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Research Article

PREVALENCE OF METALLO β LACTAMASES PRODUCING PSEUDOMONAS SPP. AND ACINETOBACTER SPP. IN A TERTIARY CARE TEACHING HOSPITAL

Subbalakshmi.E[1], Madhumathi[2], Ramaswamy Rajendran[3]

[1]Asst professor, Department of Microbiology, Sambhram Institute of Medical Sciences and Research, KGF, Karnataka

[2]Asst Professor, Department of Microbiology, Vydehi Institute of Medical Sciences and Research, White fileld, Bangalore.

[3]Professor, Head of the department, Vydehi Institute of Medical sciences and research centre, white field, Bangalore.

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ABSTRACT

INTRODUCTION: Metallo β lactamases (MBL) are metallo enzymes of Ambler class B and are resistant to clavulanic acid. They require zinc as co-factor for enzymatic activity and their activity is inhibited by ethylene diamine tetra acetic acid (EDTA) and other metal ion chelating agents. The present study was undertaken to determine the multidrug resistance pattern and the prevalence of MBL in *Pseudomonas* spp. and *Acinetobacter* spp. in a tertiary care teaching hospital.

Materials and Methods: The present study was carried out in the Department of Microbiology, Vydehi Institute of Medical Sciences and Research Centre, Bangalore from January 2014-December 2014. Consecutive 200 multidrug resistant isolates of *Pseudomonas* spp. and *Acinetobacter* spp were isolated and identified by standard microbiological procedures. Antimicrobial susceptibility testing was carried out by Kirby Bauer disc diffusion method and MBL detection was done by Imipenem / Imipenem+EDTA disc diffusion method and E strip method for Imipenem / Meropenem resistant isolates.

RESULTS: Prevalence of MBL production in *Pseudomonas* spp and *Acinetobacter* spp was 29.5%. MBL producer were seen in 16 out of 25 (64%) isolates of *Acinetobacter* spp. whereas in *Pseudomonas* spp. 43 of 175 (24.6%) isolates showed MBL production. Highest prevalence of MBL was found in aspirated fluid (n=1,100%) pleural fluid (n=1,100%), urine (n=8, 47.1%) followed by pus, catheter tip, blood, BAL fluid and sputum. The maximum MBL was found in the age group 0-5, 11-18 and then > 60 years. The highest number of MBL production was observed in Orthopaedic wards followed by surgical wards and then ICUs.

CONCLUSIONS: The prevalence of MBL production was more in Orthopaedic wards, surgical wards and ICUs suggesting nosocomial occurrence of infections due to hospital stay. The emergence of MBL and their broad spectrums and unrivalled drug resistance is creating a therapeutic challenge for clinicians and microbiologists.

KEY WORDS: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, MBL

INTRODUCTION

Pseudomonas and *Acinetobacter* have emerged as an important nosocomial pathogens. They are widely distributed in nature and their presence in the hospital environment poses a special risk on debilitated patients especially those in intensive care units who are at risk of opportunistic infections by Multidrug resistant Organisms (MDROs) *Pseudomonas* spp. and *Acinetobacter* spp. are important multidrug resistant nosocomial pathogens.[1] Acquired Metallo β lactamases (MBL) have emerged as one of the most significant resistance mechanisms owing to their capacity to hydrolyse all β lactams including Carbapenems with the exception of Monobactams. Their genes are also carried on highly mobile elements, allowing easy dissemination resulting in resistance to multiple antibiotics. [2] The Carbapenems arevital in the management of hospital acquired gram negative infections, because of their broad spectrum of activity and stability to hydrolysis by most of the β lactamases, including extended spectrum β lactamases (ESBLs). Nosocomial outbreaks of Carbapenem resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp.due to metallo β lactamases (MBLs) production have been reported from different regions. The emergence of these MBL in gram negative bacilli is becoming a therapeutic challenge as these enzymes possess high hydrolytic activity that

*Corresponding author: Subbalakshmi.E // E-mail: subhalakshmi69@gmail.com

leads to degradation of higher generation cephalosporins. [3] Carbapenamases are β lactamases, which include serine β lactamase (KPC, OXA, GES, etc.) and metallo β lactamases (MBLs). The latter require metal ion zinc for their activity, which is inhibited by metal chelators like EDTA and thiol based compounds but not by sulbactam, tazobactam and clavulanic acid. [4] MBL production is typically associated with resistance to aminoglycosides and fluoroquinolones, further compromising therapeutic options. Unlike Carbapenem resistance due to several other mechanisms, the resistance due to MBL and other Carbapenamase production has a potential for rapid dissemination as it is often plasmid mediated. Consequently, the rapid detection of Carbapenamase production is necessary to initiate effective infection control measures to prevent their dissemination. [5] The aim of this study was to study the prevalence of metallo β lactamase producing *Pseudomonas* spp.and *Acinetobacter* spp. in a tertiary care teaching hospital.

