinton agar (MHA). At the center of the plate, 10 µg meropenem was placed after the previous *Escherichia coli* inoculum was dried out and the tested strain was streaked from the edge of the disk to the periphery of the plate in four different directions. We incubated the plates overnight at 37°C. Positive results showed cloverleaf shaped zone of inhibition due to carbapenemase production. [19]

EDTA disc synergy (EDS)

We adjusted the turbidity of overnight liquid culture of the test isolate to 0.5 McFarland standards then cultured on a MHA plate. We placed a 10 µg meropenem disk or 30 µg ceftazidime on the plate. A blank disk (6 mm in diameter, Whatmann filter kept on the inner surface of the lid of the MHA plate and 10 µl of 0.5 M EDTA added to it. We transferred the EDTA disk to the surface of the plate and kept 10 mm edge-to edge apart from the meropenem or ceftazidime disk.
The plates were incubated overnight at 37°C. Positive results indicated by the presence of an expanded growth inhibition zone between the two disks. [20]

Amp C disk test:

This test was done for detection of AmpC β-lactamases. The culture of *E.coli* ATCC 25922 from an overnight culture suspension was adjusted to 0.5 McFarland standards on MHA plate. A 30 µg cefoxitin disk was kept on the surface of the agar. A blank disc 6 mm in diameter Whatman filter paper was moistened with sterile saline and was inoculated with a few colonies of the test strain. The plate was incubated overnight at 37°C. Positive results were indicated by a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the disk with test strain. An undistorted zone was considered as negative. [20]

Phenotypic expression of MBL

This was detected by EDTA-imipenem-microbiological (EIM) assay[21]. We detected MBL activity in crude cell extracts of the bacteria. Crude cell extract was prepared by disrupting bacterial cells by freezing and thawing. Micro-organisms were collected from the surface of a fresh overnight growth on MHA and were transferred to a pre-weighed micro-centrifuge tubes until the equivalent of 100 mg of bacterial wet weight was obtained. One ml of 50 mm Tris-HCL (pH 8) was added to it. We centrifuged the micro centrifuge tubes at 5000 rpm for 10 min and the pellet was frozen at -20°C for 15 min and was thawed at 37°C for 10 min. This freezing and thawing procedure was repeated 10 times. Finally we centrifuged the tube at 10000 rpm for

10 min and the supernatant was assayed for detection of MBL activity.

For detection of MBL activity in crude cell extract, indicator strain, *Escherichia coli* ATCC 25922 was cultured on MHA plate after adjusting its turbidity to 0.5 McFarland. The seeded plate was kept at room temperature for 5-10 min to allow the inoculum to dry. Within 15 min of inoculation, a 10 µg imipenem (oxoid) disc was placed in the center of the agar surface and four blank filter paper discs were applied around the central imipenem disc in such a manner that these discs had an edge-to-edge distance of 2 mm from the central disc. The four peripheral filter paper discs were labelled as 'S', 'S/Zn', 'S/E' and 'B'. Disc 'S' was loaded with 20 µl of the prepared crude extract, disc 'S/Zn' with 20 µl of crude extract supplemented with 0.1 mM ZnSO 4 , disc 'S/E' with 20 µl of crude extract supplemented with 20 mM EDTA and disc 'B' with 20 µl of 50 mM Tris-HCL only. The plate was incubated overnight at 37°C. After overnight incubation growth pattern of indicator strain *E. coli* within the sensitivity zone of imipenem around the four discs ('S', 'S/Zn', 'S/E' and 'B') was observed. Disc B was considered as a negative test control and no growth of indicator strain was observed around this disc.

Interpretation: Growth of indicator strain *E. coli* around 'S' and 'S/Zn' disc only, indicated MBL activity in crude cell extract and was considered as MBL positive. No growth around 'S', 'S/Zn' and S/E disc indicated that there is no β-lactamase activity and was considered as MBL negative and growth around 'S', 'S/Zn' and 'S/ E' disc indicated production of non-metallo enzyme.

