

## Original Research Article

# Occurrence and Antibiotic Resistance profiles of Metallo beta lactamase producing *Pseudomonas aeruginosa* among clinical isolates in Enugu Metropolis, Nigeria

### ABSTRACT

**Introduction:** Antibiotic resistance is rising worldwide, posing a challenge for contemporary medicine. Metallo-beta-lactamases (MBLs) confer resistance to beta-lactam antibiotics other than monobactams. It threatens public health because of its vast scope of action and quick spread. The study was undertaken to assess the occurrence of MBL in clinical isolates of *Pseudomonas aeruginosa* in the Enugu metropolis and to determine their resistance profile. **Material and Methods:** The work was conducted from October 2020 to July 2021 in the microbiology laboratory of the University of Nigeria Teaching Hospital, Ituku-Ozalla. A total of 127 non-duplicate bacterial isolates recovered from clinical samples including wounds, urine, sputum, ear discharge, and catheter tip processed in the microbiology laboratory of four referral hospitals within the Enugu metropolis was used for the study. Isolates were identified and characterized using standard microbiology protocols. Antimicrobial susceptibility was done using the Kirby-Bauer disc diffusion method. Phenotypic detection of Metallo-beta-lactamase production was done using the Combined Disk Diffusion Test (CDDT). **Results:** Of the 127 isolates, 68 (53.5%) were resistant to imipenem. Among these, 35 strains were positive for MBL production while 33 isolates were non-MBL producers. The highest prevalence of MBL producers was recorded from wound swabs (20 strains) followed by urine 11 strains. Both MBL producers and non-MBL producers displayed high resistance to most of the antibiotics used except Aztreonam. **Conclusion:** The overall occurrence of MBL among *Pseudomonas aeruginosa* in our study was found to be 27.6%. MBL-producing strains showed higher resistance than the non-MBL-producing strains. Aztreonam was the most potent antibiotic. The most effective approaches to combating this organism include early detection, stringent antibiotic regimens, and adherence to infection control measures.

**Keywords:** Antibiotic resistance, Carbapenemase, Metallo-beta-lactamase, Phenotypic detection, Combined disc diffusion Test, *Pseudomonas aeruginosa*

### 1. Introduction

*Pseudomonas aeruginosa* is an opportunistic organism that is known to cause nosocomial infections and different diseases such as urinary tract infection, pneumonia, sepsis, and soft-tissue infections [1]. The greatest threat challenging the healthcare sectors in developing nations like Nigeria is the rise and spread of multidrug-resistant (MDR) microorganisms [2]. MDR *P. aeruginosa* infections result in considerable morbidity and mortality. *P. aeruginosa* has a very high level of intrinsic and acquired resistance to numerous antibiotics, making it difficult to treat and limiting therapy options. *P. aeruginosa* exhibits practically all known resistance mechanisms; nevertheless, enzyme production is the predominant mode of acquired resistance, particularly beta-lactamase production [1]. Reliable susceptibility evaluation, cautious use of currently available antibiotics, and assessment of the prevalence of MDR are necessary for reducing the potential challenges associated with diseases caused by multidrug-resistant strains (2). Carbapenems are the last resort for the treatment of *P. aeruginosa* infections. Nevertheless, the high rise in carbapenem resistance causes grave concern in the management of infections caused by *P. aeruginosa* [3]. The World Health Organization (WHO) has recognized the 12 most frequent microorganisms that threaten human health. Carbapenem-resistant *P. aeruginosa* has been identified as one of the most critical, posing a major challenge to patients who need catheters and ventilators [4]. Beta-lactamases are divided into four classes: A, C, and D that use a serine-based mechanism and metallo-beta-lactamase (MBL), while class B needs a bivalent metal ion to function [5]. Metallo-lactamases (MBLs) are a class of

carbapenemases that are highly important to healthcare because of their potential to spread over the world and the resulting restricted number of available therapeutic alternatives [6]. These class B carbapenemases require zinc or another heavy metal to catalyze their reactions. MBLs have a wide spectrum of substrates that can catalyze the hydrolysis of almost all beta-lactam antibiotics [7]. Moreover, they are prevented by chelating compounds like ethylenediamine tetraacetic acid (EDTA) [2]. The rising incidence of *P. aeruginosa*-acquired MBL is very disturbing and constitutes a major threat during therapy and infection management [8]. MBL-producing microorganisms can hydrolyze penicillin, cephamycins, cephalosporins, and carbapenems, but not monobactams [9]. *P. aeruginosa* bacteria that produce MBL also have antibiotic resistance genes for other classes of antibiotics. MBL genes are found in mobile genetic elements such as plasmids, transposons, integrons, or linked with insertion sequences, and they can move within and between species. [9].