MATERIALS AND METHODS: A prospective study was carried out in the Department of Microbiology, Vydehi Institute of Medical Sciences and Research Centre, Bangalore from January 2014-December 2014. Consecutive 200 multidrug resistant isolates of *Pseudomonas* spp. and *Acinetobacter* spp. were isolated and identified by standard microbiological procedures.This work was approved by the ethical committee of the Institute. Various clinical samples-urine, sputum, pus, blood and other body fluids (Pleural fluid, BAL fluid, CSF etc.) were received at the microbiology laboratory from inpatients who were admitted in Vydehi Institute of Medical Sciences and Research Centre, Bangalore. *Pseudomonas* spp.were identified as non-lactose fermenting colonies, beta hemolysis on blood agar and on nutrient agar plates, large irregular colonies, and greenish pigmented colour colonies with musty mawkish odour. Speciation were done by biochemical reactions. *Acinetobacter* sppwere identified by pale lactose fermenting colonies on Macconkey agar plates. On Blood agar plates, based on haemolysis speciation was done. Antibiotic susceptibility testing of the isolated strains of *Pseudomonas species*. and*Acinetobacter species*. was carried out by the Standardised modified Kirby Bauer disc diffusion technique. The antibiotic discs were used according to CLSI guidelines 2014 and the potency of the drug used were as mentioned in CLSI guidelines 2014.The diameter of the zone of inhibition was measured and interpreted according to CLSI guidelines 2014.[6] A strain was considered to be multidrug resistant if it was resistant to three or four drugs. Following antimicrobial sensitivity testing, all isolates of *Pseudomonas* sppand *Acinetobacter*spp.showing resistance to Imipenem/Meropenem were selected for MBL production.

IMP-EDTA double disk synergy test

Test strains were adjusted to the McFarland (0.5) standard and were inoculated on Muller Hinton agar. One Imipenem (10µg) disk and one Imipenem-EDTA disk (Imipenem 10µg+750µg EDTA) (commercially available with Hi-Media) were placed on the plate. After aerobic incubation at 37° overnight, the inhibition zone size of Imipenem and Imipenem-EDTA discs was measured and compared. An increase in inhibition zone with Imipenem EDTA of ≥7mm, than the Imipenem disc alone was considered as MBL positive. The quality control strain used was in house MBL positive control strain of *Pseudomonas aeruginosa* and *Acinetobacter baumanii*.

2. E Strip test

Mueller Hinton agar plates were uniformly surface inoculated with bacterial suspension matched to 0.5 McFarland standard. E-test strips containing Meropenem and Meropenem-EDTA were placed on the agar surface and incubated at 37°C for 24 hours. The presence of MBL was confirmed when there was clearing of zone only with Meropenem-EDTA and not with Meropenem. The diameter of the zone of inhibition was measured and interpreted according to the guidelines of CLSI. The positive control strain used was in house MBL positive control strain of *Pseudomonas aeruginosa* and *Acinetobacter baumanii*.

RESULTS:

Multidrug resistant isolates of *Pseudomonas* spp175 (87.5 %) and *Acinetobacter* spp. 25 (12.5 %) were isolated from various clinical samples such as pus 72(36%), sputum 58(29%), urine 17(8.5%), tracheal aspirate 17(8.5%), catheter tip 13(6.5%), Blood 8(4%), Broncho alveolar lavage (BAL) 4(2%), pleural fluid 1(0.5%), aspirated fluid 1(0.5%), vaginal swab 1(0.5%). *Pseudomonas* sppwas isolated from 56 (32%) female patients and 119 (68%) male patients.*Pseudomonas*

aeruginosa was isolated from pus samples 65 (37.1%), sputum 52 (30%), tracheal aspirate 23 (13.1%), urine 12 (6.9%), catheter tip 12 (6.9%), blood 6 (3.4%), BAL fluid 3 (1.8 %), vaginal swab 1 (0.6%). However, *Pseudomonas fluorescens* was isolated from one pus sample.

