Original Research Article

Prevalence of MBL producing pseudomonas aeruginosa in various clinical specimens in tertiary care hospital, Karimnagar

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Abstract

Aim: To find the prevalence of MBL Producing *Pseudomonas aeruginosa* from various clinical specimen. **Material and Methods:** A total 114 cases from which *Pseudomonas aeruginosa* has been isolated from Swab, Urine, pus, Sputum, Bal, Foley's catheter, E.T. Secretion received from various clinical departments. The study was carried outovera period of Six months. The isolates were tested by IPM-EDTA Combined Disc Test (CDT) and Imipenem-EDTA double disc synergy test (DDST). Descriptive statistics and chi-square is used with the help of MS Excel and SPSS Version 25 **Results:** Among 114 *Pseudomonas aeruginosa* isolated from various clinical specimens, 16 (14.03%) imipenem-resistant Pseudomonas isolates. Out of 16 IPM resistant, 15(93.75%) were positive for MBL by CDT-IPM method and 11 (68.75%) were positive by Imipenem-EDTA (DDST) method, respectively. **Conclusion:** Increase in the resistant pattern of antibiotics can lead to increased morbidity, mortality and economic burden on patients. So it is necessary to detect MBL producing *Pseudomonasaeruginosa* by simple and effective methods.

Keywords: Pseudomonas aeruginosa, Combined Disc Test, Imipenem-EDTA double disc synergy test**,** metallo-β-lactamases, Imipenem (IPM)

Introduction

Pseudomonas aeruginosa is a large group of aerobic, non sporing gram negative bacilli, which are motile and are found in water, soil and other moist environment. It is the major pathogen in hospitalized patients [1, 2]. Imipenem is a derivative of thienamycin which is extremely potent and has broad range of activity against gram positive and gram-negative organisms [3]. It can be up regulation of active efflux pump system of cytoplasmic membrane or alteration in PBPs or it may be due to metallo-β- lactamases (MBLs) in the development of resistance to carbapenemases [4]. Government of India in 2017 included Pseudomonas aeruginosa as an important pathogen in national pathogen for the containment of antimicrobial resistance in 12th 5 year plan. Pseudomonas aeruginosa was 2nd among the critical pathogens which are multidrug resistant bacteria that pose a particular threat in hospitals, nursing homes, and among patients whose care requires devices such as ventilators and blood catheters, was published by WHO in 2017.

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Molecular study has shown bla VIM and bla NDM to be predominant antimicrobial resistant determinants which are contributing for carbapenemases resistance [5]. Mortality rate is higher among the infection cause by Pseudomonas aeruginosa producing MBLs [6]. Further adding to the trouble MBLs can be spread from Pseudomonas aeruginosa to enterobacteraciae [7].

Although molecular detection is more reliable for detection of MBLs, but possible only in reference laboratories. So our aim is to detect MBLs producing Pseudomonas aeruginosa by different phenotypic methods currently available.

Materials and Method

Place and Type of Study: It's a prospective analytical study, carried out in Department of Microbiology from 1st august to 31 January for a period of 6 monthsin a tertiary care hospital in Karimnagar, Telangana,

Sampling Method: A total 114 cases from which *Pseudomonas aeruginosa* has been isolated which were received include wound swabs, sputum, urine, Broncho-

alveolar lavage (BAL), blood, and indwelling catheters. Samples were processed under complete aseptic conditions.

Sample Collection: A total 114 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 test [8].

Antimicrobial sensitivity testing was performed by the disk diffusion method on Muller-Hinton agar plates by Kirby-Bauer method using antibiotics (Hi-Media, India) piperacillin/tazobactam(100µg/10 µg), ceftazidime (30 µg), amikacin(30µg), Ofloxacin (5 µg), imipenem (10µg), gentamicin(10µg), polymyxin-B (300 units) according to CLSI guidelines [9].

Different phenotypic tests are done for detection of MBL production in P. aeruginosa.
Detection ofMetallo-beta- lactamases (MBLs) bytwo Methods-

1. Imipenem (IPM)-EDTA combined disc test: This method was performed according to the description by Yong 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 37^0C. An increase in the zone diameter of ≥ 7mm around imipenem+EDTA disc in comparison to imipenem disc alone indicated production of MBL [10].

2. Imipenem-EDTA double disc synergy test (DDST): The IMP-EDTA double disk synergy test was performed according to the procedure described by Lee et al.. An imipenem (10 µg) disc was placed 20m. center to center from a blank disc containing 10 µL of 0.5 M EDTA (750 µg).

