Original article

Antibiotic resistance pattern of Pseudomonas aeruginosa with special reference to Imipenem and Metallo-beta lactamase Production

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Abstract:

Introduction: Pseudomonasaeruginosa is one of the most common pathogen causing nosocomial infections. Cephalosporins ,Carbapenem , Imipenem are the most effective treatment options for Pseudomonas aeruginosa. But now resistance to these drugs is also reported from many hospitals. Resistance is often mediated by Metallo-Beta-lactamases (MBL) production by Pseudomonas aeruginosa.The appearance of MBL genes and their spread among bacterial pathogens is a matter of concern with regard to the future of antimicrobial therapy. Rapid detection of metallo-beta- lactamases production is necessary to modify therapy and to initiate effective infection control to prevent their dissemination.

Material & Method: Pseudomonas aeruginosaisolates obtained from various clinical specimens were identified by conventional microbiological techniques. All these isolates were tested for antimicrobial susceptibility on Muller-Hinton's agar by Kirby-Bauer disk diffusion method as per CLSI guidelines.

Pseudomonas aeruginosaisolates weretested for susceptibility or resistance to Imipenem (10 mcg) &Metallo- Beta-lactamases production bydisc potentiation test with EDTA impregnated Imipenem disc.

Results: Out of 100 Pseudomonas aeruginosa strains,19(19%) wereImipenemresistant, 41(41 %) were MBL producers & 57(57%) were MDR strains .

Conclusion: The prevalence of MBL producers in our study is 41 %which calls for the implementation of continuous surveillance and the judicious selection of antibiotics in clinical practice.Early detection & prompt infection control is important to prevent further spread of MBLs to other gram negative bacilli .

Keywords: Pseudomonas aeruginosa ,Imipenem, MBL production

Introduction

Pseudomonas aeruginosa isolates are responsible for outbreaks of nosocomial infection in different parts of the world. These isolates have also been responsible for serious infections such as septicemia and pneumonia.[1]

Multiple factors contribute to make Pseudomonas aeruginosa a nosocomial pathogen, for example injudicious use of broad spectrum antibiotics, instrumentation and intrinsic resistance of micro-organisms to numerous antimicrobial agents[2].

The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by beta-lactam resistant bacteria.

However carbapenem resistance has been observed frequently innonfermenting bacilli such as Pseudomonas aeruginosa and Acinetobacter spp. The

common form of resistance is mediated by lack of drug penetration (i.e. porin mutation & efflux pump)& carbapenem hydrolyzing beta-lactamases [3].Resistance tocarbapenem is predominantly mediated byMBL, a class B type of beta-lactamases that recognize bivalent metal ions, usually zinc for their activity.[1][4]

The emergence of these MBLs in gram negative bacilli is becoming therapeutic challenge as these enzymes possess high hydrolytic activity that leads to degradation of higher generation cephalosporins.[5][6].Rapid detection of metallo-beta-lactamases production is necessary to modify therapy and to initiate effective infection control to prevent their dissemination. Since Pseudomonas aeruginosais the most common MBL producers & there is no data on prevalence of MBL producers in clinical isolates in our area so the present study was undertaken to know the antibiotic sensitivity pattern , imipenem resistance & the prevalence of metallo-betalactamase (MBL) producing Pseudomonas aeruginosa among various clinical specimens in our hospital.

Materials and Methods:

This study was conducted during Jan 2011 to April 2013.Total 100 strain of Pseudomonas aeruginosa isolated from different samples like Pus, Swab, Urine, Blood ,Body fluid, sputum etc. were identified to the species level by standard microbiological methods .[16]

The isolates were subjected to susceptibility testing against various antibiotics(discs from Himedia, Mumbai) like ceftazidime (30 µg), ticarcillin(75 µg), piperacillin(100 µg), amikacin (30 µg), cefepime (30 µg), cefoperazone (75 µg), ciprofloxacin(5µg), tobramycin (10 µg), netillin(30 µg), gentamicin (10 µg), Levofloxacin (5 µg), meropenem (10 µg) by disc diffusion test &the results were expressed as susceptible or resistant according to interpretative zone diameters recommended by the Clinical and Laboratory Standards Institute (CLSI).[6][16][17]*E.coli* ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as control strains.

All the isolates were selected for the detection of MBL production by disc potentiation test and evaluated for Imipenem resistance & MBL production . No NCCLS recommendations exist for MBL detection and reporting.[8] Various methods has been recommoned for screening MBL producing strains like the modified Hodge test, the double disc synergy test (DDST) and the disc potentiation test .In our study, we used disc potentiation test with EDTA impregnated disc as recommended in recent studies as IMP + EDTA has highest specificity & sensitivity for detection of MBLs as compared to the others.[1][11] For MIC detection of Imipenem the E test strip & micro dilution plate method is recommended[4]

THE DISC POTENTIATION TEST [9]

We used the a lawn culture of the test strain which was done on Mueller Hinton (MH) agar plates (opacity adjusted to 0.5 McFarland's standard). Two imipenem discs were placed on inoculated plates wide apart i.e. EDTA impregnated Imipenem disc (10/750ug) and imipenem only disc (10ug). After overnight incubation, an increase in zone size of ≥ 7mm around the Imipenem-EDTA disc as compared to the Imipenem only disc was recorded as a positive result for the presence of MBL.

