Study on Metallo-Beta Lactamase Producing *Pseudomonas* Species in Clinical Isolates of a Tertiary Care Hospital of Western Odisha

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ABSTRACT

BACKGROUND

Pseudomonas species are responsible for 10% of hospital acquired infection especially in an ICU set up and in burn patients. Metallo-beta lactamase production is the most common mechanism of resistance to carbapenem which is the most commonly used drug to treat *Pseudomonas*. Local prevalence of MBL producing *Pseudomonas* is important information to both microbiologist and clinician to formulate hospital infection control strategy. This cross-sectional descriptive study was conducted in a tertiary care hospital of western Odisha to detect MBL prevalence among clinical isolates of *Pseudomonas* species.

METHODS

187 *Pseudomonas* strains (165 *P. aeruginosa* and 22 *P. putida*) isolated in different clinical samples in Vitek 2 system were checked for imipenem resistance (MIC>8 µl/ml). All imipenem resistance strains were checked for MBL production by combined disc test with imipenem, and MBL production was confirmed by MBL E test.

RESULTS

Among 187 *Pseudomonas* strains 12.20% were carbapenem resistant and 9% were MBL producing. About 74% of carbapenem resistant *Pseudomonas* strains were MBL positive. MBL positivity rate was much higher in *Pseudomonas putida* (27.20%) compared to *Pseudomonas aeruginosa* (7%) and in ICU (14.20%) compared to IPD (9.20%) or OPD (6.80%). Colistin was the most effective (97%) antibiotic against MBL producing *Pseudomonas*.

CONCLUSIONS

It is better to prevent MBL *Pseudomonas* than to cure it as most of the antibiotics were found to be ineffective against it. In our study MBL production rate in clinical isolate of *Pseudomonas* was low (9%) compared to other studies in India.

KEY WORDS

Pseudomonas, Carbapenem, MBL, Positivity.

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DOI: 10.14260/jemds/2020/335

Financial or Other Competing Interests: None.

How to Cite This Article:
Mukherjee S, Mishra S, Tiwari S. Study on metallo-beta lactamase producing Pseudomonas species in clinical isolates of a tertiary care hospital of Western Odisha. J. Evolution Med. Dent. Sci. 2020;9(19): 1533-1538, DOI: 10.14260/jemds/2020/335

Submission 11-03-2020,
Peer Review 24-04-2020,
Acceptance 30-04-2020,
Published 11-05-2020.

BACKGROUND

"In Wine there is Truth, in Beer there is Strength, in Water there are *Pseudomonas*." (adaption of German proverb) *Pseudomonas* are 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 members 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]

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 are 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 sulbactam[8]. 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 Western Odisha.

METHODS

This is a descriptive cross-sectional study conducted in the Department of Microbiology, of Hitech Medical College, Rourkela, Odisha, over a period of 1 year (March 2019 to Feb. 2020). 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

Total 187 isolates of *Pseudomonas* species were isolated. A mong which 149 were from urine and 20 were from pus. 13 were from another sites like sputum, ear swab, bronchoalveolar lavage (BAL), endotracheal tube (ET) aspirate etc.

