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Detection of Metallo-β-Lactamase (MBL) Producing Acinetobacter at 3 Hospitals in Iran, Tehran

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Abstract: *Acinetobacter* sp. can cause serious health care-associated infactions. Metallo-β-Lactamase (MBL) producing acinetobacters (MBL) are responsible for worldwide nosocomial infections. Acinetobacter species almost are resistant to all antibiotics Multi-Drug-Resistant (MDR). Therefore, detection of these organisms is very important. This study was carried out on 107 isolate of acinatobacter from patients admitted in 3 hospitals in Tehran (August, 2012 to May, 2013). Antimicrobial susceptibility test was performed by Kirby-Bauer method (disc diffusion agar or DDA) for: Amikacin (30 μg), imipenem (10 μg), meropenem (10 μg), piperacillin (100 μg), p-tazobactam (110 μg), cefotaxime (30 μg), ceftazidime (30 μg), cefepime (30 μg), ciprofloxacin (5 μg), tetracycline (30 μg), ticarcillin (75 μg), tobramycin (10 μg), gentamicin (120 μg), cotrimoxazole (25 μg) and levofloxacin (5 μg). In Antimicrobial Susceptibility Test (AST), 100% of strains were resistant to cefotaxime, 98.13% to cotrimoxazole, 97.19% to piperacillin, cefepime and ticarcillin, 96.26% top-tazobactam, 95.3% to imipenem, ceftazidime and ciprofloxacin, 94.3% to meropenem, 93.4 to levofloxacin, 87.5% to amikacin, 64.4% to tobramycin, 61.68% to tetracyclin and 51.4% to gentamicin. All tests were performed on muller hinton agar. Later MIC of colistin was performed according to CLSI protocol . That showed 93.5% sensitivity of isolates. MBLs was detected by CDDT (combind disc diffusion agar method). Finaly, the results releved that 92.5% of imipenem and meropenem rasistant acinetobacter were MBL positive (showed significant zone around imipenem-EDTA disc) that is alarming and indicates necessity of proper planning for antibiotic prescription and the prevention of overuse antibiotic consumption in order to prevention of spreading these important resistant bacteria.

Key words: Acinetobacter, carbapenamases, minimum inhibitory concentration, metalobetalactamase, antimicrobial susceptibility test

INTRODUCTION

Pathogenic bacteria have increasingly been resisting to antimicrobial therapy. Recently, resistance problem has been relatively much worsened in gram negative bacilli. *Acinetobacter* sp. are typical nosocomial pathogens causing infections and high mortality, almost exclusively in compromised hospital patients (Lee *et al*., 2011). Multi-Drug Resistant (MDR) acinetobacter is a significant pathogen in health care settings where it causes a multitude of infections that include bacteremia, pneumonia, meningitis, urinary tract and wound infections. Its ability to survive under a wide range of environmental conditions makes it a frequent cause of outbreaks of infection and an endemic health care-associated pathogen. Acinetobacter is often resistant to a wide variety of antimicrobial agents, including carbapenems. Carbapenem resistance in acinetobacter is due to a variety of combined mechanisms, such as hydrolysis by β-lactamases, alterations in the outer membrane protein, penicillin-binding proteins and increased activity of efflux pumps. Acquired resistance to carbapenems, mediated by the Ambler class D β-lactamases or OXA-type carbapenamases and Ambler class B metallo-β-lactamases are of greatest concern, as they are encoded by genes which are transmissible and account for most of the resistance to carbapenems (Amudhan *et al*., 2011). Metallo-β-lactamases are resistance determinants of increasing clinical relevance in gram negative bacteria. Because of their broad range, potent carbapenemase activity and resistance to inhibitors, these enzymes can confer resistance to almost all β-lactams, most *MBL* genes (VIM, IPM and NDM-1) are found as gene cassettes on class 1 integrons. Since

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the 1990s, several metallo-β-lactamases encoded by mobile DNA have emerged in important gram negative pathogens (that is in enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*) (Ye *et al*., 2012). The Metallo-β-Lactamases (MBL) or carbapenemases which inhibit carbapenems belongs to the Ambler classification group B and they depend on heavy metals like Zn^{++} for hydrolysis of β-lactam ring. They are susceptible to iron chelators like Ethylene Diamine Tetra-Acetic Acid (EDTA). They are resistant to well-known β-lactamase inhibitors like clavulanic acid, sulbactam and tazobactam and confer resistance to all β-lactam antibiotics except monobactams. They are distinct from other β-lactamases in that they do not compete with PBPs for their mode of action (Misra, 2012).

