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DETECTION OF IMIPENEM RESISTANT AND METALLO BETA LACTAMASE PRODUCING *PSEUDOMONAS AERUGINOSA* IN A TERTIARY CARE HOSPITAL.

Microbiology

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ABSTRACT

Context- *Pseudomonas aeruginosa* is acquiring drug resistance even with imipenem according to recent studies and therefore a study was required to know the drug resistance status in the area .

Aims: The objectives of the study are to isolate the imipenem resistant *Pseudomonas aeruginosa* and Metallo beta lactamase producing *Pseudomonas aeruginosa* in tertiary care hospital

Methods and Material: 200 isolates of *Pseudomonas aeruginosa* were studied. The colonies were tested for biochemical tests, identification was done and then put to Antibiotic susceptibility test and MBL confirmation done by combined disc method.

Results: It may be observed from the study that *Pseudomonas aeruginosa* have only 10.5% imipenem resistance and out of this only 23.80% strains were Metallo beta lactamase producers.The imipenem resistance and MBL producing *Pseudomonas aeruginosa* were found more prevalent in male patients. The imipenemresistane was observed maximum in 21-40 years age bracket where as maximum MBL producers strains were detected in 41-60 years age of patients. Imipenem resistance was maximum found in the isolates drawn from surgery dept. whereas Urology dept. recorded highest percentage of MBL producer. Urine and Tissue specimens had maximum imipenem resistant isolates where as Blood samples were highest MBL producers in the study.

Conclusions: It may be concluded from the study that *Pseudomonas aeruginosa* have only 10.5% imipenem resistance in the area and out of this only 23.80% strains were Metallo beta lactamase producers. The age wise analysis of imipenem resistance and MBL producers may also provide an idea to clinicians .

KEYWORDS

MBL, Metallo beta lactamase, *Pseudomonas aeruginosa*

Introduction:

Pseudomonas aeruginosa is recognized as an emerging opportunistic pathogen of clinical relevance that causes infections in hospitalized patients particularly in burn patients, orthopedic related infections, respiratory disease, immunosuppressed and catheterized patients.[1] *Pseudomonas aeruginosa* is one of the important bacterial pathogens causing various infections in hospitals.[2]

Carbapenems are the antibiotics of choice for severe Pseudomonal infections.[3] However the resistance to this novel antibiotic is increasing worldwide.[4] Carbapenem resistance in *Pseudomonas aeruginosa* is most commonly due to production of metallo beta lactamase.[5]MBLs are broad spectrum enzymes that hydrolyze most beta lactam antibiotics except monobactums[6], Emergence of MBL producing *Pseudomonas aeruginosa* in the hospitals is alarming. Mortality rates are significantly higher in MBL producing *Pseudomonas aruginosa* compared to non MBL Pseudomonas aeruginosa.[7]

Knowing the resistance pattern of *Pseudomonas aeruginosa* and prevalence of MBLs producing strains will guide the clinicians in prescribing proper antibiotics and controlling infections caused by *P.aeruginosa.*[8]

Subjects and Methods: **Study design**-Cross sectional
Study area-Microbiology laboratory of a tertiary care center where Clinical samples were processed.

Period of study- 18 months
Sample size-Study was carried out on 200 isolates of *Pseudomonas aeruginosa* isolated from various clinical samples.

Processing of samples:
Method: All the clinical samples including pus, respiratory samples, tissue, urine and blood received in the Microbiology laboratory for culture and sensitivity were processed. After initial Gram staining ,they were inoculated on blood agar and MacConkey's agar. The inoculated culture plates were incubated aerobically at 37 ^{0} Cfor 24 hours.[12] Antibiotic susceptibility was performed on MH agar plates by Kirby baur's disc diffusion method as per CLSI guidelines[9,10]

All isolates of *Pseudomonas aeruginosa* were tested for antibiotic sensitivity testing with Piperacillin, Pipercillin+Tazobactum, Ceftazidime, Ceftrioxone, Cefepime, Gentamicin, Tobramycin, Netilmycin , Amikacin, , Ciprofloxacin, Imipenem, Merpenem, Colistin and Polymyxin.

Imipenem resistant isolates were further tested for Metallo beta lactamase production.[11]

Specimens processed were blood, urine, pus, wound swab, body fluids etc. The results were recorded and interpreted as per CLSI guidelines.[9,10] *Pseudomonas aeruginosa* ATCC 27853 was used as a quality control.

