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Full Length Research Paper

Detection of metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa* at a tertiary care hospital in Kashmir

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Metallo-beta-lactamases (MBL) producing *Pseudomonas aeruginosa* 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 producing *P. aeruginosa* is crucial for optimal treatment of critically ill patient's and to prevent the spread of resistance. Current study was undertaken with the aim of optimizing the choice, dose and duration in MBL producing *P. aeruginosa* infections in a tertiary care center in Kashmir for various types of treatment modalities. Aims and objectives are now specific and not like introduction. Various clinical samples were obtained from patients admitted in hospital or attending the OPD between January 2007 to June 2008. Antimicrobial sensitivity was performed by Kirby-Bauer disk diffusion method. Minimum inhibitory concentration (MIC) of Imipenem resistant isolates was done by agar dilution method. Metallo-beta-lactamase production was detected by combined disk method, MIC reduction of imipenem in presence of EDTA and by Epsilometer test (E-test). The intergroup comparison and risk estimation was performed by using Fisher's exact test and Odd's ratio. Out of 283 *P. aeruginosa* isolates, 38 (13.42%) were resistant to Imipenem. Thirty three (11.66%) were found to be MBL producers by combined disk test and all of them showed reduction in MIC in the presence of imipenem-EDTA in E-test. The number of MBL positive isolates from ICU was statistically significant (p=0.027). The hospital stay was significantly longer (p=0.000) among patients infected with MBL producers than MBL non producers. Statistically significant association of antineoplastic chemotherapy, urinary catherization with MBL production was found. All MBL producers were resistant to commonly used antibiotics. However, they were sensitive to polymyxin B(100%), piperacillin/tazobactum (18.2%), amikacin and ciprofloxacin (9.1%). MIC reduction is a cumbersome, laborious method and given the cost constraints of E-test a simple screening test like combined disk test may be used. In absence of therapeutic MBL inhibitors, polymyxins, aminoglycoside or fluoroquinolone molecule that may have retained some activity against the isolate may be used for the treatment of MDR *P. aeruginosa* infections.

Key words: *Pseudomonas aeruginosa*, combined disc test, Epsilometer test, Metallo-beta-lactamase.

INTRODUCTION

Carbapenems are the drug of choice for treatment of infections by penicillin or cephalosporin resistant gram negative bacilli especially in extended spectrum beta lactamase (ESBL) producing gram negative infections. However, over the past few years resistance to carbapenems due to production of carbapenemases have been reported. Carbapenemases may be defined

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as beta-latamases that significantly hydrolyze at least imipenem or meropenem. Carbapenemases involved in acquired resistance are of Ambler molecular classes A, B and D. Class B or the metallo-enzymes (MBL) are the most significant carbapenemases (Nordmann and Poirel, 2002; Walsh et al., 2005). Over the last decade MBL producing isolates have emerged particularly in *Pseudomonas aeruginosa*. These isolates have been responsible for serious infections such as septicemia and pneumonia and have been associated with failure of therapy with carbapenems. In recent years MBL genes have spread from *P. aeruginosa* to *Enterobacteriaceae,* and a clinical scenario appears to be developing that could simulate the global spread of extended-spectrum-beta- lactamases (Pitout et al., 2007). 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 (Marra et al., 2006). Therefore, detection of MBL producing gram negative bacilli especially *P. aeruginosa* is crucial for optimal treatment of patients particularly critically ill and hospitalized patients and to control the spread of resistance. The present study was planned to find out the prevalence of MBL producing *P. aeruginosa*, to study the risk factors associated with MBL producing *P. aeruginosa* infections, to optimize the therapeutic use of antimicrobials for treating metallo-beta-lactamase producing hospital based *P. aeruginosa* infections and to formulate antibiotic policy accordingly.

MATERIALS AND METHODS

Study period and clinical samples

Samples like blood, urine, sputum, wound swabs, catheter tips, pus and other body fluids obtained from patients admitted in hospital or attending the OPD between January 2007 to June 2008 were processed as per the standard microbiological procedures (Koneman et al., 2006). Identification of *P. aeruginosa* was done on the basis of Gram staining, colony morphologies on MacConkey's agar, motility, pigment production, oxidase reaction, growth at 42°C and polymyxin B sensitivity.

