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Occurrence of Metallo-B-Lactmases Producing *Pseudomonas aeruginosa* among Clinical Isolates

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Original Research Article

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Abstract: Pseudomonas aeruginosa is an increasingly prevalent opportunistic human pathogen and the most common gram negative bacterium found in nosocomial infection. Production of metallo-β- lactamase enzyme in Pseudomonas aeruginosa usually results in high level resistance to most β-lactamases and a rapid spread of MBL producing major gram negative pathogens is a matter of particular concern worldwide. To identify the metallo –β-lactmase production by double disk synergy test, combined disk diffusion method and modified hodge test as described further. Out of 514 samples, on the basis of gram staining , 108(21.01%) gram positive cocci, 296(57.58%) gram negative bacilli, 89(17.31%) no organism seen and 21(4.08%) were yeast.On the basis of culture of 89 samples , 11 (12.35%) were gram positive cocci, 23 (25.84%) were gram negative bacilli, 55(61.81%) were showed no growth. In 319 samples of gram negative bacilli, 100 were isolates of Pseudomonas aeruginosa and in which 18 were found to be producing metallo β-lactmases. This study suggests metallo-beta-lactmase production in pseudomonas aeruginosa is emerging threat in hospital isolates because it is crucial for the optimal treatment of patients.
Keywords: Metallo-beta-lactamase & Pseudomonas.

INTRODUCTION

Pseudomonas aeruginosa is increasingly prevalent opportunistic human pathogen and the most common gram-negative bacterium found in nosocomial infection. Despite improvement in antbiotic therapy, *Pseudomonas aeruginosa* is intrinsically resistant to a number of antimicrobial agents [1].

Being an opportunistic human pathogen, *Ps. aeruginosa* is also an opportunistic pathogen of plants [2]. *Ps. aeruginosa* is the most common species of the genus *Pseudomonas* [3].*Ps. Aeruginosa* produces a variety of pigments, including pyocyanin (blue green), pyoverdine (yellow green and fluorescein) and pyorubin (red brown), pyomelenine(black). King Ward and Raney developed *Pseudomanas* Agar P (King A medium) for enhancing pyocyanin and pyorubin production and *Pseudomonas* Agar F (King B medium) for enhancing fluorescein production [4].

Pseudomonas aeruginosa is gram negative aerobic non fermentative oxidase positive bacilli which is motile by polar flagella. It produces distinctive bluish-green water soluble pigment pyocyanin which distinguishes it from other fluorescent groups [5,6].

Ps. aeruginosa is important causative agent of nosocomial infection due to following reasons:

- It is resistant to commonly used antibiotics and antiseptics.
- It can survive and multiply even with minimal nutrients, if moisture is available.
- It can contaminate equipment such as respirators, endoscopes and articles such as bed pans and medicines such as lotions, ointments and eye drops.

Ps. aeruginosa causes external otitis, folliculitis acquired in swimming pools, keratitis following contact lens use of minor trauma, endocarditis in intravenous drugs abusers, opportunistic blood stream infection and hospital acquired infections like ventilator-associated pneumonia (VAP) in intubated patients, wound infections in burn patients

and *Ps. aeruginosa* bacteraemia and septicaemia which may lead to the death of the patients[6,7].

Gram-negative bacilli can develop resistance to β-lactam antibiotics by three mechanisms:

- Alteration of the antimicrobial target receptor molecule in the bacteria.
- Decreasing the accessibility of the antimicrobial to the target by altering the entry of the antimicrobial into the cell or increasing the removal of the antimicrobial from the cell.
- Destruction or inactivation of the antimicrobial [8].

Carbapenem are often used as antibiotics of last resort for treating infections due to multi-drug resistant Gram-negative bacilli. But, this scenario is changing with the emergence of metallo-β-lactamase (MBL) producing strains. The MBL_S can efficiently hydrolyze all β-lactam antibiotics except aztreonam. MBL producing Gram negative bacilli, especially *Pseudomonas aeruginosa* have been increasingly reported in Asia, Europe, Latin American and the United States. Therefore, detection of MBL producing Gram negative bacilli is crucial for the optimal treatment of patients and to control the spread of resistance [9-11].

