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PREVALENCE OF METALLO BETA LACTAMASE PRODUCING PSEUDOMONAS AERUGINOSA & ANTIBIOTIC SENSITIVITY PATTERN IN A TERTIARY CARE HOSPITAL, NORTHERN INDIA

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ABSTRACT

Introduction: *Pseudomonas aeruginosa* is the most frequent Gram-Negative bacteria detected in nosocomial infections and is an increasingly widespread opportunistic human disease. *Pseudomonas aeruginosa* is the causative agent of many different diseases, such as gastrointestinal tract infections, respiratory tract infections, skin irritation, soft tissue infections, bacteremia, skeletal and joint infections, and psoriasis.
Methods: In this study from July 2023 to July 2024. A total number of 156 cases of various clinical samples of *Pseudomonas aeruginosa* were isolated in Department of Microbiology, School of Health Sciences, C.S.J.M.U. Kanpur Sample were inoculated on Blood agar and MacConkey agar and Brain Heart Infusion Broth media were used for blood culture. The *pseudomonas aeruginosa* isolates were subjected to antibiotic susceptibility testing and Kirby-Bauer disc diffusion method for technique.
Results: A total number of 156 cases were, the study period from July 2023 to July 2024. 85 (54.48%) cases were find out positive ,out of them 49 (57.65%) positive cases were male and 36 (42.35%) positive cases were female This study further divided into different age group distribution , 10 - 20 age group obtained 8 (9.41%) positive cases and in age group of 51-60 is 9 (10.59%) cases turned out to be positive. In which the total number of MBL (metallo-beta-lactamase) positive cases are 28 (32.94%)
Conclusion: The study recommends incorporating MBL (metallo-beta-lactamase) detection methods into routine clinical laboratories due to the emergence and spread of MBL-producing pathogens. In the study results demonstrate the serious therapeutic and epidemiological threat of the spread of metallo-beta-lactamase producing *Pseudomons aeruginosa.* In this study, combined disc test (CDT) found to be best confirmatory tests as compared to double disc synergy test (DDST) in this part of country.

Keywords: *Pseudomonas aeruginosa*, Metallo-Beta-Lactamase, Gram-negative bacteria, Imipenem,

