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Occurrence of Metallo-Beta-Lactamase producing Pseudomonas aeruginosa isolates from clinical samples in a Tertiary Care Hospital

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Abstract
Background: *Pseudomonas aeruginosa is a challenging and prominent nosocomial pathogen, its infection associated with high morbidity, mortality and cost of therapy. Apart from multidrug resistance in Pseudomonas, presence of Metallo-Beta-lactamase (MBL) producing strains has added to treatment failures in Healthcare Institutions. The present study aimed at determining the occurrence of MBL producing Pseudomonas isolated from various clinical samples and their antibiogram pattern, along with their clinico-bacteriological correlation.*
Materials and Methods: *Pseudomonas aeruginosa isolates were subjected to antimicrobial susceptibility testing, using CLSI guidelines (2021). Strains resistant to Imipenem were tested for MBL production by Imipenem-EDTA combined disc test, Imipenem-EDTA DDST, EDTA Disc Potentiation test and modified HODGE test.*
Results: *A total of 200 isolates of Pseudomonas aeruginosa were tested. Imipenem resistance was 23%. Prevalence of MBL production among Imipenem resistant isolates was 53.2%, while overall it was 12.5% (25 out of 200). Male: female ratio was 2.1 :1. A high prevalence was seen in age group 51-60 years (32.5%). Diabetic foot infection was seen in 28% patients while sepsis and subacute bacterial peritonitis was seen in 16% patients yielding MBL producing Pseudomonas. Wound swabs yielded 44% of MBL positive Pseudomonas, followed by blood and peritoneal fluid (16%).*
Conclusion: *Early detection of MBL producing Pseudomonas is essential in a Healthcare setting to initiate appropriate antibiotic therapy and implement strict infection control practices.*
Keyword*: Pseudomonas aeruginosa, Metallo-beta-lactamase, resistance*

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I. INTRODUCTION

Pseudomonas aeruginosa, an aerobic Gram-negative bacterium is a prominent nosocomial pathogen. It is responsible for a large spectrum of invasive diseases in Healthcare settings, including pneumonia, Urinary Tract Infection, bacteremia and burn wound infection.[1]

Antimicrobial therapy becomes a difficult task for treating Pseudomonas infections as the organism shows natural resistance to many drugs and acquired resistance to multiple antimicrobials during treatment.[2]

Carbapenems once used as antibiotics for treating Pseudomonas infections are now experiencing treatment failures due to production of metallo-beta-lactamases (MBLs).[3] The genes coding for MBLs are located on plasmids and transposons, thus enabling a dangerous and widespread dissemination.[1]

The prevalence of MBLs in Pseudomonas aeruginosa in India, ranges from 11% to 25%.[4] The prevalence may be higher in ICU settings, among neonates and immunocompromised patients.[2]

Although MIC detection is the gold standard phenotypic test, various other methods have been investigated which are comparable with the former and at the same time, are simple, reliable, less cumbersome and cheap. These include the Imipenem EDTA combined disc Test, Imipenem EDTA double disc synergy test (DDST), EDTA Disc potentiation test using Ceftazidime, Cefepime and Cefotaxime and HODGE Test.[1]

The present study was undertaken to determine the presence of drug resistance in Pseudomonas aeruginosa isolates, with special reference to Imipenem and Meropenem. The study also sought to determine the occurrence of metallo-beta-lactamase producing Pseudomonas.

II. MATERIAL AND METHODS

The present study was undertaken in the Department of Microbiology, Goa Medical College, Bambolim, Goa, over a one year period (2021).

All samples received for cultural analysis were subjected to primary gram staining and subsequently cultured on solid media e.g. Blood Agar and MacConkey and liquid media i.e. Glucose Broth. After 18-24 hours of incubation at 37^0C, the inoculated media were examined for bacterial growth. Suspected colonies of Pseudomonas were identified using standard laboratory techniques, which included colony morphology, gram staining and biochemical reactions.[5]

Antimicrobial susceptibility testing was performed, using Kirby Bauer Disc diffusion method, as per CLSI Guidelines, using antipseudomonal antibiotics.[6]

All Pseudomonas aeruginosa isolates showing resistance or decreased susceptibility to Imipenem were subjected to testing for MBL production.

MBL production was assessed using four phenotypic methods which included Imipenem EDTA combined disc Test, Imipenem EDTA double disc synergy test (DDST), EDTA Disc potentiation test using Ceftazadime, Cefepime and Cefotaxime and HODGE Test.[6]

III. RESULTS

The study evaluated MBL production among Imipenem resistant Pseudomonas aeruginosa isolates obtained from various clinical samples of indoor and outdoor patients. A total of 36761 samples were processed for culture of bacterial pathogens. Bacterial growth was obtained in 6929 cases (18.8%). Pseudomonas aeruginosa was isolated in 1179 samples (17.01%). A random selection was made by including every 6^{th} case yielding Pseudomonas aeruginosa in the study. Thus 200 isolates of Psedomonas aeruginosa formed the material for the study.

Antimicrobial resistance pattern of the Pseudomonas isolates can be seen in Table no. 1.

