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Evaluation of three Different Phenotypic Tests for Detection of Metallo beta Lactamase in Imipenem Resistant Pseudomonas Aeruginosa from Clinical Samples

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Abstract

Background: Pseudomonas aeruginosa is one of the most important opportunistic pathogens that has been associated with community and hospital-acquired infections. Carbapenem-resistant Pseudomonas aeruginosa is one of the major concerns in clinical settings impelling a great challenge to antimicrobial therapy for patients with infections caused by the pathogen. The aim of this study was to detect the metallo-β-lactamase (MBL) production in Imipenem resistant P. aeruginosa collected from various clinical samples. **Method:** Pseudomonas aeruginosa strains isolated from various clinical specimens at a tertiary care hospital in Bhopal. A total of 225 non-repetitive isolates of P. aeruginosa recovered from various clinical samples were screened for MBL production by Three phenotypic tests such as Modified hodge test, IPM EDTA combined disc test and E test. **Results:** Of the 255 Pseudomonas aeruginosa isolates obtained during the study period, 98 (38.43%) were resistant to imipenem. Out of 98 Imipenem resistant, 26(26.53%) were positive for MBL by CDT-IPM method and E test while 20(20.40%) were positive for MBL by Modified hodge test. maximum MBL producers were obtained from pus (12.24%) followed by urine (8.16%). 100% sensitivity to Colistin and Polymixin B was observed in Pseudomonas aeruginosa isolates. **Conclusion:** In present study IPM EDTA Combined Disc Test and E test (Sensitivity =100%, specificity=100%) was found to be a better method compared to Modified Hodge. IPM EDTA Combined disc test and E test is simple to perfom and interpret. It is performed as routine antimicrobial susceptibility method as it can be easily introduced into the workflow of a clinical laboratory.

Keywords: Metallo-beta-lactamase, P.aeruginosa, Imipenem resistance.

Introduction

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen responsible for a wide range of nosocomial infections, including surgical-site infections, septicaemia, urinary tract infections and lower respiratory tract infections.[1,2] Carbapenems, including imipenem and meropenem, are the most potent antibacterial agents used for the treatment of infections initiated by multidrug-resistant gram-negative bacilli.[3] carbapenem Resistance in P. aeruginosa is due to decreased outer membrane permeability, increased efflux system, alteration of penicillin-binding protein and carbapenem-hydrolyzing enzymes carbapenemases.[4,5] However, the clinical utility of these antimicrobials is under threat with the emergence of carbapenemases, particularly metallo-β-lactamases (MBLs). MBLs belong to Ambler class B and have the ability to hydrolyze a wide

variety of β-lactam agents, such as penicillins, cephalosporins, and carbapenems.[6]

MBLs producing Pseudomonas aeruginosa was first reported from Japan in 1991 and since then has been described from various parts of the world, including Asia, Europe, Australia, South America, and North America.[6]

MBLs in Carbapenem-resistant Pseudomonas aeruginosa can be detected by different phenotypic methods and these methods are based on the ability of metal chelators to inhibit the activity of MBLs such as EDTA and thiol-based compounds. These include Combined Disk Test (CDT) using EDTA with imipenem (IPM), Modified Hodge test(MHT) and MBL Epsilometer test (E-test).[7] The aim of this study was to detect Metallo β Lactamase in Imipenem resistant Pseudomonas aeruginosa from clinical samples, compare and to evaluate the accuracy of three phenotypic test currently in use and determine antibiograms to guide clinicians in prescribing proper antibiotic and controlling hospital infection.

Materials and Methods

Study Type & Place: It is a prospective study carried out in department of Microbiology, RKDF Medical college, hospital & research centre, SRK University, Bhopal M.P over a period of 2 years.

Inclusion criteria: All samples received are processed which includes both icu & admitted patients.

Exclusion criteria: Outdoor patients & less than 48 hrs admitted patients.

Sample Collection: A total of 255 non duplicate Pseudomonas aeruginosa isolates were obtained from various clinical samples such as pus,urine,sputum,ET aspirates and blood of admitted patients.