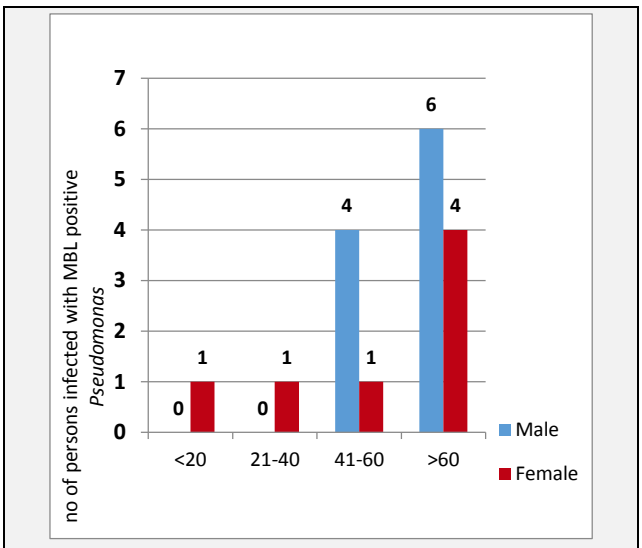

Figure 1. Age and Sex Distribution of MBL Positive Pseudomonas Species

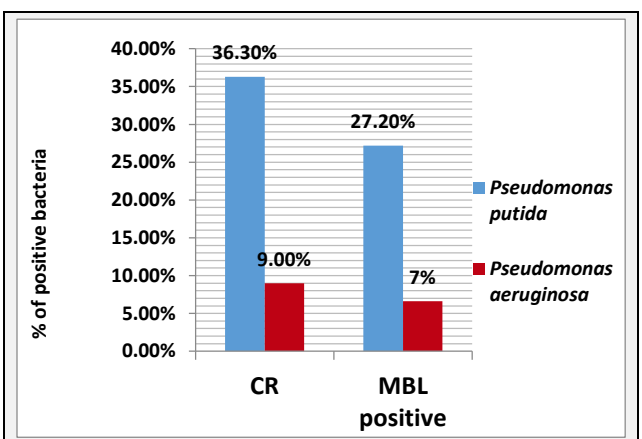

Figure 2. Carbapenem and MBL Positivity Rate in Different Species of Pseudomonas

Among 187 samples 165 were *P. aeruginosa* and 87 were *P. putida*. 87 *Pseudomonas* strains were isolated from OPD, 65 were from IPD and 35 were from ICU. Carbapenem resistance rate was 12.2% and MBL positivity rate was 9%.74% of carbapenem resistance *Pseudomonas* was MBL positive. Among 17 MBL producing strains 10 were from male patients and 7 were from female patients. Age and sex distribution of MBL positive patients was showed in Fig 1. Carbapenem resistance and MBL positivity rate of two species of *Pseudomonas* was showed in Fig 2. Sample wise CR and MBL positivity rate was depicted in Fig 3. Ward wise CR and MBL positivity rate in *Pseudomonas* was showed in Fig 4. Antibiogram of MBL positive and MBL negative *Pseudomonas* species was compared in Table 1.

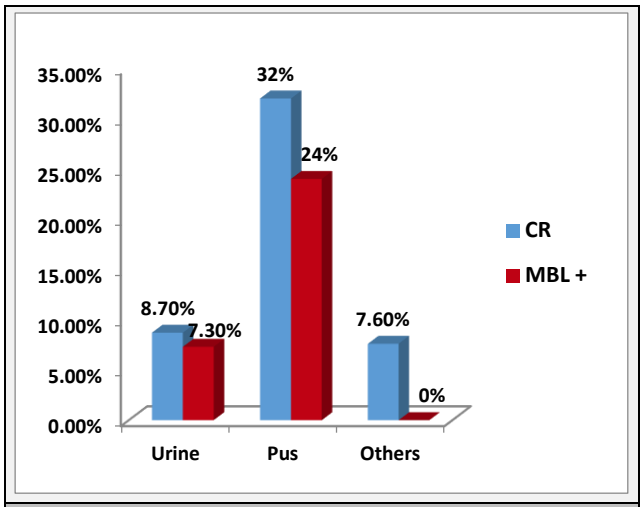

Figure 3. Sample Wise carbapenem and MBL Positivity Rate in Pseudomonas

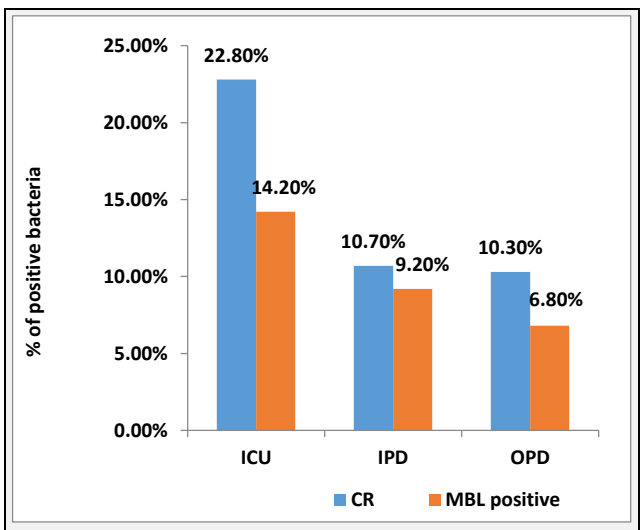

Figure 4. Ward Wise carbapenem and MBL Positivity Rate in Pseudomonas

Antibiotics	Sensitivity Shown by MBL Positive *Pseudomonas* spp. (%)	Sensitivity Shown by MBL Negative *Pseudomonas* spp. (%)
Ampicillin	10.4	40.29
Amoxycillin-clavulanic acid	18.10	66.76
Piperacillin-Tazobactam	42.12	90
Cefuroxime	2.3	40.78
Ceftazidime	20	66.7
Cefoperazone sulbactam	40	88.82
Cefepime	2.21	67.29
Amikacin	25.10	93.64
Gentamicin	21.21	83.64
Ciprofloxacin	26.84	66.29
Tigecycline	12.52	80.64
Nitrofurantoin (urine)	15.57	75.29
Colistin	97	98.11
Trimethoprim/Sulfamethoxazole	22.67	44.82

