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Original Research Article

Phenotypic Detection of Metallo β-lactamase Enzyme among Gram Negative Bacteria Isolated From Burn Patients In Sulaimani, Iraq

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Keywords

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A B S T R A C T

Metallo β-lactamase has emerged worldwide as powerful resistance determinants in Gram-negative bacteria. They hydrolyze virtually all classes of β-lactams except Monobactam, including Carbapenems, which often represent the last option for the treatment of infections with multidrug resistant Gram-negative bacteria. The aim of this study was to screen for metallo β-lactamase enzyme by four different phenotypic tests among infected burn wound patients in Sulaimani city /Iraq. This prospective study was carried out in the Burn and Plastic Surgery Hospital in Sulaimani city at a period from April - October 2011. All 177 isolated gram negative bacteria were tested by four phenotypic methods such as double disk synergy test, combined disk test, E-test and modified hodge test, all the data were analyzed by using SPSS program version 18 Inc., Evanston and p value of <0.05 regarded as significant . One hundred seventy seven Gram negative bacteria were isolated and identified from infected burn wound, out of 177 isolated strains , metallo β-lactamase were detected in 31.07% by double disk synergy test , 28.8% by combined disk test and 20.9% were recorded for modified hodge test while the less sensitive test was E-test (1.12%). Metallo β-lactamase enzyme can be detected among Gram negative bacteria by using several methods in which Combined disk test assay was found to be simple, inexpensive phenotypic resources for the detection of MBL that could be easily incorporated into the routines of clinical practice.

Introduction

Metallo β-lactamase has emerged worldwide as powerful resistance determinants in Gram-negative bacteria. They hydrolyze virtually all classes of β-lactams except Monobactam, including Carbapenems, which often represent the last option for the treatment of infections with multidrug resistant Gram-negative

bacteria (Bonomo 2011, Fard *et al.,* 2012).

MBL are metallo enzymes of Ambler class B which are Clavulanic acid resistance enzymes. They require divalent cation of zinc as co-factors for enzymatic activity and are universally inhibited by EDTA as well as other chelating agents of divalent cation (Butt *et al.,* 2005; Walsh and Toleremen 2005).

There are two dominant types of transferable MBL genes among clinical isolates, *bla* $_{IMP}$ and *bla* $_{VIM}$, which are frequently present on gene cassettes inserted into integrons located on the chromosome or on plasmids (Walsh 2011; Fard *et al.,* 2012). Other types of MBLs such as *bla* $_{AIM}$, *bla* $_{GIM}$, and *bla* $_{SPM}$ are found only sporadically in some geographic regions (Athanassios *et al.,* 2009).

The worldwide spread of acquired MBL in clinically important pathogens such as *Pseudomonas* spp., *Acinetobacter* spp. and members of Enterobacteriaceae has become a great concern (Tam *et al.,* 2007) and detection of MBL producing organisms in the clinical microbiology laboratory is a matter of major importance for the choice of appropriate therapeutic schemes and the implementation of infection control measures (Miriagou *etal.,* 2010).

There are few inhibitor tests which help to identify MBL producers in which the test seeks synergy between Carbapenems and EDTA, the inhibitor used is mostly EDTA which chelates the zinc ions and thus there is loss of Carbapenemase activity (Rolian *et al.,* 2010). Detection of Carbapenemases is difficult. It can be detected by phenotypic as well as genotypic methods. Among phenotypic tests, Modified Hodge test MHT is a relatively easy and simple test to be performed in a laboratory (Tenover, 2006).

Materials and Methods

Different gram negative bacteria were isolated in this study by culturing wound swab taken from infected burn wound on different culture media such as blood agar, MacConkey agar, Nutrient agar, and Eosin methylen blue, identification was based on colonial morphology, biochemical identification by using Api system and Vitek 2 compact system. All bacterial isolates were screened for metallo β-lactamase (MBL) enzymes by four different phenotypic methods in which 10 μg meropenem antibiotic disk was selected to be used in all phenotypic methods.

Double disk synergy test (DDST)

This test is performed by inoculating the tested organism onto MHA plate as recommended by(C.L.S.I, 2011). A 10 μg meropenem disk and a blank filter paper disk 6 mm in diameter were placed 10 mm apart from edge to edge, then, 10 μl of 0.5 molar EDTA solution was applied to the blank disk, after 18hours of incubation at 37ºC, the presence of extension of zone towards the impregnated EDTA disk was interpreted as EDTA synergy test positive (Saderi *et al.,* 2008)

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Combined EDTA disk test (CMDT).

