



## ANTIBIOTIC SUSCEPTIBILITY PROFILE AND DETECTION OF METALLO – BETA LACTAMASE IN PSEUDOMONAS AERUGINOSA ISOLATES FROM CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL

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**ABSTRACT** **Background and Purpose:** *Pseudomonas aeruginosa* is an important cause of morbidity and mortality in hospitalized patients. The selection of the most appropriate antibiotic is complicated by the ability of *P.aeruginosa* to develop resistance to multiple classes of antibacterial agents.

**Methods:** The study was conducted for a period of 18 months in a tertiary care hospital. Two hundred isolates of *Pseudomonas aeruginosa* were subjected to antimicrobial susceptibility test. Imipenem –EDTA combined disc method was used as confirmatory test for MBL production.

**Results:** 200 clinical isolates of *P. aeruginosa* were tested for antibiotic susceptibility. The highest resistance (36.5%) was noted for Ceftazidime and Piperacillin and the lowest (3.5%) resistance was observed in Polymyxin – B. Out of 21 isolates of *Pseudomonas aeruginosa* which showed resistance to imipenem, 5 (23.80%) were Metallo-Beta Lactamase (MBL) producers.

**Conclusions:** Knowing the antibiotic susceptibility pattern of *P aeruginosa* will guide the physician in treating the infections caused by *P aeruginosa*.

**KEYWORDS :** *Pseudomonas aeruginosa*, MBL producer, antibacterial agents

### INTRODUCTION

*Pseudomonas aeruginosa* is one of the important bacterial opportunistic pathogens causing various infections in hospitalized patients. Despite advances in medical and surgical care and introduction of wide variety of antimicrobial agents having anti-pseudomonal activities, *Pseudomonas aeruginosa* continues to cause complications in hospital acquired infections which become life threatening. It is increasingly 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.<sup>2</sup>

*P.aeruginosa* is a serious therapeutic challenge for treatment of both community and nosocomial infections and selection of the most appropriate antibiotic is complicated by the ability of *P.aeruginosa* to develop resistance to multiple classes of antibacterial agents even during the course of treatment.<sup>3</sup> Knowing the resistance pattern of *Pseudomonas aeruginosa* and prevalence of Metallo-beta lactamase producing strains will guide the clinicians in prescribing proper antibiotics and controlling infections caused by *P.aeruginosa*.<sup>4</sup>

### MATERIAL AND METHODS

This was a cross sectional study conducted in a Microbiology laboratory at a tertiary care hospital for a period of 18 months. Study was carried out on 200 isolates of *Pseudomonas aeruginosa* from various clinical samples.

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 °C for 24 hours. Following the appearance of bacterial growth, only isolates of *Pseudomonas aeruginosa* were included in the study. Identification was done by Gram stain, colony morphology and standard biochemical tests.

### Antibiotic susceptibility test:

All the isolates of *Pseudomonas aeruginosa* included in the study were subjected to antimicrobial susceptibility test by Kirby Bauer's disc diffusion method as per the CLSI guideline.<sup>5,6</sup>

Following antibiotic discs from HI MEDIA were used for antibiotic susceptibility test:

Piperacillin(10 µg), Piperacillin +Tazobactam(100/10 µg), Ceftazidime(30 µg), Cefepime (10 µg), Imipenem (10 µg),

Meropenem (10 µg), Amikacin (30 µg), Gentamicin (10 µg), Ciprofloxacin(5 µg), Colistin (10 µg), Polymixin

Imipenem –EDTA combined disc method was used as confirmatory test for MBL production.<sup>7,8</sup>

ATCC 27853 strain of *Pseudomonas aeruginosa* was used for quality control.

### OBSERVATIONS-

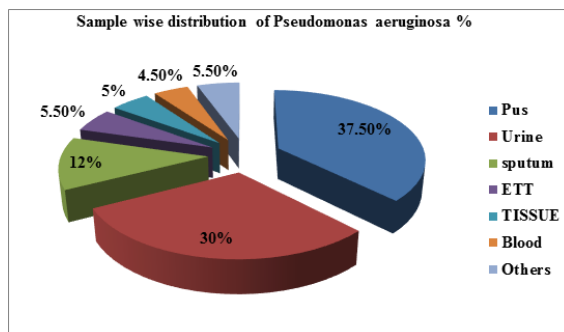
A total number of 200 *Pseudomonas aeruginosa* isolated from different clinical samples were studied. The specimens were non-repetitive and random, demographically distributed. The specimens belonged to all age groups, Male or Female. The clinical specimens received in the Microbiology laboratory of a tertiary health care center were processed.