MATERIALS AND METHODS

The study was done in the International Branch of Shahid Beheshti University of Medical Sciences and Health Services (2012-2013) 107 isolate from 3 hospital (Taleghani, Motahhari and Milad) in Iran Tehran were collected. The study protocol was approved in ethics committee of Shahid Beheshti University of Medical Sciences. Antimicrobial susceptibility test was carried out using 15 antibiotics: Amikacin 30, imipenem 10, meropenem 10, piperacillin 100, p-tazobactam 110, cefotaxime 30, ceftazidime 30, cefepime 30, ciprofloxacin 5, tetracycline 30, ticarcillin 75, tobramycin 10, gentamicin 120, cotrimoxazole 25 and levofloxacin 5. First antimicrobial susceptibility was carried out with disk diffusion agar as a routine method by use of the Clinical and Laboratory Standards Institute (CLSI, 2012). In this method, suspensions of pure cultures were prepared in bacterial suspensions and were adjusted to give inoculums with an equivalent cell density to 0.5 McFarland turbidity standards (~1.5×108 cfu mL^{-1}). Cell suspensions were then swabbed evenly onto muller hinton plates and allowed to dry. Antibiotic disks were placed on the surface of the plate using sterile forceps. The degree of resistance or susceptibility was determined by measuring the inhibited growth areas around the disc after 24 h of incubating the plates in 37°, according to CLSI guidelines (Erfani *et al*., 2008, 2011, 2012). Second antimicrobial susceptibility test was carried out with microdilution method for colistin in accordance with CLSI (2012). Later, MBLs was detected by CDDT (Manoharan *et al*., 2010). CDDT is a method for investigation of MBPs and many studies have been done about it (Behara *et al*., 2008; Mochon *et al*., 2011).

Inoculum preparation for AST: Inocula were obtained from an overnight agar culture of the test organism. Inoculum for the MIC test was prepared by taking at least 3-5 well-isolated colonies of the same morphology from an agar plate culture. The top of each colony was touched with a sterile loop and the growth was transfered into a tube containing 4-5 mL of normal saline until it achieved the turbidity of the 0.5 McFarland standards (direct method). This results in a suspension containing approximately 1-2×108 cfu mL^{-1}. The turbidity of the suspention was adjusted with sterile broth to obtain turbidity comparable to that of the 0.5 McFarland standards (Islam *et al*., 2008).

Turbidity standard for AST inoculum preparation: To standardize the inoculum density for a susceptibility test, $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standards was used. A 0.5 McFarland standard was prepared as described in CLSI, M7-A7. About 1% V/V solution of sulfuric acid was prepared by adding 1 mL of concentrated sulfuric acid to 99 mL of water and mixed well. A 1.175% W/V solution of barium chloride was prepared by dissolving 2.35 g of dehydrated barium chloride ($BaCl_2.H_2O$) in 200 mL of distilled water. To make the turbidity standard, 0.5 mL of the barium chloride solution was added to 1% 99.5 mL sulfuric acid solution and mixed well. A small volume of those turbid solutions was transferred to a screw-capped tube of the same type as used for preparing the control inocula and stored in the dark at room temperature.

Antimicrobial susceptibility test: Antimicrobial susceptibility test of the isolated organisms was done by disc diffusion method using the Kirby-Bauer technique (Bauer *et al*., 1966) according to the recommendation of CLSI (2012). All tests were performed on mueller-hinton agar. The surface was lightly and uniformly inoculated by cotton swab. Prior to inoculation, the swab stick was dipped into bacterial suspension having visually equivalent turbidity to 0.5 McFarland standards. The swab stick was then took out and squeezed on the wall of the test tube to discard extra suspension. Inoculated plates were incubated at 35°C for 24 h. On the next day, plates were read by taking measurement of zone of inhibition. Results were recorded and graded as Resistant (R), Intermediate (I) and Sensitive (S), according to the reference zone of inhibition of particular antibiotic (Fig. 1 and 2). Figure 2 shows a microtiter plate with 96 wells. The Minimum Inhibitory Concentration (MIC) in row E is 4 and C is 2. The zone of inhibition were interpretated by antibiotic zone size interpretative chart as recommended by CLSI (Islam *et al*., 2008).