INTRODUCTION

Pseudomonas aeruginosa is the most frequent Gram-Negative bacteria detected in nosocomial infections and is an increasingly widespread opportunistic human disease, it is generally found in warm, humid environments, which includes sinks, drains, respirators, humidifiers, and sanitation solutions, as a saprophyte.[1] It is availing benefit of a defect in the host's barriers which simulates an infection whereas *Pseudomonas* is the causative agent of many different diseases, such as gastrointestinal tract infections, respiratory tract infections, skin irritation, soft tissue infections, bacteremia, skeletal and joint infections, and psoriasis.[2] The combination of *Pseudomonas aeruginosa* innate resistance to numerous drug classes and its capacity to develop resistance to all essential therapies makes it a particularly hazardous microorganism although Carbapenems are still among the best treatment choices, but the spread of multidrug-resistant clones and the introduction of strains that hydrolyze and produce enzymes against them pose a challenge to their continued use.
[3] In accordance with the worldwide priority list of developing antibiotic-resistant bacteria, *Pseudomonas aeruginosa* is an extremely important pathogen which requires to be thoroughly investigated. [4] The main cause of Gram-Negative bacteria's resistance to Carbapenem is the hydrolyzing enzymes called Carbapenemases.[5,6] Clinically effective against *Pseudomonas aeruginosa* include the antibiotics in the Carbapenem group, which consist of Imipenem, Meropenem, Ertrapenem, and Doripenem.[7,8] In general, resistance to Penicillin, first- and second-generation Cephalosporins, third-generation Oxyimino Cephalosporins, including Ceftazidime and Ceftriaxone, as well as Monobactam (Aztreonam), has been identified in bacteria that produce beta lactamases.[8] Prolonged antibiotic treatment duration and earlier use of drugs that have important antipseudomonal activity represent two of the main risk factors leading to the establishment of resistant *Pseudomonas aeruginosa* strains.[9] Metallo-β-lactamase (MBL)-mediated Carbapenem resistance is becoming steadily more common in *Pseudomonas* isolates. Some of the most worrisome methods of resistance in *Pseudomonas* species currently is acquired MBL (Metallo-Beta-Lactamase).[10] Although they possess appropriate inhibitors and can hydrolyze virtually every beta-lactam molecule, zinc-dependent enzymes called Metallo-β-Lactamases (MBLs) have been receiving an excessive amount of investigation.[11] These strains showed transferable attributes toward various types of bacteria species and resistance to multiple varieties of antimicrobial drugs, although there are minimal alternatives of treatment, infections carried by Metallo-Beta-Lactamase(MBL)-producing organisms are related to high rates of mortality and morbidity and increased health care expenditures,due to its inherent susceptibility to a wide range of antimicrobial drug classes, as well as its capacity to develop resistance through mutation and the horizontal transfer of resistance factors.[12,13,14] Around the world, resistance to antibiotics has been rising, particularly in Gram-Negative bacteria, which represents a threat to the advancement of medicine and Asia is the predominant region of concern for metallo-β-lactamase (MBL) enzymes, which are becoming more widely distributed globally whereas few apparent therapy alternatives are available for many mettalo-beta-lactamase (MBL) producers who have multiple prescription medication resistances but this is more hopeful, mettalo-beta-lactamases (MBLs) might not be as powerful as they are in vitro at causing Carbapenem resistance when present in vivo when zinc levels are low.[15] By cleaving the amide link of the β-lactam ring, metallo-β-lactamase promotes resistance to β-lactams. β-lactam antibiotics possess the most widespread application globally due to their beneficial attributes.[16,17] Penicillin binding proteins or PBPs, are a group of transpeptidase enzymes that are necessary for the formation of the peptidoglycan layer found in bacterial cell walls and these enzymes are inhibited by these antibiotics by blocking their activity.[18] That gives β-lactam antibiotics their antibacterial properties is their inhibition of peptidoglycan formation, which kills off developing bacteria to withstand the deadly effects of these medications, micro-organisms have developed defense mechanisms in reaction.[19] Bacterial resistance has been rising as a result of the extended use of β-lactam antibiotics, and it currently poses

a major risk to the therapy of antibiotics.[20] MBLs (mettalo-beta-lactamases) are classified into two categories: the one which is encoded by transferable genes and the other mediated by

