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Antimicrobial resistance patterns and phenotypic detection of Metallo-β-lactamase among imipenem-resistant *Pseudomonas aeruginosa* isolates

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ABSTRACT:

Background: *Pseudomonas aeruginosa* is an important nosocomial pathogen with increasing resistance against β-lactam antibiotics. This is mainly caused by Metallo- β-lactamase **(**MBL) production and it poses a major therapeutic challenge. **Aim:** To analyse antimicrobial resistance patterns and detect MBL production among imipenem-resistant *P. aeruginosa* isolates. **Methodology:** A total of 250 *P. aeruginosa* isolates obtained from clinical specimens were identified using standard microbiological methods. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method as per CLSI guidelines. Imipenem-resistant isolates were screened for MBL production using the Double Disk Synergy Test (DDST). **Results:** Of the 250 *P. aeruginosa* isolates processed, 67 (26.8%) were imipenem resistant. Among these, 30 (44.8%) were confirmed as MBL producers. Most isolates were recovered from pus and respiratory samples and were more common among inpatients. Resistance was highest to carbapenems, cephalosporins, and fluoroquinolones, while aminoglycosides showed lowest resistance. **Conclusion:** The high prevalence of imipenem resistance and MBL production emphasize the need for early detection, antimicrobial stewardship, and continuous surveillance.

INTRODUCTION:

Pseudomonas aeruginosa is an important Gram-negative opportunistic pathogen and a major cause of healthcare-associated infections, particularly among immunocompromised individuals and patients with chronic respiratory diseases, burns, malignancies, sepsis, ventilator-associated pneumonia, or those undergoing organ transplantation. Its ability to survive on moist surfaces and colonize medical devices contributes significantly to its persistence in hospital environments and its role in nosocomial infections. (1)

The management of *P. aeruginosa* infections has become increasingly challenging due to the rapid emergence of antimicrobial resistance. This organism possesses remarkable genetic adaptability, enabling it to develop resistance to multiple classes of antibiotics and limiting available therapeutic options. (2)

Among the antimicrobial agents used against *P. aeruginosa*, β-lactam antibiotics, particularly carbapenems, have traditionally been considered highly effective because of their broad spectrum of activity and excellent penetration through the outer membrane of Gram-negative bacteria. (3) However, the increasing prevalence of multidrug-resistant (MDR) strains has compromised the efficacy of these agents. One of the most important mechanisms responsible for carbapenem resistance is the production of Metallo-β-lactamases (MBLs), enzymes capable of hydrolysing a wide range of β-lactam antibiotics. The emergence of MBL-producing *P. aeruginosa* has become a significant therapeutic and epidemiological concern, often resulting in increased morbidity, mortality, and healthcare costs. (4)

MBLs are particularly important because they can inactivate penicillins, cephalosporins, and carbapenems and are not inhibited by conventional β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. As carbapenems are frequently reserved for the treatment of severe infections caused by resistant organisms, the spread of MBL-producing strains poses a serious threat to patient care. (5)

Early detection of MBL-producing *P. aeruginosa* is therefore essential for appropriate antimicrobial therapy, implementation of infection control measures, and prevention of further dissemination within healthcare settings. The present study is aimed to isolate *P. aeruginosa* from clinical samples, analyse their antimicrobial resistance patterns, and detect MBL production among imipenem-resistant isolates using the Double Disk Synergy Test (DDST) in a tertiary care hospital in Navi Mumbai.

MATERIALS AND METHODS:

Study Design and Setting:

A prospective cross-sectional study was conducted over a period of 1.5years (July 2024 to December 2025) in the Department of Microbiology at MGM Medical College and Hospital. The study was approved by the Institutional Ethics Committee (Approval No. DHR-EC/SC/2024/07/67).

Sample Collection and Processing:

A total of 250 non-duplicate clinical isolates of Pseudomonas aeruginosa obtained from various clinical specimens including urine, pus, sputum, blood, endotracheal secretions, body fluids, tissue samples, and ear swabs were included in the study. Repeat isolates from the same patient and site were excluded.
Samples were collected using standard aseptic precautions and processed according to standard microbiological procedures. Primary isolation was performed on Blood agar, MacConkey agar, and Nutrient agar followed by incubation at 37°C for 18–24 hours (6,7).