TABLE NO. 1: ANTIMICROBIAL RESISTANCE PATTERN OF PSEUDOMONAS AERUGINOSA ISOLATES INCLUDED IN THE STUDY

Sr. No	Antibiotic	No. Resistant	%
1.	Imipenem	47	23.5
2.	Meropenem	64	32
3.	Amikacin	110	55
4.	Gentamicin	115	57.5
5.	Netilmycin	107	53.5
6.	Ciprofloxacin	149	74.5
7.	Levofloxacin	149	74.5
8.	Aztreonam	100	50
9.	Ceftazidime	124	62
10.	Cefepime	124	62
11.	Cefotaxime	124	62
12.	Piperacillin	120	60
13.	Piperacillin-Tazobactam	74	37
14.	Cefoperazone-Sulbactam	80	40

Among all Pseudomonas aeruginosa isolates tested, Imipenem and Meropenem resistance was seen in 23.5% and 32% cases respectively. Aminoglycoside resistance was 53.5% with Netilmycin. Amikacin resistance was to the tune of 55%, while Gentamicin resistance was seen in 57.5% isolates. Quinolone resistance indicated 74.5% resistance to Ciprofloxacin and Levofloxacin. Among the Cephalosporins, Cefepime, Cefotaxime and Ceftazidime, each exhibited 62% resistance. The Pseudomonas aeruginosa isolates, resistant to Imipenem were tested for MBL production i.e. 47 strains (23.5%).

Table no. 2 depicts the result of MBL production in Pseudomonas aeruginosa isolates using four phenotypic tests.

TABLE NO. 2: RESULT OF PHENOTYPIC SCREENING TESTS FOR DETECTION OF MBL PRODUCTION IN PSEUDOMONAS AERUGINOSA ISOLATES

Phenotypic test	No. positive	Percentage
Imipenem-EDTA combined disc test	25	53.2
Imipenem-EDTA DDST	15	31.9
EDTA disc potentiation test	18	38.3
Modified HODGE test	13	27.7

Imipenem-EDTA combined disc test detected maximum MBL producing isolates i.e. in 53.2% cases, followed by EDTA disc potentiation test (38.3%). Imipenem-EDTA DDST detected 15 cases out of 47 strains studied (31.9%), while the modified HODGE test was helpful in 27.7% cases (13 out of 47). MBL production was negative in 22 cases i.e. 46.8% of all Pseudomonas aeruginosa isolated and resistant to Imipenem. There were no isolates whose MBL production was detected by Imipenem-EDTA DDST, EDTA disc potentiation test and modified HODGE test and not by Imipenem-EDTA combined disc test. The overall prevalence of MBL production among Pseudomonas aeruginosa was 12.5% (25 out of 200 isolates).

The age and sex distribution of patients yielding MBL producing Pseudomonas aeruginosa can be observed in Table no. 3. The Male:Female ratio was 2.1:1. The occurrence of MBL production was 32% in the age group 51-60 years, followed by age group 41-50 years (24%) and 31- 40 years (20%).

TABLE NO.3: DISTRIBUTION OF PATIENTS YIELDING MBL PRODUCING PSEUDOMONAS AERUGINOSA

Age in years	Males		Females		Total	
	No.	%	No.	%	No.	%
0-10	0	0	1	100	1	4
11-20	0	0	0	0	0	0
21-30	1	100	0	0	1	4
31-40	3	60	2	40	5	20
41-50	5	83.3	1	16.7	6	24
51-60	5	62.5	3	37.5	8	32
61 and above	3	75	1	25	4	16
Total	17	68	8	32	25	100

All MBL producing Pseudomonas aeruginosa (100%) were isolated from patients admitted in various clinical wards of the hospital.

Among patients yielding MBL positive Pseudomonas aeruginosa, diabetic foot infection was present in 28% patients (7 out of 25), while sepsis and subacute bacterial peritonitis as a clinical diagnosis was made in 4 out of 25 patients (16%). Urinary Tract Infection and Pneumonia was evident in 12% patients (3 out of 25). (Table No. 4).

TABLE NO. 4: CLINICAL DIAGNOSIS OF SUBJECTS YIELDING MBL PRODUCING PSEUDOMONAS

Clinical diagnosis of patients	No. of MBL producers	Percentage
Diabetic foot infection	7	28
Sepsis	4	16
Subacute bacterial peritonitis	4	16
Urinary tract infection	3	12
Pneumonia	3	12
Burns	2	8
Surgical site infection	1	4
Post traumatic wound infection	1	4
Total	25	100

Analysis of clinical samples revealed that wound swabs yielded 44% (11 out of 25) of MBL positive Pseudomonas, followed by blood and peritoneal fluid (16%; 4 out of 25). In three patients (12% each), Endotracheal aspirate and urine yielded the Pseudomonas aeruginosa. (Table no. 5).