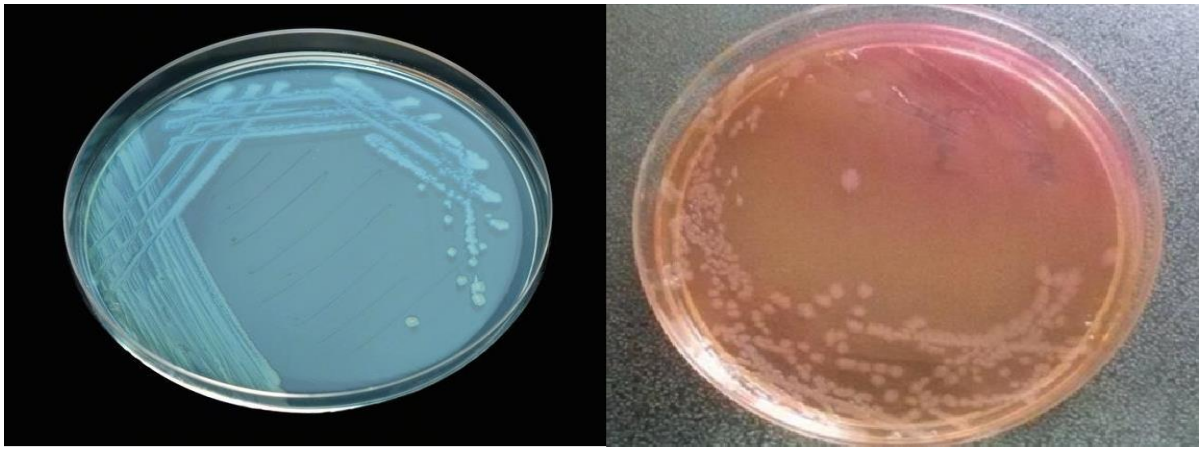
chromosomes.[21] There are now six recognized types of mobile MBLs: IMP, VIM, SPM, GIM, SIM, and AIM, every type has a different hydrolysis procedure.[22] The two main products of *Pesudomonas aeruginosa* are known to be IMP and VIM type MBL (mettalo-beta-lactamase).[23] Plasmids allow the IMP and VIM genes, which synthesize MBL, to transfer horizontally and quickly to other bacteria.[24] IMP, VIM, and NDM-encoding bacteria have been detected in a variety of community, healthcare, and environmental contexts.[25] The enzyme groups which were most frequently identified in prior studies were IMP and VIM.[26] Additionally, Asia is the region where both enzymes are most common which is depending on the nation, they are more or less common and more significant than serine Carbapenemases , the effulx pump, diminished outer membrane permeability, target alteration, and inactivation by β-lactamases constitute some of the techniques by which bacteria diminish the effects of antibiotics.[27]The major resistance strategy of Gram-Negative bacteria against beta-lactam antibiotics is the production of the enzyme beta-lactamase.[28] The majority of them are immobilized by EDTA (ethylenediaminetetraacetic acid), although they are resistant to beta lactamase inhibitors while other beta-lactamases do not contain a metallic element in their structure, they are incapable to express this susceptibility.[29,30] It is feasible to determine MBL manufacturers using molecular approaches.[29] However, smaller centers don't carry these. Imipenem (IMP)-EDTA combination disc test is a sensitive and specific method among the easy and less expensive ways to assess MBL (metallo-beta-lactamase) production. [31]

MATERIALS AND METHODS

In this study from July 2023 to July 2024. A total number of 156 cases of various clinical samples of *Pseudomonas aeruginosa* were isolated in C.S.J.M.U. Kanpur , U.P. and were studied for detection of Metallo beta lactamase, production including their Antibiogram. All samples were collected to the laboratory as aseptic precautions and transported to the laboratory. Sample were inoculated on Blood agar and MacConkey agar and Brain Heart Infusion Broth media were used for blood culture. The *pseudomonas aeruginosa* isolates were subjected to antibiotic susceptibility testing and Kirby-Bauer disc diffusion method for technique. According to CLSI (Clinical and Laboratory Standards Institute) guidelines 2020. All the experiments were treated in **HIMEDIA Private Limited (** Mumbai,India). The reagents used for blood agar media is Himedia Blood agar , for MacConkey agar media - Himedia MacConkey agar is used and for Brain Heart Infusion Broth - Himedia BHI agar is used. All the agar are used as per manufacturers's instruction .

RESULTS

A total number of 156 cases were treated in the sanitarium with clinical history of suggestive *Pseudomonas aeruginosa* strains were isolated from patients attending OPD during the study period from July 2023 to July 2024. 85 (54.48%) cases were find out positive ,out of them 49 (57.65%) positive cases were male and 36 (42.35%) positive cases were female This study further divided into different age group distribution , 10 - 20 age group obtained 8 (9.41%) positive cases and in age group of 51-60 is 9 (10.59%) cases turned out to be positive. In which the total number of MBL (metallo-beta-lactamase) positive cases are 28 (32.94%).



***Pseudomonas aeruginosa* on cled Agar** ***Pseudomonas aeruginosa* on MacConkey Agar**

Table No: 1 Distribution of isolates.