So the aim of the study was to isolate and identify *Pseudomonas aeruginosa* from various clinical samples. To compare 3 phenotypic methods in detection of MBL producing *Pseudomonas aeruginosa* namely Imipenem-EDTA combined disk test, Imipenem-EDTA double disk synergy test and modified Hodge test. To detect the Metallo-β-lactamase production in *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Study site

The study was conducted in the bacteriology section of Microbiology department at TMMC& RC MORADABAD on 100 blood samples which were collected from suspected malaria cases from january 2016 to june 2017.

The various Samples were collected with all aseptic precautions & transported to the microbiology laboratory & cultured as per standard protocol. Pseudomonas aeruginosa strains were identified by conventional phenotypic identification scheme.

The samples were inoculated on MacConkey agar, Blood agar, and Nutrient agar and incubated for 24 hours at 37^0 C. After 24 hours of incubation the culture plates were examined for growth. From these colonies smear was prepared for Gram's staining & Oxidase test was done and oxidase positive colonies were further processed.

Several phenotypic methods are available for the detection of MBL producing bacteria. All these methods are based on the ability of metal chelators such as EDTA and thiol-based compounds including mercaptoacetic acid, 2-mercaptopropionic acid, and mercaptoethanol were used, because these agents have been reported to block metallo-β-lactamase. Inhibition of enzyme activity by EDTA is an important characteristic used to distinguish MBLs from other β-lactamases [12].

The following tests were done for detection of metallo-β-lactamase production:-

- The double disk synergy test[13]
- Combined disc diffusion method[14]
- The modified hodge test (mht)[15]

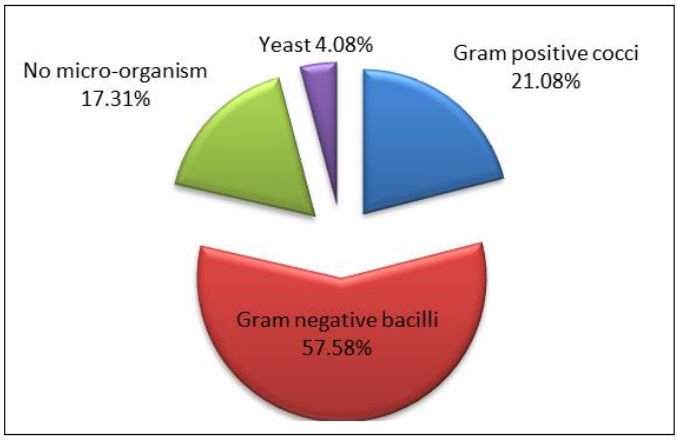
RESULTS

Out of 514 samples, on the basis of gram staining, they were categorized into 108(21.01%) gram positive cocci, 296(57.58%) gram negative bacilli, 89(17.31%) no organism seen and 21(4.08%) were yeast.(See Table 1, graph1).On the basis of culture of 89 samples , 11 (12.35%) were gram positive cocci, 23 (25.84%) were gram negative bacilli, 55(61.81%) were showed no growth. (See Table 2, graph 2). In 319 isolates of gram negative bacilli. 100 isolates of *Ps. aeruginosa*, 58 (58%) were male patients and 42 (42%) were female patients. (See Table3, Graph3) and the majority of patients distributed among age groups between 21- 40 years of age 42(42%), followed by 28(28%) among age groups more than 60years, 17(17%) were between 41-60 years of age and 13(13%) were less than 20 years of age (See Table4, Graph4). In 100 isolates the maximum no. of strains were isolated from IPD 68(68%) especially from general ward 42(42%) followed by burn patients 21(21%), ICU 5(5%) and in OPD 32(32%) strains were isolated. (See Table 5, Graph 5). Out of 100 isolates of *Pseudomonas aeruginosa* in OPD 32(32%) majority of strains from pus & other wound discharges 8(25%) followed by swab from burn patients 4(12.50%), sputum 4(12.50%), ear swabs 12(37.50%), urine 4(12.50%) and in IPD 68(68%) majority of strains from pus & other wound discharges 32(47.05%) followed by swab from burn patients 20(29.41%), sputum 10(14.70%), blood culture 4(5.88%), throat swab 2(2.94%)(See Table 6,Graph 6). Incidence of MBL positivity was 18 %(See Table 7,Graph 7).

