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"Phenotypic Detection Of MBL In Meropenem Resistant *P. Aeruginosa* Among Clinical Isolatesin A Tertiary Care Hospital."

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Abstract:

CONTEXT: Carbapenems, including imipenem (IPM) and meropenem (MRP), are the most potent antibacterial agents used for the treatment of infections initiated by multidrug-resistant gram-negative bacilli. *Pseudomonas aeruginosa*, is a commonly encountered nosocomial pathogen, especially in immunocompromised patients, thus, inflicting significant morbidity and mortality, worldwide. The emergence of MBL-producing *Pseudomonas aeruginosa* is a challenge to microbiology laboratories because there are no standardized guidelines available to detect them. This study aimed to compare four phenotypic methods to detect MBL production in *Pseudomonas aeruginosa* and to determine the antibiotic sensitivity of MBL-producing isolates.

MATERIALS AND METHODS: A total of 200 clinical isolates of *Pseudomonas aeruginosa* were tested for MBL production. Meropenem (MRP) resistant *Pseudomonas aeruginosa* were taken as MBL screening. MBL detection was done by three phenotypic methods 1) Combined disk synergy test (CDST). 2) Double disk synergy test (DDST) and 3) E-test.

RESULTS: out of 200 *Pseudomonas aeruginosa*, 73 were resistant to Meropenem as screening positive. Out of 73, 34(46.5%) isolates were MBL positive by CDST methods, 33(45%) by DDST method and 33(45%) by E-test. Colistin and polymyxin B were found to be 100% sensitive.

CONCLUSION: The study result demonstrates the antibiotic sensitivity pattern of MBL-positive isolates suggests that early detection of MBL-producing*P. aeruginosa* and determination of their antibiotic sensitivity are of crucial importance to start appropriate treatment. It was observed in this present study that the MBL E-test followed by DDST were effective options for MBL detection in this part of the country.

Keywords: *Pseudomonas aeruginosa*, Metallo-β-lactamase, CDST, DDST and E-test, antibiotic sensitivity.

Introduction:

Carbapenems, including imipenem (IPM) and meropenem (MRP), are the most potent antibacterial agents used for the treatment of infections initiated by multidrug-resistant gram-negative bacilli [(1)]. *Pseudomonas aeruginosa*, a commonly encountered nosocomial pathogen, is a potentially troublesome cause of wound infection, urinary tract infection, and respiratory tract infection, especially in immunocompromised patients, thus, inflicting significant morbidity and mortality, worldwide [(2)]. However, acquired resistance to carbapenems has been increasingly reported globally, which can be attributed to the evolution of divergent *β*-lactamases in numerous gram-negative bacteria (including *Pseudomonasaeruginosa*). *P. aeruginosa* is an important nosocomial pathogen that is intrinsically resistant to multiple antibiotics. In *P. aeruginosa*, resistance to carbapenems mediated by*the* production of Metallo beta-lactamases (MBLs) is being increasingly reported [(3)]. Genes encoding for MBL were shown to be carried on large transferable plasmids or were associated with transposons, allowing horizontal transfer of these MBL genes among different bacterial genera and species [(4)]. To date, five types of acquired MBL genes (IMP, VIM, SPM, GIM, and SIM) have been identified based on their divergent protein molecular structures [(5,6,7)]. While IMP and VIM variants have been reported worldwide, members of SPM, GIM, and SIM are restricted to certain geographical regions[(8,9)]. Detection of genes coding for MBL by polymerase chain reaction (PCR) usually gives reliable and satisfactory results;however, because of the cost of the method, it is of limited practical use for routine diagnostic microbiology laboratories. Thus, a simple and inexpensive testing method for the detection of MBL producers is necessary [(10)]. The present study was undertaken to detect MBL in *P. aeruginosa* using different phenotypic methods.

Materials and Methods:

The present study was a prospective study which was conducted in the Department of Microbiology, Index Medical College, Hospital & Research Centre, Indore. A total of 200 confirmed *P. aeruginosa* isolated from various clinical samples were included over 2 years from 2019 January to 2020 December after getting approval from the ethical committee.All isolates were subjected to antibiotic susceptibility testing as per the clinical and laboratory Standard Institute guidelines[(11)].

Screening of Metallo beta-lactamase (MBL) was done by Modified carbapenem inactivation methods(mCIM) and the Isolates which gave MBL screening test positive were subjected to confirmation by three phenotypic methods.