Total Sample	Positive Isolates	Negative Isolates
156	85 (54.48 %)	71(45.51 %)

Graph No: 1 Distribution of isolates

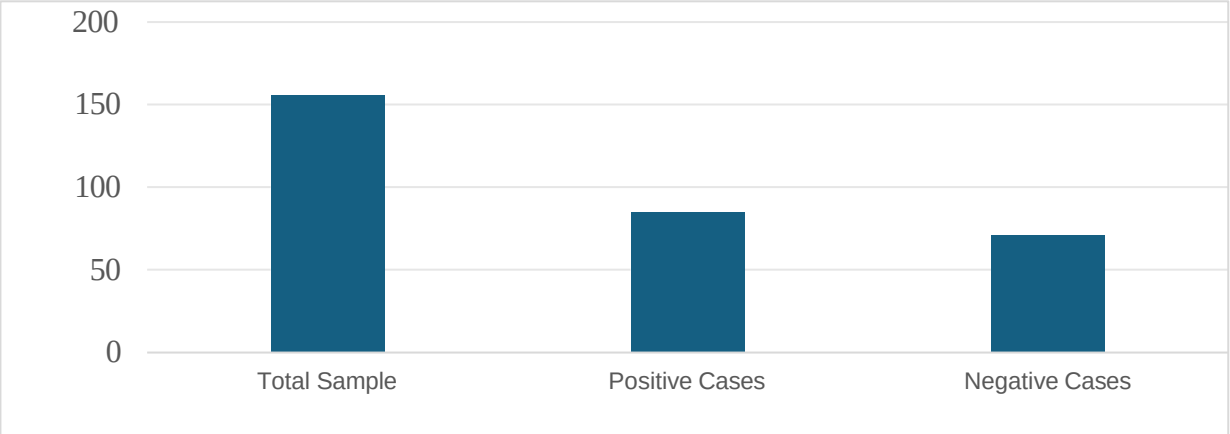


Table No: 2 Distribution of Age-wise isolates

Age Group	Isolates	(%)
10 - 20	08	(9.41 %)
21 - 30	23	(27.05 %)
31 - 40	32	(37.64 %)
41 - 50	13	(15.29 %)
51 - 60	09	(10.58 %)

Graph No: 2 Distribution of Age wise isolates.