Table-1: Showing distribution of microorganisms isolated from samples

Total samples	Gram positive cocci (%)	Gram negative bacilli (%)	No microorganism seen (%)	Yeast (%)
514	108 (21.01%)	296 (57.58%)	89 (17.31%)	21 4.08%)

Table 1 shows percentage distribution of microorganisms like gram positive cocci (21.01%), gram negative bacilli (57.58%), no organism seen (17.31%) and yeast (4.08%).

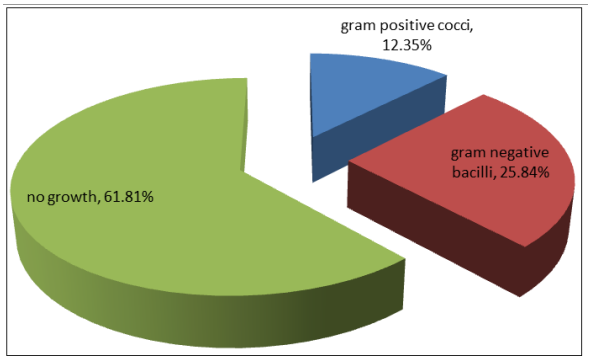


Graph 1- Pie chart showing distribution of microorganisms like gram positive cocci (21.01%), gram negative bacilli (57.58%), no organism seen (17.31%) and yeast (4.08%).

Table-2: showing distribution of organisms on the basis of culture (n=248)

No microorganisms seen (%)	Gram positive cocci (%)	Gram negative bacilli (%)	No growth (%)
89	11 (12.35%)	23 (25.84%)	55 (61.81%)

Table 2 showing percentage distribution of organisms on the basis of culture like gram positive cocci (12.35%), gram negative bacilli (25.84%) and no growth (61.81%)



Pie chart 2 shows percentage distribution of organisms on the basis of culture like gram positive cocci (12.35%), gram negative bacilli (25.84%) and no growth (61.81%).

Table-3: Sex Distribution of Patients from which *Ps. Aeruginosa* Isolated

Patients (Sex)	Number	Percentage (%)
Male	58	58%
Female	42	42%
Total	100	100%

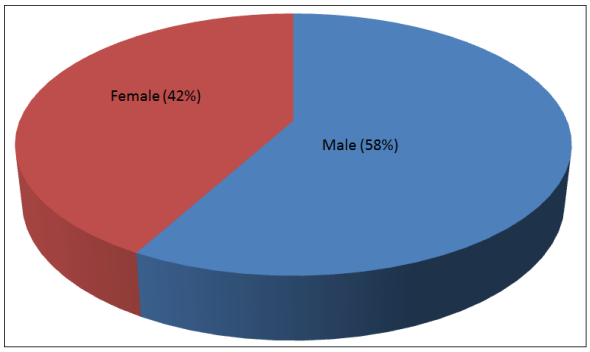


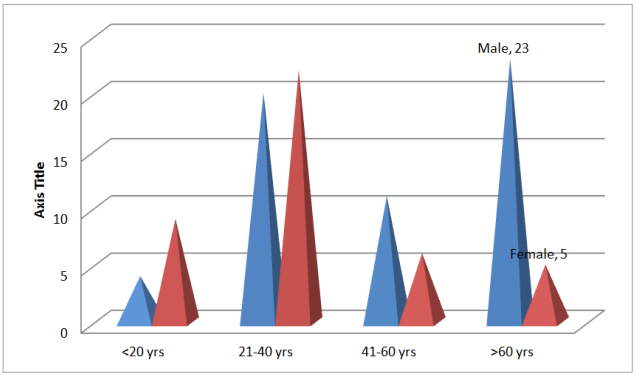
Table-3: showing, percentage distribution of patients on the basis of sex from which Ps. aeruginosa isolated like male (58%) and female (42%)

Pie chart 3 shows, percentage distribution of patients on the basis of sex from which *Ps. aeruginosa* isolated like male (58%) and female (42%).

Table 4 shows, out of 100 isolates of *Pseudomonas aeruginosa* majority of patients distributed among age groups between 21- 40 years of age 42(42%), followed by 28(28%) among age groups more than 60years, 17(17%) were between 41-60 years of age and 13(13%) were less than 20 years of age.

