Original Research Article

A Study On Metallo-Beta Lactamase Producing Pseudomonas Aeruginosa Species In Clinical Isolates Of A Tertiary Care Hospital Of Bihar

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Abstract

Introduction: Metallo-beta lactamase production is the most common mechanism of carbapenem resistance. Metallo-beta lactamase is a zinc dependent enzyme belonging to Ambler class B that can hydrolyse all beta lactam antibiotics including carbapenem. Keeping it in mind we have conducted a research to find out MBL positivity rate in clinical isolates of Pseudomonas in a tertiary care hospital of Bihar. **Methodology:** This is a descriptive cross-sectional study conducted in the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Bhagalpur, Bihar, India over a period of 1 year from May 2020 to April 2021. Depending upon site of infection samples were collected like urine, pus, sputum, BAL, ear swab etc in sterile container. Carbapenem resistance was suspected when either imipenem or meropenem was resistant (mic>8 µl/ml).MBL production was tested in all carbapenem resistant Pseudomonas species by Combined disc test with imipenem and was confirmed by MBL- E test. **Results**: A total of 1357 various body fluids were obtained from various wards of the hospital over the study period, out of which, Pseudomonas species were isolated from 207 samples. Among 207 samples, 181 were P. aeruginosa and 26 were P. putida. Majority of the samples were obtained from OPD followed by IPD and ICU. MBL production (MBL +) was noted among 13.5% (28/207) of isolates and carbapenem resistance was observed among 14.9% (31/207). When both these characters were matched, it was noted that almost two-third (74.2%) of carbapenem resistance (CR) Pseudomonas were MBL positive. Both these characteristics were more common among p. putida as compared with P. aeruginosa. **Conclusion:** MBL producing Pseudomonas is difficult to treat but easy to prevent by proper hospital infection control measures and antibiotic policy.

Key Words: Metallo-Beta Lactamase, Pseudomonas aeruginosa Species

Introduction

Pseudomonas is the gram-negative bacilli that are strict aerobes, motile with one or two flagella, utilise glucose oxidatively, and are oxidase positive. It belongs to Pseudomonadaceae family and in molecular taxonomy to fluorescent group of r RNA group I[1]. Another member of this Fluorescent group are Pseudomonas fluorescence and Pseudomonas putida that are rarely involved in clinical diseases in human[2]. With the help of different virulent factors like pyocyanin, exotoxin A, exoenzyme S, protease, phospholipase, rhamnolipids they produce both community acquired infections like otitis externa, keratitis, varicose vein ulcer and hospital acquired infection like Catheter associated urinary tract infection (CAUTI), Ventilator associated pneumonia (VAP), burn infection, bedsore, septicaemia and necrotising pneumonia in cystic fibrosis patients etc[2]. They have highly evolved Quorum sensing mechanism by which they can easily form biofilm and prevent attack of antibiotic[2].

Most important factor that makes it so much dominant in hospital environment that it can resist or even can utilise some disinfectants/ antiseptics like cetrimide for their nutrition[1], so they easily grow in hospital environment and ICU. They are responsible for 10% of all hospital acquired infection[2]. Aminoglycoside (gentamicin, amikacin, tobramycin), antipseudomonal penicillin (piperacillin, ticarcillin) and cephalosporin like Ceftazidime are used to treat Pseudomonal infection but resistance against these antibiotics are common today[2].