Antimicrobial susceptibility testing

Antimicrobial sensitivity was performed on Mueller Hinton agar plates by Kirby-Bauer disk diffusion method according to CLSI full guidelines (Clinical and Lab Standards Institute (CLSI), 2006). Following antibiotic disks(Hi media, Mumbai, India) were used gentamicin-10 µg, ofloxacin-5 µg, ciprofloxacin-5 µg, cefoperazone-75 µg, amikacin-30 µg, tobramycin-10 µg, ceftazidime-30 µg, carbenicillin-100 µg, ceftriaxone-30 µg, imipenem-10 µg, meropenem-10 µg, polymyxin B-300 units, cefoperazone-75 µg plus sulbactum-30 µg, ceftazidime-30-µg plus clavulanic acid-10µg. *P. aeruginosa* ATCC 27853 was used as control. Isolates were considered to be imipenem resistant when the zone around imipenem was = 13 mm, intermediate 14-15 mm and sensitive =16 mm.

MIC of Imipenem resistant isolates by agar dilution method

Imipenem powder (Sigma) was serially diluted to obtain concentrations ranging from 512-0.06 µg/ml. One milliliter of appropriate dilution of imipenem and 19 ml of Mueller Hinton agar cooled to 55°C was added to the corresponding labeled Petri dish after mixing thoroughly. The inoculum was prepared from overnight grown cultures of the test strains. The turbidity was matched to 0.5 Mc Farland's standards. Each plate was divided into sixteen quadrants and 2 µl of inoculum was delivered to the surface of agar plate. The plates were incubated for 16 to18 h at 37°C. The highest dilution showing no visible growth was taken as minimum inhibitory concentration (MIC) of imipenem for that particular strain. Imipenem resistant isolates were subjected to following tests for detection of metallo-beta-lactamases (National Committee for Clinical Laboratory Standards (NCCLS), 2000).

Combined-disk method

Ethylenediaminetetraacetic acid (EDTA)

0.5 M EDTA (Sigma Chemicals, St. Louis, MO) solution was prepared by dissolving 186.1g of disodium EDTA.$2H_2O$ in 1000 ml of distilled water and its pH was adjusted to 8.0 by using NaOH. The mixture was then sterilized by autoclaving. EDTA sol (4 µl) was poured on imipenem and ceftazidime disks to obtain a desired concentration of 750 µg per disk. The EDTA impregnated antibiotic disks were dried immediately in an incubator and stored at -20°C in airtight vials without dessicant until used.

An overnight broth culture of test strain (opacity adjusted to 0.5 Mc Farland opacity standards) was inoculated on a plate of Mueller Hinton agar. One 10 µg imipenem and one 30 µg ceftazidime disks were placed on the agar plate. One each of EDTA impregnated imipenem and ceftazidime disks were also placed on same agar plate. The plate was incubated at 37°C for 16 to 18 h. An increase in the zone size of at least 7 mm around the imipenem-EDTA disk or ceftazidime-EDTA compared to imipenem or ceftazidime disks without EDTA was recorded as an MBL producing strain.

MIC of imipenem plus EDTA combination

By agar dilution

1 ml of EDTA sol was added to 1 ml of imipenem solution using similar concentrations as were used for MIC to imipenem alone. Each 2 ml of EDTA and imipenem in graded concentration was added to 18 ml of Muller Hinton agar plates. The inoculum was prepared from overnight grown cultures of the test strains. The turbidity was matched to 0.5 Mc Farland standards. Using a micropipette 2 µl of each inoculum was delivered to the surface of agar plate. The inoculum was allowed to be absorbed into the agar plate before incubating at 37°C for 16 to 18 h. The highest dilution which inhibited the growth of organisms was taken as MIC of imipenem plus EDTA combination and was compared to MIC of imipenem alone. A minimum four fold reduction in MIC with imipenem-EDTA combination was interpreted as being suggestive of MBL production.

E-test

E-test metallo-beta-lactamase strips (Ab Biodisk Solna, Sweden) consist of a double sided seven dilution range of imipenem IP (4 to 256 µg/ml) and IP (1 to 64 µg/ml) overlaid with a constant gradient of EDTA. Individual colonies were picked from overnight agar plates and suspended in 0.85% saline to a turbidity of 0.5% Mc Farland's

standard. A sterile cotton swab was dipped into the inoculum suspension. The excess moisture was allowed to be absorbed for about 15 min before the E-test MBL strip was applied.(Rephrased.) Plates were incubated for 16 to 18 h at 37°C. The MIC end points were read where the inhibition ellipses intersected the strip. A reduction of imipenem MIC=3 two folds in the presence of EDTA was interpreted as being suggestive of MBL production.