Identification of Isolates:

Identification of P. aeruginosa was carried out based on colony morphology, pigment production, Gram staining characteristics, motility testing, and standard biochemical reactions including oxidase test, catalase test, Triple Sugar Iron (TSI) test, citrate utilization test, oxidative-fermentative (OF) test, nitrate reduction test, and decarboxylase reactions, as per standard microbiological guidelines (6, 7, 8).

Table 1: Biochemical characteristics of *Pseudomonas aeruginosa*

Biochemical Test	**Expected Result**	***P. aeruginosa***
Gram Stain Morphology	Gram-negative rod	POSITIVE (pink rods)
Motility (Hanging Drop)	Motile (polar flagella)	POSITIVE
Oxidase	Positive	POSITIVE
Catalase	Positive	POSITIVE
TSI (Slant/Butt/Gas/H_2S)	K/NC, No gas, No H_2S	K/NC
Indole	Negative	NEGATIVE
Citrate (Simmons)	Positive	POSITIVE
Urease	Negative/Weakly positive	NEGATIVE/WEAKLY POSITIVE
Nitrate Reduction (Denitrification)	Positive (to N_2 gas)	POSITIVE
Methyl Red (MR)	Negative	NEGATIVE
Voges-Proskauer (VP)	Negative	NEGATIVE
OF Test — Glucose	Oxidative only (O/NC)	OXIDATIVE
Lysine Decarboxylase (LDC)	Negative	NEGATIVE

Ornithine Decarboxylase (ODC)	Negative	NEGATIVE
Arginine Dihydrolase (ADH)	Positive	POSITIVE
Pyocyanin Pigment (BA, NA)	Blue-green pigment	POSITIVE (most strains)
Growth at 42°C	Positive	POSITIVE

Antimicrobial Susceptibility Testing:
Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method on Mueller-Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines, 2024 (9). Antibiotics tested included antipseudomonal β-lactams, aminoglycosides, fluoroquinolones, and carbapenems. Results were interpreted as susceptible, intermediate, or resistant according to CLSI breakpoints.

Table 2: Antibiotic panel tested of *P. aeruginosa* isolates

Antibiotic Class	Antibiotic (Code)	Disk Content (µg)	S ≥mm	R ≤mm
Carbapenems	Imipenem (IPM)	10	19	15
Carbapenems	Meropenem (MRP)	10	19	15
Penicillin + BLI	Piperacillin/Tazobactam (PIT)	100/10	21	14
Aminoglycosides	Gentamicin (GEN)	10	15	12
Aminoglycosides	Amikacin (AK)	30	17	14
Aminoglycosides	Tobramycin (TOB)	10	15	12
Fluoroquinolones	Ciprofloxacin (CIP)	5	21	15
Cephalosporins (3rd gen)	Ceftazidime (CAZ)	30	18	14

Phenotypic Detection of Metallo-β-Lactamase Production:
Imipenem-resistant isolates were screened for Metallo-β-lactamase (MBL) production using the Double Disk Synergy Test (DDST) with imipenem and imipenem-EDTA disks. Enhancement of the inhibition zone towards the EDTA disk was interpreted as positive for MBL production.