An overnight broth culture of the test strain with an opacity adjusted to 0.5 McFarland standards was used to inoculate a plate of Mueller-Hinton agar as recommended by (C.L.S.I 2011). 4 μml of the sterilized EDTA solution was added to

10 µg Meropenem disk, then the EDTA impregnated antibiotic disks were dried immediately in an incubator and stored at -20 ºC in airtight vials without any desiccants until used (Samatha and Parveen 2011).

After drying of MHA plate, a 10 µg Meropenem disk and meropenem disk combined with EDTA was placed 20 mm apart (Behera *etal.,* 2008). After 24 hours incubation at 37 ºC an increase in the zone size of at least 7 mm around the Meropenem combined EDTA impregnated disk compared to Meropenem disks alone was recorded as MBL producing strains (Supriya *et al.,* 2010).

E-Test

Metallo β-lactamase enzyme and MIC of Meropenem were tested by using E-test which consists of a plastic strip. One half of the strip was impregnated with Meropenem gradient against seven dilution (0.125, 0.19, 0.25, 0.38, 1.0, 1.5, 2, 3 , 4, 8 µg/ml) and the other end of the strip was impregnated with Meropenem overlapped with constant concentration of EDTA ranging from 0.032-2 µg/ml. Tested colonies from overnight culture were suspended with 0.85% of normal saline (NaCl) to a turbidity of 0.5 McFarland's standard according to manufacturer's recommendation (kumar *et al*., 2012b).

A sterile cotton swab was used to produce a uniform layer on a Mueller-Hinton agar plate and the excess moisture was allowed to be absorbed for about 15 min before the E-test MBL strip was applied. The plate was incubated for 16 to 18 h at 37º C and the MIC end points were read where the inhibition ellipses intersected the strip (Bashir *et al.,* 2011).

MIC ratio of Meropenem/Meropenem +EDTA (MEP/MEPI) was calculated and a positive metalloβ-lactamase test was decided if the value of (MEP/MEPI) is > 8 or if there is zone of deformation insensitive area along the strip or appearance of phantom zone along the strip according to the manufacturer recommendation (kumar *et al*., 2012b).

Modified Hodge test (MHT)

All tested Gram negative bacteria were subjected to MHT test according to what was described by Lee *et al.,* (2001) ; An overnight culture suspension of pan sensitive strain of *E.coli* was prepared by adding two to three isolated colonies of *E.coli* strain to 5 ml of normal saline, and the suspension was further diluted by adding 1 ml of suspension to 4 ml of 0.85% NaCl and the mixture was adjusted to 0.5 McFarland's standard and this suspension was streaked across the entire plate of MHA plate. After drying 10 µg of Meropenem disk was placed at the center of the plate and up to 4 different isolates of Gram negative bacteria were streaked linearly from the periphery of the plate into the direction of Meropenem disk at the center and the test plate was incubated for 18 hours at 37ºC. The presence of a 'cloverleaf shaped' zone of inhibition around each tested strain is interpreted as Carbapenemases producing strain or a positive result (Amjad *et al.,* 2011).

Results and Discussion

Phenotypic detection of MBL enzyme by using double disk synergy test (DDST)

DDST were performed by observing synergism between Meropenem disk and EDTA disk (figure 1), and this test showed that 51 (28.81 %) bacteria from all isolated

bacteria isolated from burn patients were positive by this test, and *Acinetobacter* species (15.82%) were the most frequently isolated bacteria observed to be positive by this test among all Gram negative bacteria (table 1) followed by *pseudomonas* species (8.47%).

Phenotypic detection of MBL enzyme by using combined EDTA disk test (CMDT).

This test was performed to detect MBL production. If the zone of inhibition around Meropenem with EDTA was more than 7 mm compared to Meropenem disk alone, the test is considered to be positive (figure 2).

Among 177 isolates, 55 (31.07 %) were found to be positive by this test, and 95% of positive bacteria by this test showed a high rate of zone of inhibition > 20 mm and *Acinetobacter* species were the most common bacteria observed to be positive by this test (14.69%) , followed by *Pseudomonas* species (14.12), as observed in table 2.

Phenotypic detection of MBL enzyme by E-Test

E-test was used for all isolated bacteria by using different concentrations of Meropenem (MP) at one end of the E-test strip and EDTA mixed with meropenem (MPI) at the opposite end.