Enhancement of the zone of inhibition in the area between imipenem and the EDTA disc in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result [11].

It is now a known fact that there is increase in the antibiotic resistance among different pathogenic bacteria worldwide. One the mechanism which bacteria become resistant to antibiotic is release of enzymes.

Inclusion criteria: All samples received are processed which includes both in-patient and outpatient department

Exclusion criteria: Repeat isolates from the same patients.

Statistical Methods: Statistics of the study is done by using Descriptive statistics shown by proportion and chi-square test with the help of Microsoft Excel 2010 and SPSS Version 25.

Ethical Consideration and Permission: The necessary approval to conduct this study was obtained from the Institutional Ethics Committee (IEC) of the college before starting the study. In the present study no any scoring system or any surgical procedure were used.

Result

Table-1: Sample wise distribution of P. aeruginosa isolates

Sample	Pseudomonas	Screened for IMI in Routs AST (16) 14.03%	CDST (15)	DDST (11)
Pus	58	9	8	6
Sputum	43	4	4	2
Urine	8	2	2	2
BAL	3	1	1	1
Foley's catheter	1	0	0	0
E.T. Secretion	1	0	0	0
Total	**114**	**16**	**15**	**11**

A total of 114 consecutive Non-repetitive isolates of *Pseudomonas aeruginosa* obtained from various clinical samples over a period of 6 months were included in the study, out of which 58 were isolated from pus, 43 from sputum, 8 from urine, 3 from BAL, 1 from Foley's catheter, 1 from E.T. secretion. Out of 16 Imipenem-resistant Pseudomonas isolates, 15(93.75%) were positive for MBL by CDT-IPM method, whereas 11 (68.75%) were positive by DDST-IPM method and difference between these two test statistically not significant at 5% level of significance, respectively depicted in Table 1.

Table-2: Antibiotic Resistance patterns of P. aeruginosa isolates

Antibiotic	Resistant	Percentage
Imipenem(IPM)	16	14.03
Cefoperazone sulbactam (CFS)	26	22.80
Piperacillin/Tazobactom (PIT)	19	16.66
Carbenicillin (CB)	18	15.78
Amikacin (AK)	25	21.92
Gentamycin (GEM)	28	24.56
Ofloxacin (OF)	45	39.47
Ceftazidime (CAZ)	41	35.96

A total of 114 consecutive Non-repetitive isolates of *Pseudomonas aeruginosa* obtained from various clinical samples, 14.03%ofisolates were resistant to Imipenem, similarly 22.80% to CFS, 16.66% to PIT, 15.78% to CB, 21.92% to AK, 24.56%to GEN, 39.47% to OF and 35.96 % to CAZ are found to be resistant.

Table-3: Antibiotic Resistivity pattern of *P. aeruginosa* isolates

Antibiotic	Resistance Shown by Imipenem resistant Pseudomonas aeruginosa (n = 16) (%)	Resistance Shown by Imipenem Sensitive Pseudomonas aeruginosa (n=98) (%)
Cefoperazone sulbactam	7 (43.75)	19(19.38)
Piperacillin/Tazobactom	4 (25)	15(15.30)
Carbenicillin	4 (25)	15(15.30)
Amikacin	5 (31.25)	20(20.40)
Gentamycin	6 (37.5)	22(22.44)
Ofloxacin	10 (62.5)	35(35.71)
Ceftazidime	6 (37.5)	35(35.71)

Of the total number of 114 *Pseudomonas* isolates, a total of 16 (14.03%) *Pseudomonas* spp. were found resistant to imipenem and the rest 98 (85.97%) were sensitive to imipenem.

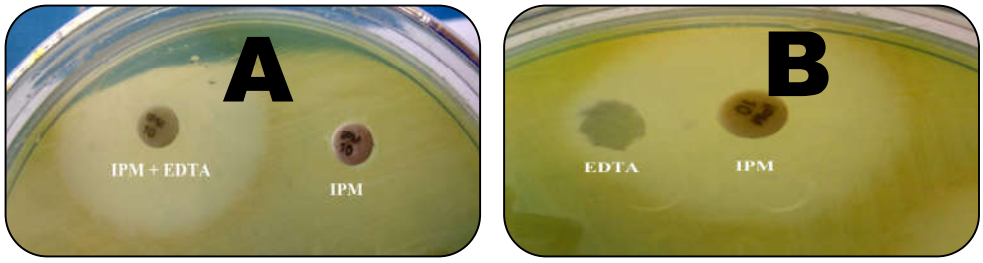

Figure-1: Screening of metallobetalactamase production by imipenem-EDTA CDT (A) and imipenem-EDTA DDST (B)