The antibiotic resistance pattern of MBL positive *Pseudomonas aeruginosa* was Ceftazidime (100%), Gentamicin (28.7%), Amikacin (62.6%), Aztreonam (96%), Cefepime (94.8%), Ciprofloxacin (55.7%), Imipenem (10.3%), Meropenem (26.4%), Piperacillin/tazobactam (81%), Ticarcillin/clavulanic acid (81.6%), Colistin (2.3%), and Polymyxin B (0%). For urinary isolates the resistance pattern to Norfloxacin, was 100%.

Acinetobacter sppwas isolated from 13 (52%) female patients and 12 (48%) male patients. *Acinetobacter baumannii* was isolated from pus samples 6 (24%), sputum 6 (24%), urine 3 (12%), tracheal aspirate 2 (8%), Blood 2 (8%), aspirated fluid 1(4%), BAL fluid 1(4%), catheter tip 1(4%), pleural fluid 1(4%). Two isolates of *Acinetobacter lwoffi*were isolated from urine sample. [Table 1].

The antibiotic resistance pattern of MBL positive *Acinetobacter* spp. was Ceftazidime (100%), Gentamicin (52.2%), Amikacin (87%), Cefepime (95.7%), Ciprofloxacin (56.5%), Imipenem (39.1%), Meropenem (65.2%), Piperacillin /Tazobactam (91.3%), Ticarcillin/clavulanic acid (78.3%), Co-trimoxazole (100%) and Tetracycline (100%). [Table 2.]

All the 200 isolates of *Pseudomonas* spp and *Acinetobacter*spp were screened for MBL production. MBL producers were seen in 59 (29.5%) isolates whereas 141 (70.5%) were non MBL producers. MBL producers were seen in 43 of 175 (25%) isolates of *Pseudomonas* spp. and 16 of 25 (64%) isolates of *Acinetobacter* spp. [Table 3].

The isolates which showed resistance to Imipenem/Meropenem were tested for MBL production using Imipenem/Imipenem+EDTA disc diffusion method and E strip test using Meropenem/Meropenem+EDTA method. Both the tests were equally effective and all the Imipenem/Meropenem resistant isolates showed MBL production (100%)

The MBL positive isolates were seen more in the age group of 0-5 followed by 11-18 age group and >60 age group. The MBL positive isolates were detectable least in the age group from 11-40 and 41-60 years, the rate of isolation being 45.5% followed by 11-18 age group (43%) and >60 years (36%).

MBL production was detected in aspirated fluid (n=1,100%), pleural fluid (n=1,100%), urine (n=8, 47.1%) in pus (n=27, 37.5%), catheter tip (n=4, 30.8%), BAL fluid (n=1, 25%), Blood (n=2, 25%), sputum (n=12, 20.7%), tracheal aspirate (n=3, 12%). [Chart1]. 52.6% of the MBL positive isolates were from Orthopaedic wards. Surgical wards, and ICUs accounted for 31.3%, and 27.1% respectively. In case of Medical wards the rate of isolation was 27.1%, TB wards (20%), Paediatric wards (22.2%), OBGs (16.7%) respectively. [Chart 2].

Table 1 – Isolation of *Pseudomonas Species* & *Acinetobacter Species*

ORGANISM NAME	PUS	SPUTUM	URINE	BLOOD	ASP FLUID	BAL FLUID	CATHETER TIP	PLEURAL FLUID	TRACHEAL ASP	VAGINAL SWAB	TOTAL
Pseudomonas aeruginosa	65	52	12	6	0	3	12	0	23	1	174
Pseudomonas fluorescens	1	0	0	0	0	0	0	0	0	0	1
Acinetobacter baumannii	6	6	3	2	1	1	1	1	2	0	23
Acinetobacter lwoffi	0	0	2	0	0	0	0	0	0	0	2
Total	72	58	17	8	1	4	13	1	25	1	200

Maximum number of *Pseudomonas spp* and *Acinetobacter spp*. were isolated from pus samples followed by sputum and then tracheal aspirate and urine.