Imipenem-EDTA Combined disc method - Used as confirmatory test for MBL production.[11,12] A lawn culture of the isolate was prepared. After allowing it to dry for 5 minutes, Two Imipenem discs, one with 0.5 M EDTA (Hi media) and the other plain Imipenem disc were placed on the surface of agar plates approximately 30 mm apart. Plates were incubated for 24 hours at 37 $^{0C.}$ An increase in the zone diameter = or > 7mm around Imipenem + EDTA disc in comparison to Imipenem disc alone has been considered as production of MBL[11,13]

Results: Out of 200 isolates, 21 isolates of *Pseudomonas aeruginosa* showed resistance to Imipenem antibiotic(10.5%).(**Table 1**) Out of 21 imipenem resistant ,only 5 (23.80%) were found Metallo beta lactamase producers.(**Table 2)** In present study 11.11% were male and 9.23% were female patients showed imipenem resistance(**Table3**).The maximum 18.18% imipenem resistance was observed in 21-40 years age of patients followed by 12.34% in more than 60 years age and 4% in 41-60 years and 0-20 years patients . (**Table 4)** Surgery dept. recorded maximum 21.05% imipenem resistance, followed by ICU(15.51%),Urology 9.09%,Medicine 6.06% and ENT 4%(**Table 5**).Urine and Tissue samples showed maximum 20% imipenem resistance followed by blood (11.11%),ETT (9.09%),Pus 6.67% (**Table 6).** In the present study 4 out of 15 (26.67%) were male and 1 out 6 (16.67%) were female patients ,which were imipenem resistant and MBL producers(**Table 3**).The highest 50% MBL producers were in the age group of 41-60 years followed by 37.5% in age group of 21-40 years and 10% in the age above 60 years (**Table 4**). Urology dept. recorded maximum 100%MBL producers followed by Surgery dept. (37.5%) and then ICU (11.11%)(**Table 5).**The maximum 100% MBL producers were found in blood sample followed by Urine (25%) and then Pus (20%) samples**.(Table 6**)

Discussion: *Pseudomonas aeruginosa* has one of the broadest ranges of infectivity among all pathogenic microorganisms. In the past few decades, it has been increasingly recognized as a pathogen in a variety of serious infections in hospitalized patients especially with impaired immune defenses .It exhibits intrinsic resistance to a variety of antimicrobials including beta lactams. Metallo-β-lactamase (MBL)

producing *P. aeruginosa* is an emerging threat and a cause of concern for treating physicians.

In order to select the correct antibiotic for treatment and to prevent dissemination of such infection, it is needed to study the antimicrobial susceptibility pattern in *P. aeruginosa* isolates.[14]

Imipenem resistance in Pseudomonas aeruginosa:

Pseudomonas aeruginosa is one of the most frequent nosocomial pathogens and the infections due to these are often difficult to treat due to antibiotic resistance.[15] carbapenem are beta lactam antibiotics, presently considered as the most potent agents of treatment of multidrug resistant gram negative bacterial infection due to the stability of these agents against the majority of β-lactamases and their high rate of permeation through bacterial outer membranes.[16] The carbapenemes have been drug of choice for the treatment of serious infection caused by gram negative bacterial infections.[17] Pseudomonas shows resistance to carbapenem due to decrease outer membrane permeability, increased efflux system, alteration penicillin binding proteins and carbapenem hydrolyzing enzymes carbapenemase.[18]

In various studies across the world, varying rates of resistance (4-60%) have been reported for imipenem.[19] In the present study the imipenem resistance was found to be 10.5%(**Table 1**) which is close to the study carried out Bimla banjare et al. In their study on 239 *P.aeruginosa* isolates, they found 10.46% resistance to imipenem.[20] In 2008 Agrawal G., R.B Lodhi et al. [14] in their study reported 8.05 % imipenem resistance. The resistance with imipenem antibiotic was 16.0% in the study carried out by Hemlatha V.Uma Sekar et al. in the year 2004**.** [21]