Antimicrobial Susceptibility testing:

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method as per CLSI guidelines[(11)]. In the present study the susceptibility testing was carried out against the following antibiotics:- ceftazidime(30µg), cefepime(30µg), ceftriaxone(30µg), cefoxitin(30µg), imipenem(10µg), meropenem(10µg), piperacillin/tazobactam(100/10µg), ticarcillin/clavulanic

acid(100/75µg), aztreonam(30µg), gentamycin(10µg), amikacin(10µg), netilmicin(30µg), polymyxin B(300units), colistin(10µg). in case of urinary isolates, ofloxacin(5µg) and norfloxacin(10µg) were also included. *P. aeruginosa* ATCC 27853 strain was employed as the control strain[12].

Phenotypic methods were used for confirmation of MBL production.

Imipenem(IMP)-EDTA combined disc test(CDST): - The IMP-EDTA combined disk test wasperformed as described by Yong et al. Test organisms were inoculated onto plates with Mueller Hinton agar as recommended by the CLSI. A 0.5 M EDTA solution was prepared by dissolving 18.61g in 100ml of distilled water and adjusting pH to 8.0 by using NaOH. The mixture was sterilized by autoclaving. Two 10µg imipenem disks (Becton Dickinson) were placed on the plate, and appropriate amounts of 10µl of EDTA solution were added to one of them to obtain the desired concentration (750µg). The inhibition zones of the Imipenem and Imipenem-EDTA disks were compared after 16 to 18 hours of incubation in the air at 35°C. In the combined disc test, if the increase in inhibition zone with the Imipenem and EDTA disc will be≥ 7mm than the Imipenem disc alone, it will be considered MBL positive[13].

Double Disk Synergy Test (DDST): - This test was performed with an overnight broth culture of the test strain inoculated on the MHA plate and allowed to dry. 10µl of the 0.5M EDTA solution was added to a 6mm blank filter paper disk (Whatman filter paper no.1) which contained approximately 750µg of EDTA. A 10µg imipenem disk was placed in the centre of the plate flanked by an EDTA disk at a distance of 20 mm Centre to Centre from a blank disc containing 10µl of 0.5M EDTA (750µg). After overnight incubation, the presence of an enlarged zone of inhibition towards the EDTA disk was interpreted as positive for an MBL producer [14].

E-Test: E-test Metallo-beta-lactamase strips consisted of a double-sided seven dilution range of imipenem IP (4 to 256µg/ml) and IP (1 to 64µg/ml) overlaid with a constant gradient of EDTA. Individual colonies were picked from overnight agar plates and suspended in 0.85% saline to a turbidity of 0.5 McFarland's standard. A sterile cotton swab was dipped into the inoculum suspension, and a lawn culture of inoculums was done on the MHA plate. The excess moisture was allowed to be absorbed for about 15 min before the E-test MBL (AB bioMerieux) strip was applied. Plates were incubated for 16 to 18 h at 37°C. The MIC endpoints were read where the inhibition ellipses intersected the strip. A reduction of imipenem MIC=3 two folds in the presence of EDTA will be interpreted as being suggestive of MBL production [15].

Results:

Out of 200 clinical isolates of *P. aeruginosa,*73 wereresistant to meropenem which was considered MBL screening positive.

All 73 Meropenem-resistant clinical isolates were further processed for confirmation by different phenotypic methods for MBL detection. Among the three tests, DDST and E test was founded

100% sensitive and specific whereas CDST was found to be specificity 98% but sensitivity was 97% (Table 1).

Table 1: Comparison of screening and confirmatory test in the phenotypic detection of MBL

Organisms	Total number of meropenem-resistant*P. aeruginosa isolates out of 200*	Imp-EDTA combined disc test	Double Disc Synergy test	E test
Pseudomonas aeruginosa	73	34 Sensitivity=97% Specificity=98	33 Sensitivity=100% Specificity=100%	33 Sensitivity=100% Specificity=100%

The Antibiotic sensitivity pattern amongst the isolates. 81% of isolates wereresistant to ofloxacin and Gentamycin and 19% of isolates were sensitive respectively. There were 67.5% of isolates were sensitive to cefepime and 32.5% were resistant. 66% of isolates were resistant to Amikacin and 34% of isolates were sensitive. All the samples were sensitive to Colistin & Polymyxin. 33.5% of samples were resistant to Ticarcillin/Clavulanic Acid and 66.5% of samples that sensitive. 53% of samples were resistant to Ceftazidime and 47% of samples that sensitive. 66.5% of samples were resistant to Ceftriaxone and 33.5% of samples were sensitive and there were 100% isolates resistant to Tigecycline **(Table 2).**