Table 2–Antibiotic resistance pattern of *Pseudomonas species*

Acinetobacter species

Organism	CAZ	G	AK	AT	CPM	CIP	IMP	MRP	PTZ	TCC
P. aeruginosa	100 %	28.7 %	62.6 %	96 %	94.8 %	55.7 %	10.3 %	26.4 %	81.0 %	81.6 %
P. fluorescens	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %
A.baumannii	100 %	52.2 %	87 %		95.7 %	56.5 %	39.1 %	65.2 %	91.3 %	78.3 %
A.lwoffi	0 %	0%	0 %		0 %	0 %	50 %	0 %	50 %	0 %

Table 4- Distribution of MBL positive isolates age wise

MBL	Age in years						Total
	0 5 yrs.	6 10 yrs.	11 18 yrs.	19 40 yrs.	41 60 yrs.	>60 yrs.	
Negative	6(54.5%)	3(75%)	4(57.1%)	50(73.5%)	53(74.6%)	25(64.1%)	141(70.5%
Positive	5(45.5%)	1(25%)	3(42.9%)	18(26.5%)	18(25.4%)	14(35.9%)	59(29.5%
Total	11(100%)	4(100%)	7(100%)	68(100%)	71(100%)	39(100%)	200(100%

P=0.553, Not significant, Fisher Exact test

Table 5; MBL isolation of *Pseudomonas* and *Acinetobacter* gender wise

MBL	Gender		Total
	Female	Male	
Negative	49(71%)	92(70.2%)	141(70.5%)
Positive	20(29%)	39(29.8%)	59(29.5%)
Total	69(100%)	131(100%)	200(100%)

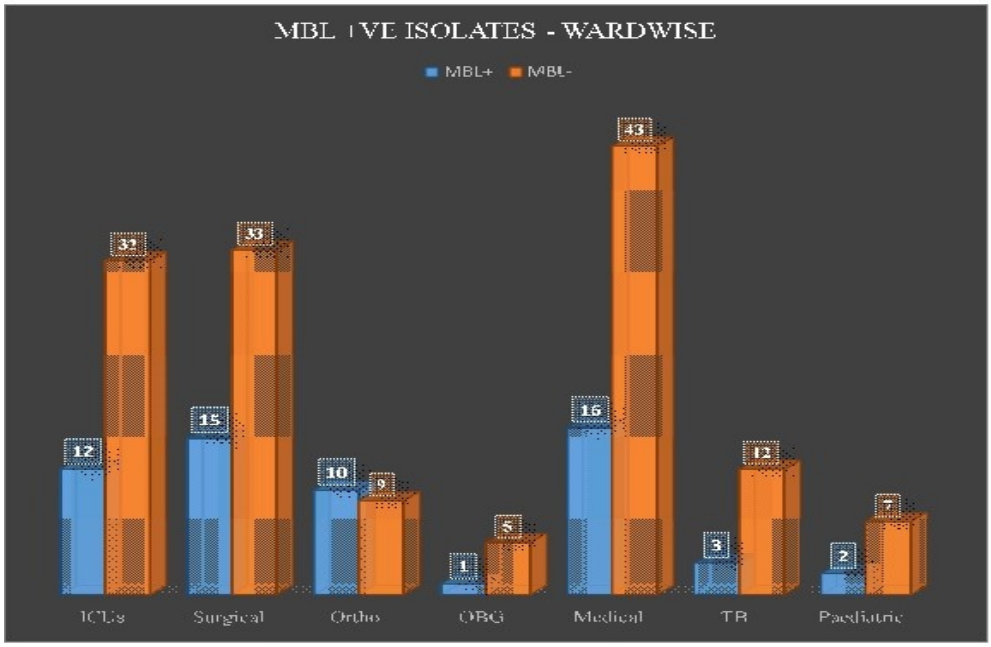
P=0.908, Not significant, Chi-Square test

Table 3. MBL isolation of *Pseudomonas spp* and *Acinetobacter spp*

MBL	Organism		Total
	Pseudomonas spp	*Acinetobacter spp*	
Negative	132(75.4%)	9(36%)	141(70.5%)
Positive	43(24.6%)	16(64%)	59(29.5%)
Total	175(100%)	25(100%)	200(100%)