Genotypic detection of the *bla*-IMP and *bla* -VIM genes

This was carried out by using Polymerase Chain Reaction after DNA extraction in all the carbapenem resistant isolates. *bla*-IMP primers:
bla-IMP-F (5′-GAATAGAATGGTTAACTCTC-3′)
bla-IMP-R(5′-CCAAACCACTAGGTTATC-3′) and
bla-VIM primers:
bla-VIM-F (5′- GTTTGGTCGCATATCGCAAC-3′)
bla-VIM-R(5′-AATGCGCAGCACCAGGAT AG-3′)
were used.[22] For DNA Extraction, a total of 4–5 identical colonies of were re-suspended in 500 µl of sterile saline in 1.5 ml Eppendorf tube. This was boiled at 100°C for 10 min, centrifuged at 8000 rpm for 5 min and the supernatant containing DNA was used for further processing. The PCR mixture used was as follows: 1 µl DNA template in a 49 µl mixture containing 10 mM Tris/HCl (pH 8.8), 50 mM KCl, 4 mM MgC l2, 200 µM each dNTP (Fermentas Genetix Biotech Pvt. Ltd., New Delhi), 1 µl of each of the forward and reverse primers (Bangalore genei) and 1 unit Taq DNA polymerase (Fermentas Genetix Biotech Pvt. Ltd., New Delhi). The PCR conditions included: Initial denaturation at94°C for 5 min followed by 33 cycles each of 94°C for 25 s, 53°C for 40 s and 72°C for 50 s, followed by a single final elongation step at 72°C for 6 min. The PCR product of188 bp for *bla*-IMP and 382 bp for *bla*-VIM was visualised by 1.5% agarose gel electrophoresis containing ethidium bromide 0.5 µg/ml (Bangalore Genei)

RESULTS

As shown in table (1) the incidence of *Acinetobacter baumannii* was 36 % (54/150) among the clinical collected samples. 88.9% (48/54) of *Acinetobacter baumannii* isolates were found carbapenem resistant by disc diffusion method. The resistant strains were tested phenotypically for the carbapenem resistance activity. Out of these isolates 54.2% (26/48) were found positive for carbapenenase by modified Hodge test, 70.8% (34/48) were positive by EDTA disc synergy. While 85.4% (41/48) found positive by Amp C disk test. 43.75% (21/48) of the resistant strains were positive to both modified Hodge test and EDTA disc synergy. Concerning EDTA-imipenem-microbiological (EIM) assay, 77% (37/48) strains were positive. Regarding PCR results, bla-VIM was detected in 12.5% (6/48) of the resistant isolates while bla-IMP was detected in 33.3% (16/48) of the resistant strains.

Table 1: Results of PCR and different phenotypic methods for detection of resistant *Acinetobacters*

***Acinetobacter baumannii* (N= 54/ 150)**	**Resistant**	**Non resistant**	**Total Number**
KirbyBauer disk diffusion (N= 54)	48 (88.9%)	6 (11.1%)	54 (100%)
a- Modified Hodge test	26 (54.2%)	22 (45.8%)	48(100%)
b- EDTA disc synergy	34 (70.8%)	14 (29.2%)	48(100%)
c- Amp C disk test	41 (85.4%)	7 (14.6 %)	48(100%)
d- +ve for both **Modified Hodge test** & **EDTA disc synergy**	21 (43.75%)	27 (56.25%)	48(100%)
e- +ve for EDTA-imipenum – microbiological assay	37(77%)	11 (33%)	48(100%)
PCR *bla*-IMP	16 (33.3%)	32 (66.7%)	48(100%)
PCR *bla*-VIM	6 (12.5 %)	42 (87.5 %)	48(100%)