Bacterial Isolates: A total 255 cases from which Pseudomonas aeruginosa has been isolated. Identification of Pseudomonas aeruginosa was done by conventional methods like, colony morphology on Blood agar and Mac Conkey'agar, pigment production, oxidase test, sugar fermentation, TSI reaction, IMViC reactions, and urease test8.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was done in Mueller Hinton agar by Kirby Bauer disc diffusion method and the result was interpreted as per the 2021 CLSI 9guidelines.The following antibiotics were tested by disc diffusion method, Imipenem (10ug), Meropenem (10ug), Ceftazidime (30ug), Cefepime (30ug), Piperacillin (100ug), Piperacillin/ Tazobactum (100ug), Gentamicin (10ug), Tobramicin (10ug), Amikacin (10ug), Levofloxacin (5ug), Ciprofloxacin (5ug), Azteronam (0ug), Colistin (10ug) and Polymixin B (10ug).[10]

MBL Screening: The following methods used for the screening of MBL producing Imipenem resistance P.aeruginosa isolates.

Figure 1: Modified Hodge Test

Modified Hodge Test: A lawn culture of 1:10 dilution of 0.5 McFarland's standard Escherichia coli ATCC 25922 was done on a Muller Hinton (MH) agar. A 10 µg imipenem disc (Hi media) was placed in the center of the plate. Imipenem resistant P. aeruginosa (test isolates) were streaked from the edge of the disc to the periphery of the plate in four different directions. After

overnight incubation, the plates were observed for the presence of a "clover leaf" shaped zone of inhibition which was interpreted as MHT positive.[4,10,11]

Imipenem EDTA Combined disc test: This method was performed according to the description byYong et al. two imipenem discs one with 0.5 M EDTA and the other plain were placed on the surface of lawn culture of isolate with discs being 30mm apart. The plates were incubated overnight at 370C. An increase in the zone diameter of ≥ 7mm around imipenem+ EDTA disc in comparison to imipenem disc alone indicated production of MBL.[12,13]

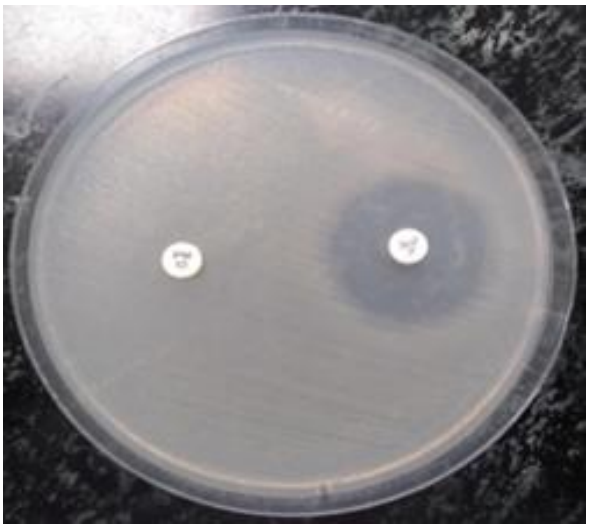


Figure 2: Imipenem-EDTA combined disc test

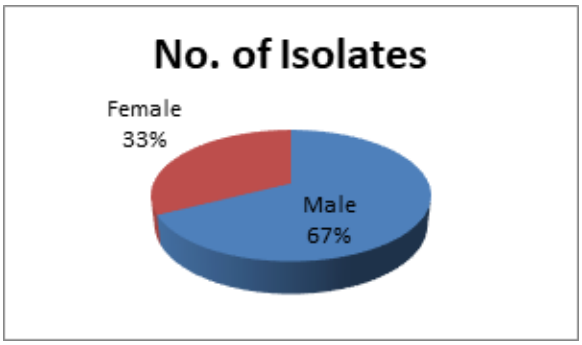
Figure 3: Epsilometer Test

MBL Epsilometer Test (E test): E-test MBL strips consist of a double-sided seven-dilution range of Imipenem IP (4–256 µg/mL) and IP (1–64 µg/mL) overlaid with a constant 36 gradient EDTA. Individual colonies were picked from overnight agar plates and suspended in 0.85% saline to a turbidity of 0.5 Mc Farmland's. A sterile cotton swab was dipped into the inoculum suspension, and a lawn culture of the inoculum was performed on an MHA plate. Excess moisture was allowed to be absorbed for approximately 15 minutes before the E-test MBL strip was applied. The plates were incubated for 16–18 h at 37°C. The MIC endpoints were read where the inhibition ellipses intersected the strip. A reduction of imipenem MIC=3 two-fold in the presence of EDTA was interpreted as being suggestive of MBL production.[14]

Results

A total of 255 isolates of P.aeruginosa were collected from various clinical specimens over a period of 2 years. Of the 255 Pseudomonas aeruginosa isolates, 98 were Imipenem resistant, of which 26 were MBL producers, and the remaining non-MBL producers.