P<0.001**, significant, Chi-Square test

Chart 2- Distribution of MBL positive isolates ward wise

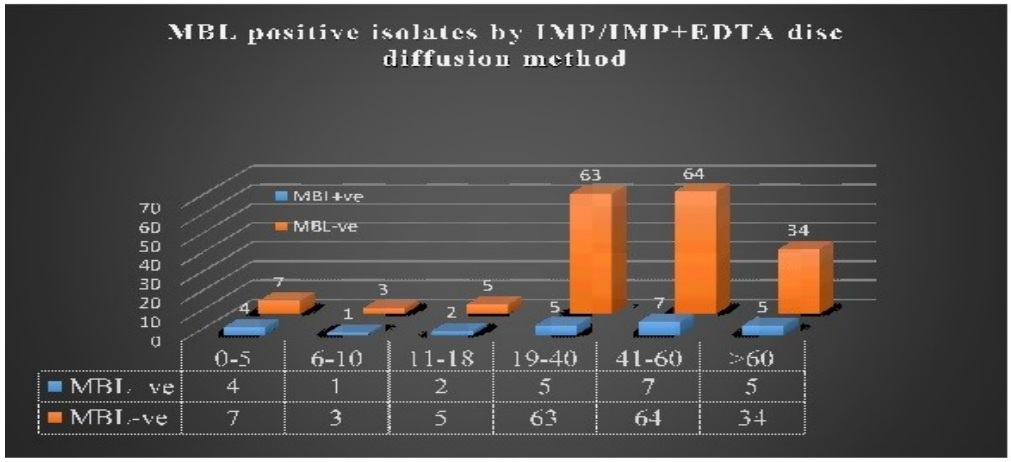


Source	ICUs	Surgical	Ortho	OBG	Medical	TB	Paediatric
MBL +ve %	27.1%	31.3%	52.6%	16.7%	27.1%	20.0%	22.2%

MBL positive isolates were more from Orthopaedic wards followed by Surgical wards and ICUs.

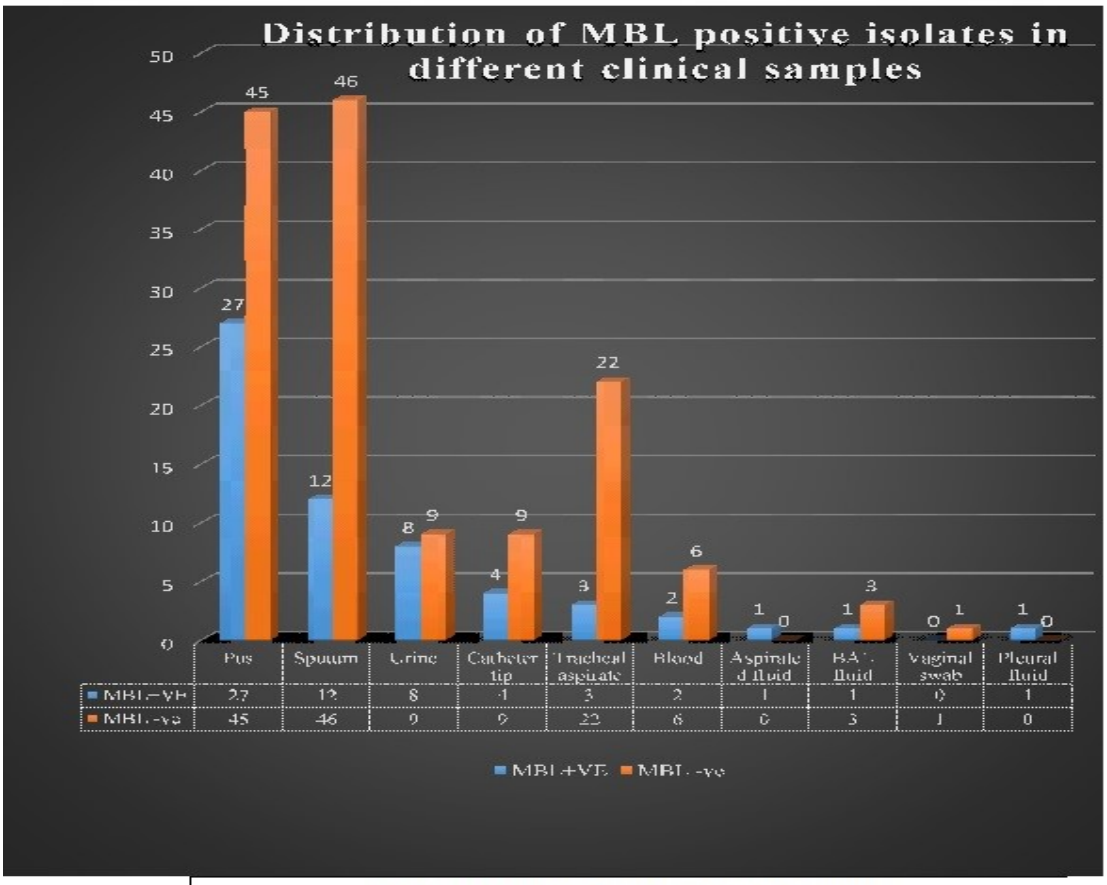
ICUs include (MICU, NICU, NUCU, PICU, and CTIC). Surgical wards include (MSW, FSW, FCTW, MCTW, NMSW, PASW and MPSW). Orthopaedic wards includes (MOR, FOR, MMOW, FMOW). TB wards (MTB, FTB, MSDB). Medical wards (MMW, FMW, MRW, FRW, PVT 1, SPW1).