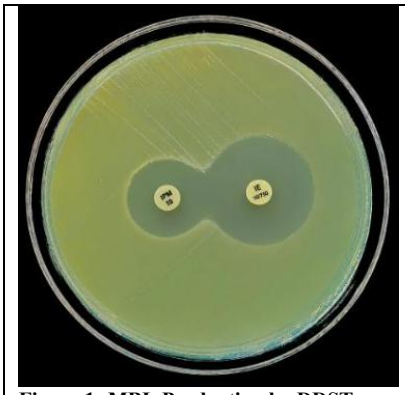


Figure 1: MBL Production by DDST

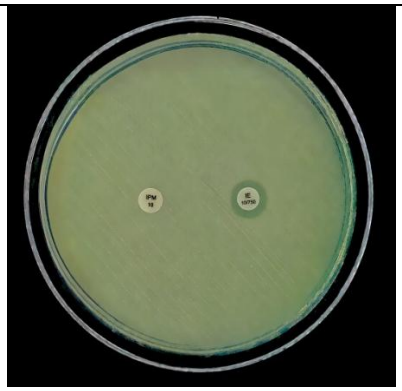


Figure 2: Non MBL producer by DDST

Data were entered in Microsoft Excel and analysed using descriptive statistics. Categorical variables were expressed as frequencies and percentages.

RESULTS:

In the present study, total 5127 clinical specimens were received in laboratory of which, 2207 (43.05%) samples were sterile and 2920 (56.95%) showed significant bacterial growth. Among these culture positive isolates, P. aeruginosa was isolated from 250 non-duplicate samples. So, in this study, the prevalence of *P. aeruginosa* (among culture positive samples) in this tertiary care hospital is 8.56%.
Among 250 P. aeruginosa isolates, 67 (26.8%) were imipenem resistant. Of these, 30 (44.8%) were confirmed as MBL producers by DDST.
Gender-wise distribution: Out of 250 isolates, 158 (63.2%) were from male patients and 92 (36.8%) were from female patients.

Table 3. Gender-wise distribution of *Pseudomonas aeruginosa* isolates (n=250)

Gender	Frequency (n)	Percentage (%)
Female	92	36.8
Male	158	63.2
Total	**250**	**100.0**

Age-wise distribution: P. aeruginosa infection was seen among all age groups, but was more frequent in middle-aged and elderly patients. Highest number of isolates belonged to the 41–60 years age group, and accounted for 84 (33.6%) cases, followed closely by the >60 years age group with 78 (31.2%) cases.

Table 4. Age-wise distribution of *Pseudomonas aeruginosa* isolates (n=250)

Age group	Frequency (n)	Percentage (%)
≤20 years	22	8.8
21–40 years	66	26.4
41–60 years	84	33.6
>60 years	78	31.2
Total	**250**	**100.0**

Sample-wise distribution: Respiratory and wound related specimens contributed the largest proportion of isolates.

Table 5. Sample-wise distribution of *Pseudomonas aeruginosa* isolates (n=250)

Sample type	Frequency (n)	Percentage (%)
Blood	6	2.4
Pus	60	24
Urine	30	12
Respiratory specimen:	**118**	**47.2**
─ BAL	8	3.2
─ Sputum	54	21.6
─ ET secretion	56	22.4
Swabs	13	5.2
Other body fluids[$]	5	2
Tissue	13	5.2
Miscellaneous[#]	5	2
Total	250	100

$ Other body fluids include: Bile, Peritoneal fluid, Pleural fluid, Gastric lavage
# Miscellaneous includes: Tips, Nasal discharge

Department-wise distribution: In the present study higher isolates were obtained from inpatients and lower from the outpatients. Also, highest number were received from the ICU, which contributed 71 (28.4%). This was followed by the Surgery and allied departments, which contributed 61 (24.4%) isolates.

Table 6. Department-wise distribution of *Pseudomonas aeruginosa* isolates (n=250)