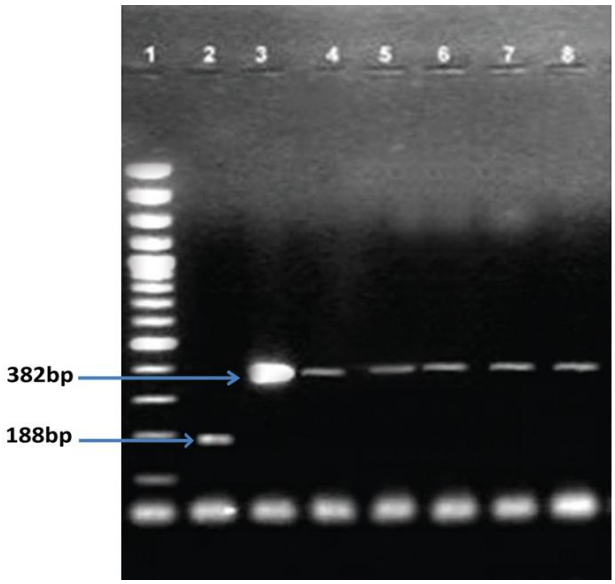

Fig. 1: PCR detection of *bla*-IMP and *bla*-VIM genes.
Lane-1. 100-1500bp ladder (Fermentas)
Lane-2. Control *bla*-IMP (188bp)
Lane-3. Control *bla* –VIM (382bp)
Lane-4. test strain 1(382bp)
Lane-5. test strain 2(382bp)
Lane-6. test strain 3(382bp)
Lane-7. test strain 4(382bp)
Lane-8. test strain 5(382bp)

DISCUSSION

Acinetobacter baumannii is a major problem in hospital acquired infections.[23] Such as bacteremia, pneumonia, meningitis, urinary tract infection, and wound infection.[24] So, among 150 clinical samples collected from ICU in Tanta University Hospitals 36 % (54/150) were *Acinetobacter baumannii*. This result was higher than that of Fouad et al.[25] and Ajlan et al.[26]. In both studies *Acinetobacter baumannii* represented 10% of their total isolates. While the study of Banerjee el al. showed that the prevalence of Acinetobacter spp was 42% in which 96.94% of them were *Acinetobacter baumannii*, which is closely related to the results of the present study. [27] The higher prevalence rate could be due to the decreased adherence to infection control measures.

There is high prevalence of carbapenems resistant *Acinetobacters*.[23] There are many mechanisms of this resistance.[28,29,30] In our study, 88.9% of the *Acinetobacter baumannii* isolates were found to be carbapenem resistant by disc diffusion method. These results were contributed to the high rate of carbapenem misuse in ICU in Tanta University Hospitals. Antibiotics prescriptions were not guided by culture sensitivity to the isolated organisms in many cases; also there was decreased adherence to the antibiotic policy. Our results were in parallel to the results of Fouad et al.[25] and Uwingabiye et al.[31]

Carbapenems resistant *Acinetobacter* associated with much serious hospital acquired infections.[32, 33] so, sensitive means of laboratory detection of MBL-producing isolates are urgently required if we are to prevent the ongoing spread of these problematic organisms in Egypt. In this study carbapenamases production was screened by Modified Hodge Test and MBL production by inhibitor-based methods using EDTA as the inhibitor. It was found that 70.8 %(34/48) of the tested isolates were MHT positive, indicating the production of carbapenamases. While EDTA disc synergy test resulted in 54.2 % (26/48) positive. The remaining negative isolates by MHT (14 isolates) were suggested to have other resistant mechanisms such as loss of porins or upregulation of efflux pumps.[29]

The EDTA-imipenem-microbiological assay, which differentiates metalloenzymes from non–metalloenzymes was used in this study and it was found that 77% (37/48) of the isolates were metalloenzymes producers while 23% (11/48) of the isolates were non metalloenzymes producers. EDTA-imipenem-microbiological assay was evaluated for the first time in *A. baumanii* in the study of Mendiratta, D et al.[34] who found that 4 isolates (9.3%) were MBL producers and 14 (32.56%) were non-metallo enzyme producers among the 43 screen test positive. The high rate of metallo enzymes producers in our study could be due to the miss use of imipenem and the heavy empirical antibiotics used in ICUs in Tanta University Hospitals. The results of this study revealed differences between the MβL tests used. This variation may be due to methodological aspects of the tests, or the types of metallo-enzymes involved.

However, of the 4 MBL positives in Mendiratta et al.[34]. Study, only 2 showed presence of *bla*-VIM and none of the 14 non-metalloenzyme producers showed presence of either *bla*-IMP or *bla*-VIM. In our study 6(12.5%) of these isolates were *bla*-VIM positive and 16(33.3%) isolates were *bla*-IMP positive. The difference between the screening methods and PCR could be due to presence of either other MBL encoding gene like SIM, SPM, GIM, AIM or variants of IMP and VIM which were not tested in our study[35] and it is very costly to do all these genes in every case tested for carbapenem resistant *Acinetobacter*.