Table 2: antibiotic-resistance& sensitivity pattern among isolates

Antibiotic	Resistant		Sensitive	
	Number	Percentage	Number	Percentage
Amikacin	132	66%	68	34%
Aztreonam	58	29%	142	71%
Ceftazidime	106	53%	94	47%
Cefepime	135	67.5%	65	32.5%
Ceftriaxone	133	66.5%	67	33.5%
Colistin	0	0%	200	100%
Gentamycin	162	81%	38	19%
Imipenem	73	36.5%	127	63.5%
Meropenem	73	36.5%	127	63.5%
Ofloxacin	162	81%	38	19%
Pipracillin/Tazobactam	105	52.5%	95	47.5%
Polymyxin B	0	0%	200	100%
Ticarcillin/clavulanic acid	67	33.5 %	133	66.5%

All MBL-producing isolates were resistant to Imipenem and meropenem by disc diffusion test. MBL producers also showed 100% resistance to Aztreonam,Ceftazidime,Cefepime, and Ceftriaxone and all MBL isolates were sensitive to Colistin and PolymyxinB. Among other drugs, Amikacin was founded to be the second most effective drug. (Table 3)

Table 3: antibiotics sensitive & resistant pattern for MBL (N=33)

	Sensitive	Resistant
Amikacin	25(76%)	8(24%)
Aztreonam	0(0%)	0(100%)
Ceftazidime	0(0%)	33(100%)
Cefepime	0(0%)	33(100%)
Ceftriaxone	0(0%)	33(100%)
Colistin	23(100%)	0(0%)
Gentamycin	15(43%)	18(57%)
Imipenem	0(0%)	0(100%)
Meropenem	0(0%)	0(100%)
Ofloxacin	10(30%)	23(70%)
Pipracillin/Tazobactam	13(39%)	20(61%)
Polymyxin B	33(100%)	0(0%)
Ticarcillin/clavulanic acid	22(66.6%)	11(33.3%)

Discussion:

Pseudomonas aeruginosa is one of the most frequent nosocomial pathogens and the infection caused is most often difficult to treat due to antibiotic resistance. Acquired drug resistance is frequent in nosocomial isolates of Pseudomonas spp. Carbapenems have a broad spectrum of antibacterial activity. Acquired Metallo-β-lactamases (MBL) in pseudomonas spp. has recently emerged as one of the most worrisome resistance mechanisms as capable to hydrolyze all beta-lactam antibiotics including penicillins, cephalosporins and carbapenems, apart from Aztreonam. There has been an increase in reports of Carbapenem resistance in *P. aeruginosa* worldwide. Detection of MBL by PCR usually gives reliable and satisfactory results; however, because of the cost of the method, it is of limited practical use for routine diagnostic microbiology laboratories. Thus, a simple and inexpensive testing method for the detection of MBL producers is necessary [(16)].

In the evaluation of three selected MBL phenotypic assays (CDST, DDST and E-test), two methods were shown a 100% sensitivity and specificity whereasthe CDST MBL detection method was shown with 97% sensitivity and 98% specificity (Table 1).

Moststudies have used IMP-EDTA combined disc (CDST), double-disc synergy test (DDST) and E-test for MBL detection. After the review of the literature and the experience of

contemporary workers, the above three methods (CDST, DDST & E-test) were employed in the present study [17]. It was observed in the present study that MBL E-test followed by DDST were effective options for MBL detection. However, DDST was cost-effective.

The antibiogram of *P. aeruginosa isolates (*Kirby-Bauer disc diffusion method) shows 81% isolates that were resistant to ofloxacin and Gentamycin and 19% isolatesthat were sensitive respectively. There were 67.5% of isolates were sensitive to cefepime and 32.5% were resistant. 66% of isolates were resistant to Amikacin and 34% of isolates were sensitive. All the samples were sensitive to Colistin & Polymyxin. 33.5% of samples were resistant to Ticarcillin/Clavulanic Acid and 66.5% of samples that sensitive. 53% of samples were resistant to Ceftazidime and 47% of samples that sensitive. 66.5% samples were resistant to Ceftriaxone and 33.5% samples that sensitive and there were 100% isolates resistant to Tigecycline (Table 2). MBL-positive isolates showed 100% resistance to Aztreonam,Ceftazidime,Cefepime, and Ceftriaxone and all MBL isolates were sensitive to Colistin and PolymyxinB. Among other drugs, Amikacin was founded to be the second most effective drug (Table 3).

In conclusion, the study result demonstrates the antibiotic sensitivity pattern of MBL-positive isolates suggesting that early detection of MBL-producing*P. aeruginosa* and determination of their antibiotic sensitivity are of crucial importance to start appropriatetreatment.It was observed in this present study that the MBL E-test followed by DDST were effective options for MBL detection in this part of the country.

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