Detailed information of the patients and isolates was recorded in a separate proforma. The medical record of the patients was reviewed and age, sex, days of hospitalization, underlying illness, presence of malignant disease, antineoplastic chemotherapy, any surgical procedure, source from which isolate was recovered, urinary catherization, previous antibiotic use was recorded.

Statistical analysis

The data was expressed as mean ±SD and the percentage. The intergroup comparison and risk estimation was performed by using Fisher's exact test and Odd's ratio. Various statistical packages of SPSS, Minitab and Java stat calculators were used for analysis.

RESULTS

A total of 283 *P. aeruginosa* strains were isolated from patients admitted or attending OPD during the study period. Out of them 155 (54.8%) were male and 128 (45.2%) were female with a male: female ratio of 1.2: 1. The number of *P. aeruginosa* strains isolated from patients were 132 (46.6%) followed by 37 (13.1%), 37 (13.1%), 34 (12.0%), 19 (6.7%), 16 (5.7%) and 8 (2.8%) in the age group =60 years, 50-59 years, 40-49 years, 0-9 years, 30-39 years, 20-29 years and 10-19 years respectively. Highest number of strains were isolated from plastic surgery 124 (43.8%) followed by ICU 35 (12.4%), neurosurgery 14 (4.9%), POW 13 (4.6%), neonatology 11 (3.9%), nephrology 11 (3.9%), CVTS 10 (3.5%), oncology 7 (2.5%), endocrinology 7 (2.5%), haematology 6 (2.1%), neurology 6 (2.1%), general medicine 5 (1.8%), gastroenterology 5 (1.8%), paediatric surgery 5 (1.8%), general surgery 4 (1.4%), accident and emergency 3 (1.0%), observation 2 (0.7%) and surgical gastroenterology 2 (0.7%). Thirteen (4.6%) isolates were from OPD patients. Maximum number of *P. aeruginosa* strains were isolated from wound swab 131 (46.3%) followed by blood 59 (20.8%), urine 35 (12.4%), CSF 18 (6.4%), pus 17(6.0%), sputum 10 (3.5%), bile 6 (2.1%), endotracheal tip 5 (1.8%) and ear discharge 2 (0.7%).

Out of 283 *P. aeruginosa* isolates, 38 (13.42%) were resistant to imipenem by disk-diffusion method. Thirty three (11.66%) were found to be MBL producers by combined disk test (Figure 1). Five imipenem resistant isolates were negative for MBL by combined disk test. Out of 33 isolates which were positive in the combined-disk test (CDT), 25 (75.75%) showed zone of enhancement around both imipenem plus EDTA and ceftazidime plus EDTA (Figure 2), 5 (15.15%) isolates showed zone enhancement only around imipenem plus EDTA and 3 (9.1%) only around ceftazidime plus EDTA. The ATCC *P. aeruginosa* 27853 did not exhibit any zone size enhancement with EDTA-impregnated imipenem or ceftazidime disks (Figure 3). All the 33 isolates which were positive in the CDT showed reduction in MIC of more than four- fold in the presence of imipenem -EDTA combination by agar dilution method (Table 1). E- test was performed on 20 representative strains and all isolates showed a reduction in MIC of imipenem = 3 two-fold dilutions in the presence of EDTA (Table1 and Figure 4).

The mean age of the patients from whom MBL producers were isolated, was 50.66 years (range 1 week-81 years). Highest number of cases were above 60 years of age 17 (51.5%) followed by age group 50-59 years 4 (12.1%), 0 to 9 years 4 (12.1%), 40- 49 years 3 (9.1%), 20-29 years 2 (6.1%), 30-39 years 2 (6.1%) and 10-19 years 1 (3.0%). No statistically significant ($p>0.05$) association of a particular age group with MBL production was seen. Out of 33 patients from whom MBL producers were isolated 17 (51.5%) were male and 16 (48.5%) were females which was not statistically significant ($p>0.05$) when compared to gender -distribution of patients from whom MBL negative strains were isolated.

Of the 33 MBL positive isolates, 8 (24.2%) were from ICU, 7 (21.2%) from plastic surgery, 3 (9.1%) from neurosurgery, 2 (6.1%) from neonatology, 2 (6.1%) from nephrology, 2 (6.1%) from oncology, 2 (6.1%) from haematology, 2 (6.1%) from endocrinology, 1 (3.0%) from post- operative ward, 1 (3%) from general medicine, 1 (3%) from general surgery, 1 (3%) from CVTS and 1 (3%) from neurology. The number of MBL positive isolates from ICU was statistically significant ($p=0.027$) compared to MBL negative isolates.