Table 1. Antibiotic Sensitivity Pattern of Pseudomonas Species

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 metallo-beta lactamase enzyme they become antibiotic resistant[9]. Lots of phenotypic tests are available for detection of metallo-beta 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] These tests include Combined disc test (CDT) using EDTA with imipenem or ceftazidime, Double disc synergy test (DDST), EDTA disc potentiation test (PT) using ceftazidime or cefotaxime, Modified Hodge test, Carba NP test, Modified carbapenem inactivation method (mCIM) etc.[5] These all tests are used for screening purpose. 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 187 isolated *Pseudomonas* strains, 12,20% were carbapenem resistant which was almost similar with study done by Kanungo et al[19] (10.9%) but was lower than study done by Rajput et al[3](17.2%), Ranjan et al[5] (21.3%), Choudhary et al[9] (33.88%), Mishra et al[10] (58%), Biradar et al[13] (32%) and Varaiya et al[20] (26%). Implementation of strict antibiotic policy in our hospital might be responsible for such low level of carbapenem resistance in our study. Among carbapenem resistant *Pseudomonas*, 73.9% 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 Mishra et al[10] (100%),Chand et al[21] (94.52%), Attal et al[22] (88.89%), Fam et al[23] (87.5%) and Irfan et al[24] (100%).In our study MBL positivity rate was 9% which was lower than Rajput et al[3] (12%), Kaur et al[8] (21.8%), Choudhary et al[9] (20%), Mishra et al[10] (58%), Qu et al[12] (9.1%), Biradar study[13] (25%), Rit et al[14] (41%), Behera et al[15] (39.56%), Khosravi et al[16](19.5%), Varaiya et al[20] (14.3%), Navneet et al[25] (12%), Hemlata et al[26] (14%), Castanheira et al[27] (34%), Owlia et al[28] (19.7%), Manoharan et al[29] (42.6%), Kumar et al[30] (26.9%) and Kali et al[31] (22.4%). Lower MBL positivity rate than our study (9%) were reported by few studies like Mandiratta et al[32] (8.2%), Agarwal et al[33] (8.05%) Pitout et al[34] (7.65%), Ibukin et al[35] (4.12%) and Choudhary et al[36] (6.12%). 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 testing[5]. 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. Similar Male preponderance was reported by Ranjan et al,[5] Choudhary et al.[9] and Biradar et al[13] but in a study in Nepal[37] slight female preponderance was noted. Highest no of MBL positive isolates (10 out of 17) came from older age group (>60) in our study. In contrast, 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 (24%) followed by urine isolates (7.3%).Rajput et al[3], Choudhary et al,[9] Mishra et al al,[10] Biradar et al[13] and Chand et al[21] had reported similar finding. 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 (14.20%) compared to IPD (9.20%) and OPD (6.80%).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 patients[8] all might be responsible for high MBL positivity rate in ICU compared to IPD and OPD in our study. 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. We had found that 27.20% of *P. putida* and 7% of *P. aeruginosa* strains were MBL positive. MBL positivity was about 4 times 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*.[38]

In antibiotic sensitivity test, MBL positive *Pseudomonas* species showed poor sensitivity against most of the antibiotics like ampicillin (10.4%), amoxiclav (18.10%), Cefuroxime (2.3%), Ceftazidime (20%), Cefoperazone sulbactum (40%), amikacin (25.10%) gentamicin (21.21%) ciprofloxacin (26.84%), cotrimoxazole (22.67%), Nitrofurantoin (15.57%) etc compared to MBL negative strains. This finding was consistent with another studies like Choudhary et al,[9] Mishra et al[10] and Biradar et al.[13] However, in our study sensitivity towards piperacillin tazobactam in MBL positive strains was 42.12% that was almost same with the study done by Mishra et al[10] (48.42%), Biradar et al[13] (38%) and Chand et al[21] (47.80%) and was higher than Choudhary et al[9] (19.5%). In our study colistin resistance was seen in 3% of MBL positive strains like study by Choudhary et al[9] (2.7%) but not like Mishra etal[10] where very high colistin resistance (58.95%) was reported in capital city of Odisha. In contrast, Biradar et al[13] from Kashmir had reported that 100% sensitivity to colistin in MBL positive

Pseudomonas species. Although colistin is the last resort against MBL positive *Pseudomonas* it cannot be used randomly due nephrotoxic side effect.[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.

CONCLUSIONS

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 (9%) in *Pseudomonas* was lower when compared to most of the similar studies in India. MBL prevalence in *Pseudomonas putida* (27.2%) was four times higher than *Pseudomonas aeruginosa* (7%). Colistin was the only antibiotic with good sensitivity (97%) against this dangerous superbug.

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