Table-4: Age Distribution among Patients with MDRPA Infection

Age Group	Total patients	Male		Female	
		Case	Percentage (%)	Case	Percentage (%)
<20 yrs.	13	4	6.89%	9	21.42%
21-40 yrs.	42	20	34.48%	22	52.38%
41-60yrs.	17	11	18.96%	6	14.28%
>60yrs.	28	23	39.65%	5	11.90%
Total	100	58	58%	42	42%



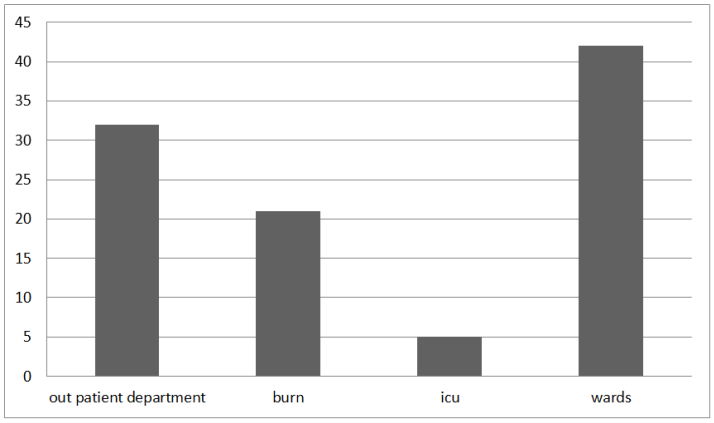
Bar chart 4 showing, out of 100 isolates of *Pseudomonas aeruginosa* majority of patients distributed among age groups between 21- 40 years of age 42(42%), followed by 28(28%) among age groups more than 60years, 17(17%) were between 41-60 years of age and 13(13%) were less than 20 years of age.

Table-5: Distribution of Pseudomonas among Patients of IPD/OPD/ ICU/Burn (n= number of strains)

Organism	Out Patient Department	In Patient Department		
		Burn	ICU*	Wards
Ps. aeruginosa (n=50)	32 (32%)	21 (21%)	5 (5%)	42 (42%)
Total	32(32%)	68(68%)		

***ICU- Intensive Care Unit**

Table 5 shows, out of 100 isolates of *Pseudomonas aeruginosa* the maximum no. of strains were isolated from IPD 68(68%) especially from general ward 42(42%) followed by burn patients 21(21%), ICU 5(5%) and in OPD 32(32%) strains were isolated.

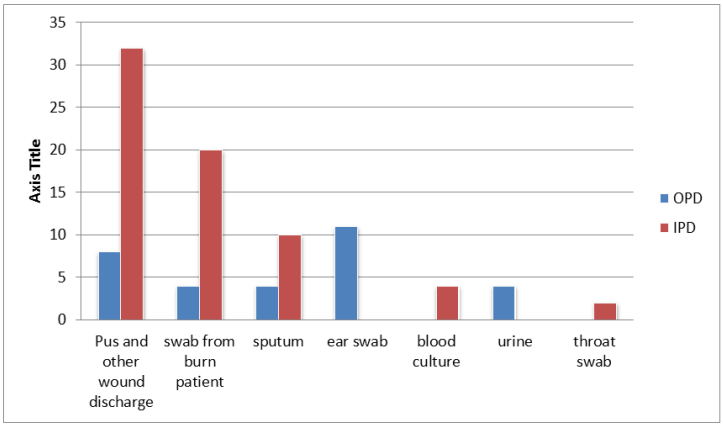


Bar chart 5 showing, out of 100 isolates of *Pseudomonas aeruginosa* maximum no. of strains were isolated from IPD 68(68%) especially from general ward 42(42%) followed by burn patients 21(21%), ICU 5(5%) and in OPD 32(32%) strains were isolated.