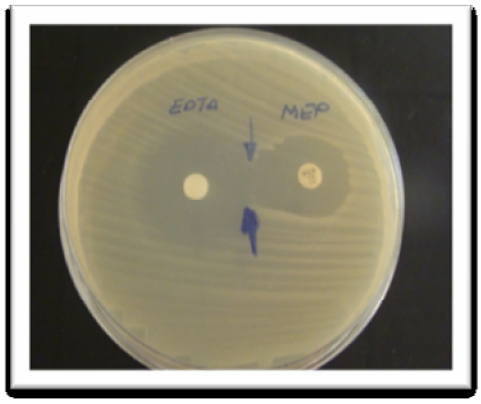
Positive results were recorded if the zone of inhibition around Meropenem divided by zone of inhibition around combined Meropenem with EDTA is > 8 according to manufacturer's recommendation (Biomeruiex. France), while negative results were observed if the value is <8 in which there was a zone of inhibition along both sites (figure 3 and 4). Out of the 177 isolated bacteria tested by this method, only 2 isolates were found to be positive in which both of the strains were Meropenem resistant, while 175 samples were negative, but 51 isolates showed entire growth along the strip and this will be regarded as undermined results and all these 51 strains were Meropenem resistant strains.

Phenotypic detection of MBL by Modified Hodge test (MHT)

Modified Hodge test was performed as a screening test for all Gram negative bacilli isolated from burn patients including both meropenem resistant and sensitive strains. Positive test was recorded as indentation of the zone of inhibition along the tested bacteria towards the Meropenem disk (figure 5). MHT test also was detected in 37 (20.9 %) out of all 177 Gram negative bacteria , from which only 6 isolates were from Meropenem resistant strains and the reaming 31 samples were Meropenem sensitive strains (table 3). The prevalence rate of MBL enzyme was differing according to each phenotypic test which was not significant statistically (*p value* >0.05). The most sensitive methods was combined disk test which account for 31.07% followed by double disk synergy tests (28.8%), while the least sensitive methods was E-test as observed in table 4.

The spread of Carbapenemase in Enterobacteriaceae is among the most important issues in the antimicrobial resistance. The rapid and recent diffusion of class A and B Carbapenemase determined the need of specific diagnostic tests able to detect with high sensitivity this type of resistance and to discriminate between the different enzymes (Ambretti *et al.,* 2013).

Figure.1 Double disk synergy test for MBL.



Positive MBL test indicated by synergy between Meropenem disk and EDTA disk (Blue arrow)

Figure.2 Combined EDTA disk test

MEP+ EDTA mixed disk, MEP: meropenem disk, positive MBL test indicated by zone of inhibition around combined disk is > 7mm to that of meropenem disk alone.