Chart 3- MBL positive isolates detection by Imipenem / Imipenem+EDTA disk diffusion method



MBL +ve %	36.4%	25.0%	28.6%	7.4%	9.9%
MBL -ve %	63.6%	75.0%	71.4%	92.6%	90.1%

Chart 1- Distribution of MBL positive isolates in different clinical samples



MBL +vet % of Isolates (Sample wise)									
Pus	Sputum	Urine	Catheter tip	Tracheal aspirate	Blood	Aspirated fluid	BAL fluid	Vaginal swab	Pleural fluid
37.5 %	20.7 %	47. 1%	30.8 %	12.0 %	25. 0%	100 %	25 %	0%	10 00 %

DISCUSSION: *Pseudomonas aeruginosa* and *Acinetobacter baumanii* are associated with nosocomial infections in immunocompromised patients.Carbapenems are the drug of choice for these multidrug resistant pathogens. However, there is a tremendous increase in the reports of carbapenem resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumanii.* In the present study, among all the clinical isolates, multidrug resistant (resistant to 4 or more classes of antimicrobials including carbapenems) were screened for phenotypic detection of metalloβ lactamase producing strains. A similar study was done by Chauhan et al [7]. In our study, the infections caused by *Pseudomonas* aeruginosa were more in males 119 (68%) than in females 56 (32%). This was compatible with the study by Peshattiwar PD et al. [8] where in the rate of isolation of*Pseudomonas aeruginosa* in males was 81 (64.28%) and females 45 (35.71%). Similar findings were seen in Senthamarai et al [9] and Rakesh Kumar et al [10]. The outdoor activity, personal habits, nature of work and exposure to soil, water and other areas which are inhabited by organism could be the reason for male preponderance.

Pseudomonas aeruginosa was common in the age group 41-60 years followed by 19-40 years. This observation was compatible with the study by Rakesh Kumar et al [10] and Senthamarai et al [9] where the isolation of *Pseudomonas aeruginosa* was common in the age group 11-20 years and 21-40 years.

The rate of isolation of *P. aeruginosa* correlated with the duration of the hospital stay and was directly proportional to a higher prevalence of the infection.[Table-4].The isolation of *Pseudomonas aeruginosa* was more from pus n=65, (37.4%) followed by sputum n=50, (30%) whereas in Senthamarai et al [9]*P.aeruginosa*was isolated predominantly in pus sample, the rate of isolation being 47.11% followed by sputum 36.5%. Wound infection and respiratory infections were found to be commonly affected by *P.aeruginosa*.

To isolate multidrug resistant pathogens we included drugs of different classes for antibiotic sensitivities namely cephalosporins (3rd generations), Aminogycosides, carbapenems and monobactems.

First, with reference to 3rd generation antibiotics, we observed 100% resistance with Ceftazidime and 95.7% with cefepime. The maximum number of resistance to Ceftazidime was observed with Peshattiwar PD et al [8]study (53.17%) and Senthamarai et al study [9] (65.38%).Indiscriminate use of 3rd generation cephalosporin as broad spectrum empirical therapy production and dissemination of ESBL enzymes has contributed to such a surge in resistance pattern. [Table-5].

