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Original Research Paper

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PHENOTYPIC DETECTION OF METALLO-β-LACTAMASE (MBL) AMONG IMIPENEM SENSITIVE & RESISTANT PSEUDOMONAS AERUGINOSA ISOLATES

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ABSTRACT

Background: Metallo-β-Lactamase (MBL) producing *Pseudomonas aeruginosa* is a major cause of nosocomial infections and poses newer diagnostic and therapeutic challenges. We studied MBL production in imipenem sensitive & resistant P. aeruginosa isolates by phenotypic methods. **Material & Methods:** 63 clinical isolates of P. aeruginosa were obtained from samples received in Department of Microbiology, RNT Medical College, Udaipur (Raj). Their antibiotic susceptibility tests were performed according to the Clinical & Laboratory Standard Institute (CLSI) guidelines. MBL production was detected by phenotypic methods – Imipenem EDTA double disk synergy test (DDST) & Imipenem EDTA combined disk test (CDT). **Result:** Out of 63 P. aeruginosa isolates 48 (76.19%) were sensitive, 11 (17.46%) were resistant and 04 (6.34%) were intermediate to imipenem. 21 (43.75%) sensitive isolates, 10 (90.90%) resistant isolates and 02 (50%) intermediate isolates were found to be MBL positive. **Conclusion:** The present study highlights the necessity to identify the MBL positive P. aeruginosa strains for effective treatment, irrespective of imipenem sensitivity. Phenotypic tests for MBL detection are simple, easy to perform and can be done along with routine antibiotic sensitivity testing.

KEYWORDS : Metallo-β-Lactamase (MBL), Pseudomonas aeruginosa, Imipenem EDTA combined disk test (CDT).

INTRODUCTION:-

The emergence of carbapenemases is increasing now days. Carbapenems such as Imipenem & meropenem are the last resort for the treatment of infections caused by MDR gram negative bacilli [1]. *P. aeruginosa* is a major cause of a variety of nosocomial infections. *P. aeruginosa* shows resistant to carbapenems by the production of Metallo-β-Lactamase (MBL). MBL has the ability to hydrolyze a wide variety of β-lactam antibiotics, such as penicillins, cephalosporins & carbapenems [2],[3],[4]. Genes responsible for MBL are present on transferable plasmids or associated with transposons [5]. MBLs are inhibited by metal chelators, like ethylene diamine tetra acetic acid (EDTA) & thiol based compounds. Detection of MBL genes with polymerase chain reaction (PCR) is the gold standard method, but it is not routinely used due to its high cost [6]. As P. aeruginosa is intrinsically resistant to a variety of antibiotics, so early detection of MBL positive isolates are necessary for better treatment & to prevent dissemination of these genes from one bacterium to another. The aim of this study was to detect MBL by phenotypic methods in carbapeneme susceptible & resistant *P. aeruginosa* obtained from various clinical samples.

MATERIAL AND METHODS:-

The present study was a retrospective study which was conducted in the Department of Microbiology, RNT Medical College and associated Hospitals, Udaipur, Rajasthan, India. 63 confirmed *P. aeruginosa* obtained from various clinical samples were included after getting approval from Institutional Ethical Committee. Their antibiotic susceptibility tests were performed according to the Clinical & Laboratory Standard Institute (CLSI) guidelines [7].

MBL production was detected in both imipenem sensitive and imipenem resistant isolates by phenotypic methods, such as Imipenem EDTA double disk synergy test (DDST) [8] & Imipenem EDTA combined disk test (CDT) [9].

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was performed by Kirby- Bauer disc diffusion method as per CLSI guideline [7]. Colonies was inoculated into peptone water and turbidity was adjusted at 0.5 McFarland standard. Broth culture was spread on the plate to make lawn culture on Mueller Hilton Agar (MHA). In this study the antimicrobial susceptibility testing was carried out against the following antibiotics: - Ceftazidime (30μg), Gentamycin (10μg), Piperacillin/Tazobactum (100/10μg), Amikacin (30μg), Ciprofloxacin (5μg), Imipenem (10μg), cefepime (30μg), aztreonam (30μg) and colistin (10μg). P. aeruginosa ATCC 27853 strain was employed as the control strain [7].

Phenotypic methods used for detection of MBL production.

Imipenem (IMP)-ethylene diamine tetra acetic acid combined disc test (CDT):

The IMP-EDTA combined disc test was performed as described by Yong et al. The test culture were made on the MHA recommended by CLSI guidelines. Commercially available Imipenem (10μg) and Imipenem EDTA (10+ 750μg) discs were placed on MHA at a distance of 20mm each other. After incubation period of 16-18 hours at 37°C, the increase in inhibition zone ≥7mm around the IMP/EDTA disc than IMP alone was considered as positive for MBL production [8].