Determination of MIC by broth microdilution method: Microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) of an antimicrobial agent.

Interpretation of results: The MIC represents the concentration of antimicrobial at which there is complete inhibition of growth. In reading the end points, a barely visible haze of growth or a single colony is disregarded. The results were interpreted according to the recommendation chart of CLSI (2012). The strains were referred to as Resistant (R) and Susceptible (S) in relation to the MIC of antimicrobial tested as per Table 1.

Fig. 1: Disc diffusion agar multi-drug-resistant acinetobacter

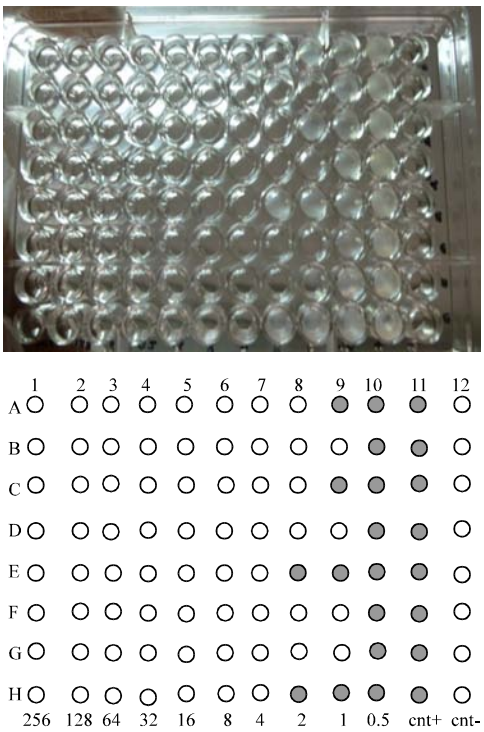

Fig. 2: This slide shows a broth microdilution antimicrobial susceptibility test. This method is the most accurate method of determining the antimicrobial susceptibility of acinetobacter to colistin

Preparation of antimicrobial agents: Colistin antibiotic were dissolved in solvents (water) and diluents (water) for preparation of stock solutions of antimicrobial agent (CLSI, 2012). In order to preparation of serial dilution for colistin use CLSI (2012) method (9, 1, 3, 7, ...) for preparation dilutions of antimicrobial agents was used (Table 2 and 3).

Phenotypic detection of MBLs: The isolates were evaluated phenotypically for the presence of a Metallo-β-Lactamase (MBL), using the metal chelating agent EDTA. Identification of MBL activity was performed by carbapenem-EDTA combined disk method. Two Imipenem (IMP; 10 μg) discs were applied to the inoculated plates and 10 μL of a sterile 0.5 M EDTA (pH 8.0) solution was applied to one disk. A sterile 6 mm filter paper disc to which 10 μL of 0.5 M EDTA was applied was used to determine, if EDTA alone might inhibit the growth of the test isolates. The plates were incubated at 35°C under ambient air for 18 h. The zones of inhibition around the IMP and IMP-EDTA discs were measured and zone increases of ≥7 mm in the presence of EDTA were noted and interpreted as indicative of an MBL phenotype on the basis of criteria described previously (Fig. 3a, b).