114(44.70%) P.aeruginosa isolates were obtained from ICU Patients whereas maximum number of P. aeruginosa isolates 141(55.29%) were obtained from IPD patients [Table 1].



Graph 1: Gender Wise distribution

[Graph 1] shows the gender wise distribution of patients. (172)67% of the total Pseudomonas

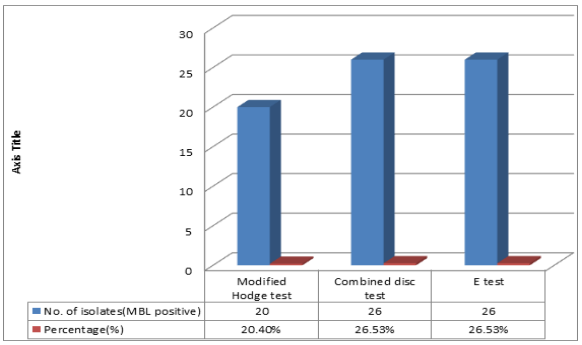
aeruginosa isolates were obtained from male patients and 83(33%) from female patients.

[Table 2] Shows the age wise distribution of patients. The isolates were obtained from patients from various age groups, ranging from 1 to 80 years. Maximum number of isolates (87) were from the age group of 21-30years followed by 31-40 age group (70) and least number of isolates (04) from 71-80 years. No isolates were found in patients aged more than 80 years.

Out of the 98/255(38.43%)Imipenem resistant isolates were obtained in which maximum number of Imipenem resistant P.aeruginosa isolates 43(16.86%) were obtained from Pus sample followed by 23(9.01%) from urine sample and least number of Imipenem resistant isolates were obtained from Blood sample 02(0.78%) respectively [Table 3].

Of the 255 Pseudomonas aeruginosa isolates, 98 were Imipenem-resistant, of which 26(26.53%) were MBL producers.

In the present study 26 MBL were isolated from 98 Imipenem-resistant isolates; therefore, the percentage of MBL in Imipenem-resistant isolates was 26.53%. Maximum MBL-producing isolates were obtained from Pus sample 12(12.24%) followed by urine 08 (8.16%). No MBL were obtained from Blood sample. [Table 4]



Graph 2: Comparison of MBL detection by 3 different phenotypic methods

All Imipenem-resistant isolates were screened for MBL production using three different phenotypic tests such as Modified Hodge test, Combined disc test and MBL E test. 26 isolates that showed positive for MBL production in both Combined Disc test and E test. whereas 20 isolates were positive for MBL production by Modified Hodge test. [Graph 2]

[Table 5] Shows the antibiotic resistant profile of Pseudomonas aeruginosa isolates. Of the 255 isolates of P.aeruginosa, 98(38.43%) were highly resistance to Imipenem followed by Tobramicin 89(34.90%), Amikacin 82(32.15%), Ceftazidime 86(33.72%), Ciprofloxacin 79(30.98%), whereas 100% sensitivity was found towards Polymixin B and colistin.

Table 1: Ward wise distribution of Isolates

Ward name	No. of Isolates	Percentage %
ICU	114	44.70
IPD	141	55.29

Table 2: Age wise distribution of P.aeruginosa isolates

Age group	Isolates no.	Percentage (%)
1-10	06	2.35%
11-20	15	5.88%
21-30	87	34.11%
31-40	70	27.45%
41-50	36	14.11%
51-60	28	10.98%
61-70	09	3.52%
71-80	04	1.56%
≥80	00	00%
Total	255	

Table 3: Sample Wise distribution of isolates

Specimen	Total no.& % of Isolates	Total no.&% of Imipenem resistant isolates
Pus	106(41.56%)	43(16.86%)
Urine	69(27.05%)	23(9.01%)
Sputum	41(16.07%)	08(3.13%)
ET aspirates	27(10.58%)	19(7.45%)
Blood	04(1.56%)	02(0.78%)
Others	08(3.13%)	03(1.17%)
Total	255(100%)	98(38.43%)

Table 4: Distribution of MBL among Imipenem resistant P.aeruginosa in various clinical samples.