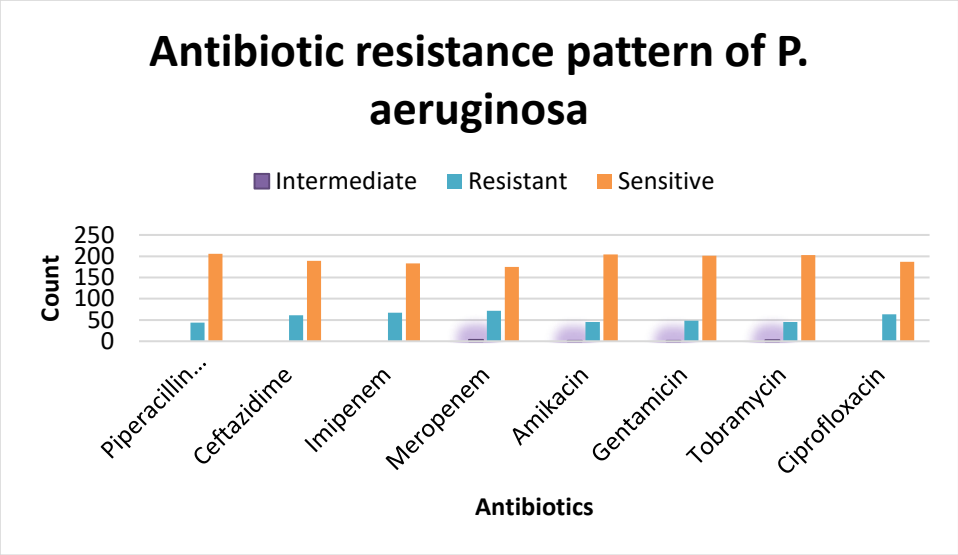
Department	Inpatients	Outpatients	Total	Percentage (%)
Dermatology	0	1	1	0.4
EMS	24	2	26	10.4
ENT	6	5	11	4.4
Gynaecology	2	0	2	0.8
Medicine allied	28	5	33	13.2
Orthopaedics	15	2	17	6.8
Respiratory Medicine	18	10	28	11.2
Surgery allied	49	12	61	24.4
ICU	71	0	71	28.4
Total	213	37	250	100

Antibiotic resistance pattern: The highest resistance was observed against meropenem, where 72 (28.8%) isolates were resistant, followed by imipenem with 67 (26.8%), ciprofloxacin with 63 (25.2%), and ceftazidime with 61 (24.4%) resistant isolates. Piperacillin–tazobactam showed the highest susceptibility, with 206 (82.4%) isolates being sensitive. Resistance to aminoglycosides was comparatively lower. Overall, carbapenem resistance was noted as a major concern in the present study.

Table 7. Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolates (n=250)

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Piperacillin–Tazobactam	206 (82.4)	0 (0.0)	44 (17.6)
Ceftazidime	189 (75.6)	0 (0.0)	61 (24.4)
Imipenem	183 (73.2)	0 (0.0)	67 (26.8)
Meropenem	175 (70.0)	3 (1.2)	72 (28.8)
Amikacin	204 (81.6)	1 (0.4)	45 (18.0)
Gentamicin	201 (80.4)	1 (0.4)	48 (19.2)

Tobramycin	203 (81.2)	2 (0.8)	45 (18.0)
Ciprofloxacin	187 (74.8)	0 (0.0)	63 (25.2)



Graph 1. Antibiotic resistance pattern of *P. aeruginosa*

Imipenem susceptibility profile: Among 250 isolates, 67 (26.8%) were found to be resistant to imipenem.

Table 8. Gender wise distribution to imipenem resistance in Pseudomonas aeruginosa isolates (n=67)

Gender	Imipenem Resistant (n)	Percentage (%)
Female	21	31.3
Male	46	68.4
Total	67	100

Table 9. Age wise distribution to imipenem resistance in Pseudomonas aeruginosa isolates (n=67)

Age	Imipenem Resistant (n)	Percentage (%)
≤20 years	7	10.4
21–40 years	18	26.9
41–60 years	18	26.9
>60 years	24	35.8
Total	67	100

Table 10. Sample wise distribution to imipenem resistance in Pseudomonas aeruginosa isolates (n=67)

Sample type	Imipenem Resistant (n)	Percentage (%)
Blood	1	1.5
Pus	12	17.9
Urine	17	25.3
Respiratory specimen:	**24**	**35.8**
─ BAL	1	1.5
─ Sputum	6	9
─ ET secretion	17	25.3
Swabs	6	9
Other body fluids[$]	1	1.5
Tissue	2	3
Miscellaneous[#]	4	6
Total	67	100

MBL positivity among imipenem-resistant *Pseudomonas aeruginosa* isolates by DDST(n=67):
Among the 67 imipenem-resistant *P. aeruginosa* isolates, 30 (44.8%) were positive for Metallo-β-lactamase (MBL) production, while 37 (55.2%) were MBL negative.