While a higher incidence (42.8%) was reported by Gladestone *et al* among *P.aeruginosa* isolates.[18]Awari Abhijit et al also noted a higher incidence (55%) among their Pseudomonas isolates.[22] This could be due to the use of a large number of broad spectrum antibiotics in their patients. Comparatively the percentage is less (10.5%) in the present study which signifies the controlled use of broad spectrum antibiotics, large share of samples from outdoor patients .The other reasons for imipenem resistance variation are isolate-to-isolate variations, suggesting an adaptation of the strain to local or individual selective conditions. Some of the genetic and phenotypic variations are also associated with drug resistance.[23]

In present study 11.11% were male and 9.23% were female patients showed imipenem resistance(**Table3**).21-40 years age group recorded maximum(18.18%) imipenem resistance followed by 12.34% in >60 years patients**.(Table 4)** Surgery dept. recorded maximum 21.05% imipenem resistance, followed by ICU(15.51%),Urology 9.09%,Medicine 6.06% and ENT 4%(**Table 5**).Urine and Tissue samples showed maximum 20% imipenem resistance followed by blood (11.11%),ETT (9.09%),Pus 6.67% (**Table 6)**

Metallo-beta-lactamase producing Imipenem resistant *Pseudomonas aeruginosa*

P. aeruginosa is also notoriously known worldwide for developing antimicrobial resistance, thus jeopardizing the treatment option and sealing the patient's fate. Like many other gram negative bacteria which have developed multi drug resistance, Carbapenems became the mainstay of therapy to treat this pathogen.[24]

However million dollars of pharmaceutical research were undone by swift strokes of genetic evolution in *P. aeruginosa*, and they developed Carbapenem resistance.

To resist carbapenems, these gram negative bacilli have started producing two types of enzymes: serine carbapenemases, and metallo-beta-lactamases (MBLs).[25] These enzymes can hydrolyze not only carbapenems but many β lactams as well .[26] The genes responsible for production of MBLs lie on a plasmid, and hence can be horizontally transferred easily, efficiently and rapidly to other bacteria.

In our study out of 21 Imipenem resistant Pseudomonas aeruginosa 5 (23.8%) were metallo-beta –lactamase producers.(**Table 2**) In a recent study by Nisha et al in 2016 [27] almost similar incidene (23%) was reported, while Arunva Kali et al.[28] reported very high incidence (72.70%) of MBL producers in their imipenem resistant *P.aeruginosa*.

Comparative Studies on Imipenem Resistance Pattern and MBL Producing Pattern in *Pseudomonas aeruginosa*

Study series	Year of study	Imipenem resistance %	MBL+ VE %
Present study	2017	10.5%	23.80%
Nisha .karanje, Soumya singh,. Kolhapur[27]	2016	-	23.0%
ArunvaKali, Sreenivasan S. et al Pondicherry [28]	2013	22.4%	72.70%
Awari Abhijit, Nighute Sunita. Ahmedabad [22]	2012	55.0%	69.09%
Varaiya Ami, Nikhil kulkarni, Mumbai 2007[15]	2008	25.0%	20.80%
Agrawal G,R.B. Lodhi et al[14]	2008	8.05%	85.71%
Hemlatha V.Uma Sekar et al Chennai [21]	2004	16.0%	87.5%

In the present study 4 out of 15 (26.67%) were male and 1 out 6 (16.67%) were female patients ,which were imipenem resistant and MBL producers(**Table 3**).The highest 50% MBL producers were in the age group of 41-60 years followed by 37.5% in age group of 21-40 years and 10% in the age above 60 years (**Table 4**). Urology dept. recorded maximum 100%MBL producers followed by Surgery dept. (37.5%) and then ICU (11.11%)(**Table 5**).The maximum 100% MBL producers were found in blood sample followed by Urine (25%) and then Pus (20%) samples**.(Table 6**)

Conclusion- The genetic evolution of *Pseudomonas aeruginosa* is taking place causing drug resistance to almost all antibiotics including carbapenems which is a cause of concern to the clinicians. The resistance rate of imipenem varies place to place depending upon rationality in use of antibiotics , isolate-to-isolate variations, an adaptation of the strain to local or individual selective conditions.

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Table 1- Imipenem resistant *Pseudomonas aeruginosa-*

Total No of isolates	Isolates sensitive to imipenem No. (%)	Isolates resistance to Imipenem No. (%)
200	179 (89.5%)	21 (10.5%)

Note - Only 21 out of 200 (10.5%) isolates were imipenem resistant .