Antibiotic treatment for serious infections caused by MBL-producing *P. aeruginosa* usually results in a poor clinical outcome. With a worldwide increase in incidence, early detection is critical for establishing effective antibiotic regimens and infection control practices. PCR-based genotyping remains the gold standard for MBL detection and classification; nevertheless, diagnostic laboratories still need to do culture-based phenotypic testing to promptly identify MBL activity [10]. The MBL enzyme can be detected phenotypically in pathogenic organisms. The acknowledged methods include the Modified Hodge Test (MHT), Double-Disc Synergy Test (DDST), Combined Disc Diffusion Test with Imipenem and EDTA (CDDT), and MBL E-Test [11, 12]. Keeping this in mind, the current study was conducted to determine the percentage occurrence of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* strains in our setting, as well as their antibiotic sensitivity pattern, so that an appropriate infection management plan and antibiotic policy can be developed to prevent their spread.

## 2. MATERIALS AND METHODS

### 2.2 Study Design

This study was carried out from October 2020 to July 2021 in the microbiology laboratory of the University of Nigeria Teaching Hospital, Ituku-Ozalla in Enugu State, Nigeria

### 2.2 Study Area

Enugu Metropolis is located in Enugu State, Nigeria's South-East geopolitical zone. It is surrounded by the states of Kogi and Benue to the north, Ebony to the east, Abia to the south, and Anambra to the west. It is situated at the foot of the Udi Plateau. Enugu State has 17 local governments. The Metropolis has four Tertiary Health Care Centers: the University of Nigeria Teaching Hospital Enugu (UNTH), the Enugu State University Teaching Hospital (ESUTH), the National Orthopedic Hospital Enugu (NOHE), and the Federal Neuropsychiatric Hospital Enugu (FNHE). They function as referral and specialist centers for the southeast geopolitical zone. This was a descriptive cross-sectional research. A total of 127 *Pseudomonas aeruginosa* isolates were recovered from various clinical samples, including wounds, swabs, urine, sputum, ear discharge, and catheter tips from the University of Nigeria Teaching Hospital (UNTH), National Orthopaedic Hospital, Parklane Hospital, and the Enugu Metropolis Division Hospital.

### 2.3 Collection of Sample

Isolates previously processed in the microbiology laboratories of the University of Nigeria Teaching Hospital Enugu (UNTH), Enugu State University Teaching Hospital (ESUTH), National Orthopedic Hospital Enugu (NOHE), and some private laboratories in Enugu Metropolis were collected randomly and aseptically and inoculated on nutrient agar slants. The isolates were obtained from urine, wounds, ear discharge, and catheter tips. They were taken to the Microbiology Laboratory at the University of Nigeria Teaching Hospital, Enugu.

### 2.4 Isolation of Bacteria

All isolated organisms were transported to the laboratory and maintained in nutrient agar slants. They were sub-cultured onto nutrient agar and incubated at 37<sup>o</sup> C for 24 hours. The isolates were sub-cultured onto blood agar, MacConkey (Central Drug House (P) Ltd), and Centrimide agar plates (HiMedia, MH024, IND) and incubated at 37<sup>o</sup> C for 24 hours.

### 2.5 Identification of Bacteria Isolates

The isolates were identified using growth on Centrimide, colonial morphology, odor, Gram stain, oxidase test, sugar fermentation, and IMViC reactions [13].

## 2.6 Antimicrobial Susceptibility Testing

The antibiotic susceptibilities of the isolates were evaluated using Kirby Bauer's disk diffusion method, as specified in the Clinical Laboratory Standard Institute (CLSI) guidelines [14]. The antimicrobials used were Imipenem (10µg), ceftazidime (30µg), ofloxacin (5 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amoxicillin/clavulanate (20/10 µg), nitrofurantoin (50 µg), ceftazidime (30 µg), cefixime (30 µg), cefotaxime (30 µg), and cefuroxime (30 µg). Mueller-Hinton agar plates were inoculated with a standardized suspension of the isolates comparable to 0.5 McFarland turbidity standards, and the antibiotic discs were aseptically placed on the agar plates. The plates were then incubated at 37 °C for 18 to 24 hours. The Inhibition Zone Diameter (IZD) was measured and recorded following incubation by CLSI standards [15].