Name of samples	No. of MBL producing isolates	Percentage(%)
Pus	12	12.24%
Urine	08	8.16%
Sputum	01	1.02%
ET aspirates	04	4.08%
Blood	00	00%
Others	01	1.02%
Total	26	26.53%

Table 5: Antibiotic Resistant Pattern of P.aeruginosa

Name of Antibiotic	Resistance	Percentage(%)
Imipenem(10ug)	98	38.43%
Ceftazidime(30ug)	86	33.72%
Cefepime(30ug)	74	29.01%
Piperacillin(100ug)	65	25.49%
Piperacillin/Tazobactum(100ug)	71	27.84%
Gentamicin(10ug)	73	28.62%
Tobramycin(10ug)	89	34.90%
Amikacin(30ug)	82	32.15%
Ciprofloxacin(5ug)	79	30.98%
Levofloxacin(5ug)	66	25.88%
Polymixin B(10ug)	00	00%
Colistin(10ug)	00	00%

Discussion

Pseudomonas aeruginosa is a leading cause of nosocomial infections. For treatment of these infections, carbapenems, especially imipenem, are used. However, the prevalence of imipenem resistance to P. aeruginosa has been increasing worldwide.[15] Carbapenem-resistant Pseudomonas aeruginosa is listed as an organism posing a serious threat by the Centers for Disease Control and Prevention. In India, up to 40% P. aeruginosa isolates are shown to be carbapenem resistant.[16] In India first case of MBL producing Pseudomonas was reported in 2002.[17] Metallo-beta lactamases comprise a group of beta-lactamases that are highly active hydrolysers of carbapenems, and have potent but variable enzymic activity against other beta lactam antibiotics, except the monobactams.[18]

In the present study maximum isolates were obtained from IPD (55.29%) then ICU patients (44.70%). Similarly, in a study by Viswamohanan et al,[19] 2019 and Kothari et al 2022,[20] majority of the isolates were obtained from the wards patients (49%) and

(64.5%).whereas in contrast to this, in a study by Gupta, et al,[21] 2016 and Mahesh C. Sahu et al.2012,[22] who had reported that highest isolation rate of P.aeruginosa from ICU samples to be (28%) and (20.38%) respectively.

In our study, the rate of isolation was more common in men 172(67%) than women 83(33%), A similar finding was reported by Senthamarai S. et al 2014,[24] from tertiary care hospital at Kanchipuram, Tamil nadu, India, which showed maximum isolates (55.76%) in male then (44.23%) in female.

One other study conducted by Bindu & Saikumar from a Tertiary Hospital in Chennai, reported maximum isolates from male (54.05%) then female(45.9%) patients.[24]

The number of P. aeruginosa isolates was bigger in the age group of 21-60 years, whereas minimum isolates were obtained from age group of 71-80 years in our study. A similar observation was made by Ranjan et al 2010,[25] from Haryana, Javiya et al 2008,[26] from Gujarat and Radhika et al 2022,[14] from Bibinagar, India,in which showed that maximun samples were isolated in the age group of 21-60 years.

In this study, out of the 98/255 Imipenem resistant isolates were obtained in which maximum number of isolates 43(43.87%) were found from Pus sample respectively.

In the present study, the prevalence of MBL in imipenem-resistant isolates was 26.53%. These findings differ from those reported by Behera from AIIMS, New Delhi,[27] who showed that MBL production in Imipenem-resistant isolates was 64.28%, and Nadya Ameen et al.[28] from Pakistan, who showed that (64.9%) Imipenem-resistant isolates produced MBL.

In the present study, MBL producers were mostly found in Pus (12.24%), followed by urine (8.16%), sputum (1.02%), ET (4.08%), Blood (00%) and other (1.02%). which was in accordance to the findings of Shanthi et al 2009,[29] from Chennai, who showed 41.8% of MBL producers, followed by urinary tract (25.5%), wound swab (20%), and blood (12.7%). This study differs from that conducted by Basak et al 2010,[30] from JNMC, Wardha, which showed that among MBL producers, wound swabs accounted for 43.7%, followed by urine (37.5%), sputum (6.2%), endotracheal tube secretions (6.2%) and body fluids (6.2%).