Table 1: Zone diameter interpretative standards and equivalent Minimum Inhibitory Concentration (MIC) breakpoints for acinetobacter

	MIC value (μg mL^{-1})	
Name of antibiotic	Susceptible (S)	Resistant (R)
Colistin	≤2	≥4

Table 2: Scheme for preparing dilutions of Antimicrobial agent to be used in Broth Microdilution Susceptibility tests (CLSI, 2012)

Steps	Concentration (μg mL^{-1})	Source	---Antimicrobial solution*---			Log2
1	5120	Stock	1	9	512.000	9
2	512	Step 1	1	1	256.000	8
3	512	Step 1	1	3	128.000	7
4	512	Step 1	1	7	64.000	6
5	64	Step 4	1	1	32.000	5
6	64	Step 4	1	3	16.000	4
7	64	Step 4	1	7	8.000	3
8	8	Step 7	1	1	4.000	2
9	8	Step 7	1	3	2.000	1
10	8	Step 7	1	7	1.000	0
11	1	Step 10	1	1	0.500	-1
12	1	Step 10	1	3	0.250	-2
13	1	Step 10	1	7	0.125	-3

*Volume (mL)+CAMHB volume (mL) = Final concentration (μg mL^{-1}); CAMHB = Ction-Adjusted Mueller-Hinton Broth

Table 3: The Minimum Inhibitory Concentration (MIC) of the colistin

Sample	Dilution of colistin (μg mL^{-1})								
No.	256	128	64	32	16	8	4	2	1
39	-	-	-	-	-	-	-	-	+
61	-	-	-	-	-	-	-	+	+
7	-	-	-	-	-	-	+	+	+

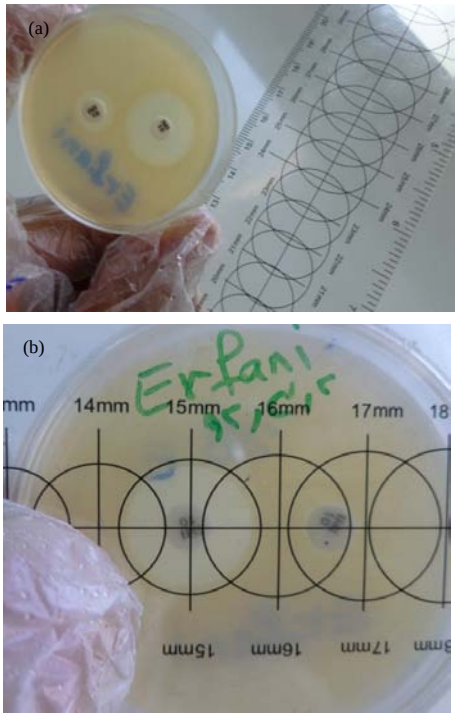

Fig. 3: a) Detection of MBLs producihg acinetobacter. Combination Disc Diffusion Test (CDDT): Imipenem and imipenem-EDTA disc; b) An increase in the inhibition zone atleast 7 mm around the imipenem-EDTA disc is MBL positive

Acinetobacter grew up to the EDTA disc. Figure 3 illustrates the MBL activity as determined using the carbapenem-EDTA combined disk method (Behera *et al*., 2008; Mochon *et al*., 2011; Amudhan *et al*., 2011; Bashir *et al*., 2011).

RESULTS AND DISCUSSION

Carbapenem-resistant acinetobacter often are resistant to additional classes of antibiotics (MDR bacteria). The antimicrobial susceptibility of the 107 imipenem and meropenem-resistant acinetobacter isolates investigated in this study showed that these isolates were MDR (Mohamed and Raafat, 2011) (Table 4).

In present study, 100% of strains were resistant to cefotaxime, 98.13% to cotrimoxazole, 97.19% to piperacillin, cefepime and ticarcillin, 96.26% top-tazobactam, 95.3% to imipenem, ceftazidime and ciprofloxacin, 94.3% to meropenem, 93.4 to levofloxacin, 87.5% to amikacin, 64.4% to tobramycin, 61.68% to tetracyclin and 51.4% to gentamicin. Over 43.92% of strains sensitive to gentamicin, 33.6% to tobramycin, 15.8% to tetracycline, 5.6% to meropenem, 4.67% to levofloxacin, ceftazidime, 3.7% to ciprofloxacin, 2.8% to imipenem and p-tazobactam 0.9% to cotrimoxazol, piperacilin, cefepime and ticarcillin and 0% to cefotaxime (Table 4). In Table 4, MIC of colistin 93.5% of acinetobacters were sensitive (7 strain from 107 acinetobacter). MBLs was detected by CDDT (combind disc diffusion agar method) results that 92.5% of acinetobacter which rasistant to imipenem and meropenem were MBL positive (showed significant zone around imipenem-EDTA disc).