Out of 200 isolates of *Pseudomonas aeruginosa* 135 (67.50%) were from Male patients and 65 isolates (32.50%) from Female patients. Maximum number of *Pseudomonas aeruginosa* were isolated from the age group of above 60 years (40.50%), followed by 41 to 60 years (25%).

The maximum number of *Pseudomonas aeruginosa* (29%) were from ICU,

Out of 200 samples from which *Pseudomonas aeruginosa* was isolated, 75(37.50%) were pus samples, 60 (30%) urine, 24(12%) Sputum, ETT secretions 11 (5.50%), tissue 10 (5%) and blood 9 (4.50%).(Figure -1)

**Figure -1 :Percentage of sample wise distribution of *Pseudomonas aeruginosa***



**ABST was performed with 13 different antibiotics** 1- Penicillins (Piperacillin, Piptazo) 2-Cephems (Ceftazidime, Cefepime) 3- Aminoglycosides ( Gentamycin, Tobramycin ,Amikacin ,Netilmycin) 4-Quinolones (Ciprofloxacin) 5- Carbapenems (Imepenem, Meropenem) 6- Lipopeptides( Colistin, Polymyxin B)

Ceftazidime and Piperacillin antibiotics showed maximum (36.50%) resistance to *Pseudomonas aeruginosa* followed by Netilmycin (32%), Cefepime and Tobramycin each (29.50%), Gentamycin 26.50%, Amikacin 25% , Piperacillin-tazobactam (23%), carbapenem (10%), colistin 4.5% and Polymyxin 3.5% resistance ( Table -1)

**Table-1 Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa***

S. No.	Name of the Antibiotic	Total No Isolates	Sensitive No.	Sensitive %	Resistant No.	Resistant %
1	Piperacillin	200	127	63.50 %	73	36.50%
2	Piperacillin +Tazobactam	200	154	77.00 %	46	23.00 %
3	Ceftazidime	200	127	63.50 %	73	36.50 %
4	Cefepime	200	141	70.50 %	59	29.50 %
5	Gentamycin	200	147	73.50 %	53	26.50 %
6	Tobramycin	200	141	70.50 %	59	29.50 %
7	Netilmycin	200	136	68.00 %	64	32.00 %
8	Amikacin	200	150	75.00%	50	25.00 %
9	Ciprofloxacin	200	146	73.00 %	54	27.00 %
10	Imipenem	200	180	90.00 %	20	10.00 %
11	Meropenem	200	180	90.00%	20	10.00 %
12	Colistin	200	191	95.50 %	09	04.50 %
13	Polymyxin	200	193	96.50 %	07	03.50 %

Out of 200 isolates of *Pseudomonas aeruginosa* 21(10.5%) were Imipenem resistant

Out of 21 Imipenem resistant *Pseudomonas aeruginosa* 5 (23.8%) were metallo-beta-lactamase producers.

## DISCUSSION

The present study was aimed to determine the antibiotic susceptibility pattern of *Pseudomonas aeruginosa* isolated from various clinical specimens in a tertiary care center. A special effort was made to detect metallo-beta-lactamase producing *Pseudomonas aeruginosa*.

Infections caused by *P. aeruginosa* are frequently life threatening and often difficult to treat, due to the multiplicity of mechanisms of resistance. Its general resistance is due to a combination of factors. It is intrinsically resistant to antimicrobial agents, due to the low permeability of its cell wall. It has the genetic capacity to express a wide repertoire of resistance mechanisms. It can become resistant through mutations in the chromosomal genes which regulate the resistance genes. It can acquire additional resistance genes from other organisms via plasmids, transposons and bacteriophages and become resistant during a therapeutic course.<sup>9</sup>

Regional variations in the antibiotic resistance exist for different organisms, including *P. aeruginosa* and this may be related to the difference in the antibiotic prescribing habits.