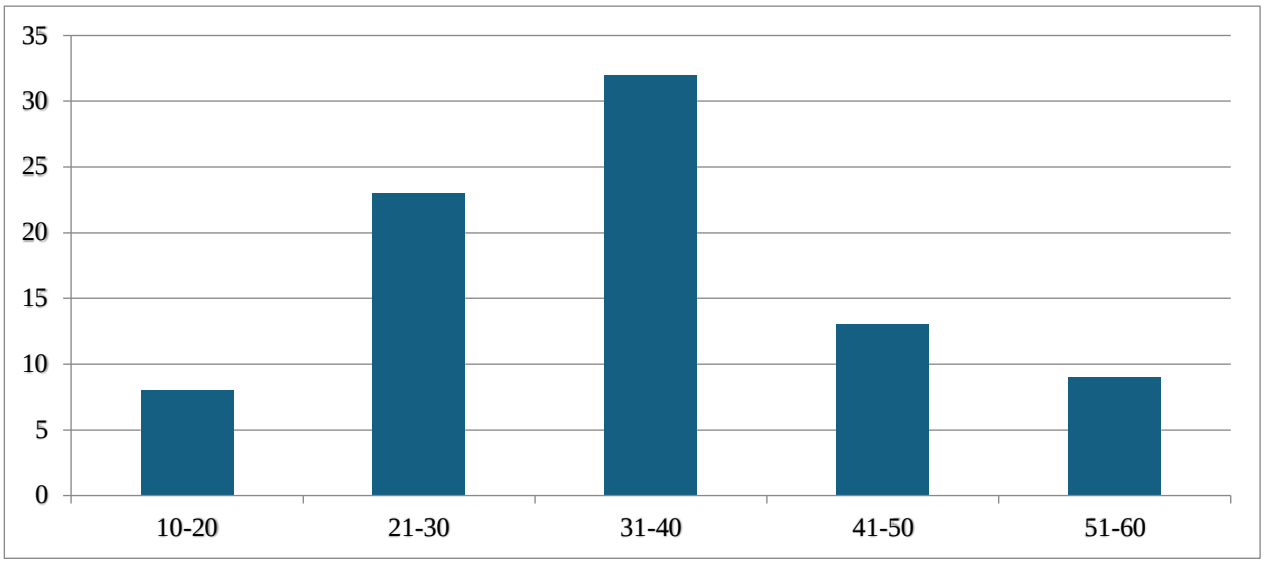


Table No: 3 Clinical specimens of *Pseudomonas aeruginosa.*

Clinical Specimens	***Pseudomonas aeruginosa* (85)**	**(%)**
Wound Swab	27	(31.76 %)
Urine	20	(23.53 %)
Blood	17	(20 %)
Sputum	13	(15.29 %)
Pus	8	(9.41 %)

Table No: 4 Percentage of MBL and Non- MBL Producers among Imipenem Resistant Isolates

Organism	**Total of imipenem resistant isolates**	**MBL producers**	**Non - MBL producer**
Pseudomonas aeruginosa	85	20 (23.53 %)	65 (76.47 %)

Table No: 5 Comparison of methods for detection of MBL in *Pseudomonas aeruginosa.*

Tests	**ATCC 27853 *(P.aeruginosa)***	**No. of MBL positive (n=20)**	**Percentage positivity**
CDT	Negative	20	(100 %)
DDST	Negative	12	(60 %)

Table No: 6 Antibiotics Susceptibility Pattern for *Pseudomonas aeruginosa.*

Antibiotics Name	Sensitivity (%)	Resistant (%)
Ceftazidime	45 (52.94 %)	40 (47.05 %)
Cefotaxime	38(44.70 %)	47 (55.29 %)
Azteronam	62 (72.94 %)	23 (27.05 %)
Meropenem	65 (76.47 %)	20 (23.52 %)
Imipenem	66 (77.64 %)	19 (22.35 %)
Doripenem	64 (75.29 %)	21 (24.70 %)
Ertapenem	62 (72.94 %)	23 (27.05 %)
Gentamicin	43 (50.58 %)	42 (49.41 %)
Tobramycin	58 (68.23 %)	27 (31.76 %)
Amikacin	55 (64.70 %)	30 (35.29 %)
Tetracycline	39 (45.88 %)	46 (54.11 %)
Netilmicin	48 (56.47 %)	37 (43.52 %)
Ciprofloxacin	62 (72.94 %)	23 (27.05 %)
Colistin	85 (100 %)	0 (0 %)
Polymyxin B	85 (100 %)	0 (0 %)
Piperacillin - tazobactam	36 (42.35 %)	49 (57.64 %)
Norfloxacin	76 (89.41 %)	9 (10.58 %)
Nitrofurantoin	80 (94.11 %)	5 (5.88 %)
Piperacillin	80 (94.11 %)	5 (5.88 %)
Cefepime	78 (91.76 %)	7 (8.23 %)
Tigecycline	85 (100 %)	0 (0 %)