Observation &Results

A total number of 100 P.aeruginosa strains were isolated from different clinical specimens during the study period.Table no:1 shows antibiotic resistance ofP.aeruginosa strains to different antibiotics.

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TABLE NO: 1

Sr. No	**Antibiotic**	**Sensitive strains**	**Resistant strains**
1	Ceftazidime	67	33
2	Ticarcillin,	58	42
3	Piperacillin	65	35
4	Amikacin	78	22
5	Cefepime	72	28
6	Cefoperazone	65	35
7	Ciprofloxacin	72	28
8	Tobramycin	76	24
9	Netillin,	63	37
10	Gentamicin	73	27
11	Leofloxacin	83	17
12	Meropenem	81	19

In our study, out of 100 Pseudomonas aeruginosa strains , 81 wereImipenem sensitive & the prevalence of MDR P. aeruginosa strains were 57 %.Among the 100 P.aeruginosa clinical isolates, 41 (41%) were confirmed to be MBL producers bythe disc potentiation test. Out of the 41MBL producing P.aeruginosa strains, 26(63.4%) were isolated from pus and wound swab and 6(14.6%) were isolated from urine &5(12.1%)from sputum samples.Table no: 2 shows the isolation of MBL producing P.aeruginosa strains from different clinical isolates.

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TABLE NO: 2

Sr. No	Sample	No. Of samples	MBL producing strains
1	Pus &swab	58	26
2	Urine	17	6
3	Blood	10	2
4	Sputum	9	5
5	Bone biopsy	1	0
6	Pleural fluid	4	2
7	Gastric aspirate	1	0
Total		100	41

Discussion

Pseudomonasaeruginosa producing MBLs was first reported from Japan in 1991.Since then they have been reported from various parts of the world including Asia, Europe, Australia.[6]MBL production is a significant problem in hospital isolates of P.aeruginosa. With increasing isolation of ESBL-producing isolates in the hospitals needs the use of carbapenemes , problem of MBL production is increasing.[17]

Out of 100 pseudomonas strains, high drug resistance i.e.42% was found toticarcillin while B.Behera repo-rted highest i.e. 63% resistance toticarcillin. Out of 100 pseudomonas strains, 37 (37%) were resistant tonetillin , 35(35%) were resistant to piperacillin & cefoperazone. In our study 33(33%) pseudomonas strains were resistant to ceftazidime which correlates with Pitt.et. al[8],who reported 39.6% resistance to ceftazidime.Among the total isolates the less resistance was found to cefepime& ciprofloxacin (28%), gentamicin (27%),

Tobramycin (24%) . Moniri R et al [14]reported 27.9 % resistanceto gentamicin which correlates to our study.Out of the 100 isolates, the lowest resistance was found to amikacin (22%) &mero-penem(19%),Levofloxacin(17 %) . Moniri R et al[14] reported 23.2 % resistance to amikacin ,which correlates to our study.Agarwal et al reported 8.05% resistance to Imipenem .Madhu Sharma et al[7] reported high drug resistance i.e. 37.9 % to Imipenem in their study. In various studies across the world varying resistance(4-60%) has been seen towards imipenem and meropenem[6]

In our study the prevalence of MDR P.aeruginosa strains were 57 %, which is nearby to studies conducted by Madhu Sharma et al[7]&Moniri R et al[14] i.e. 62% & 73.9 % respectively. Out of 57 MDR strains ,19 were resistance to meropenem. Of 57 MDR strains 17 were resistant to only2 to 3 antimicrobial agents ,20 were resistant to 4 to 7 antimicrobial agents & 20 were resistant to 8 or more antimicrobial agents .This is an emerging threat & a matter of concern for physicians.

Among the 100 P.aeruginosa clinical isolates, 41 (41%) were confirmed to be MBL producers bythe disc potentiation test.[9]Attal RO et al [9] reported lowest (11.4%) MBL producers in their study whileB.Behera et al [11]&Madhu Sharma et al[7] reported highest i.e.61.5% & 69.5% MBL producers in their study . All 19 strains which were resistance to meropenem showed MBL Production.

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To conclude there is emergence of multidrug resistant strainsof Pseudomonas aeruginosa(57%)in our hospital, some of them are Imipenemresistant (19 %)& there is emergence of MBL producers(41%) P.aeruginosain our hospital.This calls for the implementation of continuous surveillance and the judicious selection of antibiotics in clinical practice.Early detection & prompt infection control is important to prevent further spread of MBLs to other gram negative bacilli .The Disc potentiation test is easy to perform, is cost effective and quite specific amongst other phenotypic methods which are used for MBL detection. The Disc potentiation test can also be done along with routine antibiotic sensitivity tests.[9]

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