Discussion

Metallo-β-lactamase-producing *Pseudomonas aeruginosa* (MPPA) is an important nosocomial pathogen that shows resistance to all β-lactam antibiotics except monobactams. This has been reported in several countries [12]. Carbapenem hydrolysing Metallo beta lactamases which is able to hydrolyse carbapenem is important mechanism for imipenem resistance Mehta A et al[13]. In our study, P. aeruginosa is most frequently isolated organism from pus sample which is about 50.9 % which is in accordance to study conducted by kali A et. al. in Pondicherry in 2013[2], but change was noted in study done by Sood MS et. al [12] where imipenem-resistant Pseudomonas was most commonly isolated from respiratory secretions.

P. aeruginosa-producing MBL was first reported in India in 2002 [7,14]. Present study shows 14.03% MBL positive Imipenam resistant *Pseudomonas aeruginosa* cases which is quite less than the study done by Peleg et al who have described a two year study from Alfred hospital, showing 55.8% MBL positive isolates, Doguen young et al from Korea showed 50% of MBL

production in *Pseudomonas aeruginosa* [15, 16] but was little less compared to Soumya S et. al., Sujatha R et alwho found 26.6% and 22% respectively [17,18].

CLSI recommends to carry out MBL detection by Modified hodge test(MHT), though few reliable methods are published, but two or more methods has to be carriedto conform the resistance related to MBL production. Out of 16 (14.03%) Imipenam-resistant *Pseudomonas* isolates 15 (93.75%) were positive for MBL by CDST-IPM method and 11 (68.75%) were positive by DDST-IPM method. This is comparable to study of Sood et al (100%), Irfan et al.10 (100%), Attal et al (88.89%), and Fam et al (87.5%) [12, 19-21].

Jesudason MV et al found that 75% organism were MBL producers by EDTA disc synergy test but our study showed slightly higher which is 93.75% [22]. Study done at Mathura in 2016 and Belagavi in 2017 showed CDT is better interpreter of MBL than DDST which we have seen in our study [12,17] but study by John S et. al [23] showed DDST to be better choice for phenotypic detection may be due to the differences in population structure of MBL genes between different geographical areas studied.

Imipenem resistant isolates may show resistance to other antibiotics also as the location of MBL genes encoded on plasmids also encodes resistance to other antibiotics such as to aminoglycosides, betalactams, and fluroquinolones. It is observed in our study that Amikacin and ceftazidime were resistant in 31.25% and 37.5 % Imipenam-resistant *Pseudomonas* isolates which is less compared to work done by kali A et. al. which is about 72.7% and 81.1% respectively [2]. Mehta A et al observed PIT and AK resistance in 20% and 26.67% which is comparable with our present study which is about 15. 78% and 21.92% of P. aeruginosa isolates respectively. Study done by Ghasemian at al. in 2018 found MBL prevalence little higher, it may be due to the use of molecular methods for detection [24].

Franklin C et al reported phenotypic MBL detection system is highly sensitive (100%) and specific (98%) in detecting MBL producing organisms and also the method is simple to perform, and the materials used are cheap, nontoxic, and easily accessible so that it can be used routinely in clinical laboratories [25].

Conclusion

It is a known fact that the major contributory factor in developing drug resistance is unethical use of antibiotics. Soit's our effort to highlight the importance of resistance pattern which will help the clinicians to make appropriate antibiotic choice and also timely introduction of appropriate infection control procedures thereby preventing hospital spread of resistant strains. Further similar studies needed in future to know the trends in MBL resistant organisms.

Contribution by different authors- For this manuscript, study was done by Dr. Amar C. Sajjan, Statistics and manuscript prepared by Mr. Sachin Gurnule with the help of Dr. Amar and Data collection done by Dr. B. Aparna

What this study adds to existing knowledge? From our study we came to know that CDT is more sensitive than DDST, but no single method is reliable. So need two or more methods to detect all MBL Producing Strains.

Findings: Nil; **Conflict of Interest**: None initiated
Permission from IRB: Yes
Ethical approval: The study was approved by the Institutional Ethics Committee

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