DISCUSSION

Carbapenems have a broad range of antibacterial exertion. Hence, they're frequently used as a last resort in treatment. These are resistant to hydrolysis by utmost beta- lactamases and there has been an increase in reports of Carbapenem resistance in *P. aeruginosa* worldwide.The combined disc test (CDT) showed 20 isolates to be producing MBL, while double disc synergy testin g (DDST) detected in only 12 (60 %) isolates. About (47.05 %) of isolates of *Pseudomonas aeruginosa* were resistant to Ceftazidime followed by Cefotaxime (55.29 %). Amongst Aminoglycisides, least resistance was noted against Tobramycin (31.76 %), while higher resistance was noted for Netilmicin (43.52 %), Gentamicin (49.41 %) and Amikacin (35.29 %). While resistance to Imipenem (22.35 %) and Meropenem (23.52 %) cases was noted, while Piperacillin - tazobactum for (57.64 %) of resistance. The resistance pattern of *Pseudomonas* isolates is shown in table no.6. In the present study, an attempt was made to know the antibiotic profile of *Pseudomonas aeruginosa* with special concern to find MBL producing strains. Incidence is higher in males (57.65 %) as compare to females (42.35 %). Wound swab constituted majority of specimens accounting for (31.76 %), followed by urine (23.53 %), blood (20 %), sputum (15.29 %) and pus (9.41 %). These parameters indicate that most of these *Pseudomonas* isolates are nosocomial in origin. All Imipenem resistant isolates were strained for MBL production by combined disc test and double disc synergy test. MBL production in combined disc test is found in 20 isolates out of 85 Imipenem resistant isolates and in double disc synergy test MBL production was noticed in only 12 isolates. Prevalence of metallo beta lactamase in Imipenem resistant isolates is 19(23.35 %) and is a major mechanism of Carbapenem resistance. Prevalence of metallo beta lactamase in *Pseudomonas aeruginosa* isolates is 85(54.49 %). The combined disc test (CDT) was set up to be superior to double disc synergy test (DDST). This is an agreement with other published studies, which have set up combined disc test to be one of the most

sensitive ways for detecting MBL. Combined disc test could be used as a accessible screening method in the clinical microbiology laboratories.Present study underlines the unique problem with MBLs, because of their broad spectrum and inimitable drug resistance, creating a remedial challenge for clinicians and microbiologists. Hence we imply the detection of MBL in *Pseudomonas aeruginosa* should be a routine practice. To conquer the problem of emergence and the spread of multidrug resistant *Pseudomonas aeruginosa* combined relation and cooperation of microbiologists, clinicians and the infection control crew is needed. We suggest the ordinary surveillance of antibiotic resistant in the sanitarium.In the present study, total 156 isolates of *P. aeruginosa* isolated from various specimens were studied out of which 85 isolates are positive. Majority of samples were from male patients. Pseudomonal infections were more common in age group 31 to 40 years. Wound swabs and urine were the most common samples. Combined disc test (CDT) is simple, reliable and superior to double disc synergy test (DDST) in detection of metallo beta lactamases. Prevalence of metallo beta lactamase in *Pseudomonas aeruginosa* isolates was 85 (54.49 %) in our hospital. Metallo beta lactamase in Imipenem resistant isolates was 23.35 % and was a major mechanism of Carbapenem resistance.To conquer the problem of emergence and the spread of multidrug resistant *Pseudomonas aeruginosa*, the infection regulates the practices, aggresssive ordinary superintendency of antibiotic resistant and the rational use of antibiotic procedure in the medical center should be administered.

CONCLUSION

The study, carried out between July 2023 to July 2024, identified 85 *Pseudomonas aeruginosa* isolates from various clinical samples. Among these isolates:

- 23.53 % were positive in the screening test for MBL using Imipenem, Meropenem, and third-generation Cephalosporins.
- 100 % were confirmed as MBL-positive using the Imipenem–combined disc test (CDT).
- 60 % were positive by the Double disc synergy test (DDST).

The study recommends incorporating MBL (metallo beta lactamase) detection methods into routine clinical laboratories due to the emergence and spread of MBL-producing pathogens.In the study results demonstrate the serious therapeutic and epidemiological threat of the spread of metallo beta lactamase producing *Pseudomons aeruginosa.* In this study, combined disc test (CDT) found to be best confirmatory tests as compared to double disc synergy test (DDST) in this part of country.

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Conflicts of Interest: These are no conflicts of interest.

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