Increasing emergence of multidrug resistant bacteria impose a challenge for their selection and appropriate treatment (Rossolini and Mantengoli, 2008). Over prescription of different classes of antibiotics in hospital and community acquired infections is proposed as a possible mechanism for their development (Johson *et al*., 1999). The dissiemination of antibiotic resistance genes among bacterial strains is becoming a crucial problem in infectious diseases (Ye *et al*., 2012). Therefore, optimizing laboratory methods for finding resistant strains is nassesery (Erfani *et al*., 2011). Carbapenem-resistant acinetobacter often are resistant to additional classes of antibiotics, such as cephalosporins, aminoglycosides and fluoroquinolones (MDR bacteria) (Lee *et al*., 2005; Bratu and Quale, 2006; Purohit *et al*., 2012; Irfan *et al*., 2008). The antimicrobial susceptibility of the 107 imipenem and meropenem-resistant acinetobacter isolates investigated in this study showed that these isolates were MDR (Table 4). A mechanism of this resistance is being characterized by the production of a specific enzyme called Metallo-β-Lactamases (MBL). These enzymes (VIM, IMP and NDM-1) belong to Ambler class B β-lactamases based on their amino acid sequence homology and to group 3, according to the Bush classification based on their substrate profiles (imipenem hydrolysis). The genes responsible for MBL production may be chromosomal or plasmid mediated (Purohit *et al*., 2012). MBL enzymes are inhibited by Ethylene Diamine Tetra-Acetic acid (EDTA). The rapid detection of MBL positive isolates is necessary to control infection and to prevent their dissemination (Kabbaj *et al*., 2013). Resistance to both imipenem to be a better indicator of MBL production (Purohit *et al*., 2012). In the Middle East, the occurrence of imipenem-resistant *A. baumannii* is alarmingly recognized. In Saudi Arabia, the susceptibility rate of *A. baumannii* isolated from a tertiary care hospital to imipenem was reported to be as low as 10%. In Bahrain, 58% of 454 *A. baumannii* isolated from a 1000-bed tertiary care centre were resistant to imipenem. In United Arab Emirates and Qatar, 100% resistance to imipenem was observed in the tested

Table 4: Antibiogram of acinetobacter strains (n = 106) (MAST disc)

Antibiotics groups	Antibiotics	Sensitive [n (%)]	Intermediate [n (%)]	Resistant [n (%)]
Penicillins	Piperacillin 100	1 (0.90)	2 (1.87)	104 (97.20)
	Ticarcillin 75	1 (0.90)	2 (1.87)	104 (97.20)
β-lactam β-lactamse inhibitor combinations	P-tazobactam 110	3 (2.80)	1 (0.90)	103 (96.27)
Cephems	Ceftazidime 30	5 (4.67)	-	102 (95.33)
	Cefepime 30	1 (0.90)	2 (1.87)	104 (97.20)
	Cefotaxime 30	-	-	107 (100.00)
Carbapenems	Imipenem 10	3 (2.80)	2 (1.87)	102 (95.33)
	Meropenem 10	6 (5.60)	-	101 (94.40)
Aminoglycosides	Gentamicin 120	47 (43.92)	5 (4.67)	55 (51.40)
	Tobramycin 10	36 (33.64)	2 (1.87)	69 (64.48)
	Amikacin 30	18 (16.80)	5 (4.67)	84 (87.50)
Tetracyclines	Tetracycline 3	17 (15.89)	24 (22.43)	66 (61.68)
Fluoroquinolones	Ciprofloxacin 5	4 (3.73)	1 (0.90)	102 (95.33)
	Levofloxacin 5	5 (4.67)	2 (1.87)	100 (93.46)
Folate pathway inhibitors	Cotrimoxazol 25	1 (0.90)	1 (0.90)	105 (98.13)