The predominant source of MBL positive strains was urinary tract 9 (27.3%) followed by wound swab 8 (24.2%), blood 6 (18.2%), sputum 4 (12.1%), CSF 3 (9.1%), pus 2 (6.1%), endotracheal tip 1 (3%). Isolation of MBL positive isolates from urinary tract ($p=0.006$) and respiratory tract ($p=0.004$) was statistically significant.

Of the MBL positive, 8 (24.2%) were isolated from patients with malignancy, 5 (15.2%) from patients with diabetes, 5 (15.2%) from patients with sepsis, 4 (12.1%) from patients with cardiovascular disease, 4 (12.1%) from patients with CNS infections, 4 (12.1%) from patients with trauma and 3 (9.1%) from patients with burns.

The interval between admission to hospital and isolation of pathogen was longer among patients infected with MBL producers (mean 28.8 days, SD± 12.3 range 6-46 days) than patients infected with MBL non producers (mean 20.2 days, SD ±10.1, range 0-39 days) and the difference was statistically significant ($p=0.000$).

Fourteen (42.4%) MBL positive strains were isolated from patients who had H/O surgery however this was not statistically significant ($p=0.852$). Eight (24.2%) patients infected with MBL producers had malignancy however no statistically significant association ($p=0.292$) of malignancy with MBL production was seen. Five (15.2%) MBL positive strains were isolated from patients who had received antineoplastic chemotherapy which was statistically significant ($p=0.039$) when compared with non

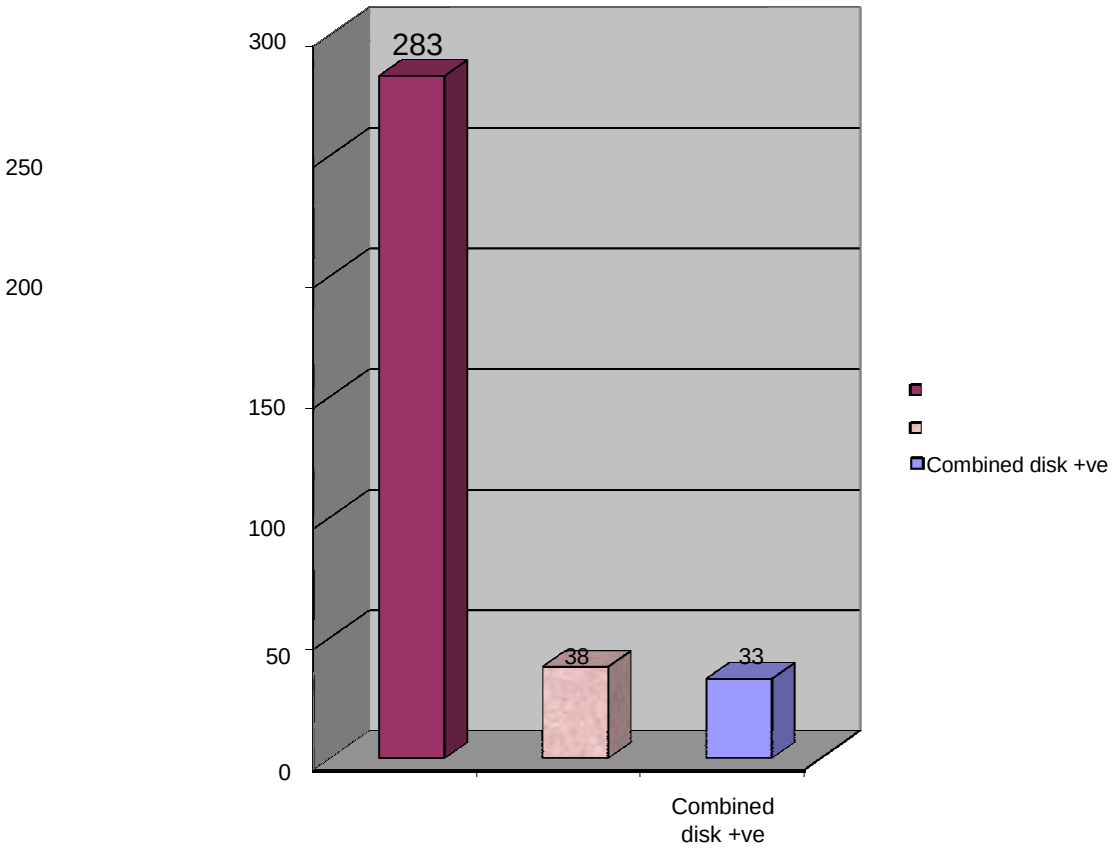

Figure 1. Shows number of imipenem resistant and metallobetalactamase +ve *P. aeruginosa* isolates.