Imipenem-EDTA Double disk synergy test (DDST):

The test culture was made on the MHA as CLSI guidelines. 10 μl 0.5M EDTA solution was added to a 6mm blank filter paper disk which contains 750 μg of EDTA. An imipenem (10μg) disk and a blank disk containing 10μl of 0.5 M EDTA (750μg) was placed on MHA at a distance of 20mm each other. After incubation period of 16-18 hours at 37°C, the enhancement of zone of inhibition in the area between imipenem and EDTA disk in comparison with the zone of inhibition on the far side of the drug was interpreted as positive for MBL production [9].

RESULTS:

Out of 63 *P. aeruginosa* isolates 48 (76.19%) were sensitive, 11 (17.46%) were resistant and 04 (6.34%) were intermediate to imipenem. All 63 isolates irrespective of imipenem sensitivity were processed by phenotypic methods for MBL detection. Out of 63 P. aeruginosa isolates 33 (52.38%) were found MBL positive. 21 (43.75%) sensitive isolates, 10 (90.90%) resistant isolates and 02 (50%) intermediate isolates were found MBL positive. 33 (52.38%) were found positive by CDT, 21 (33.33%) were found positive by DDST.

Highest resistant seen in ceftazidime (70%), followed by pipracillin/tazobctam (63.5%). The sensitivity for gentamycin, cefepime & ciprofloxacin was 67%. 78% isolates were sensitive to amikacin. 76.19% isolates were sensitive to imipenem. Sensitivity to aztreonam was 56%. All isolates were sensitive to colistin (Table 1).

Table 1 Antibiotic sensitivity & resistant pattern in P.aeruginosa isolates (N= 63)

Antibiotic	Sensitive	Resistant
Ceftazidime	19 (30%)	44 (70%)
Gentamycin	42 (67%)	21 (33%)
Amikacin	49 (78%)	14 (22%)
Pipracillin / Tazobactam	23 (36.5%)	40 (63.5%)
Cefepime	42 (67%)	21 (33%)
Imipenem	48 (76.19%)	11 (17.46%)
Ciprofloxacin	42 (67%)	21 (33%)
Aztreonam	35 (56%)	28 (44%)
Colistin	63 (100%)	0 (0%)

Out of 33 MBL positive isolates, 27 (82%) were resistant to ceftazidime. The sensitivity for gentamycin & ciprofloxacin was 67%. Cefepime, imipenem & aztreonam were equally sensitive among MBL positive isolates and sensitivity was 64%. 79% isolates were sensitive to amikacin & 42% were sensitive to pipracillin/tazobactam (Table 2)

Table 2 Antibiotic sensitivity & resistant pattern in MBL positive isolates (N=33)

Antibiotic	Sensitive	Resistant
Ceftazidime	06 (18%)	27 (82%)
Gentamycin	22 (67%)	11 (33%)
Amikacin	26 (79%)	07 (21%)
Pipracillin / Tazobactam	14 (42%)	19 (58%)
Cefepime	21 (64%)	12 (36%)
Imipenem	21 (64%)	11 (33%)
Ciprofloxacin	22 (67%)	11 (33%)
Aztreonam	21 (64%)	12 (36%)
Colistin	33 (100%)	0 (0%)

DISCUSSION:

Pseudomonas aeruginosa is the most frequent pathogen causing Hospital Acquired Infections (HAI). Further, acquired drug resistance is common in nosocomial isolates of *Pseudomonas spp* [10]. *P. aeruginosa* has a unique property to develop drug resistance during treatment. The MBL production & antibiotic sensitivity pattern among *P. aeruginosa* have a diversity in different geographic area, due to unempirical uses of antibiotics. Therefore, it is necessary to detect antibiotic sensitivity pattern and MBL producing isolates by simple & rapid phenotypic methods for better treatment & to control antibiotic resistance. MBL is not only produced by carbapenem resistant isolates, but has also been produced by sensitive isolates [11],[12].

In this study, 52.38% *P. aeruginosa* isolates were found positive for MBL production. The results are similar to Sadhna et al [13], Madhu et al [14], and Behara et al [15], they had reported MBL production in *P. aeruginosa* as 41%, 61.5% & 69.5% respectively. Aggarwal et al [16] & Kaur et al [17], had reported MBL production as 11.4% & 21.8% respectively, which is lesser to as compared to this study. In this study we detected MBL production in *P. aeruginosa* isolates irrespective of imipenem sensitivity. 21 (43.75%) sensitive isolates, 10 (90.90%) resistant isolates and 02 (50%) intermediate isolates were found MBL positive. Similar studies were carried out by other workers [11],[12],[18].

CONCLUSION:

The present study highlights the necessity to identify the MBL positive *P. aeruginosa* strains for effective treatment, irrespective of imipenem sensitivity. Screening of only carbapenem susceptible isolates for MBL detection is insufficient. Carbapenem sensitive organisms carrying hidden MBL genes, which may spread unnoticed & may lead to infection control problems [4]. Some guidelines must be designed to select out isolates for MBL detection. MBL detection must be done in all diagnostic laboratories to prevent the emergence & spread of the resistance mechanism. Phenotypic tests for MBL detection are simple, easy to perform and can be done along with routine antibiotic sensitivity testing.

Conflicts of interest: No conflicts of interest regarding the publication of this paper.

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