A. baumannii isolates (Mohamed and Raafat, 2011). In the study by Purohit *et al.* (2012), 9.3% MBL producers detected by using EDTA-Imipenem-Microbiological assay (EIM). In other study by Irfan *et al.* (2008), MBL was produced by 96.6% of imipenem-resistant acinetobacter isolates whereas 100% imipenem-resistant pseudomonas aeruginosa isolates were MBL producers (Irfan *et al.*, 2008). The frequency of MBL acinetobacter baumanii is higher by 84 and 96% (Kabbaj *et al.*, 2013). In the study, analyzed the prevalence of MBL production in Imipenem-resistant acinetobacter Baumanii isolated from the main university hospital based in Alexandria, Egypt, phenotypic detection of MBLs by Combined Disc Test (CDDT) identified a proportion of 70% as MBL producers (Mohamed and Raafat, 2011). In the study, 107 isolates of acinetobacter were collected from different clinical specimens of patients. MBLs was detected by CDDT (Combind Disc Diffusion agar method) CDDT is a method for investigation of MBPs and many studies have been done about it (Behera *et al.*, 2008; Mochon *et al.*, 2011; Amudhan *et al.*, 2011; Bashir *et al.*, 2011). Finaly, the results releved that 92.5% of imipenem and meropenem rasistant acinetobacter were MBL positive (showed significant zone around imipenem-EDTA disc) (Fig. 2). Due to fast distribution, acinetobacters are serious challenge for infection control team and physicians in hospital settings and increase the incidence of these pathogens causes restrictions in therapeutic programs. Many worldwide studies show the incidence of MBL positive acinetobacters, the emergence of this enzymes is an indicator for spreading of this enzymes in this species. Therefore, rapid isolation of acinetobacters in order to prevent nosocomial infections with this organisms is valuable so proper planning of antibiotic prescription and the prevention of overuse antibiotic consumption is useful for prevention of spreading these important resistant bacteria.

In the study, 107 isolates of acinetobacter were collected from different clinical specimens of patients. MBLs was detected by CDDT. Finaly, the results releved that 92.5% of imipenem and meropenem rasistant acinetobacter were MBL positive (showed significant zone around imipenem-EDTA disc) (Fig. 2). The separation of the MBL positive acinetobacters is warning and as a result of the antibiotics overuse.

CONCLUSION

By using Imipenem-EDTA disc method, as a screening test for metallo-β-lactamase production, researchers found a very high percentage of metallo-β-lactamase producing isolates among multidrug resistant acinetobacter isolates. The findings strongly suggest that there is a need detection of MBL producers is necessary to prevent further spread of these organisms.

REFERENCES

Amudhan, S.M., U. Sekar, K. Arunagiri and B. Sekar, 2011. OXA â-lactamase-mediated carbapenem resistance in *Acinetobacter baumannii*. Indian J. Med. Microbiol., 29: 269-274.

Bashir, D., M.A. Thokar, B.A. Fomda, G. Bashir, D. Zahoor, S. Ahmad and A.S. Toboli, 2011. Detection of metallo-beta-lactamase (MBL) producing Pseudomonas aeruginosa at a tertiary care hospital in Kashmir. Afr. J. Microbiol. Res., 5: 164-172.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Behera, B., P. Mathur, A. Das, A. Kapil and V. Sharma, 2008. An evaluation of four different phenotypic techniques for detection of metallo-β-lactamase producing *Pseudomonas aeruginosa*. Indian J. Med. Microbiol., 26: 233-237.

Bratu, S. and J. Quale, 2006. Global emergence of nosocomial gram-negative pathogens possessing carbapenem-hydrolyzing β-lactamases. J. Biol. Sci., 6: 434-445.

CLSI, 2012. Performance standards for antimicrobial susceptibility testing. Twentieth Informational Supplement, Document M100-S20, Clinical and Laboratory Standards Institute, Wayne, PA,. USA.

Erfani, Y., H. Choobineh, R. Safdari, A. Rasti and S. Alizadeh, 2008. Comparison of E. test and disk diffusion agar in antibiotic susceptibility of *Escherichia coli* isolated from patients with urinary tract infection in Shariati Hospital (Iran). Res. Biol. Sci., 3: 24-27.

