National Journal of Basic Medical Sciences | ORIGINAL ARTICLE | Volume - IV, Issue-2

METALLOBETALACTAMASES PRODUCING CLINICL ISOLATES OF PSEUDOMONAS AND ACINETOBACTER SPECIES IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction: Metallo-beta-lactamases (MBLS) mediated resistance is an emerging threat in clinical isolates of *Pseudomonas spp.* and *Acinetobacter spp..* These strains are responsible for several nosocomial outbreaks in tertiary care centers across the world. It is well known that poor outcome occurs when patients with serious infections due to MBL producing organisms are treated with antibiotics to which the organism is completely resistant. Therefore, detection of these MBL producers is crucial for optimal treatment of critically ill patient's and to prevent the spread of resistance.

Material and methods: The present study was undertaken to determine the incidence of MBL production in 400 clinical isolates of *Pseudomonas spp.* and *Acinetobacter spp.* by IMP-Ethylene diamine tetraacetic acid (EDTA) disk synergy test and to study the antibiotic susceptibility pattern of MBL producers by Kirby Bauer disk diffusion method .

Observation: Of the 23 imipenem resistant isolates 17(73.91%) were found to be MBL producers. MBL producers were found to be 100% susceptible to colistin and polymyxin B. 47.06% to piperacillin/ tazobactam, 35.29% to amikacin, 23.53% to ciprofloxacin and 05.88% to gentamicin.

Conclusion: Our findings showed that there is a need to do surveillance to detect MBL producers, judiciously use carbapenems to prevent their spread and use effective antibiotics, such as piperacillin-tazobactum, amikacin, ciprofloxacin and gentamicin, after sensitivity testing for treatment.

Key words: *Pseudomonas, Acinetobacter,* Metallo beta lactamase (MBL).

INTRODUCTION

The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by beta lactam resistant bacteria. Due to broad spectrum of activity and stability to hydrolysis by most beta lactamases, the carbepenems have been the drug of choice for treatment of infections caused by penicillin or cephalosporin resistant gram negative bacilli especially, extended spectrum β-lactamases(ESBL) producing gram negative infections[1]. However, carbapenem resistance has been observed frequently in non fermenting bacilli *Pseudomonas Aeruginosa* and *Acinetobacter spp.* Resistance to carbapenems is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins and carbapenem hydrolyzing enzymes-carbapenemase[2].

Metallo beta lactamase (MBL) belongs to a group B β-lacamase which requires divalent cations of zinc as cofactors for enzyme activity[3]. The gene responsible for MBL production may be chromosomally or plasmid mediated and hence poses a threat to spread of resistance by gene transfer among gram negative bacteria[2]. Moreover the treatment alternatives are unavailable or expensive/toxic with poor outcome[4]. Therefore, rapid detection of metallo beta lactamases production is necessary to modify therapy and to initiate effective infection control to prevent their dissemination.

The purpose of our study was to detect metallo beta lactamase production in clinical isolates of *Pseudomonas spp*. and *Acinetobacter spp.* and to study their antibiotic susceptibility pattern.

MATERIAL AND METHODS

The prospective study was conducted in the department of Microbiology of the Govt. Medical College, Amritsar. from June 2010-December 2012. 400 clinical isolates of *Pseudomonas spp.* and *Acinetobacter spp.* from various clinical specimens were studied for metallo beta lactamases production.

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All isolates of *Pseudomonas spp.* and *Acinetobacter* spp. were characterised to species level using standard procedures[5,6]. Susceptibility testing against Imipenems(IMP), cephalosporins and other CLSI recommended antibiotics was performed by Kirby Bauer disk diffusion method[7]. To identify MBL production in these isolates, IMP-Ethylene diamine tetraacetic acid (EDTA) disk synergy test was used[8]. *E. coli* ATCC 25922 and *P. Aeruginosa* ATCC 27853 were used as control strains.

IMP- EDTA Disk synergy test: All these clinical isolates were lawn cultured on Muller Hinton agar and those found to be resistant to imipenem and cephalosporins were subjected to IMP EDTA dusk synergy test. A 10µg imipenem disk and imipenem plus EDTA 750 µg were placed on Mueller Hinton Agar and incubated at 37˚c for overnight.