AmpC b-lactamases were detected phenotypically in this study; it was found that 85.4% (41/48) of Carbapenem resistance in *Acinetobacter baumannii* were found positive by Amp C disk test. The study of Martins et al.[36] detected that (79.6%) of the phenotypically tested strains were positive for cephalosporinase (AmpC), this result[36] agree with our

study. This results indicated that AmpC is the main method of carbapenem resistance in this study, also these results agreed with the results of Sinha et al[37] 48.78% (20/41) of AmpC phenotypically positive isolates were also positive for MBL by MHT, EDTA disc synergy test and EDTA-imipenem-microbiological assay. The coexistence of AmpC and MBL was detected in different studies.

bla-IMP and *bla*-VIM are the most common genes encoding carbapenamases production.[38,39] In our study 6(12.5%) of these isolates were *bla*-VIM positive and 16 (33.3%) isolates were *bla*-IMP positive. Our results were in parallel with the study done by Amudhan et al.[40], they found that among *Acinetobacter baumannii*, (MBL) production has been reported to be high with MBL producing *Acinetobacter baumannii* with *bla*-VIM than those with *bla*-IMP. While Acinetobacter spp., (MBL) production has been reported to be high with *bla*-IMP as the most prevalent gene in another study of Karthika et al. [41] Also, the studies of Amudhan et al.[40] in India found that carbapenemase encoding genes 89% for *bla*-IMP higher than that for *bla*-VIM, which was 51%. While the study of Fattouh et al.[42] in Egypt, in which they tried to identify the presence of both *bla*-IMP-1and *bla*-VIM-1 using PCR among 19 clinical isolates of *A. baumannii*. But none of *bla*-IMP -1and *bla*-VIM-1 was detected in their isolates.

CONCLUSION

Carbapenem resistant is a major problem in hospital acquired infections. As it is one of the important treatment options for *Acinetobacter baumannii*, so detection of carbapenem resistance mechanisms in *Acinetobacter baumannii* by phenotypic methods is a trial to save this treatment option and prevent its misuse in Egypt. MBL and AmpC play an important role in carbapenem resistant in *Acinetobacter baumannii*, our study recommends the phenotypic methods used for the detection of MBL production and AmpC to the diagnostic laboratories for detection of carbapenem resistant Acinetobacter baumannii to avoid therapeutic failures and nosocomial outbreaks.

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- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
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REFERENCES

1. Maravić A, Skočibušić M, Fredotović Ž, et al. Urban riverine environment is a source of multidrug-resistant and ESBL-producing clinically important Acinetobacter spp. Environ Sci Pollut Res Int. 2016;23(4): 3525–3535.
2. Al Atrouni A, Joly-Guillou M-L, Hamze M, Kempf M. Reservoirs of non-baumannii acinetobacter species. Front Microbiol. 2016; 7:49.
3. Evans BA, Hamouda A, Amyes SG. The rise of carbapenem-resistant Acinetobacter baumannii. Curr Pharm Des. 2013; 19(2):223–238.
4. Wisplinghoff H, Paulus T, Lugenheim M, et al. Nosocomial bloodstream infections due to Acinetobacter baumannii, Acinetobacter pittii and Acinetobacter nosocomialis in the United States. J Infect 2012; 64:282–90.
5. Karah N, Haldorsen B, Hegstad K, Simonsen GS, Sundsfjord A, Samuelsen Ø; Norwegian Study Group of Acinetobacter. Species identification and molecular characterization of Acinetobacter spp. blood culture isolates from Norway. J Antimicrob Chemother 2011; 66:738–44.
6. Yang Y, Wang J, Fu Y, Ruan Z, Yu Y. Acinetobacter seifertii isolated from China: Genomic sequence and molecular epidemiology analyses. Medicine (Baltimore) 2016; 95:e2937. Back to cited text no. 1
7. Sengstock DM, Thyagarajan R, Apalara J, Mira A, Chopra T, Kaye KS, et al. Multidrug-resistant Acinetobacter baumannii: An emerging pathogen among older adults in community hospitals and nursing homes. Clin Infect Dis 2010;50:1611-6.
8. Organization WHO. WHO Publishes List of Bacteria for which New Antibiotics are urgently Needed. (WHO, Geneva, 2017). http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/.
9. Antunes LC, Visca P, Towner KJ. Acinetobacter baumannii: evolution of a global pathogen. Pathog. Dis.2014; 71, 292–301.
10. Arezzo D, S. et al. Epidemic multidrug-resistant Acinetobacter baumannii related to European clonal types I and II in Rome (Italy). Clin. Microbiol. Infect. 2009;15, 347–357 .
11. Potron, A., Poirel, L. & Nordmann, P. Emerging broad-spectrum resistance in Pseudomonas aeruginosa and Acinetobacter baumannii: mechanisms and epidemiology. Int. J. Antimicrob. Agents .2015; 45, 568–585.