Table 2- MBL producing Pseudomonas aeruginosa out of Imipenem resistant isolates .

Total Imipenem resistant *Pseudomonas aeruginosa*	MBL Non producers No. (%)	MBL Producers No. (%)
21(100%)	16 (76.20%)	05 (23.80%)

Note - Out of 21 Imipenem resistant Pseudomonas aeruginosa 5 (23.8%) were MBL producers.

Table 3- Sex wise Imipenem Resistance and MBL *producing Pseudomonas aeruginosa*

Gender	No. of samples	ImipenemResist.(No)	Imipenem Resist(%)	MBL +ve (No)	MBL +VE (%)
Male	135	15	11.11%	04	26.67%
Female	65	06	09.23%	01	16.67%
Total	200	21	10.50%	05	23.80%

Note -15 out of 135(11.11%) isolates of male patients were imipenem resistant, 4 Out of 15(26.67 %)were MBL producers . 6 out of 65(9.23 %) isolates were imipenem resistant in female patients , only 1out of 6 (16.67%) was MBL producer.

Table 4-Age based Imipenem resistant and MBL producing *Pseudomonas aeruginosa*

AGE IN YEARS	Total Sample (No)	Imipenem Resist. (No)	Imipenem Resist (%)	MBL +VE (No)	MBL +VE (%)
0- 20	25	01	04.00%	00	00.00%
21-40	44	08	18.18%	03	37.5%
41 -60	50	02	04.00%	01	50.00%
>60	81	10	12.34%	01	10.00%
TOTAL	200	21	10.50%	05	23.80%

Note- The maximum 8 out of 44 (18.18%) imipenem resistant were detected in the patients in the age group of 21-40 years.10 out of 81 (12.34%)were detected form more than 60 years age of patients. The lowest 1 out of 25(4%) imipenem resistant were detected from 0-20 years patients.The maximum 1 out of 2 (50%) imipenem resistant isolates were MBL producers in 41-60 years age . 3 out of 8(37.5%) imipenem resistant isolates were MBL producers in 21-40 years age of patients . 1 out of 10 (10%) in > 60 years age was MBL producer .There was no MBL producer detected up to 20 years age .

Table-5 Department wise Imipenem Resistant samples and MBL producers *Pseudomonas aeruginosa*

S.No	Depart.	Total sample	Imipenem Resist (NO)	Imipenem Resist.%	MBL +VE (No)	MBL +VE (%)
1	ICU	58	09	15.51%	01	11.11%
2	Surgery	38	08	21.05%	03	37.5%
3	Medicine	33	02	06.06%	00	00%
4	ENT	25	01	04.00%	00	00%
5	Urology	11	01	09.09%	01	100%
6	OBG	11	00	00.00%	00	00%
7	TB Chest	15	00	00%	00	00%
8	Others	09	00	00%	00	00%
	Total	200	21	10.50%	05	23.80%

Note- Imipenem resistance detected maximum 21.05% in cases of surgery department followed by 15.51% in ICU . No imipenem resistant were detected from OBG and TB Chest department. 100% MBLproducers isolates detected from the imipenem resistant isolates from Urology dept.37.5% imipenem resistant isolates from surgery dept. were MBL producers. ICU recorded 11.11% MBL producers

Table-6 Imipenem Resistance and MBL producing *Pseudomonas aeruginosa* based on specimens

Sample	Total No	Imipenem Resistant (NO)	Imipenem Resistant (%)	MBL +VE (No)	MBL +VE (%)
Pus	75	05	06.67%	01	20%
Urine	60	12	20.00%	03	25%
Sputum	24	00	00.00%	00	00%
ETT	11	01	09.09%	00	00%
Tissue	10	02	20.00%	00	00%
Blood	09	01	11.11%	01	100%
others	11	00	00.00%	00	00%
TOTAL	200	21	10.50%	05	23.80%

Note-Imipenem resistance 12 out of 60 samples(20%) from urine, 5 out of 75 (6.67%) from Pus .2 out of 10 (20%) from Tissue and 1 out of 9 (11.11%) from blood sample were detected. 03 out of 12 (25%) imipenem resistant found MBL+VE in Urine sample. One each sample from blood and pus were MBL+VE.

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