Figure.3 E-test positive result

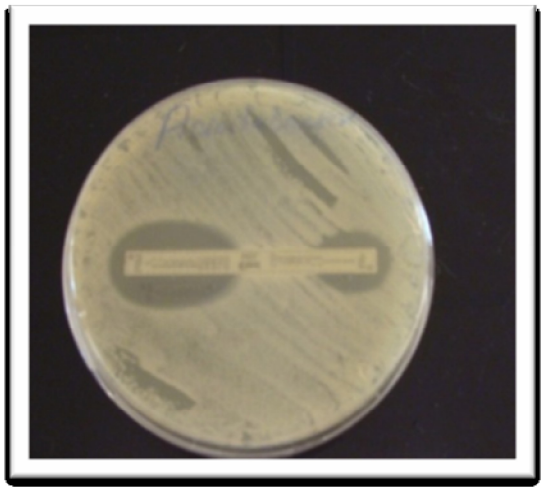


Figure.4 E-test negative result

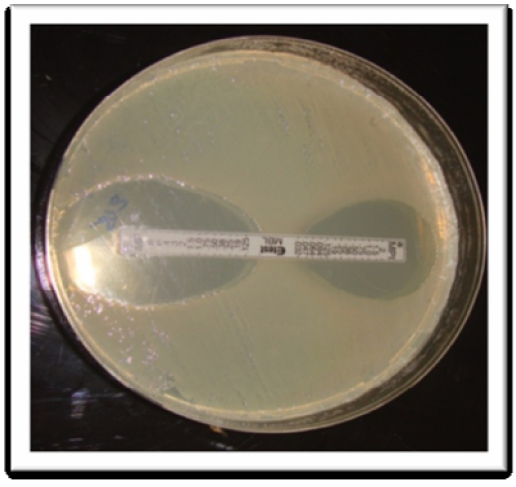
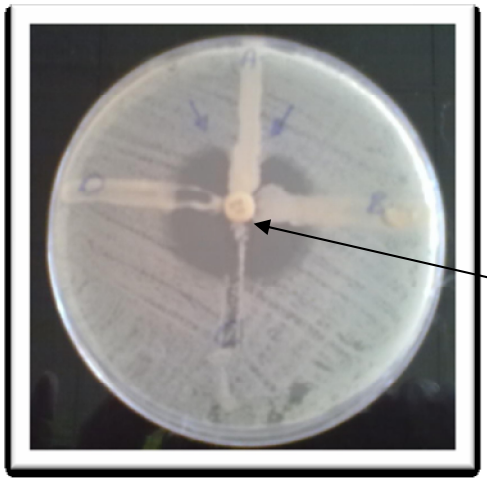


Figure.5 Photograph of Modified Hodge test (MHT)



A: Klebsiella pneumoniae (positive), B: Acinetobacter baumannii (negative), C: Pseudomonas aeruginosa (negative), D: E. coli (negative). Positive MBL: indentation of the zone of inhibition along the tested bacteria(A) designated by black arrow towards Meropenem disk.

Table.1 Numbers and percentages of MBL producing Gram negative bacteria double disk synergy test (DDST)

Gram negative bacterial species	**Positive DDST**	**Negative DDST**	**Total No. of bacteria**
	No. %	**No. %**	**No. %**
Pseudomonas species	15 (8.47%)	33 (18.64)	48 (27.12)
Acinetobacter species	28 (15.82)	16 (9)	44 (24.86)
Klebsiella pneumoniae	7 (3.95)	37 (20.9)	44 (24.86)
Enterobacter cloacae	-	18 (10.16)	18 (10.17)
Escherichia coli	1(0.56)	10 (5.64)	11 (6.21)
Morganella morganii	-	4 (2.25)	4 (2.25)
Providencia rettegeri	-	3(1.69)	3 (1.69)
Proteus mirabilis	-	2 (1.12)	2 (1.12)
Burkholderia pseudomallei	-	1 (0.56)	1 (0.56)
Achromobacter xylosoxidans	-	1(0.56)	1 (0.56)
Ralastonia paucula	-	1(0.56)	1 (0.56)
Total	51 (28. 81)	126 (71.18)	177 (100)

DDST: Double disk synergy test

Table.2 Numbers and percentages of MBL producing Gram negative bacteria by combined disk test (CMDT)

Gram negative bacterial species	Positive CMDT	Negative CMDT	Total No. of bacteria
	No. %	No. %	No. %
Pseudomonas species	25 (14.12)	23 (12.99)	48 (27.12)
Acinetobacter species	26 (14.69)	18 (10.16)	44 (24.86)
Klebsiella pneumoniae	3 (1.69)	41 (23.16)	44 (24.86)
Enterobacter cloacae	-	18 (10.16)	18 (10.17)
Escherichia coli	1 (0.56)	10 (5.64)	11 (6.21)
Morganella morganii	-	4 (2.25)	4 (2.25)
Providencia rettegeri	-	3 (1.69)	3 (1.69)
Proteus mirabilis	-	2 (1.12)	2 (1.12)
Burkholderia pseudomallei	-	1 (0.56)	1 (0.56)
Achromobacter xylosoxidans	-	1 (0.56)	1 (0.56)
Ralastonia paucula	-	1 (0.56)	1 (0.56)
Total	55 (31.07)	122 (68.92)	177 (100)

Table.3 Numbers and percentages of MBL producing Gram negative bacteria by Modified Hodge test (MHT)

Gram negative bacterial species	Positive MHT	Negative MHT	Total No. of bacteria
	No. %	No. %	No. %
Pseudomonas species	8(4.52)	40 (22.59)	48(27.12)
Acinetobacter species	9 (5.08)	35 (19.77)	44(24.86)
Klebsiella pneumoniae	18(10.16)	26 (14.68)	44(24.86)
Enterobacter cloacae	1(0.56)	17 (9.6)	18(10.17)
Escherichia coli	1(0.56)	10 (5.64)	11(6.21)
Morganella morganii	-	4 (2.25)	4(2.25)
Providencia rettegeri	-	3 (1.69)	3(1.69)
Proteus mirabilis	-	2 (1.12)	2(1.12)
Burkholderia pseudomallei	-	1 (0.56)	1(0.56)
Achromobacter xylosoxidans	-	1 (0.56)	1(0.56)
Ralastonia paucula	-	1 (0.56)	1(0.56)
Total	37 (20.9)	140 (79.09)	177 (100)