12. Amudhan SM, Sekar U, Arunagiri K, Sekar B. OXA β- lactamase mediated carbapenem resistance in Acinetobacter baumannii . Indian J Med Microbio.2011; l29:269-74.
13. Varaiya A, Kulkarni N, Kulkarni M, Bhalekar P, Dogra J. Incidence of metallo-β-lactamase producing Pseudomonas aeruginosa in ICU patients. Indian J Med Res. 2008; 127:398-402.
14. Picão RC, Andrade SS, Nicoletti AG, Campana EH, Moraes GC, Mendes RE. Metallo-β-lactamase detection: Comparative evaluation of double-disk synergy versus combined disk tests for IMP-, GIM-, SIM-, SPM-, or VIM- producing isolates. J ClinMicrobiol.2008; 46:2028-37.
15. Bergogne BE, Towner, KJ. Acinetobacter spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin Microbiol Rev 1996; 9:148–165.
16. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology.1966; 45, 493-496.
17. CLSI Performance standard for Antimicrobial susptibility testing; twenty third informational supplement. M100-S26. 2016; Vol 36 No.1
18. Clinical and Laboratory Standards Institute CLSI. Screening and Confirmatory Tests for Suspected Carbapenemases Production. 2010; 20 supplemental table 2A-S2.
19. Amjad A, Mirza Ia, Abbasi S, Farwa U, Malik N, Zia F. Modified Hodge test: A simple and effective test for detection of carbapenemase production. Iran J Microbiol. 2011;3(4):189–193.
20. Joseph, Noyal & Harish, Belgode & Menezes, Dr. Godfred & Sistla, Sujatha & Parija, Subhash. . Detection of carbapenemases. Clin Microbiol Rev 2008; 50:100–119.
21. Purohit M, Mendiratta DK, Deotale VS, Madhan M, Manoharan A, Narang P. Detection of metallo-β-lactamases producing Acinetobacter baumannii using microbiological assay, disc synergy test and PCR. Indian J Med Microbiol. 2012 Oct-Dec; 30(4):456-61.
22. Mostachio AK, van der Heidjen I, Rossi F, Levin AS, Costa SF. Costa. Multiplex PCR for rapid detection of genes encoding oxacillinases and metallo-β-lactamases in carbapenem resistant Acinetobacter spp. J Med Microbiol 2009; 58:1522-4.
23. Laurent Poirel and Patrice Nordmann . Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin. Microbiol. Infect.2006; 12(9):826-36.
24. Lisa L. Maragakis and Trish M. Perl. Acinetobacter baumannii: Epidemiology, Antimicrobial Resistance, and Treatment Options. Clinical Infectious Diseases 2008; 46:1254–63
25. Fouad, M., Attia, A., Tawakkol, W. and Hashem, A. Emergence of carbapenem-resistant Acinetobacter baumannii harboring the OXA-23 carbapenemase in intensive care units of Egyptian hospitals. International Journal of Infectious Diseases.2013; 17(12): e1252-e1254.
26. Ajlan S, Baker A, Makled A, Salem E, Salama A. Phenotypic and molecular characterization of clinical Acinetobacter isolates from Menoufia University Hospitals. Menoufia Medical Journal.2017; 30(4):1030.
27. Banerjee T, Mishra A, Das A, Sharma S, Barman H, Yadav G. High Prevalence and Endemicity of Multidrug Resistant Acinetobacter spp. in Intensive Care Unit of a Tertiary Care Hospital, Varanasi, India. Journal of Pathogens. 2018; 1-8.
28. Walsh TR, Toleman MA, Poirel L, Nordmann P .Metallo-β-lactamases: the quite before the storm? Clin Microbiol Rev. 2005; 18: 306-325.
29. Karthika RU, Rao RS, Sahoo S, Shashikala P, Kanungo R, Jayachandran S, Prashanth K. Phenotypic and genotypic assays for detecting the prevalence of metallo-β- lactamases in clinical isolates of Acinetobacter baumannii from a south Indian tertiary care hospital. J Med Microbiol .2009; 58: 430-435.
30. Wirth FW, Picoli SU, Cantarelli VV, Goncalves AL, BrustFR, Santos LM, Barreto MF . Metallo-β-lactamase producing Pseudomonas aeruginosa in two hospitals from Southern Brazil. Braz J Infect Dis.2009; 13: 170-172.
31. Uwingabiye J, Frikh M, Lemnouer A, Bssaibis F, Belefquih B, Maleb A, Dahraoui S, Belyamani L, Bait A, Haimeur C, Louzi L, Ibrahmi A, Elouennass M. Acinetobacter infections prevalence and frequency of the antibiotics resistance: comparative study of intensive care units versus other hospital units. Pan African Medical Journal.2016; 23.
32. De AS, Kumar SH, Baveja SM . Prevalence of metallo- β-lactamase producing Pseudomonas aeruginosa and Acinetobacter baumannii in intensive care areas in a tertiary care hospital. Indian J Crit Care Med .2010; 14: 217-19.
33. Pitout JD, Gregson DB, Poirel L ,McClure JA, Le P, Church DL . Detection of Pseudomonas aeruginosa producing metallo-β-lactamases in a large centralized laboratory. J Clin Microbiol .2005; 43: 3129-3135.