Table 11. Phenotypically confirmed MBL positivity among imipenem-resistant

MBL status	Frequency (n)	Percentage (%)
MBL negative	37	55.2
MBL positive	30	44.8

Total	67	100.0

DISCUSSION:

In the present study, 250 non-duplicate Pseudomonas aeruginosa isolates were recovered from 2920 culture-positive samples, accounting for a prevalence of 8.56%. This finding is comparable with reports by Maharjan et al. (10) and Khan et al. (11), who documented prevalence rates of 6.48% and 6.67%, respectively. The slight variations observed among studies may be attributed to differences in patient populations, healthcare settings, antimicrobial usage practices, and infection control measures.

A predominance of isolates was observed among male patients and in the middle-aged and elderly age groups. Similar observations have been reported by Radhika et al. (1), Acharya et al. (12), Strateva et al. (13), and Hirsch et al. (14), who attributed the increased frequency of infection in these groups to greater healthcare exposure, underlying comorbidities, and age-related decline in immune function.

Respiratory specimens constituted the major source of P. aeruginosa isolates, followed by pus and urine samples. These findings are consistent with previous studies by Pitout et al. (15) and Qureshi et al. (16), highlighting the important role of P. aeruginosa in ventilator-associated pneumonia, lower respiratory tract infections, and wound infections. The highest isolation rates were observed in intensive care units and surgical wards, supporting observations by Sheikh et al. (17) and Sachdeva et al. (18) that invasive procedures, prolonged hospitalization, and extensive antibiotic exposure contribute significantly to colonization and infection by this organism.

Antimicrobial susceptibility testing demonstrated considerable resistance to carbapenems, with resistance rates of 26.8% and 28.8% for imipenem and meropenem, respectively. Comparable resistance patterns have been reported by Sadeghi et al. (19). Resistance to ciprofloxacin and ceftazidime was also notable, whereas piperacillin–tazobactam exhibited the highest susceptibility (82.4%), consistent with findings reported by Bunyan et al. (20) and Azimi et al. (21). These findings emphasize the importance of antibiotic stewardship programs to guide empirical therapy.

Among the imipenem-resistant isolates, majority belonged to elderly patients indicating a possibility of increase in resistance with comorbidities and age.

Among the 67 imipenem-resistant isolates, 44.8% were identified as Metallo-β-lactamase (MBL) producers by the **Double Disk Synergy Test**. This prevalence is comparable to that reported by Pitout et al. (15) but lower than rates described by Kabir et al. (22). The moderate prevalence observed in the present study suggests that, in addition to MBL production, alternative mechanisms such as porin loss and efflux pump overexpression may contribute to carbapenem resistance, as previously described by Lister et al. (23).

The predominance of MBL-producing isolates among hospitalized patients and from respiratory specimens is suggestive of hospital-associated transmission. These findings reinforce the need for antimicrobial stewardship, routine surveillance, and strict infection prevention measures, particularly in intensive care settings.

The major strengths of this study include the analysis of a substantial number of non-duplicate isolates. Furthermore, the correlation of Imipenem resistance with patient and sample distribution provides additional epidemiological insights that have been inadequately explored in previous studies.

LIMITATIONS: The study has certain limitations. Phenotypic methods may have lower sensitivity and specificity compared with molecular techniques for the detection of MBL-producing isolates. Molecular characterization was not investigated which is a major limitation.

FUTURE DIRECTIONS: Future studies should include **molecular detection panels** covering a broader range of carbapenemase genes, to understand which genes are responsible for resistance. That will help elucidate transmission dynamics and identify potential outbreaks.

CONCLUSION:

The present study demonstrated a considerable prevalence of imipenem resistance and MBL production among *P. aeruginosa* isolates, particularly in surgical and critically ill patients. The high level of resistance to multiple

antibiotic classes emphasizes the need for judicious antibiotic use, early detection of MBL producers, and strengthened infection control practices.Routine surveillance and antibiotic stewardship programs are essential to stop the spread of multidrug-resistant *P. aeruginosa* in healthcare settings.