Table-6: Distribution of Samples Received from OPD's (n= 32) and IPD's (n=68)

S. No	Sample	Samples from OPD		Samples from IPD	
1		Number	Percentage (%)	Number	Percentage (%)
2	Pus & other wound discharges	8	25%	32	47.05%
3	Swab from burn patients	4	12.50%	20	29.41%
4	Sputum	4	12.50%	10	14.70%
5	Ear swabs	12	37.50%	-	-
6	Blood Culture	-	-	4	5.88%
7	Urine	4	12.50%	-	-
8	Throat Swab	-	-	2	2.94%
	Total	32	32%	68	68%

Table 6 shows, out of 100 isolates of *Pseudomonas aeruginosa* in OPD 32(32%) majority of strains from pus & other wound discharges 8(25%) followed by swab from burn patients 4(12.50%), sputum 4(12.50%), ear swabs 12(37.50%), urine 4(12.50%) and in IPD 68(68%) majority of strains from pus & other wound discharges 32(47.05%) followed by swab from burn patients 20(29.41%), sputum 10(14.70%), blood culture 4(5.88%), throat swab 2(2.94%).



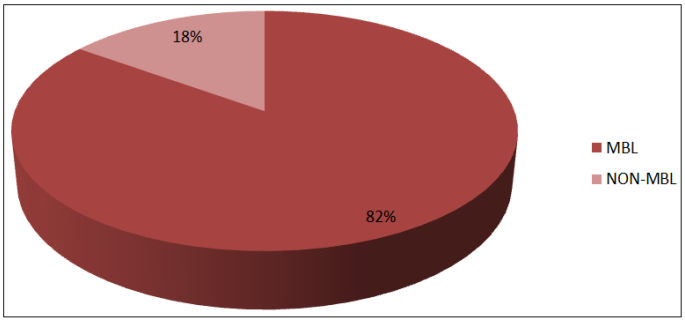
Bar chart 6 showing, out of 100 isolates of *Pseudomonas aeruginosa* in OPD 32(32%) majority of strains from pus & other wound discharges 8(25%) followed by swab from burn patients 4(12.50%), sputum 4(12.50%), ear swabs 12(37.50%), urine 4(12.50%) and in IPD 68(68%) majority of strains from

pus & other wound discharges 32(47.05%) followed by swab from burn patients 20(29.41%), sputum 10(14.70%), blood culture 4(5.88%), throat swab 2(2.94%).

Table 7 shows, out of 100 isolates of *Pseudomonas aeruginosa* incidence of MBL positivity was 18%.

Table-7: Prevalence Of MBL Producing Ps. aeruginosa

Total number of strain	Positive for MBL number	Percentage for MBL (%)
100	18	18%



Pie chart 7 shows out of 100 isolates of *Pseudomonas aeruginosa* incidence of MBL positivity was 18%.

DISCUSSION

Out of 100 isolates of *Pseudomonas aeruginosa*, 18 isolates were found to be positive for metallo beta lactamases production which is similar to the 16% MBL production studies by Hemalata *et al.* [16] 58(58%) males and 42(42%) females were positive in 100 isolates of *Pseudomonas aeruginosa* and which is similar to the study Chickmagalure Shivaswamy[17] Vinod Kumar, *et al*. incidence of *Pseudomonas aeruginosa* among male 32 (59.3%) is higher than female 22 (40.7%) and Deeba Bashir *et al*. [18], incidence of Ps. *aeruginosa* strains among male 155 (54.8%) is higher than female 128 (45.2%). In our study majority of *Pseudomonas aeruginosa* strains were isolated from IPDs (68%), which were mainly from pus and other wound discharge (47.05%), than followed by swab from burn patients (29.41%), sputum (14.70%), blood culture (5.88%), throat swab (2.94%), which is similar to the study of jay kumar *et al* [19]. & Behera *et al* [20]. In our study the majority of patients distributed among age groups between 21- 40 years of age 42(42%), followed by 28(28%) among age groups more than 60years, 17(17%) were between 41-60 years of age and 13(13%) were less than 20 years of age. which is similar to the study of Ramprasad balikaran *et al* [21].

CONCLUSION

Out of 100 strains majority of *Ps. aeruginosa* were isolated from male patients. From inpatient department (IPD) more numbers of *Pseudomonas aeruginosa* strains were isolasted then outpatient department (OPD). In OPD samples maximum incidence of *Ps. aeruginosa* was found in ear swab while in IPD it was maximum in swab from pus. MBL production was seen in 18 out of 100 strains of *Pseudomonas aeruginosa*. Metallo-beta-lactmases production in pseudomonas aeruginosa is emerging threat in hospital isolates so detection of metallo-beta-lactmases production in pseudomonas aeruginosa is crucial for the optimal treatment of patients.

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