Interpretation:

After overnight incubation, the established zone diameter difference of ⩾ 7 mm between imipenem disk and imipenem plus EDTA was interpreted as EDTA synergy positive.

OBSERVATIONS

The present study was conducted on 400 isolates of *Pseudomonas and Acinetobacter* species from clinical specimens received in the department of Microbiology, Govt. Medical College Amritsar.

In our study maximum isolates were from pus 271(67.75%) followed by urine 47(11.75%), sputum 45(11.25%), blood 12(03.00%) and majority of the isolates were of *Pseudomonas aeruginosa* 319(79.75%) followed by *Acinetobacter baumannii* 26(06.50%) as shown in Table 1. Among *Pseudomonas spp.* majority of the isolates were of *Pseudomonas aeruginosa* 319(79.75%) followed by P. stutzeri 23(05.75%), P.putida 12(03.00%), P.fluorescens 9(02.25%).

Out of these majority were from hospitalized patients 367(91.75%) and the rest 33(08.25%) were from out patients.

Clinical isolates of *Pseudomonas sp.* and *Acinetobacter sp.* had showed maximum sensitivity to imipenem (94.25%) followed by sulbactam cefoperazone (83.25%) and amikacin (81.75%) as shown in Table 2.

Twenty three imipenem resistant isolates were tested for metallo beta lactamase (MBL) production and seventeen (73.91%) were found to be MBL producers as shown in Figure 1 and picture 1.

Table - 1 : DISTRIBUTION OF ISOLATES AMONG VARIOUS SAMPLES

Isolate	Pus n/%	Urine n/%	Vaginal swab n/%	Blood n/%	Sputum n/%	Ear swab n/%	Throat swab n/%	Nasal swab n/%	Cerebro spinal Fluid n/%
[1]P.aeruginosa n=319	216/67.71	41/12.85	5/01.57	5/01.57	34/10.66	8/02.50	4/01.25	4/01.25	2/00.63
[1]P. stutzeri n=23	13/56.52	3/13.04	-	2/08.69	5/21.74	-		-	-
[1]P.putida n=12	8/66.67	1/08.33	-	1/08.33	2/16.67	-	-	-	-
[1]P.fluorescens n=9	2/22.22	2/22.22	-	1/11.11	4/44.44	-	-	-	-
[2]A.baumannii n=26	22/84.61	-	-	2/07.69	-	-	2/07.69	-	-
[2]A.lwoffii n=11	10/90.91	-	-	1/09.09	-	-	-	-	-
Total isolates n+400	271/67.75	47/11.75	5/01.25	12/03.00	45/11.25	8/02.00	6/01.50	4/01.00	2/00.50

Maximum numbers of isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were from pus i.e.67.71% and 84.61% respectively.

Maximum numbers of MBL producers were isolated from pus sample i.e. 13(76.47%) followed by blood i.e. 2(11.76%) as shown in Table 3. No MBL producers were isolated from ear swab, throat swab, nasal swab and cerebrospinal fluid samples.

All the MBL producing isolates were 100.00% susceptible to colistin and polymyxin B. MBL producers showed 47.06% sensitivity to piperacillin/ tazobactam, 35.29% to amikacin, 23.53% to ciprofloxacin and 05.88% to gentamicin as shown in Table 4.

Table - 2 : ANTIBIOTIC SENSITIVITY PATTERN OF VARIOUS ISOLATES

Isolate	AK	G	CF	CT	CTN	CZ	PCTZ	TOBRA	I	SBCP
	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%
[1]P.aeruginosa n=319	269/84.33	157/49.21	118/36.99	110/34.48	120/37.62	188/58.93	237/74.29	216/67.71	302/94.67	275/86.21
[1]P. stutzeri n=23	17/73.91	11/47.83	6/26.09	12/52.17	9/39.13	11/47.83	15/65.22	8/34.78	21/91.30	18/78.26
[1]P.putida n=12	9/75.00	5/41.67	3/25.00	4/33.33	6/50.00	7/58.33	6/50.00	5/41.67	12/100.00	11/91.67
[1]P.fluorescens n=9	5/55.56	4/44.44	3/33.33	2/22.22	4/44.44	5/55.56	6/66.67	4/44.44	9/100.00	5/55.56
[2]A.baumannii n=26	19/73.08	12/46.15	13/50.00	11/42.31	12/46.15	14/53.84	16/61.54	15/57.69	24/92.30	19/73.08
[2]A.lwoffii n=11	8/72.73	4/36.36	3/27.27	4/36.36	6/54.54	5/45.45	7/63.63	8/72.73	9/81.82	5/45.45
Total n=400	327/81.75	193/48.25	146/36.50	143/35.75	157/39.25	230/57.50	287/71.75	256/64.00	377/94.25	333/83.25