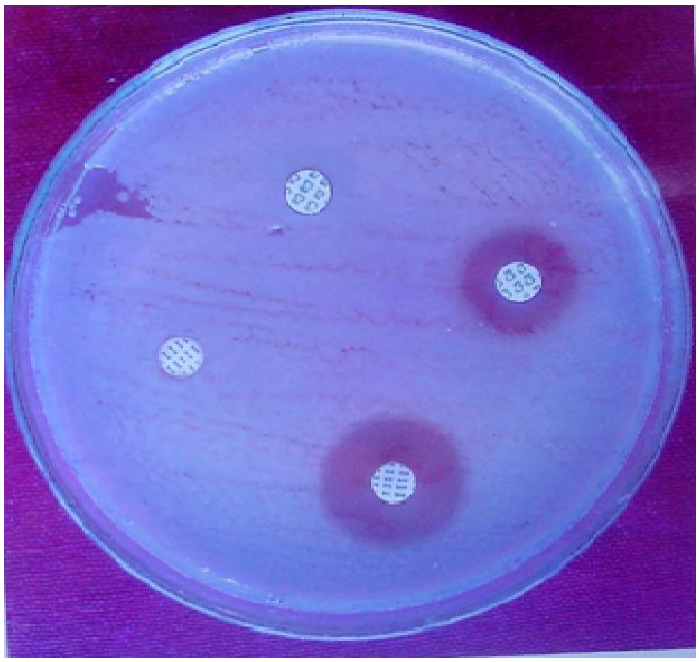

Figure 2. Shows zone of enhancement around both imipenem plus EDTA and ceftazidime plus EDTA.

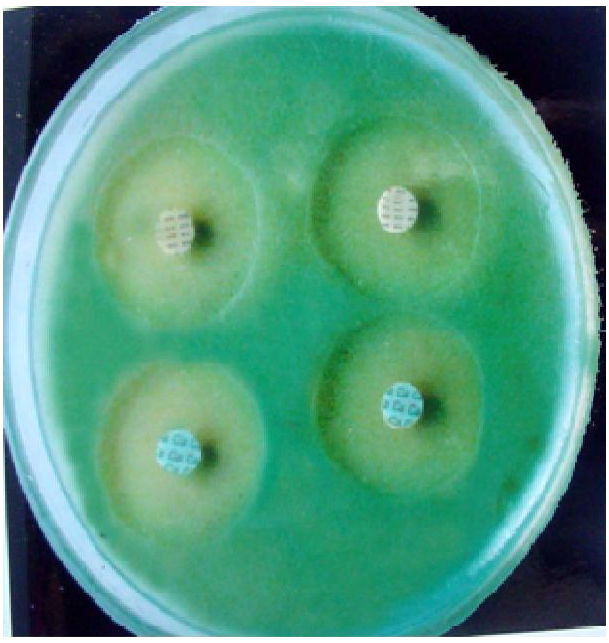

Figure 3. *P. aeruginosa* ATCC 27853 shows no zone size enhancement with EDTA-impregnated imipenem or ceftazidime disks.

Table 1. MIC (microgram/ml) of imipenem and imipenem+EDTA combination for MBL+ve strains.

Method	Agar dilution		E-test		
Serial No.	Imipenem	Imipenem+ EDTA	Imipenem	Imipenem+EDTA	
1	512	2	>256		2
2	64	0.5	64		1
3	512	16	>256		16
4	256	1	>256		1
5	128	1	128		1.5
6	512	2	>256		1.5
7	64	0.12	48		<1
8	32	0.5	24		<1
9	64	0.25	96		<1
10	512	1	>256		<1
11	256	1	>256		2
12	32	1	48		1
13	256	1	>256		1
14	256	2	>256		1
15	512	2	>256		4
16	256	2	256		2
17	128	0.25	96		1.5
18	256	2	>256		4
19	128	0.5	256		<1
20	64	0.12	64		<1
21	32	0.25			
22	128	4			
23	64	2			
24	256	8			
25	32	0.12			
26	512	8			
27	64	1			
28	128	16			
29	256	4			
30	512	1			
31	64	0.12			
32	64	4			
33	128	2			

producing strains. Twenty six (78.8%) MBL positive cases had H/O urinary catherization. The difference from non-producers was statistically significant (p=0.010). Thirty-one (93.9%) patients infected with MBL producing *P. aeruginosa* gave history of ß-lactam consumption in the preceding two weeks. The difference from patients infected with non-producing strains was not statistically significant (p=0.222).