## 2.7 Screening for carbapenemase production

MBL-producing *E. coli* and *K. pneumoniae* resistant to imipenem were evaluated for MBL production. Isolates that had reduced susceptibility to imipenem with an inhibition zone diameter of  $\leq 21$  mm were used as cut-off values according to CLSI guidelines [14].

## 2.8 Detection of metallo-β-lactamase production

### 2.8.1 Combined Disc Diffusion Test (CDDT)

MBL production was detected using the Combined Disc Diffusion Test as per Franklin *et al* with slight modifications [16]. Two 10µg imipenem discs (one impregnated with 10µl of 750µg EDTA Sigma Chemicals, St. Louis, MO) were placed at a distance of 25mm apart on the Mueller Hinton (MH) agar medium inoculated with test organism standardized with 0.5 McFarland standards. After 24 hours of incubation at 37°C, inhibition zones around imipenem and imipenem + EDTA discs were compared. MBL production was seen to have occurred when the zone diameter around the imipenem + EDTA discs increased by more than 4mm as compared to imipenem alone. Before being added to the antibiotic disk(s), EDTA was tested on the isolate(s) alone to ensure that it did not inhibit the test organism and provide a false positive [2].

## 2.7 Statistical Analysis

All statistical analyses were carried out using SPSS for Windows version 22 (SPSS, Chicago, IL, USA). Descriptive statistics were employed to describe categorical variables (frequency and percentage). The Chi-square ( $X^2$ ) test (at 95% confidence intervals) was used to compare variables. A p-value of  $\leq 0.05$  was taken as statistically significant.

## 2. RESULTS

In Table 1, results show that out of the 127 *Pseudomonas aeruginosa* isolated from diverse sample sources, wound swabs provided the highest number of *Pseudomonas aeruginosa* isolates with a total of 71 (55.9%), followed by Urine: 36 (28.3%) and Sputum: 10 (7.9%). Ear discharge produced 7 (5.5%) isolates of *Pseudomonas aeruginosa*, while the catheter tip produced only 3 (2.4%).

Out of the 127 *P. aeruginosa* isolates, 68 (53.5%) were imipenem-resistant. Of the 68 (53.5%) imipenem-resistant isolates, 35 (51.5%) produced MBL, while 33 (48.5%) did not produce MBL.

The highest rate of imipenem resistance was found in catheter tips 66.7% (2/3), followed by urine 63.9% (23/36), and the lowest ear discharge 42.4% (3/7). There was no statistical significance between the source of the isolates and imipenem resistance  $p= 0.435$ .

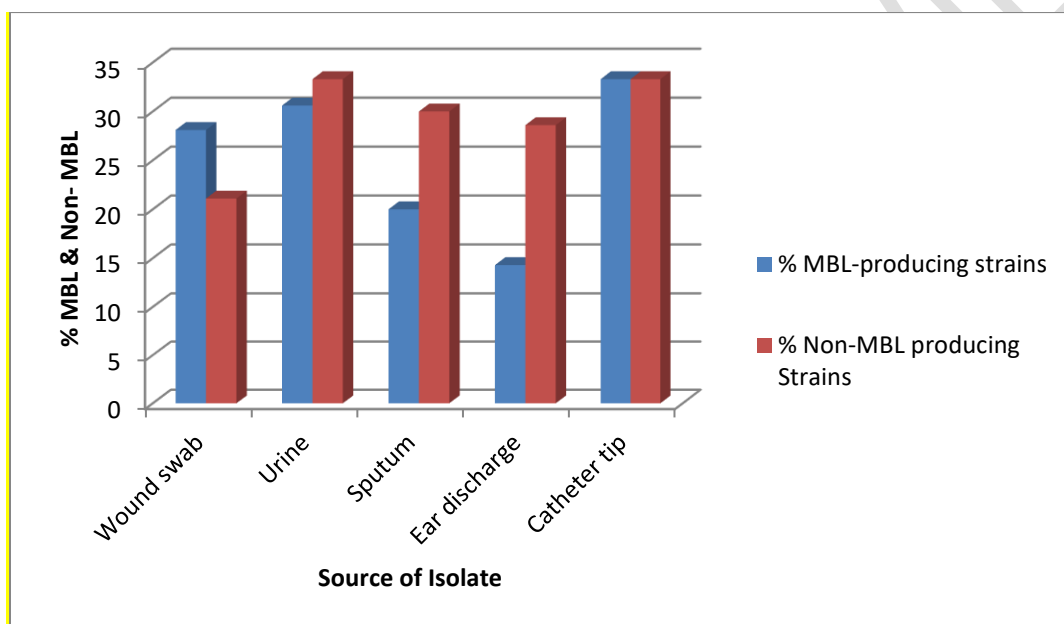
Fig 1 shows the distribution of MBL and Non-MBL producers among the isolates. The highest occurrence of MBL was again obtained from catheter tips 33.3% (1/3) while the lowest was from ear discharge 14.3% (1/7). There was no statistical significance association between the source of isolates and MBL production  $p= 0.252$ .