Table.4 Prevalence rate of MBL producing enzyme by four different phenotypic assays among burn patients

Gram negative bacteria	positive phenotypic test of MBL				Total No. of Gram negative
	No. (%)				
	DDST	CMDT	MHT	E-test	
Pseudomonas species	15(8.47)	25(14.12)	8(4.51)	2 (1.12)	48 (27.12)
Acinetobacter species	28(15.82)	26(14.69)	9 (5.08)		44 (24.86)
Klebsiella pneumoniae	7 (3.95)	3 (1.69)	18 (10.17)	-	44 (24.86)
Enterobacter cloacae		-	1 (0.56)	-	18 (10.17)
Escherichia species	1 0.56)	1 (0.56)	1 (0.56)	-	11 (6.21)
Morganella morganii	-	-	-	-	4 (2.26)
Providencia rettegeri	-	-	-	-	3 (1.69)
Proteus mirabilis	-	-	-	-	2 (1.12)
Burkholderia pseudomallei	-	-	-	-	1 (0.56)
Achromobacter xylosoxidans	-	-	-	-	1 (0.56)
Ralastonia paucula	-	-	-	-	1 (0.56)
Total	51 (28.8)	55 (31.07)	37 (20.9)	2 (1.12)	177 (100)

DDST: Double disk synergy test, CMDT: combined disk test, MHT: Modified hodge test, E-test: Episilometer test. $p > 0.05$

MBL enzymes were identified from clinical isolates worldwide with increasing frequency over the past few years and strains producing these enzymes are responsible for prolonged nosocomial outbreaks that were accompanied by serious infections (Amin *et al.*, 2010).

Although for the first time in Kurdistan region different phenotypic tests were used in an attempt to detect MBL enzymes among hospitalized burn patients and to know which test was better to be used for MBL detection.

DDST detect MBL in 51(28.8%) out of all Gram negative bacterial strains in which 95% of Meropenem resistant isolates showed a significant zone of enhancement toward EDTA disk which was in agreement with a study done in Ahwaz and Teheran (Khosravi and Mihahi 2008; Saderi *et al.*, 2010) which detected 26.5 %, but higher prevalence rates of DDST (45%) and (33%) were detected in other studies carried out in India and Tunisia (45% , 33% and 30%) by Amin, (2010), Bashir *et al.*, (2011) and Zoghlami *et al.*, (2012).

Beside using DDST, other tests, such as CMDT, were used which show high positive results in 98% of Meropenem resistance strains with zone of inhibition > 7 mm. Interpretation of the CMDT assay results is more objective than that of the DDST results according to what was concluded previously (Bhalerao *et al.*, 2010), because the DDST depends upon the technician's expertise in discriminating true synergism from intersection of

inhibition zone whereas CMDT is easy to be interpreted as it needs no synergisms just an increase zone of inhibition around the combined Meropenem disk by EDTA. The prevalence rate of CMDT in the current study was 55(31.07%) among all Gram negative bacteria which was in agreement with that done by Eser *et al.,* (2009) which found 76% positive CMDT among Gram negative bacteria but it disagrees with what was recorded by Gupta *et al.,* (2012) in which the sensitivity of CMDT was equal to that of E-test.

Another newer method for the detection of MBL in this study is E-test strip. This test can be used to determine MIC of Meropenem resistant strains, and was evaluated against Meropenem disk combined with inhibitors EDTA.

The test was used in several studies and found to be a sensitive 100% in detecting of MBL enzyme (Khosravi *et al.,* 2012) which disagrees with this study in that only 2 strains of isolated Gram negative bacteria were positive by this test and both of strains were Meropenem resistance but 51 strains out of all isolated species were found to give undetermined results (there was a growth along entire E-test strip at Meropenem pole of strip). The cause of this result are due to inability to detect MBL producers by this test and the need for using test strip of higher Meropenem concentration more than 8 µg/ml (Galani *et al.,* 2008).