1=Pseudomonas,2=Acinetobacte,AK=Amikacin,G=Gentamicin,CF=Ciprofloxacin,CT=Cefotaxime,CTN=Ceftriaxone,CZ=Ceftazidime,PCTZ=Piperacillin Tazobactam,TOBRA=Tobramycin,I=Imipenem,SBCP=Sulbactam Cefoperazone. Maximum numbers of isolates were susceptible to imipenem i.e. 94.25% followed by sulbactam cefperazone i.e.83.25%.

Table - 3 : Disribution Ofmbl Producing Isolates Among Various Samples

Isolate	Pus n/%	Urine n/%	Vaginal swab n/%	Blood n/%	Sputum n/%
[1]P.aeruginosa n=13	11/84.62	1/07.69	-	-	1/07.69
[1]P. stutzeri n=1	1/100	-	-	-	-
[1]P.putida n=0	-	-	-	-	-
[1]P.fluorescens n=0	-	-	-	-	-
[2]A.baumannii n=2	1/50.00	-	-	1/50.00	-
[2]A.lwoffii n=1	-	-	-	1/100.00	-
Total n=17	13/76.47	1/05.88	-	2/11.77	1/05.88

1=Pseudomonas, 2=Acinetobacter

Figure 1 : Prevalence of MBL Production in Imipenem Resistant Isolates

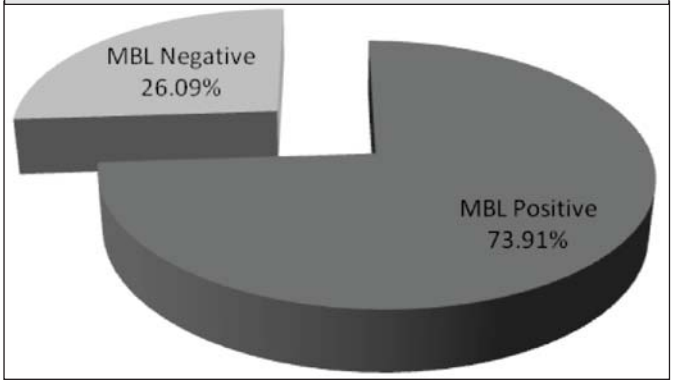


Table - 4 : ANTIBIOTIC SENSITIVITY PATTERN OF MBL PRODUCING ISOLATES

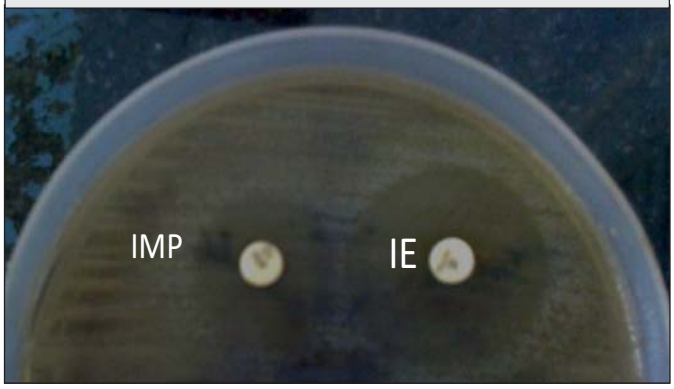
Isolate	AK	G	CF	CT	CTN	CZ	PCTZ	TOBRA	I	SBCP	C	PMB
	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%
[1]P.aeruginosa n=13	5/38.46	1/07.69	4/30.77	-	-	-	7/53.84	-	-	-	13/100.00	13/100.00
[1]P. stut zeri n=1	-	-	-	-	-	-	-	-	-	-	1/100.00	1/100.00
[1]P.putida n=0	-	-	-	-	-	-	-	-	-	-	-	-
[1]P.fluorescens n=0	-	-	-	-	-	-	-	-	-	-	-	-
[2]A.baumannii n=2	1/50.00	-	-	-	-	-	1/50.00	-	-	-	2/100.00	2/100.00
[2]A.lwoffii n=1	-	-	-	-	-	-	-	-	-	-	1/100.00	1/100.00
Tot al n=17	6/35.29	1/05.88	4/23.53	-	-	-	8/47.061	-	-	-	17/100.00	17/100.00