Table 2 shows the antimicrobial susceptibility profile of MBL and non-MBL-producing strains. Among the MBL-producers, the most potent drug was Aztreonam 54.3% followed by Fluoroquinolones (ofloxacin and ciprofloxacin) 42.9% and the least was aminoglycosides (gentamicin) 40%. MBL producers were 100% resistant to Augmentin, nitrofurantoin, ceftazidime, cefixime, cefuroxime, and ceftazidime. Non-MBL-producers were highly susceptible to Aztreonam at 87.9% and were moderately susceptible to fluoroquinolones (ofloxacin and ciprofloxacin) at 60.6% respectively and gentamicin at 57.6%. They were also 100% resistant to Augmentin, nitrofurantoin, ceftazidime, cefixime, cefuroxime, and ceftazidime.

**Table 1: Distribution of MBL according to the sources of isolates, imipenem resistance, and MBL-production**

Sample	No of isolate/%	No/% Resistant to Imipenem
Wound swab	71 (55.9)	35 (49.3)
Urine	36 (28.3)	23 (63.9)
Sputum	10 (7.9)	5 (50.0)
Ear discharge	7 (5.5)	3 (42.9)
Catheter tip	3 (2.4)	2 (66.7)
<b>Total</b>	<b>127</b>	<b>68 (53.5)</b>

**P = 0.435**

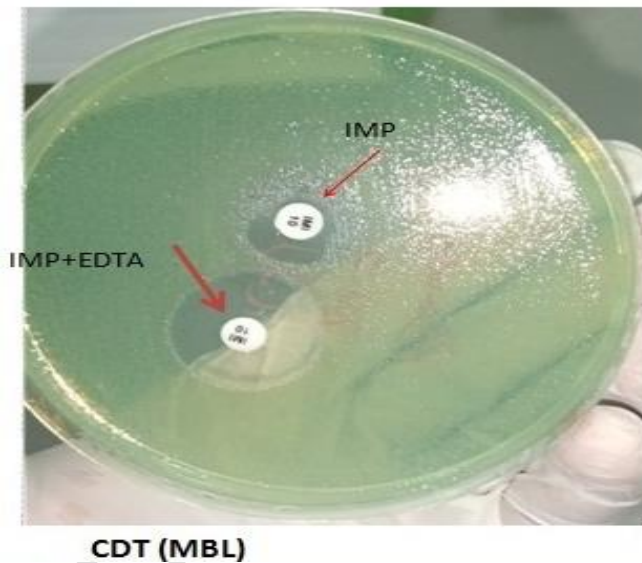


**Fig 1: Percentage distribution of MBL and Non-MBL according to the source of isolates**

**Table 2: Antimicrobial Susceptibility Tests of MBL-producing and Non-MBL-producing *P. aeruginosa* strains**

Antibiotics	MBL (% Susceptible)	MBL (% Resistant)	Non-MBL (% Susceptible)	Non –MBL (% Resistant)
Ofloxacin	15(42.9)	20 (57.1)	20 (60.6)	13 (39.4)