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Beta lactamase destroy beta lactam ring of antibiotic and make them ineffective against Pseudomonas[3]. carbapenem is the drug of choice in extended spectrum beta lactamase producing Pseudomonas[3]. This was derived from thienamycin, a naturally derived product of Streptomyces cattleya[4]. Ertopenem, Doripenem, imipenem, Meropenem and Faropenem are example of carbapenem but imipenem and Meropenem are most commonly used carbapenem in India[4].With the progress of time irrational and inappropriate use of carbapenem led to emergence of carbapenem resistant Pseudomonas- first in Japan in 1991[5] and then in different part of the world. In India first case of MBL producing Pseudomonas was reported in 2002[6, 7]. Mechanism of carbapenem resistance is mainly three types, first due to increase expression of porin in cell wall, second due to increase activity of efflux pump and third- production of metallo-beta lactamase[7]. Metallo-beta lactamase production is the most common mechanism of carbapenem resistance[7]. Metallo-beta lactamase is a zinc dependent enzyme belonging to Ambler class B that can hydrolyse all beta lactam antibiotics including carbapenem[8]. Ambler class A, C, D beta lactamases use serine as active site so they can be easily degraded by beta lactamase inhibitor like clavulanic acid or sulbactam8. But metallo-beta lactamase cannot be inhibited by clavulanic acid or sulbactum so MBL producing Pseudomonas is now emerging as a nightmare for treating physician. Besides that, resistance determinant of MBL is located in highly mobile genetic element allowing easy dissemination from patient to patient or even from patient to health care providers[2]. So, prevention is the always better option than treatment of MBL Pseudomonas infection. Clinician in every hospital should know the local prevalence of MBL producing Pseudomonas to formulate proper antibiotic policy and hospital infection control strategy to prevent outbreak of this dangerous superbug.

Keeping it in mind we have conducted a research to find out MBL positivity rate in clinical isolates of Pseudomonas in a tertiary care hospital of Bihar.

Methodology

This is a descriptive cross-sectional study conducted in the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Bhagalpur, Bihar, India over a period of 1 year from May 2020 to April 2021. Depending upon site of infection samples were collected like urine, pus, sputum, BAL, ear swab etc in sterile container.

Sample Processing

All samples were inoculated immediately into Blood agar and MacConkey agar media (HiMedia, Mumbai) and incubated for 18-24 hrs. At 37°C in incubator. Next day growth was observed, and gram stain was performed. All the positive growth which was oxidase positive selected and put in Vitek[2] identification system (Biomerieux). Identification and antibiogram of oxidase positive growth was done in fully automated Vitek[2]. Carbapenem resistance was suspected when either imipenem or meropenem was resistant (mic>8 µl/ml).MBL production was tested in all carbapenem resistant Pseudomonas species by Combined disc test with imipenem and was confirmed by MBL- E test.

Combined Disc Test[5]

Two 10 µg IPM disks were put on the MHA plate seeded with the test organism. 10 µL of EDTA solution (750 µg) was added to one of them. The plate was incubated for 16-18 hrs at 35°C. If the increase in inhibition zone with the IPM + EDTA disk was >7 mm than the IPM disk alone, it was suspected as MBL positive.

MBL Epsilometer Test (E-Test)[5]

The MBL E-strip with seven-dilution range of IPM (4-256 µg/mL) in one side and IPM plus EDTA (1-64 µg/mL) on another side was put on MHA plate seeded with test organism. The plate was then incubated in incubator at 35°C for 18-20 hrs. MIC ratio of IPM/IPM + EDTA of >8, or reduction of IPM MIC by >3log2 dilutions in the presence of EDTA confirmed MBL production.

Results

A total of 1357 various body fluids were obtained from various wards of the hospital over the study period, out of which, Pseudomonas species were isolated from 207 samples. Among which 172 were from urine, 21 were from pus and rest 14 were from another sites like sputum, ear swab, bronchoalveolar lavage (BAL), endotracheal tube (ET) aspirate etc. Among 207 samples, 181 were P. aeruginosa and 26 were P. putida. Majority of the samples were obtained from OPD followed by IPD and ICU.

MBL production (MBL +) was noted among 13.5% (28/207) of isolates and carbapenem resistance was observed among 14.9% (31/207). When both these characters were matched, it was noted that almost two-third (74.2%) of carbapenem resistance (CR) Pseudomonas were MBL positive. Both these characteristics were more common among p. putida as compared with P. aeruginosa. [Figure 1] Both MBL producing strains were more common among males than females (19:9). Majority of the MBL positive organism were isolated from the patients with age group of 40-60 years. Various factors affecting CR and MBL + has been shown in table 1 and 2. Antibiogram of MBL positive and MBL negative Pseudomonas species was compared in table 3.