MBLs are sensitive to metal chelators like EDTA and thiol based compounds and these inhibitors are exploited to detect MBL activities of the organisms. Currently, there is no recommended method for the detection of MBL in routine laboratory practice. E-test is presently the most widely accepted standardized screening test for the detection of MBL.[31]

In the present study, all Imipenem-resistant isolates were screened for MBL production using the Modified Hodge test, IPM EDTA combined disk test and E-test. MBL production in CDT and E-test was same (26.53%), whereas in MHT (20.40%) respectively. CDT with Imipenem and EDTA with a cut-off > 7 mm positive and negative results were clearly distinguished.

In this study, for detection of MBL, CDT and E test was a more sensitive method, Similarly, a study by Behera et al.2008[2] from Pondicherry, India who reported equal efficacy of both combined disk test and E test, In some other study Berges L, et al 2007,[32] reported CDT to be satisfactory for screening despite its low specificity as it is an easy procedure and is simple to interpret. whereas other studies Leek,et al 2001,[10] and Amjad et al 2011,[11] showed that Modified hodge test to be 100% sensitive and more easy and simple test.

Franklin et al 2006,[33] from Melbourne, Australia CDST 100% and DDST 79% and P. Pandya et al 2011,[34] from Gujarat, India CDST 96.3% and DDST 81.48%.

Above studies showed that CDT to be a more sensitive method for the detection of MBL than DDST. In the present study, the Imipenem-EDTA combined disc test and Imipenem-EDTA MBL E-test were equally effective for MBL detection,

which was in accordance with B Behera et al,[27] from AIIMS, New Delhi, India, who found that both combined disc and E-test were equally sensitive for MBL detection.

In the present study, the antibiogram of 255 P. aeruginosa isolates showed more resistance against Imipenem (38.43%) followed by Tobramycin (34.90%) Ceftazidime (33.72%), In contrast a study done by Radhika et al,[14] from Bibinagar, India who found resistance to Imipenem was 20%. Some other observation done by Angadi et al 2012,[35] and Bashir et al 2011,[36] from Srinagar, showing an Imipenem resistance of 21.6% and 13.42%, respectively.

Another studies published by Behera et al,[27] from AIIMS, New Delhi, Radhika et al from Bibinagar, Tavajjohi Z,[37] from Iran and Dwivedi et al,[38] from Lucknow, who found a resistance against Ceftazidime(67%), (55%), (35%) and (85%).

In this study, Piperacillin/Tazobactam was found to be 27.84% resistance. A study by Peshattawar PD, et al,[39] from Bijapur, India, showed a Piperacillin/Tazobactam resistance of 20.62%, which was similar to our study.

In the present study we found a 30.98% resistance against Ciprofloxacin, In contrast highly resistance against ciprofloxacin reported by Angadi et al 2012,[35] from Dr. D. Y. Patil Medical College and Research Centre, Pimpri, Pune, India, showing a Ciprofloxacin resistance of 60%. Highly Resistance were also observe in another study conducted by KM Mohan Sundaram,[40] from Vinayak Missions Medical College, Salem, and Senthamarai S. et al 2014,[41] from Tamilnadu, India showing a Ciprofloxacin resistance of 62.5% and 61.53%.

Tobramycin was found to be (34.90%) resistance in our study, whereas a study published by Patel et al,[42] from Barabanki, India in 2016, who reported highly resistance to Tobramycin (60.29%). One more study from Iran, P. aeruginosa was found to be 100% resistant to tobramycin.

In our study, polymyxin B and colistin showed 100% susceptibility against P. aeruginosa isolates. Similarly in a study by Suhani Gondha et al. 2022,[43] who reported (100%) sensitivity to Polymyxin B. A study by Patel et al 2021,[44] reported highly sensitive to Colistin (94.5%) respectively.

Conclusions

This study shows that MBL Detection is a challenge for routine microbiology laboratories, Till date CLSI has not given any guidelines on which test to follow for diagnosis of MBL. We had performed 3 tests and among them we found IMP & IMP EDTA combined disc test to be equally sensitive as IMP EDTA E test. However, as detected in our study, Imipenem-EDTA combined disc test and E test is the most convenient phenotypic method for detection of MBL production in GNB with high sensitivity and its advantage is that it is also less time consuming, technically less demanding as compared to MHT, therefore, less cumbersome to perform in routine microbiological laboratories.

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