<b>Ciprofloxacin</b>	15 (42.9)	20 (57.1)	20 (60.6)	13 (39.4)
<b>Gentamicin</b>	14 (40.0)	21 (60.0)	19 (57.6)	14 (42.4)
<b>Aztreonam</b>	19 (54.3)	16 (45.7)	29 (87.9)	4 (12.1)
<b>Augmentin</b>	0 (0.0)	35 (100)	0 (0.0)	33 (100)
<b>Nitrofurantoin</b>	0 (0.0)	35 (100)	0 (0.0)	33 (100)
<b>Ceftazidime</b>	0 (0.0)	35 (100)	0 (0.0)	33 (100)
<b>Cefixime</b>	0 (0.0)	35 (100)	0 (0.0)	33 (100)
<b>Cefuroxime</b>	0 (0.0)	35 (100)	0 (0.0)	33 (100)
<b>Cefoxitin</b>	0 (0.0)	35 (100)	0 (0.0)	33 (100)



**Fig 2: Imipenem-EDTA combined Disc Test**

#### **4. DISCUSSION**

Metallo- $\beta$ -lactamase-producing *Pseudomonas aeruginosa* (MPPA) is a significant nosocomial pathogen resistant to all  $\beta$ -lactam antibiotics except monobactams. This has been reported across many nations [17]. Carbapenems are beta-lactam antibiotics that are regarded as the most effective drugs for treating multidrug-resistant pseudomonas infections due to their stability against the majority of beta-lactamases and their high rate of penetration into bacterial outer membranes (18). Carbapenem hydrolyzing Metallo

beta-lactamases can hydrolyze carbapenem and it is an important mechanism for imipenem resistance [19]. The resistance to imipenem in our study was 53.5% (68/127) of the total isolates of *P. aeruginosa*. Comparable rates were found in Iran at 57.1% [20] and in Egypt at 62.5% [21]. Lower rates had been reported in Saudi Arabia at 19.0% [22], in Ethiopia at 22% [23], in Japan at 28.5% [24], and in Taiwan at 16.0% [25]. However, higher rates had been reported in China 77.5% [1], India 74.19% [26], Egypt 78.3% [27], Nepal 79.6% [28], and Pakistan 80.7% [29]. Global variations in *P. aeruginosa* resistance rates could be attributed to differences in antibiotic prescribing methods and sample size around the world.

Our study showed that of the 68 imipenem-resistant isolates of *P. aeruginosa*, 35 (51.5%) were positive for MBL and this aligns with the work of Wang and Wang in China who reported 55.2% [1]. Shrestha et al reported a lower rate of 40.3% in Nepal [28] and Sarjan et al recorded a higher rate of 68% in India [30]. The presence of MBL-positive isolates constitutes not only a therapeutic problem but also an urgent challenge to infection control management. Given that these organisms are not easy to detect, they pose a major threat, particularly due to their role in unsuspected spread throughout the institution and the possibility of participating in horizontal MBL gene transfer with other bacteria in the healthcare facility [30]. This study also recorded the highest occurrence of MBL-producers from catheter tips 33.3% (1/3). This agrees with the work of Devi et al and Viswamohan et al [18, 30]. However, Wang et al and Sood et al recorded the highest occurrence in respiratory secretions/sputum [1,17 ] and Sajjan et al and Kali et al reported highest occurrence in wound swabs [30, 32]. The observed highest occurrence in catheter tips in our study may have been due to the ability of *Pseudomonas aeruginosa* to form biofilms on indwelling medical devices thereby increasing its resistant potential.

In our study, MBL-producing isolates recorded 100% resistance to Augmentin, Nitrofurantoin, Ceftazidime, Cefixime, Cefuroxime, and cefoxitin. Many of the strains also displayed high resistance to aminoglycosides (gentamicin) 60% followed by fluoroquinolones (ofloxacin and Ciprofloxacin) 57.1% respectively. MBL genes are carried on mobile genetic elements that also encode resistance genes to aminoglycosides and fluoroquinolones. This justifies the concomitant high resistance rates to these two types of antibiotics, in addition to their production [30]. The most potent drug against MBL producers in our study was Aztreonam with an average sensitivity of 54.3%. Wang and Wang recorded Colistin as the most potent antibiotics but we did not use colistin in our panel of antibiotics. In theory, MBLs can hydrolyze all beta-lactam antibiotics except aztreonam. Aztreonam resistance in 45.7% of MBL producers could be attributed to the presence of additional resistance mechanisms.