MHT was detected in 37 (20.9%) isolated Gram negative bacteria from which 20 (30.3%) isolates were from Meropenem resistance strains and 19(17.1%) from Meropenem sensitive strains that were isolated from different units in burn hospital. These results indicate that the positive MHT test among Meropenem sensitive strains was indicative of MBL positive strains or presence of other types of β-lactamase enzymes such as class A Carbapenemase KPC which is in agreement to studies done in India and Iran (Noyal *et al.,* 2009; Hashemi *et al.,* 2012) which concluded that MHT test is a good tool for the diagnosis of MBL even in small percentages (12% and 16%).

Out of all Gram negative isolates, *K. pneumoniae* was found to be positive for MHT test at 17 (9.6%) out of all Meropenem sensitive and resistant strains. This result was similar to study done in Pakistan but with a higher MHT positive (27%) among Gram negative bacteria isolated from burn patients (Amjad *et al.,* 2011) while lower percentage of MHT positive test was observed among non fermenter group such as *Acinetobacter* and *Pseudomonas* species which were lower than *K. pneumonia* in the current study.

Although the rates of resistance to Carbapenems are low but they have been increasing especially MBL enzyme which hydrolyzes all classes of β-lactam antibiotics and their spread will be a catastrophe as there is no option for treatment by any other drugs so early diagnosis and isolating the patients may aid in preventing the spread of the enzymes among Gram negative bacteria.

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References

Ambretti, S., Gaibani,P., Berlingeri, A., Cordovana, M., Tamburini,M., Bua, G., Landini M., and Sambri, V. 2013. Evaluation of phenotypic and genotypic approaches for the detection of class A and class B Carbapenemases in Enterobacteriaceae. Microb Drug Resist. 123.

Amin, A. G., Hussain, P.B., and Hameed, A. 2009. Prevalence of antibiotic resistance among clinical isolates of *Klebsiella pneumoniae* isolated from a Tertiary Care Hospital in Pakistan . Malaysian Journal of Microbiology. 52: 81-86.

Amjad, A., Mirza, IA., Abbasi, S.A., Farwa, U., Malik, N., and Zia, F. 2011. Modifed Hodge test: A simple and effective test for detection of Carbapenemase production. Iran .J.Microbiol.34 : 189-193.

Athanassios T. , Kristo,I., Pittaras, T., Spanakis, N., and Pournaras, S.2009. Large Dissemination of VIM-2Metallo β-lactamase producing *Pseudomonas aeruginosa* strains causing health care associated community onset infections. Journal of clinical Microbiology. 4711: 3524–3529.

Bashir, D., Thokar,M.A., Fomda,B.A., Bashir,G., Zahoor,D., Ahmad, S., and Abubaker Toboli, S. 2011. Detection of metallo β-lactamase MBL producing *Pseudomonas aeruginosa* at a tertiary care hospital in Kashmir. African Journal of Microbiology Research. 52: 164-172.

Behera, B., Mathur, P.,Das, A., Kapil, A., and Sharma, V. 2008. An evaluation of four different phenotypic techniques for detection of metallo β-lactamase producing *Pseudomonas aeruginosa.* Indian J Med Microbiol. 263: 233-237.

Bhalerao, D. R., Kinikar, S., and Akhter,A.G. 2010. Study of Metallo β-lactamase producing *Pseudomonas aeruginosa* in Pravara Rural Hospital. Pravara Med Rev. 53.

Bonomo, R. A. 2011. New Delhi metallo β-lactamase and multidrug resistance. *Clin Infect Dis.* 524: 485-487.

Butt T, Usman, M., and Ahmed, R.N 2005. Emergence of Metallo β-lactamase producing *Pseudomonas aeruginosa* in Pakistan. J Pak Med Assoc. 55: 302–304.

C.L.S.I 2011. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards, Clinical and Laboratory Standards Institute .31.

Eser, O., Ergin, A., and Hasçelik, G. 2009. Antimicrobial resistance and existence of metallo β-lactamase in *Acinetobacter species* isolated from adult patients. Mikrobiyol Bul. 433: 383-390.

Fard , F., Irajian, M., Moslehi,G., Takantape Z, Fazeli H, and Salehi M. 2012. Drug resistance pattern of *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients at Isfahan AL Zahra hospital, Iran 2009-2010. Indian J Med Microbiol. 42: 94-97.

Galani, I. R., Hatzaki,P.D., Plachouras, D., Souli,M., and Giamarellou, H. 2008. Evaluation of different laboratory tests for the detection of metallo-beta-lactamase production in Enterobacteriaceae. J. Antimicrob. Chemother. 61: 548-553.