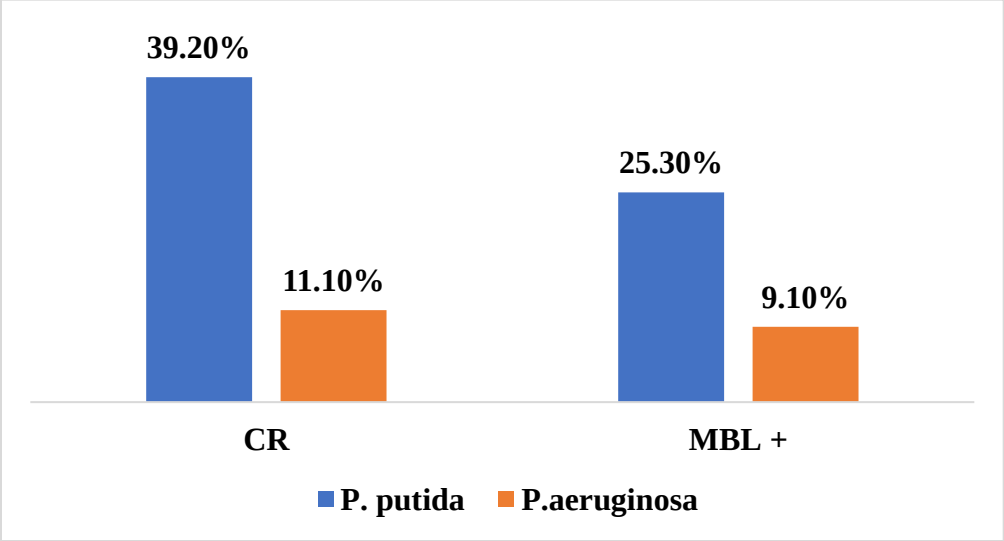

Figure 1: Carbapenem resistance and MBL Positivity Rate in both the species of Pseudomonas

Table 1: Sample wise and ward wise distribution of carbepenam resistance

Factor	Classification	Carbepenam resistance	MBL +
Type of sample	Urine	9.3%	9.1%
	Pus	34.2%	27.3%
	Others	8.9%	1.1%
Point of collection	OPD	11.1%	7.5%
	IPD	13.4%	11.2%
	ICU	24.5%	17.3%

Table 2: Antibiogram showing sensitivity of both the species to various antibiotics

Antibiotic	Sensitivity of MBL + isolates (%)	Sensitivity of MBL – isolates (%)
Ampicillin	7.2	42.1
Amoxicillin-clavulinic acid	20.2	67.5
Piperacillin-Tazobactum	40.2	91.4
Cefuroxime	2.1	44.7

Ceftazidime	20.2	67.5
Cefoperazone-sulbactum	40.2	91.4
Cefepime	2.1	66.9
Amikacin	22.1	100
Gentamycin	20.2	82.1
Ciprofloxacin	27.8	67.5
Tigecycline	12.5	82.1
Nitrofurantoin	15.4	75
Colistin	100	100
Trimethoprim/Sulfamethoxazole	20.2	44.7

Discussion

Pseudomonas has the ability to grow and multiply in moist environment and equipment including sinks, drain flower vas, hydrotherapy pools, ponds, river and even in distilled water[2]. In hospital setting it can grow in many disinfectants and pharmaceutical product posing serious problem in infection control[2]. Blue green colour pyocyanin produced by P. aeruginosa and pyoverdine produced by another Pseudomonas act as virulent factor. With the help of metallobeta lactamase enzyme they become antibiotic resistant[9]. Lots of phenotypic tests are available for detection of metallobeta lactamase production in Pseudomonas. Principal of all those test is the ability of metal ion chelator like EDTA or thiol compound to inhibit activity of MBL[10]. Among all these tests CDT showed good sensitivity (79%) than other tests like DDST (70.8%) and Disc potentiation test (54.2%) in a study done by Ranjan et al[5].