1=Pseudomonas, 2=Acinetobacter,

AK=Amikacin,G=Gentamicin,CF=Ciprofloxacin,CT=Cefotaxime,CTN=Ceftriaxone,CZ=Ceftazidime,PCTZ=Piperacillin Tazobactam, TOBRA = Tobramycin, I = Imipenem, SBCP=Sulbactam Cefoperazone, C=Colistin,PMB=Polymyxin B.

All the MBL producing isolates were 100.00% susceptible to colistin and polymyxin B.

IMP= IMIPENEM, IE= IMIPENEM + EDTA
PICTURE 1 SHOWING MBL PRODUCTION



DISCUSSION

Pseudomonas aeruginosa and *Acinetobacter baumannii* are non-fermentative gram-negative bacteria that have minimal nutritional requirements and can survive on a wide variety of surfaces and in aqueous environments. *P. aeruginosa* and *A. baumannii* rarely cause serious infections in otherwise healthy persons and are infrequently identified as normal microbial flora in healthy individuals[9,10]. Infections with *P. aeruginosa* or *A. baumannii* are of greatest concern for hospitalized patients, particularly those in intensive-care units (ICUs), where these opportunistic pathogens are capable of causing severe invasive infections in critically ill and immunocompromised patients. Rates of colonization with *P. aeruginosa* and *A. baumannii* increase in hospitalized patients, particularly in those who have been hospitalized for extended periods of time and/or have received broad-spectrum antimicrobial therapy or cancer chemotherapy[9,10].The spectrum of human infections caused by *P. aeruginosa* ranges from superficial skin infections to fulminant sepsis.

P. aeruginosa is the leading cause of nosocomial respiratory infections and is of particular concern for intubated persons and patients with ventilator-associated pneumonia[9,11] . Hospital-acquired infections with *A. baumannii* also most commonly involve the respiratory tract; like *P. aeruginosa*, *A. baumannii* also causes nosocomial urinary tract infections and wound infections, and infections may progress to septicemia[10]. *P. aeruginosa* and *A. baumannii* are resistant to antimicrobials from several different structural classes, either intrinsically or through acquisition of genetic determinants for resistance over time. Antimicrobial

resistance among clinical isolates of *P. aeruginosa* and *A. baumannii* may complicate the treatment of infections and can adversely affect clinical outcomes and patient treatment costs[12]. New antimicrobial agents with activity against *P. aeruginosa* and *A. baumannii* will not be available in the near future, making ongoing surveillance of the activities of currently available agents very important.

In the present study maximum isolates were from pus 271(67.75%) followed by urine 47(11.75%), and sputum 45(11.25%) which are consistent with the findings of Rajat et al, Saderi et al, Nagaveni et and Rashid et al[13,14,15,16]. However study done by Chaudhary *et al* maximum isolates were from sputum followed by pus and blood[17].

Majority of the isolates of *Pseudomonas sp* and *Acinetobacter sp* were from hospitalized patients 367(91.75%) and the rest 33(08.25%) from out patients. This may be because more number of samples received in our laboratory were from hospitalized patients as compared to out patients. Similar findings were shown by Bashir et al 95.40% from hospitalized and 04.60% isolates from out patients[18]. However, in the study by Rashid et al 206(70.00%) were inpatients and 88(30.00%) were out patients[16].