Next when we compared the the resistance pattern of aminoglycosides, the resistance pattern with Gentamicin was (28.7%) and Amikacin (62.6%) while in a study by Senthamarai et al [9] the resistance pattern to Amikacin (57.14%) was lower compared to Gentamicin (87.8%). In Bashir et al [11]] study 90.9%% isolates were resistant to Amikacin and 81.9% isolates were resistant to Gentamicin. We observed the resistance pattern to Ciprofloxacin in our study as 55.7% and the resistance pattern in Rakesh Kumar et al [10] study as 59%. The resistance pattern to Ticarcillin / clavulanic acid was 81.6% in the present study whereas in a study by Senthamarai et al [9] the resistance pattern was 56.7%. The ESBL enzymes are inhibited by β lactamase inhibitors via clavulanic acid and sulbactam. Hence the use of β lactamase inhibitors may be an alternative to 3rd generation cephalosporins but the effect of this combination varies depending on the subtype of ESBL present, βlactamase inhibitor resistance was 56.7% in Senthamarai et al [9]study whereas in the present study it was 81.6% which makes them less reliable for therapeutic purposes. We found in case of *Pseudomonas aeruginosa* the resistance was least with Colistin and Imipenem. Finally we compared the antibiotic resistance pattern with carbapenems.The resistance pattern was 10.3% and 26.4% to Imipenem and Meropenem where as in a study by Peshattiwar et al [8] the resistance pattern to Imipenem and Meropenem was 12.69% each whereas a study by Murugan et al [12] showed 100% resistance to Imipenem and Meropenem. Exposure to antibiotics is a significant risk factor for colonization and infection with nosocomial multidrug resistant pathogens. The unique problem with MBLs is their unrivalled broad spectrum resistance profile. In many cases MBL genes may be located on plasmids with genes encoding other antibiotic determinants. All MBL producers were resistant to many antibiotics but all of them were susceptible to Polymyxin B as seen in the present study. In the absence of therapeutic MBL inhibitors, Polymyxin B have been shown to be effective in the treatment of MDR *Pseudomonas aeruginosa* infections. [13]We observed 100% resistance with Ceftazidime, Norfloxacin and 95.7% with Cefepime. The antibiotic resistant pattern of Murugan et al [12] showed 100% resistant to Piperacillin, Meropenem, Cefepime, Cefotaxime, Aztreonam and Ticarcillin.

Acinetobacter spp. was common in the age group 19-40 years and then >60 yrs. *Acinetobacter baumannii* was isolated more from pus (26.6%) and sputum (26.6%) samples which is compatible with Smith Kumar et al.[13]

To identify multidrug resistant pathogens we included cephalosporins (3rd generations), Aminoglycosides,Quinolones, Tetracyclines and Carbapenems. In the present study of antibiotic resistance pattern of *Acinetobacter*spp highest drug resistance was observed in Ceftazidime which was compatible with a study by Safari et al[14]respectively.

When we compared the antibiotic resistance pattern of aminoglycosides the resistance to Gentamicin and Amikacin were 52.2% and 87% in our study whereas in Thipperudraswamy et al[15] study the resistance to Gentamicin was 87.75% and the antibiotic resistance to Gentamicin and Amikacin in Ahir et al [1] study was 44.87% and 32.05% respectively. Possible reasons for the variety of antibiotic resistance rates in the different studies was not understood, but it may reflect the amount of antibiotics used in various settings.

Next we compared the antibiotic resistance pattern to quinolones.The resistance to Ciprofloxacin was 56.5%.

where as KalidasRit et al [16] showed 98% resistance.

Further, the overall resistance to Imipenem and Meropenem was 39.1% and 65.2%.whereas in a study by Gupta et al [17]Imipenem and Meropenem resistance was (37.6%) and 30% respectively and was observed more in ICU patients. Besides being resistant to Imipenem, the MBL producers were characteristically resistant to third generation cephalosporins and quinolones thus limiting the therapeutic options as Polymyxin only, which also must be used judiciously and not be used as monotherapy. [1] Emergence of MBL producing *Pseudomonas aeruginosa* and *Acinetobacter* spp. in the hospitals are alarming and reflect excessive use of Carbapenems and selective antibiotic pressure. Therefore, a strict antibiotic policy should be followed in every hospital to prevent further spread of MBLs.

In the present study MBL positive isolates of *Pseudomonas* spp. was (24.6%) *Acinetobacter* spp. showed (64%) respectively. A study by Hodiwala et al [18]showedMBL positive isolates of *Pseudomonas*spp 50 % and *Acinetobacter* spp56%. Ahir et al [1] study (11.42%), (10.40%) Irfan et al [5]100% and 96.6% KalidasRit et al [16] study 41% and 22% respectively. MBL positive isolates of *Acinetobacter* were more than *Pseudomonas spp.* which was compatible with Hodiwala et al [18] and Ahir et al [1] study.

MBL positive isolates were more in the age group 0-5 years (45.5%), 11-18 years (42.9%) and then >60 years (35.9%). In Bashir et al [11] study highest number of MBL positive isolates were above 60 years (51.59%) followed by 0-9 years (12.1%).[chart 1] No statistically significant association of a particular age group with MBL production was seen.

The highest MBL positive isolates were from Orthopaedic wards (52.6%) Surgical wards (31.3%) ICUs (27.3%), Medical wards (27.1%), TB wards (20.0%), and Paediatric wards (22.2%) OBGs (16.7%) in our study, suggesting hospital as a source of infection.The prevalence of MBL positive isolates by Bashir et al [11] was 23% in ICUs Thipperudraswamy et al [15] showed MBL positive isolates from surgery ward (30.15%), followed by Medicine (25.62%) and Orthopaedics (16.08%). It is apparent that various mechanisms exist for the production of multiple β lactamases especially in high pressure units like surgery, medicine and orthopaedics where newer β lactams are being routinely prescribed.