22 (66.7%) MBL positive strains were isolated from patients who had taken extended spectrum beta lactams in the preceding two weeks. The difference from non-producers was not statistically significant (p=0.833). Four (12.1%) patients with MBL positive strains had taken carbapenems which was not statistically significant (p=0.149) compared to patients with non MBL-producing strains (Table 2).

MBL producers were 100% resistant to cefoperazone, cefoperazone plus sulbactum, ceftazidime, ceftazidime plus clavulanic acid, ofloxacin, gentamicin, carbenicillin, tobramycin, ceftriaxone, meropenem. Isolates were 100% sensitive to polymyxin B, 18.2% sensitivity was shown to piperacillin/tazobactum and 9.1% to amikacin and ciprofloxacin (Table 3).

DISCUSSION

MBL producing isolates are associated with a higher morbidity and mortality. Moreover given the fact that MBLs will hydrolyze all classes of ß-lactams and that we

Figure 4. E-test demonstrating reduction in MIC of imipenem in the presence of EDTA.

are several years away from the development of a safe therapeutic antibiotic; their continued spread would be a clinical disaster. The occurrence of an MBL positive isolate poses not only a therapeutic problem but is also a serious concern for infection control management. As a result of being difficult to detect, such organisms pose significant risks particularly due to their role in unnoticed spread within institutions and their ability to participate in horizontal MBL gene transfer, with other pathogens in the hospital (Agarwal et al., 2006). The present study was conducted with above perspective in view to know the prevalence of MBL producing *P. aeruginosa*, to find the associated risk factors and possible treatment alternatives.

Different studies have reported the use of methods like imipenem- EDTA combined disk test, double-disk synergy test using imipenem and EDTA,E- test, modified Hodge test (Behera et al., 2008; Lee et al., 2003; Arakawa et al., 2000; Yong et al., 2002; Migliavacca et al., 2002; Yan et al., 2004). In our study we used three different methods for detection of MBL producing *P. aeruginosa*; combined-disk test, reduction in MIC with imipenem plus EDTA combination and E-test. A total of 283 strains were isolated over a study period of one and a half year. Thirty-eight (13.42%) out of 283 were resistant to imipenem and 33 (11.66%) were found to be MBL producers by combined-disk test. Five out of thirty-eight were negative in the combined disk test. Our findings are similar to the results of many studies conducted in different settings. Hemlatha et al. (2005) from India reported that 16% of *P. aeruginosa* isolates were resistant to imipenem and 14% were positive for MBL production by combined disk test (Hemlatha et al., 2005). Behera et al. (2008) found 14.47% of *P. aeruginosa* resistant to imipenem and 10.53% positive for MBL production by combined-disk test. Behera et al. (2008) reported 7.2% imipenem resistance and 4.4% MBL positivity by combined-disk test among *P. aeruginosa* isolates (Berges et al., 2007).

In our study 25 (75.75%) isolates showed a significant zone enhancement of =7 around both imipenem- EDTA disk and ceftazidime-EDTA disk compared to plain disks. 5 (15.15%) isolates showed significant zone enhancement only around imipenem-EDTA disk and 3 (9.1%) around ceftazidime-EDTA disk only. In the study by Mendiratta et al. (2005) 15 MBL producers could be detected by using DDST with ceftazidime whereas with imipenem 14 isolates could be detected (Mendiratta et al., 2005). Similarly Hemlatha et al. (2005) found that EDTA-ceftazidime could pick up additional isolates of MBL producers as six MBL producers were detected with ceftazidime-EDTA and five with imipenem-EDTA (Hemlatha et al., 2005). In our study more isolates were found to be positive for MBL by imipenem-EDTA disks than ceftazidime-EDTA disks. This can be explained by the fact that MBL producing organisms might have other ceftazidime resistance mechanisms. With such strains, CDT using ceftazidime will not show MBL production as suggested by Behera et al. (2008).

All the 33 isolates which were positive in the combined-disk test showed MIC reduction of > 4 fold with imipenem-EDTA combination compared to imipenem alone by agar dilution method. A MIC reduction of 16- 512 folds was observed. Similar finding were reported by previous studies (Migliavacca et al., 2002; Hemlatha et al., 2005). Five isolates which were resistant to imipenem but were found to be negative for MBL in the combined– disk test and also showed reduction in MIC of < 4 fold with imipenem -EDTA combination might have some other mechanism of resistance to imipenem like permeability of outer membrane and /or active efflux.