The periodic testing and analysis of antibiotic resistance would enable the physicians to detect trends in resistance pattern to the commonly prescribed antibiotics in a given organism.

In the present study Piperacillin-tazobactam showed greater antibacterial activity against *P. aeruginosa* as compared to its monotherapy (i.e. piperacillin alone). Resistance rates of piperacillin-tazobactam combination were considerably lower (23%) in comparison to piperacillin alone (36.50%) as concurrent administration of a  $\beta$  lactamase inhibitor markedly expands the spectrum of activity. Bimlabanjare et al (2015).<sup>10</sup> in their study noted less resistance to piperacillin and piperacillin-tazobactam (17.45% and 7.94%), while a very high resistance to piperacillin was noted by Piyali Datta et al (2014).<sup>11</sup> Asghar et al noted 41.2 % resistance with piperacillin-tazobactam.<sup>12</sup>

Increasing resistance to beta-lactams in *P. aeruginosa* has become a serious threat, particularly against third and fourth generation

cephalosporins. Ceftazidime and cefepime are the prescribed anti-pseudomonal third and fourth generation cephalosporins, respectively. The resistance to ceftazidime, in our study, was found to be 36.50% and the resistance to cefepime was found to be 29.50%. This is comparable to a study by Bimla Banjare et al. In their study the resistance to ceftazidime and cefepime was 29.28% & 25.10% respectively.<sup>10</sup> In the study of Shahid et al<sup>13</sup> and Pitt et al.<sup>14</sup> ceftazidime resistance was 20% and 39.6% respectively. While low resistance was noted by Ayesha Ansari et al. In their study the resistance to ceftazidime and cefepime was found to be 22.03% and 16.01%.<sup>9</sup> In the study carried out by Wafa Ahmad et al (2015) noted 100% resistance to ceftazidime and 98% resistance to cefepime.<sup>15</sup>

The resistance to other antibiotics in our study was, Gentamycin 26.50%, Tobramycin 29.50%, Amikacin 25% and ciprofloxacin 27%. Banjare Bimla et al<sup>10</sup> have reported higher resistance in these antibiotics: gentamycin 46.86%, Tobramycin 25.20%, amikacin 39.33% and ciprofloxacin 28.40%. Ayesha Ansari et al.<sup>9</sup> and Rakesh M. Rajat et al.<sup>16</sup> also noted higher resistance for these drugs in their study.

In the present study 3.5% resistance was observed in Polymyxin B & 4.5 % for colistin. While Piyali Datta et al<sup>11</sup> did not find any resistance to polymyxin B and colistin in their strains. Bimla Banjare et al<sup>10</sup> also did not find any colistin resistant strain. 8.4% colistin resistant strains were found in the study carried out by S. Mohanty et al (2013).<sup>17</sup>

Polymyxin B and colistin are the most effective antimicrobial agents against *Pseudomonas aeruginosa*. However they are very costly and nephrotoxicity limits their use and therefore should be used only as a last resort.

The carbapenems have been drug of choice for the treatment of serious infection caused by gram negative bacterial infections. In the present study the imipenem resistance was found to be 10.5% which is close to the study carried out by Bimlabanjare et al. In their study on 239 *P. aeruginosa* isolates, they found 10.46% resistance to imipenem.<sup>10</sup>

To resist carbapenems, these gram negative bacilli have started producing two types of enzymes: serine carbapenemases, and metallo-beta-lactamases (MBLs).<sup>18</sup> These enzymes can hydrolyze not only carbapenems but many  $\beta$  lactams as well.<sup>19</sup> 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. In a recent study by Nisha et al in 2016<sup>20</sup> almost similar incidence (23%) was reported, while Arunva Kali et al.<sup>21</sup> reported very high incidence (72.70%) of MBL producers in their imipenem resistant *P. aeruginosa*.

## CONCLUSION

This study shows that the clinical isolates of *Pseudomonas aeruginosa* are becoming resistant to commonly used antibiotics and gaining resistance to newer antibiotics. Our study suggest that polymyxin B or colistin represent the best treatment option for MBL producing *Pseudomonas aeruginosa*.

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