In the present study MBL positive isolates were seen in aspirated fluid (100%), pleural fluid (100%), followed by urine (47.1%) and in pus (37.5%) proves that inpatients are prone for multidrug resistance.

MBL positive isolates were more in urine samples followed by pus in our study. The more number of isolation in urine samples could be due to the use of indwelling urinary catheters and long term hospitalisation which are found to be the risk factors associated with isolation of multidrug resistant pathogens. Similar observation was found in Hirakataet al [19] study and in Bashir et al [11]where isolation of MBL positive isolates in urine was (27.3%) followed by pus (24.2%).

In a study by Ahiret al [1], Kaur et al [20] and in Thipperudraswamy et al [15] MBL was more in pus (59%). Such a findings is seen in the present study where in the rate of isolation of MBL was 37.5% in pus.

Pseudomonas aeruginosa and *A.baumannii* are the predominant nosocomial pathogens in many hospitals across the world. These are notorious nosocomial pathogens, which can survive in the hospital environment and are intrinsically resistant to many antimicrobials and commonly cause infections in the ICUs. [2]

In the present study 59 isolates showed MBL production of 200 multidrug resistant isolates. MBL detection was done by Imipenem / Imipenem+EDTA disc diffusion method and E strip using Meropenem / Meropenem+EDTA for Meropenem resistant samples. Both were equally sensitive for MBL detection. The number of positive isolates by IMP/IMP+EDTA disc diffusion method was 24 and by E strip was 58. Only 23 strains were positive by both the tests. In Chauhan et al study[7]among the Carbapenem resistant strains, 17 (29.31%) were positive for MBL production by EzysMBL strip and 18 (31.3%) by combined disc test. Only 8 were positive by both the tests.

With the Imipenem and EDTA combined disc test with a cut off >7mm, the positive and negative results were more clearly discriminated. [2] One of the major disadvantages of DDST was the subjective interpretation of result in some instances. We also found the MBL E test to be very sensitive for detection of MBL on *Pseudomonas aeruginosa*and *Acinetobacter spp***.** In a study byBashir et al [11] he found E strip and combined disk test were equally sensitive in detection of MBL by Anoar et al study [21]

showed E test (1.12%) as less sensitive test than by double disc synergy test (31.1%).

However given the cost constraints of E test, a simple screening method like combined Imipenem/Imipenem+EDTA method can be used. Disc containing 10µg of Imipenem or Meropenem are not suitable for screening all strains that produce MBL, because the MICs of Imipenem and Meropenem for several MBL producers are lower than 8µg/ml. Moreover, this phenotypic contradiction may also be due to the production of large amounts of ESBL / AmpC enzymes as well as bacterial membrane alterations. Thus, the results of susceptibility testing of IMP/MER-EDTA were not enough for identification of MBL producing strains of *Pseudomonas aeruginosa* since EDTA can give false positive results due to altered OprD levels [22].

As the standard guideline for MBL producers are not clearly defined, different workers have employed different methods of detection such as Modified Hodge test and spectrophotometrically. Some workers have used screening methods which utilize metal chelators such as EDTA. In the present study we screened MBL producers among MDRs which were screened by two methods, double disc synergy test and E strip method. These phenotypic tests can be used effectively in clinical settings lacking molecular biology tools until confirmation by a reference centre. Though it is desirable to detect the MBL producers at the earliest by routine laboratory testing, one must exercise care while interpreting phenotypic results based on inhibitor synergy. The phenotypic methods need to be correlated to the genetic markers of resistance. [1]

CONCLUSIONS: Emergence of MBL producing *Pseudomonas aeruginosa* and *Acinetobacter* spp in the hospital is alarming and reflect excessive use of Carbapenems and selective antibiotic pressure. Therefore, a strict antibiotic policy should be followed in every hospital to prevent further spread of MBLs. Clinicians should be made aware of the problem of MBLs, so that they can prescribe antibiotics judiciously. As most MBL producing organisms are multidrug resistant, this might pose a therapeutic challenge to clinicians as well as to microbiologists. Timely implementation of proper infection control practices reduce, eliminate and prevent establishment of antibiotic resistant organisms and prevent cross-contamination.

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