## 5. CONCLUSION

Our study recorded the occurrence of MBL producers in clinical isolates of *P. aeruginosa* at 27.6% (35/127) in our setting. Aztreonam was the most potent drug among MBL-producers in our study. The high prevalence of MBL-producing *P. aeruginosa* in the Enugu metropolitan is of concern because it raises the prospect of reaching a treatment impasse in the absence of newly developed therapeutic MBL inhibitors. Early identification of MBL-producing isolates is crucial for reducing infection outcomes and avoiding the spread of these strains throughout the hospital. The combined disc diffusion test (CDDT) with imipenem may be utilized as a possible screening procedure for detecting MBL production in routine microbiology laboratories where molecular methods are not possible. Continuous monitoring of MBL prevalence and the adoption of suitable antibiotic policies are critical for MBL surveillance and control in hospitals worldwide.

Ethical Approval:

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

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## REFERENCES

1. Wang W, Wang X. Prevalence of metallo- $\beta$ -lactamase genes among *Pseudomonas aeruginosa* isolated from various clinical samples in China. *J. Lab. Med.* 2020; 44(4) 197-203
2. Ejikeugwu C, Nworie O, Saki M. Metallo- $\beta$ -lactamase & AmpC Genes in *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* isolates from abattoir and poultry origin in Nigeria. *BMC Microbiology* 2021; 21:124.
3. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev* 2007; 20:440–58.
4. WHO publishes list of bacteria for which new antibiotics are urgently needed [<https://www.who.int/en/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed>]
5. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother* 2010;54:969–76
6. Ugwu MC, Shariff M, Nnajide, CM, Beri K, Okezie M, Iroha IR, Esimone CO. Phenotypic and Molecular Characterization of  $\beta$ -Lactamases among Enterobacterial Uropathogens in Southeastern Nigeria. *Canadian Journal of Infectious Diseases and Medical Microbiology* 2020;9(2): 1-9
7. Halat DH, Moubareck CA . The Current Burden of Carbapenemases: Review of Significant Properties and Dissemination among Gram-Negative Bacteria. *Antibiotics* 2020;( 9): 186; doi:10.3390/antibiotics904018

8. Meletis G, Bagkeri M. *Pseudomonas aeruginosa*: multi- drug-resistance development and treatment options. In: Basak C, editor. Infection Control. Rijeka, Croatia: IntechOpen, 2013: 33–56.
9. Zhao WH, Hu ZQ. Epidemiology and genetics of VIM-type metallo-beta-lactamases in Gram-negative bacilli. *Future Microbiol* 2011;6:317–33.
10. Javed H, Ejaz H, Zafar A, Rathore AW, Haq I. Metallo-beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: A rising threat for hospitalized children. *Journal of Pakistan Medical Association* 2016; 66( 9) :1068-1071
11. Sachdeva R, Sharma B, Sharma R. Evaluation of Different Phenotypic Tests for Detection of Metallo- $\beta$ -lactamases in Imipenem-Resistant *Pseudomonas aeruginosa*. *Journal of laboratory physicians*2017; 9: 249.
12. Fazlul MK, Nazmul M.H, Najnin A, Munira B. Detection of Metallo- $\beta$ -Lactamase Encoding Genes among Clinical Isolates of *Escherichia coli* in a Tertiary Care Hospital, Malaysia. *Journal of International Pharmaceutical Research*, 2019; 11 (1): 1-8.  
<https://doi.org/10.31838/ijpr/2019.11.01.031>
13. Baron EJ, Peterson LR, Tenover FC, Tenover FC, Tenover FC. Baily and Scott's Diagnostic Microbiology 9th Edition; Mosby Inc. St Louis Baltimore; 1994: 362-385
14. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-fifth Informational Supplement M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
15. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement M100-S25. Wayne, PA: Clinical and Laboratory Standards Institute; 2015
16. Franklin C, Liolios L, Peleg AY. Phenotypic detection of carbapenem-susceptible metallo- $\beta$ -lactamase-producing gram-negative bacilli in the clinical laboratory. *Journal of clinical microbiology* 2006; 44(9): 3139-3144.
17. Sood S. Phenotypic Tests for detecting incidence of Metallo-B-Lactamase (Mbl) Producing *Pseudomonasaeruginosa* in Jaipur. *National J Laboratory Medi.*2014;3 (2):22-7