Another studies like Samuelson et al[11], Qu et al[12], Biradar et al[13] also had reported that CDT was better than all other tests. It was easy to perform and cheaper and having objective interpretation[5]. Rit et al[14] in Kolkata had reported that CDT was having same accuracy with MIC detection. So we had selected CDT as screening test for MBL detection in our study. CDT could be performed using ceftazidime or imipenem but as Pseudomonas might have other resistance mechanism other than MBL production to ceftazidime[15] we had used imipenem for CDT. For confirmation, PCR analysis of MBL gene was the gold standard but it was not feasible in routine microbiology laboratory in a developing country like India[5]. In contrast, MBL E test showed good specificity (98%) in study by Khosravi et al[16], Walsh et al[17] and Segal et al[18]. So, we had selected MBL E test as the confirmatory test for MBL detection in our study.

In our study among 207 isolated Pseudomonas strains, 14.9% were carbapenem resistant which was slight higher than Kanungo et al[19] (10.9%) but lower than many others[3, 5, 9, 10, 13, 20]. Implementation of strict antibiotic policy in our hospital might be responsible for such low level of carbapenemresistance in our study. Among carbapenem resistantPseudomonas, 74.2% were MBL producing in our study which was almost same as that of study done by Biradar et al[13] (74%) but was lower than some others[10, 21-24]. In our study MBL positivity rate was 13.5%. This is lower than many previous researchers[3, 8-10, 12-16, 20, 30-36]. Variation in MBL positivity rate in different studies might be due to several factors like Geographical area, Infection control attitude of the hospital, sample size and and method of testing5. But overall in our hospital MBL positivity rate was lower than most of the studies in India and it should be maintained in future by strict hospital infection control and antibiotic policy. Slight male preponderance was seen in in MBL positive Pseudomonal infection, but it was not significant. There are studies reporting male preponderance[5, 9, 13] and at the same time some have reported female dominance[37]. Highest no of MBL positive isolates came from older age group (>40) in our study. Choudhary et al[9] and Biradar et al[13] had reported middle age group (31-60) was the most commonly affected age group. Long hospital stay, frequent hospital admission due to age related problems and relative immunocompromised status of older age group[5] might be responsible for higher MBL positivity rate in older age group in our study.

MBL positivity in Pseudomonas was highest in pus isolates followed by urine that is similar to others[3, 9, 10, 13, 21]. Wound easily comes in contact of hospital environment leading to easy colonisation compared to bladder that requires catheter manipulation to get infected by Pseudomonas. MBL positivity of Pseudomonas in our study was highest in ICU compared to IPD and OPD. It was consistent with the study by Kaur et al[8] but not with the study done by Easwaran et al[4] where MBL positivity was highest in IPD followed by ICU. More number of invasive interventions prolong stay in ICU, serious nature of the disease in ICU patients8 all might be responsible for high MBL positivity rate in ICU. Most of the study done in India about MBL production was in Pseudomonas aeruginosa which was the most common species of Pseudomonas. As per our literature search no data was available about MBL positivity in Pseudomonas putida in India. MBL positivity was higher in P. putida than P. aeruginosa in our study. It was in consistent with a spanish study[38] where 14% of P. putida and 0.3% of P. aeruginosa was MBL positive. P putida acts as environmental reservoir of MBL resistance gene and acts as a donor of this gene to P. aeruginosa.

In antibiotic sensitivity test, MBL positive Pseudomonas species showed poor sensitivity against most of the antibiotics like ampicillin, amoxiclav, Cefuroxime, Ceftazidime, Cefoperazone sulbactum, amikacin, gentamicin, ciprofloxacin, cotrimoxazole, Nitrofurantoin compared to MBL negative strains. This finding was consistent with another studies[9, 10, 13]. In every hospital MBL positivity in Pseudomonas should be checked by Microbiologist as routine laboratory practice and local prevalence of that superbug should be kept in mind during hospital infection control policy making to prevent outbreak of this highly communicable resistance determinant.

Conclusion

MBL producing Pseudomonas is difficult to treat but easy to prevent by proper hospital infection control measures and antibiotic policy. In our study MBL positivity rate (12.5%) in Pseudomonas was lower when compared to most of the similar studies in India. MBL prevalence in Pseudomonas putida was higher than Pseudomonas aeruginosa. Colistin was the only antibiotic with good sensitivity against this dangerous superbug.

Conflict of interest

None declared by any of the authors

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