Erfani, Y., A. Rasti, A. Mirsalehian, S.M. Mirafshar and V. Ownegh, 2011. E-test versus disk diffusion method in determining multidrug resistant strains of *Escherichia coli* in urinary tract infection. Afr. J. Microbiol. Res., 5: 608-611.

Erfani, Y., A. Rasti, M. Rezaee and S. Jafari, 2012. Comparison of Iranian disc diffusion agar and epsilometer test (E-test) accuracy, in antibiotic susceptibility of Escherichia coli isolated from patients with urinary tract infection. Afr. J. Microbiol. Res., 6: 3571-3573.

Irfan, S., A. Zafar, D. Guhar, T. Ahsan and R. Hasan, 2008. Metallo-beta-lactamase-producing clinical isolates of *Acinetobacter* species and *Pseudomonas aeruginosa* from intensive care unit patients of a tertiary care hospital. Indian J. Med. Microbiol., 26: 243-245.

Islam, M.A., M.M. Alam, M.E. Choudhury, N. Kobayashi and M.U. Ahmed, 2008. Determination of Minimum Inhibitory Concentration (MIC) of cloxacillin for selected isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA) with their antibiogram. Bangladesh J. Vet. Med., 6: 121-126.

Johson, J.R., P. Delavari and M. Azar, 1999. Activities of a nitrofurazone-containing urinary catheter and a silver hydrogel catheter against multidrug-resistant bacteria characteristic of catheter-associated urinary tract infection. Antimicrob. Agents Chemother., 43:2990-2995.

Kabbaj, H., M. Seffar, B. Belefquih, D. Akka, N. Handor, M. Amor and A.E. Alaoui, 2013. Prevalence of metallo-β-Lactamases producing acinetobacter baumannii in a moroccan hospital. ISRN Infect. Dis., Vol. 2013. 10.5402/2013/154921.

Lee, K., J.H. Yum, D. Yong, H.M. Lee and H.D. Kim *et al*., 2005. Novel acquired metallo-β-Lactamase gene, bla_{SIM-1}, in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea. Antimicrob. Agents Chemother., 49: 4485-4491.

Lee, K., D. Yong, S.H. Jeong and Y. Chong, 2011. Multidrug-resistant *Acinetobacter* spp.: Increasingly problematic nosocomial pathogens. Yonsei Med. J., 52: 879-891.

Manoharan, A., S. Chatterjee and D. Mathai, 2010. Detection and characterization of metallo β lactamases producing *Pseudomonas aeruginosa*. Indian J. Med. Microbiol., 28: 241-244.

Misra, R.N., 2012. Metallo β-lactamases: A perspective and implications. Med. J. Dr DY Patil Univ., 5: 10-13.

Mochon, A.B., O.B. Garner, J.A. Hindler, P. Krogstad and K.W. Ward *et al*., 2011. New Delhi metallo-β-lactamase (NDM-1)-producing *Klebsiella pneumonia*: Case report and laboratory detection strategies. J. Clin. Microbiol., 49: 1667-1670.

Mohamed, N.M. and D. Raafat, 2011. Phenotypic and genotypic detection of metallo-β-lactamases in Imipenem-resistant *Acinetobacter baumannii* Isolated from a tertiary hospital in Alexandria, Egypt. Res. J. Microbiol., 6: 750-760.

Purohit, M., D.K. Mendiratta, V.S. Deotale, M. Madhan, A. Manoharan and P. Narang, 2012. Detection of metallo-β-lactamases producing *Acinetobacter baumannii* using microbiological assay, disc synergy test and PCR. Indian J. Med. Microbiol., 30: 456-461.

Rossolini, G.M. and E. Mantengoli, 2008. Antimicrobial resistance in Europe and its potential impact on empirical therapy. Clin. Microbiol. Infect., 14: 2-8.

Ye, Q., W. Shao, Q. Wang, X. Chen, J. Li and M. Zhou, 2012. Emergence of carbapenem-resistant *Enterobacter cloacae* carrying IMP-26 metallo-β-lactamase in class 1 integron and TEM-104 extended-spectrum β-Lactamase in conjugative plasmid. Afr. J. Microbiol. Res., 6: 3186-3191.