18. Devi PV, Reddy PS, John MS. Prevalence of Metallo-B-Lactamases producing *Pseudomonasaeruginosa* among the clinical isolates: A study from tertiary care hospital. *International Journal of Current Microbiology and Applied Sciences* 2015;4(4): 955-961
19. Mehta A, Tukaram P. Detection and characterization of metallo- $\beta$ -lactamases producing *Pseudomonasaeruginosa* clinical isolates at a tertiary care hospital of Bhopal, India. *Int J Res Med Sci* 2016; 4:4084-8
20. Safarirad S, Arzanlou M, Mohammadshahi J, Vaez H, Sahabkar et al. Prevalence and characteristics of Metallobeta-lactamase-positive and High-risk clone ST 235 *Pseudomonas aeruginosa* at Ardabil Hospitals, Jundishapur J. Microbiol 2021; 14(3): e115819. <https://doi.org/10.5812/ijm.115819>
21. Saedii AA, Ahmed AM, Mohamed OM, Carbapenem resistant strains of *Pseudomonas* species at Minia University Hospital. *Minia Journal of Medical Research* 2022; 33(4) 160-165 doi 10.21608/MJMR.2022.270692
22. Khan MA, Faiz A. Antimicrobial resistance patterns of *Pseudomonas aeruginosa* in Tertiary care hospitals of Makkah and Jeddah. *Ann Saudi Med* 2016; 36(1): 23-28
23. Abdeta A, Negeri AA, Beyene D, Adamu E, Fekede E, Fentaw S, Tesfaye M, Wakoya GK, Prevalence and Trends of Carbapenem-Resistant *Pseudomonas aeruginosa* and *Acinetobacter* Species isolated from clinical specimens at the Ethiopian Public Health Institute, Addis Ababa, Ethiopia: A Retrospective Analysis. *Infect Drug Resist.* 2023 Mar 11; 16:1381-1390. Doi:10.2147/IDR.S403360.
24. Kakeya H, Yamada K, Nakaie K, Takizawa E, Okada Y, Fujita A, et al [A comparison of susceptibility of *Pseudomonas aeruginosa* clinical isolates to carbapenem antibiotics in our hospital] *Japanese Journal of Antibiotics* 2014; 67(4):241-248
25. Lee HS, Loh YX, Lee JJ, Liu CS, Chu C. Antimicrobial consumption and resistance in five Gram-negative bacteria species in a hospital from 2003 to 2011. *J. MicrobiolImmuno Infect.* 2015; 48: 647-54
26. Chand K, Lath A, Yadav A, Sharma M, Singh NP, Kumar S, Yadav S et al Phenotypic and genotypic detection of Beta-lactamase producing *Pseudomonas aeruginosa* isolated from Haryana, India. *Journal of Applied Biology and Biotechnology* 2024; 12(4)
27. Abaza AF, El Shazil SA, Selim HS, Aly GS. Metallobeta-lactamase producing *Pseudomonas aeruginosa* in a healthcare setting in Alexandria, Egypt. *Pol J Microbiol* 2017; 66:297-308.
28. Shrestha PM, Kattel HP, Sharma S, Bista P, Basnet BK, Ghimire P, Rijal KR. Metallo-beta-lactamase-producing *Pseudomonas* isolates from Two Tertiary Care Centres in a District of Nepal: A Descriptive cross-sectional Study. *Journal of Nepal Med Assoc.* 2024; 62(271); 202-206
29. Gondal AJ, Choudhry N, Niaz A, Yasim N. Molecular Analysis of carbapenem and Aminoglycoside Resistance Genes in Carbapenem-Rsistant *Pseudomonas aeruginosa* clinical Strains. A Challenge for Tertiary Care Hospitals. *Antibiotics*;13(2): 191
30. Viswamohanan I, Lakshminarayana L, Bhargavi KH, Jitendranath A. A Study ob Metallo-beta-lactamase Mediated Resistance in Clinical Isolates of *Pseudomonas aeruginosa*. *International Journal of Current Microbiology and Applies Sciences* 2016; 5(12): 499-506
31. Sajjan AC, Gurnule SR., Aparna B. Prevalence of MBL producing *Pseudomonas aeruginosa* in various clinical specimens in tertiary care hospital, Karimnagar. *Tropical Journal of Pathology & Microbiology* 2019; 5(4): 205-209
32. Kali A, Srirangaraj S, Kumar S, et al. Detection of metallo-beta-lactamase producing *Pseudomonas aeruginosa* in intensive care units. *Australas Med J.* 2013 Dec31;6 (12):686-93. doi: 10.4066/AMJ. 2013. 1824. eCollection 2013

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