All the twenty isolates on which E-test was performed were MBL positive. Thus 100% positive results were obtained in our study which is comparable to various studies. A study conducted by Marchiario et al. (2005) showed that E-test had a sensitivity of 92% in identifying MBL positive isolates (Varaiya et al., 2008). Pitoutt (2005) reported 96% sensitivity of E- test in detecting MBL positives among imipienem non-susceptible *P. aeruginosa* (Agarwal et al., 2008) . Behera et al. (2008) found positive results in all the 30 carbapenem resistant *P. aeruginosa* strains on which E-test was performed. In our study we screened carbapenem resistant isolates only which might have accounted for very high sensitivity of the test.

Combined disk test, MIC reduction and E-test were found to be equally sensitive in detecting MBL positive

Table 2. Shows association of risk factotrs with MBL production.

Risk factor	MBL+ve	MBL-ve	p value	%	
	n(33)	%	n(250)		
1. Surgery					
Yes	14	42.4	101	40.4	p=0.852 (NS)
No	19	57.6	149	59.6	
2. Malignancy					
Yes	8	24.2	42	16.8	p=0.292 (NS)
No	25	75.8	208	83.2	
3. Antineoplastic therapy					
Yes	5	15.2	14	5.6	p=0.039 (Sig)
No	28	84.8	236	94.4	
4. Urinary catheterization					
Yes	26	78.8	138	55.2	p=0.010 (Sig)
No	7	21.2	112	44.8	
5. Beta-lactam antibiotic intake					
Yes	31	93.9	216	86.4	p=0.222 (NS)
No	2	6. 1	34	13.6	
6. Extended spectrum beta lactam intake					
Yes	22	66.7	162	64.8	p=0.833 (NS)
No	11	33.3	88	35.2	
7. Carbapenem intake					
Yes	4	12.12	14	5.6	p=0.149 (NS)
No	29	87.88	236	84.4	

Table 3. Sensitivity pattern of MBL+ve and MBL-ve isolates of *P. aeruginosa.*

Antibiotic	MBL +Ve		MBL -Ve	
	n (33)	%	n (250)	%
Amikacin*	3	9.1	98	39.2
Ciprofloxacin*	3	9.1	157	62.8
Ofloxacin*	0	0.0	73	29.2
Ceftazidime*	0	0.0	80	32.0
Ceftazidime + clavulanate*	0	0.0	84	33.6
Gentamicin*	0	0.0	73	29.2
Carbenicillin*	0	0.0	57	22.8
Tobramycin*	0	0.0	51	20.4
Imipenem*	0	0.0	245	98.0
Meropenem*	0	0.0	179	71.6
Polymixin B	33	100.0	250	100.0
Piperacillin + Tazobactum*	6	18.2	135	54.0
Cefoperazone*	0	0.0	76	30.4
Cefoperazone and Sulbactum*	0	0.0	93	37.2
*p= <0.05				

isolates. MIC reduction is a cumbersome, laborious method and given the cost constraints of E-test a simple screening test like combined disk test can be performed along with routine susceptibility testing.

In our study the overall prevalence of MBL positives among *P. aeruginosa* was 11.66%. In India prevalence ranging from 8 to 14% has been reported (Zavascki et al., 2006; Varaiya et al., 2008; Hirakata et al., 1998). All the

33 MBL producing strains were isolated from inpatients thus pointing to the fact that MBLs are largely a problem of hospitalized patients who share numerous risk factors. The major contributions were from ICU 8 (24.2%), plastic surgery 7(21.2%). Zavaski et al. (2006) reported that ICU stay increased the risk for acquisition of MBL producing *P. aeruginosa* (Hirakata et al., 2003) . In our study out of 35 *P. aeruginosa* isolate from ICU, 8 (22.8%) were found to be MBL producers and the number of MBL producers from ICU was statistically significant. These findings are consistent with the study by Varaiya et al. (2008) who report 20.8% MBL producers among *P. aeruginosa* isolates in ICU (Yan et al., 2001).