Gupta, V., Sidhu, S., and Chander, J. 2012. Metallo β-lactamase producing nonfermentative Gram negative bacteria: an increasing clinical threat among hospitalized patients. Asian Pac J Trop Med. 59: 718-721.

Hashemi, H. B., M. Farzanehkhah, A. Dolatyar, M. Imani, M. R. Farzami and M. Rahbar 2012. A study on prevalence of KPC producing from *Klebsiella pneumoniae* using Modified Hodge Test and CHROMagar in Iran. Annals of Biological Research. 312: 5659-5664.

Khosravi, A. and Mihahi, F. 2008. Detection of Metallo-β-lactamase producing *Pseudomonas aeruginosa* strains isolated from burn patients in Ahwaz, Iran. Diagn Microbiol Infect Dis. 60: 125–128.

Kumar, S. H., Baveja, S.M., and Gore, M.A. 2012b. Prevalence and Risk Factors of Metallo β-lactamase Producing *Pseudomonas aeruginosa* and *Acinetobacter* species in Burns and Surgical Wards in a Tertiary Care Hospital. Journal of Laboratory Physicians. 41: 39-42.

Lee, K., Y. Chong, H. B. Shin, Y. A. Kim, D. Yong, and J. H. Yum. 2001. Modified Hodge and EDTA-disk synergy tests to screen metalloβ-lactamase-producing strains of *Pseudomonas* and *Acinetobacte*r species. Clin. Microbiol. Infect. 7:88-91.

Miriagou, V., Cornaglia,G., Edelistin , M., Galani,I., Giske, C.G., Gniadkowski,M., Malamou-Lada, E., Martinez-Martinez, L., Navarro,F., Nordmann,P., Pexi,L., Papournaras,S., Rossolini,G.M., Tsakaris, A., Vatopoulose,A., and Canton, R. 2010. Acquired carbapenemase in Gram negative bacterial pathogenes: detection and surveillence issues. Clin Microbial infect. 16: 112-122.

Noyal, M. J. C., Menezes, G. A. , Harish, B. N. , Sujatha, S. and Parija, S.C. 2009. Simple screening tests for detection of Carbapenemases in clinical isolates of nonfermentative Gram-negative bacteria. Indian Journal of Medical Research. 1296: 707-712.

Rolain MJ, Panola P, and Cornaglia G. 2010. New Delhi metalloβ -lactamase NDM-1: towards a new pandemia? Clinical Microbiology and infection. 1612: 1699-1701.

Saderi, H., Zohreh, K. , Parviz, O. , Bahar,M.A., Baghr, S.M. 2008. Phenotypic Detection of Metallo-beta-lactamase Producing *Pseudomonas* aeruginosa strains isolated from burned patients. Iranian Journal of Pathology. 31: 20-24.

Samatha, P. and Parveen,K.V. 2011. Prevalance of ESBL Ampc B-lactamase in Gram negative clinical isolates. Journals of bioscience and technology .24: 353-357.

Supriya, U., lay R. , and Amitabha B. 2010. Presence of different β-lactamase classes among clinical isolates of *Pseudomonas aeruginosa* expressing AmpC β-lactamase enzyme. J Infect Dev Ctries. 44: 239-242.

Tam, V. H., Chang, K.T., LaRocco, M.T., Schilling, A.N. , McCauley, S.K., Poole,K., and Garey, K.W. 2007. Prevalence, Mechanisms, and risk factors of Carbapenem resistance in bloodstream isolates of *Pseudomonas aeruginosa*.Diagn. Microbiol. Infect. Dis. 58: 309–314.

Tenover, F. C. 2006. Mechanisms of Antimicrobial Resistance in Bacteria. American Journal of Medicine. 1196: 3-10.

Walsh, T. R., and Tolereman, M.A. 2005. Metalloβ- lactamase:The quite befor the storm. Clinical Microbiological Review .18: 306-325.

Walsh, T. R. 2011. New Delhi metallo-beta-lactamase-1: detection and prevention. CMAJ. 18311: 1240-1241.

Zoghlami, A., Kanzari,L., Boukadida,J., Messadi, A.A, and Ghanem, A. 2012. Epidemiological profile and antibiotic resistance of *Pseudomonas aeruginosa* isolates in burn and traumatology center in Tunisia over a three-year period. J.Tunis Med. 9011: 803-806.