In the present study clinical isolates of *Pseudomonas sp.* and *Acinetobacter sp.* had showed maximum sensitivity to imipenem (94.25%) followed by sulbactam cefoperazone (83.25%) and amikacin (81.75%). This is in accordance with study done by Agrawal et al[19]. In a study done by Javiya et al *Pseudomonas aeruginosa* was highly sensitive to imipenem (78.57%) and remarkably resistant to other group of antibiotics i.e. 67.86% for third generation cephalosporins and 52-68% for aminoglycosides[20].However Saderi et al in their study showed that only 50.78% isolates of *Pseudomonas aeruginosa* were sensitive to imipenem[13].

Out of 23 imipenem resistant isolates 17(73.91%) were found to be MBL producers which are consistent with the findings Chacko et al where 72.73% imipenem resistant isolates were found to be MBL producers[21]. However, study done by Irfan et al 96.60% of imipenem resistant *Acinetobacter* isolates and 100.00% imipenem resistant *Pseudomonas* isolates were MBL producers[22].

Maximum numbers of MBL producers were from ICU patients 9/17(52.94%), isolated in pus sample i.e. 13/177 (6.47%) followed by blood i.e. 2/17(11.76%) (Table III). Emergence of MBLs producing *Acinetobacter* spp. and *P. aeruginosa* in our clinical strains is alarming and reflects excessive use of carbapenem. Therefore, early detection and prompt instillation of infection control measures is important to prevent further spread of MBLs to other gram negative rods.

Almost similar findings were shown by Bashir et al that maximum numbers of MBL producers were isolated from pus/wound sample[18]. However in a study done by Choudhary U et al MBL producing *P. aeruginosa* were commonly isolated from urine (20%) and blood (11.43%)[17]. All the MBL producing isolates were 100.00% susceptible to colistin and polymyxin B. MBL producers showed 47.06% sensitivity to piperacillin/ tazobactam, 35.29% to amikacin, 23.53% to ciprofloxacin and 05.88% to gentamicin. Similar findings were shown by Agrawal et al and Manoharan et al where MBL producers were 100.00% sensitive to polymyxin B and colistin respectively[19,23]. Chacko et al in their study showed that MBL producing isolates were 100.00% sensitive to colistin, but sensitivity to piperacillin/tazobactam and gatifloxacin was 81.00% and 54.00% respectively[21]. Thus, it is also important to follow antibiotic restriction policies to avoid excessive use of carbapenem and other broad spectrum antibiotics.

CONCLUSION

To conclude, in our institute 100% sensitivity was observed to colistin and polymyxin B in MBL producers. The treatment option for MBL producers is limited and patients are put on colistin and polymyxin B thus there is a danger of emergence of their resistance because of their overuse. Once resistance to colistin and polymyxin B emerges, there is hardly any treatment option left after this.

Treatment of infectious diseases becomes more challenging with each passing year. This is especially true for the opportunistic pathogens *Pseudomonas aeruginosa* and *Acinetobacter baumannii.*

The rapid dissemination of carbapenem resistance is worrisome and calls for studies to detect MBL producers and the judicious selection of antibiotics after sensitivity testing in the clinical practice.