The maximum number of MBL positive isolates were obtained from urine (27.3%) followed by wound swab (24.2%), blood (18.2%), sputum (12.1%). Hirakata et al. (1998) reported in their study that the predominant source of isolation for MBL positive *P. aeruginosa* was urinary tract (40.0%) followed by respiratory tract (18.8%) and abscesses, pus and wounds (15%) (Agarwal et al., 2006). In the present study 27.3% MBL positive isolates were recovered from urine and the association was statistically significant. Most of these patients were having indwelling urinary catheter. This finding is consistent with another study reported by Hirakata et al. (2003)

Statistically significant difference was found in the duration of hospitalization in the patients infected with MBL producing and non-producing *P. aeruginosa*. Most of MBL producers were from patients with malignancy receiving chemotherapy, sepsis with multi-organ dysfunction, burn and polytrauma patients who needed prolonged hospitalization. The mean duration of hospitalization in the patients infected with MBL producers was 29.8 days (range 6-46 days) . In the study reported by Varaiya et al. (2008) the mean hospital stay of patients in who MBL producers were isolated was 25 days (range 6-44 days) . Yan et al. (2001) suggested that surgery is a risk factor for acquisition of MBL producers (Landman et al., 2005). In our study 42.4% patients infected with MBL producers had undergone surgery but the association with MBL production was not statistically significant.

In the study reported by Hirakata et al. (1998, 2003) no statistical association of surgery with MBL production was seen (Markou et al., 2003) . Hirakata et al. (1998, 2003) suggested that malignancy is a risk factor for acquisition of MBL producing *P. aeruginosa*. In their study 53.8% MBL producing *P. aeruginosa* were recovered from patients with malignancy (Varaiya et al., 2008). In our study 24.2% cases had malignant disease however the association was not statistically significant. The present study revealed a statistically significant difference in the number of patients infected with MBL producing and non-MBL producing *P. aeruginosa* who had received antineoplastic chemo-therapy. In the current study 78.8% patients infected with MBL producers had H/O urinary catheterization which was statistically significant compared with non-producers. Hirakata et al. (1998, 2003) reported that the possible risk factors for infection or colonization with MBL producers include long term hospitalization, administration of antineoplastic agents and use of indwelling urinary catheters (Markou et al., 2003). Zavaski et al. (2006) reported that exposure to beta-lactams is a significant risk factor for MBL producing *P. aeruginosa* infections (Hirakata et al., 2003). In our study of 31 out of 33 (93.9%) MBL positive patients had history of prior use of ß- lactams. However the difference from non-producers was not statistically significant. In our study only 4 of 33 patients (12.1%) had taken carbapenems. Our data is consistent with that of Hirakata et al. (1998, 2003) and Yan et al. (2001) which suggest that selective pressure from carbapenems is not always required for isolation of MBL producers (Agarwal et al., 2006, Landman et al., 2005).

In the present study MBL producers showed very high resistance to all antimicrobials except polymyxin B compared to non-producers and the difference was statistically significant ($p<0.05$). All isolate (100%) of MBL producing *P. aeruginosa* were resistance to gentamicin, ceftazidime, carbenicillin, tobramycin, ceftriaxone, ofloxacin, cefoperazone, cefoperazone plus sulbactum and ceftazidime plus clavulanic acid. Only 9.1% MBL positive isolates were sensitive to amikacin and ciprofloxacin; 18.1% were sensitive to piperacillin/tazobactum. All MBL producing *P. aeruginosa* isolates (100%) were sensitive to polymyxin B. Our findings are very much consistent with many studies reported in literature (Marchiaro et al., 2005; Navaneeth et al., 2002; Varaiya et al., 2008; Agarwal et al.,2006).

The unique problem with MBLs is their unrivalled broad spectrum resistance profile. In addition, in many cases the MBL genes may be located on plasmids with genes encoding other antibiotic resistance determinants. These MBL positive strains are usually resistant to ß-lactams, aminoglycosides and fluoroquinolones. However, they usually remain susceptible to polymyxins (Walsh et al., 2005). All the isolates in our study were sensitive to polymyxin B. In absence of therapeutic MBL inhibitors, polymyxins have shown to be effective in the treatment of MDR *P. aeruginosa* infections. It has been claimed that polymyxins are not as toxic as previously thought (Markou et al., 2003). However, they should not be used in monotherapy. A combination therapy must be preferred. An aminoglycoside or a fluoroquinolone molecule that may have retained some activity against the isolate may be chosen substantiated by rapid determination of its MIC levels for the isolate. In addition rifampin may be an interesting agent for treating multi-drug resistant *P. aeruginosa* infections (Walsh et al., 2005). In the present scenario use of polymyxin B should be judicious as strains with reduced susceptibility have emerged (Landman et al., 2005). In the absence of novel agents the spread of MBL producers may lead to

therapeutic dead ends. The early detection of MBL producing *P. aeruginosa* may avoid the future spread of these multi-drug resistant strains.

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