TABLE NO. 5: CLINICAL SAMPLES YIELDING MBL PRODUCING PSEUDOMONAS

Sample	No. of MBL producers	Percentage
Wound swab	11	44
Peritoneal fluid	4	16
Blood	4	16
Urine	3	12
Endotracheal aspirate	3	12
Total	25	100

IV. DISCUSSION

P. aeruginosa is an important opportunistic pathogen with innate resistance to many antibiotics. Inspite of availability of potent antibiotics and improvement in supportive care, P. aeruginosa infections remain one of the serious nosocomial infections, with increasing morbidity and mortality. A high mortality may be attributable to the inherent virulence of the organism, as well as the fact that it often occurs with immunosuppression and comorbid conditions. In addition, Pseudomonas aeruginosa is susceptible to a limited number of antimicrobial agents, which increases the likelihood of inappropriate empirical antimicrobial therapy.

Increasing resistance to different antipseudomonal drugs among hospital strains has become a threat to treatment options, especially among immunocompromised patients.

In the present study, 62% strains of Pseudomonas were resistant to Ceftazidime, Cefepime and Cefotaxime.

Among the Aminoglycosides, resistance to Amikacin, Gentamicin and Netilmycin was 55%, 57.5% and 53.5% respectively. The resistance to Ciprofloxacin was 74.5%. Piperacillin, when tested alone showed a resistance of 60%, while beta lactam/beta lactamase inhibitor drug Piperacillin-Tazobactam and Cefoperazone-Sulbactam showed a lower resistance of 37% and 40% respectively.

In the present study, the resistance to Imipenem was 23.5% i.e. 47 out of a total of 200 Pseudomonas isolates studied were resistant.

The antibiogram pattern in the study of Radhika et al showed 71% resistance against Cefotaxime, followed by Ceftazidime (55%) among Cephalosporins. Ciprofloxacin resistance was seen in 50% isolates while resistance to Imipenem was 20%.[1]

Resistance to Aztreonam (94.44%) and Piperacillin/Tazobactam and Cefepime (80.55% each) was observed in the study of Choudhary et al.[4] Imipenem resistance was observed in 33.88% isolates of Pseudomonas aeruginosa in their study.[4]

In the study of Agarwal et al, resistance to Amikacin was 73%, while it was 86% to Ofloxacin, Ceftazidime and Aztreonam. Imipenem resistance was observed in 100% isolates.[2]

Pseudomonas aeruginosa has limited susceptibility to antimicrobials. Gupta et al opine that overall drug resistance in Pseudomonas can lead to a serious medical disaster.[7] Carbapenems, once considered as potent Beta lactam antibiotics, have lost their potential due to emergence of Carbapenem hydrolyzing MBLs.

The occurrence of MBL producing Pseudomonas aeruginosa was 12.5% in the present study. This correlates with that obtained in the studies of Radhika et al[1] (15%), Choudhary et al[4] (20%) and Agarwal et al[2] (11.81%). In India, the prevalence rate of MBL producing Pseudomonas aeruginosa has been reported to vary from 11% to 25%.[4]

In the present study, the prevalence of MBL in Imipenem resistant strains was 53.2% (25 out of 47). Radhika et al observed a prevalence of 75% MBL among imipenem resistant strains in their study.[1]

The emergence of MBL producing Pseudomonas has become a therapeutic challenge, as these enzymes hydrolyze and degrade higher generation Cephalosporins. The spread of these enzymes to members of Enterobacteriaceae is alarming and is probably a reflection of excessive use of carbapenem.[8] Early detection and stringent infection control measures need to be implemented.

In the present study, patients whose samples yielded MBL producing Pseudomonas were in the age groups 51-60 years (32%). However, MBL producing isolates was obtained commonly from the 21-40 year age group (41.66%), followed by the 41-60 year age group (20%) in the study of Choudhary et al.[4] A higher prevalence in the elderly is probably related to decreased immunity and age-related comorbidities.

Pseudomonas aeruginosa can infect any external site or organ and is a leading cause of Healthcare associated infections, including pneumonias, urinary tract infections, bacteraemia, meningitis and wound infections. In the present study, diabetic foot infection yielded the maximum MBL producing Pseudomonas (28%) followed by Sepsis cases and Subacute Bacterial peritonitis (16%). Urinary Tract Infection and Pneumonia was present in 12% of the study subjects.

The type of sample yielding the bacterial pathogen is a reflection of the clinical diagnosis of the patients, from whom the Pseudomonas aeruginosa is isolated. In the present study, wound swabs yielded the maximum MBL producing Pseudomonas (44%). Peritoneal fluid and blood yielded 16% of the isolates followed by urine and endotracheal aspirates (12%).

In the study of Radhika et al, MBL producers were found in wound swabs (44.4%), followed by sputum (18.37%), urine (10%) and other body fluids (3.3%).[1] Similarly, Chaudhary et al isolated MBL producing Pseudomonas from pus (36.11%), endotracheal secretions (30.43%) and urine (19.44%).

V. CONCLUSION

The severity of Pseudomonas infections can be limited by early detection and appropriate antimicrobial therapy, which unfortunately has very little choice. Colistin being used alone or in combinations may serve as a salvage therapy. Alternately, 'antibiotic holiday' may be attempted , with the use of old antibiotics as a last resort.

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