34. Mendiratta D, Deotale V, Madhan M, Manoharan A, Narang P, Purohit M. Detection of metallo-β-lactamases producing Acinetobacter baumannii using microbiological assay, disc synergy test and PCR. Indian Journal of Medical Microbiology. 2012; 30(4):456.
35. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-β-lactamases: The Quiet before the storm. Clin Microbiol Rev 2005;18:306-25.
36. Martins, H., Bomfim, M., França, R., Farias, L., Carvalho, M., Serufo, J. and Santos S. Resistance Markers and Genetic Diversity in Acinetobacter baumannii Strains Recovered from Nosocomial Bloodstream Infections. International Journal of Environmental Research and Public Health.2014; 11(2):1465-1478.
37. Sinha N, Agarwal J, Srivastava S et al.: Analysis of carbapenemresistant Acinetobacter from a tertiary care setting in North India. Indian J Med Microbiol.2013;31: 60-3.
38. Upadhyay S, Sen MR, Prakash P et al. Detection and characterisation of AmpC β-lactamase-producing isolates of Acinetobacter spp. in North India. Int J Antimicrob Agents.2010; 36: 472-3.
39. Gupta V, Garg R, Garg S, Chander J, Attri AK Coexistance of extended spectrum beta lactamases, AMPC beta lactamases and metallo beta lactamases in acietobacter baumanii from burns patients: A report from a teriary care center of India. Annals of Burns and Fire Disasters .2013; vol. XXVI - n. 4.
40. Amudhan MS, Sekar U, Kamalanathan A, Balaraman S. "bla mediated carbapenem resistance in Pseudomonas and Acinetobacter species in India," The Journal of Infection in Developing Countries.2012; 6(11): 757–762.
41. KarthikaUR, Srinivasa RR, Suchismita S, Shashikala P, Kanungo R, Jayachandran S and Prashanth K .Phenotypic and genotypic assays for detecting the prevalence of metallo-b-lactamases in clinical isolates of Acinetobacter baumannii from a South Indian tertiary care hospital. Journal of Medical Microbiology .2009; 58, 430–435.
42. Fattouh M, El-din AN. Emergence of carbapenemresistant acinetobacter baumannii in the intensive care unit in Sohag University Hospital, Egypt, Int J Curr Microbiol App Sci, 2014.