REFERENCES

1. Mendiratta DK, Deotale V, Narang P. Metallo beta lactamase producing Pseudomonas aeruginosa in a hospital from rural area. Indian J Med Res. 2005;121:701-3.
2. Gladstone P, Rajendran P, Brahmadathan KN. Incidence of carbapenem resistant nonfermenting gram negative bacilli from patients with respiratory infections in the intensive care unit. Indian J Med Microbiol. 2005;23:189-91.
3. Bush K. Metallo â-lactamase: a class apart. Clin Infect Dis. 1998; 27(1):48-53.
4. Marra AR, Pereira CA, Gales AC, Menezes LC, Cal RG, de Souza JM et al. Blood stream infections with metallo-beta-lactamase producing Pseudomonas aeruginosa: Epidemiology, microbiology and clinical outcomes. Antimicrob Agents Chemother. 2006;50:388-90.
5. Govan JRW. Pseudomonas, Stenotrophomonas, Burkholderia. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology. 14th ed. New Delhi: Elsevier, a division of Reed Elsevier India Pvt. Ltd. ;2006:13-21.
6. Fallon RJ, Young H. Neisseria, Moraxella, Acinetobacter. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology. 14th ed. New Delhi: Elsevier, a division of Reed Elsevier India Pvt. Ltd. ;2006:294.
7. Wayne PA. Clinical and laboratory standard institute 2006. Performance standards for antimicrobial disc tests. Approved Standards, 9th ed.; sixteenth informational supplement. M2-A9 2006;26.
8. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol. 2002;40:3798-801.
9. Kiska DL, Gilligan PH. Pseudomonas, In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken editors. Manual of clinical microbiology, 7th ed. Washington, D.C: ASM Press; 1999. p. 517–25.
10. Schreckenberger PC, von Graevenitz A. Acinetobacter, Achromobacter, Alcaligenes, Moraxella, Methylobacterium, and other nonfermentative gram-negative rods. In: P. R. Murray, E. J. Baron, M. A.Pfaller, F. C. Tenover, and R. H. Yolken editors. Manual of clinical microbiology. 7th ed. Washington DC; ASM Press: 1999. p. 539-60.
11. Pollack M. Pseudomonas aeruginosa. In: G. L. Mandell. J. E. Bennett and R. Dolin editors. Principles and practice of infectious diseases. 5th ed. Philadelphia, Pa: Churchill Livingstone; 2000. p. 2310–35
12. Harris A, Torres-Viera C, Venkataraman L, DeGirolami P, Samore M, Carmeli Y. Epidemiology and clinical outcomes of patients with multiresistant Pseudomonas aeruginosa. Clin Infect Dis 1999;28:1128–33.
13. Saderi H, Karimi Z, Owlia P, Bahar AM, Rad ABMS. Phenotypic detection of metallo-beta-lactamase producing Pseudomonas aeruginosa strains isolated from burned patients. Iranian J Path 2008;3(1):20-4.
14. Nagaveni S, Rajeshwari H, Oli AK, Patil SA, Chandrakanth RK. Incidence of metallo beta-lactamase producing Pseudomonas Aeruginosa in clinical samples. An international quarterly journal of life sciences The Bioscan 2010;5(2):251-3.
15. Rajat RM, Ninama GL, Mistry K, Parmar R, Patel K, Vegad MM. Antibiotic resistance pattern in Pseudomonas aeruginosa species isolated at a tertiary care hospital, Ahmadabad. National journal of medical research 2012;2(2):156-9.
16. Rashid A, Chowdhury A, Rahman HZS, Begum AS, Muazzam N. Infections by Pseudomonas aeruginosa and Antibiotic Resistance Pattern of the Isolates from Dhaka Medical College Hospital. Bangladesh J Med Microbiol 2007;01(02):48-51.
17. Chaudhary U, Bhaskar H, Sharma M. Imipenem-EDTA disk method for rapid identification of metallo-β-lactamase producing Gram-negative bacteria. Indian J Med Res 2008 April;127:406-7.
18. Bashir D, Thokar MA, Fomda BA, Bashir G, Zahoor D, Ahmad S et al. Detection of metallo-beta-lactamase (MBL) producing Pseudomonas aeruginosa at a tertiary care hospital in Kashmir. African Journal of Microbiology 2011 Jan 18;5(2):164-72.
19. Agrawal G, Lodhi RB, Kamalakar UP, Khadse RK, Jalgaonkar. Study of metallo-β-lactamase production in clinical isolates of Pseudomonas aeruginosa. Indian J Med Microbiol 2008;26(4):349-51.
20. Javiya VA, Ghatak SB, Patel KR, Patel JA. Antibiotic susceptibility patterns of Pseudomonas aeruginosa at a tertiary care hospital in Gujarat, India. Indian J Pharmacol 2008;40:230-4.
21. Chacko B, Varaiya A, Dedhia B. Imipenem resistant metallo β lactamse producing Pseudomonas aeruginosa. Indian J Med Microbiol 2008;26:398.
22. Irfan S, Zafar A, Guhar D, Ahsan T, Hasan R. Metallo-β-Lactamase producing clinical isolates of Acinetobacter species and Pseudomonas aeruginosa from intensive care unit patients of a tertiary care hospital. Indian J Med Microbiol 2008;26(3):243-5.
23. Manoharan A, Chatterjee S, Mathai D, SARI Study Group. Detection and characterization of metallo beta lactamases producing Pseudomonas aeruginosa. Indian J Med Microbiol 2010;28:241-4.