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AUTHOR CONTRIBUTIONS:

V.P.: Data collection, methodology, performing research, investigation, analysis, writing original draft, funding.
A.B.H.: Conceptualization, study idea, supervision, review of draft, publication idea and research.

DECLARATION OF INTERESTS:

There are no competing interests to declare.

REFERENCES:

1. Radhika A, Lakshmi JT, Ariyanachi K, Sakthivadivel V. Detection of metallo beta-lactamase (MBL) producing *Pseudomonas aeruginosa* in a tertiary care hospital, Ghanpur, Medchal, India. *Maedica (Bucur)*. 2022;17(1):134-42.
2. Patel D. Antibiotic resistance, a global pandemic [Internet]. Knoxville (TN): University of Tennessee; 2021 [cited 2026 Jun 11]. Available from: https://trace.tennessee.edu/utk_chanhonoproj/2435
3. Bedenić B, Sardelić S. Carbapenemases. In: Mishra M, editor. *Growing and Handling of Bacterial Cultures*. London: IntechOpen; 2018. Chapter 5. doi:10.5772/intechopen.76282.
4. Ameen N, Memon Z, Shaheen S, Fatima G, Ahmed F. Imipenem resistant *Pseudomonas aeruginosa*: the fall of the final quarterback. *Pak J Med Sci*. 2015;31(3):561-5.
5. Abbas FM. Metallo-β-lactamases: a review. *Ann Rom Soc Cell Biol*. 2021;25(5):1308-16.
6. Procop GW, Church DL, Hall GS, Janda WM. Koneman'scolor atlas and textbook of diagnostic microbiology. Jones & Bartlett Learning; 2020 Jul 1.
7. Cheesbrough M. District laboratory practice in tropical countries, part 2. Cambridge university press; 2005.
8. Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney practical medical microbiology. 14th ed. Edinburgh: Churchill Livingstone; 1996.
9. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 34th ed. CLSI supplement M100. Wayne, PA: CLSI; 2024.
10. Maharjan N. Pseudomonas aeruginosa isolates among clinical samples showing growth in a tertiary care centre: a descriptive cross-sectional study. JNMA J Nepal Med Assoc. 2022;60(252):676–680. doi:10.31729/jnma.6517. PMID:36705229; PMCID: PMC9446506.
11. Khan JA, Iqbal Z, Rahman SU, Farzana K, Khan A. Prevalence and resistance pattern of Pseudomonas aeruginosa against various antibiotics. Pak J Pharm Sci. 2008;21(3):311 5. PMID:18614431.
12. Acharya M, Joshi PR, Thapa K, Aryal R, Kakshapati T, Sharma S. Detection of MBL genes among Pseudomonas aeruginosa. BMC Res Notes. 2017;10(1):1-6.
13. Strateva T, Yordanov D. Pseudomonas aeruginosa – a phenomenon of bacterial resistance. J Med Microbiol. 2009;58(9):1133-48.
14. Hirsch EB, Tam VH. Impact of multidrug-resistant Pseudomonas aeruginosa infection on patient outcomes. Expert Rev Pharmacoecon Outcomes Res. 2010;10(4):441-51.
15. Pitout JD, Gregson DB, Poirel L, McClure JA, Le P, Church DL. Detection of Pseudomonas aeruginosa producing metallo-β-lactamases. J Clin Microbiol. 2005;43(7):3129-35.
16. Qureshi R, Shafique M, Shahid M, Rasool MH, Muzammil S. Detection of blaVIM gene in carbapenem-resistant Pseudomonas aeruginosa. Pak J Pharm Sci. 2018;31(5):1949-53.
17. Sheikh AF, Rostami S, Jolodar A, Tabatabaiefar MA, Khorvash F, Saki A, Shoja S, Sheikhi R. Detection of metallo-β-lactamases among carbapenem-resistant Pseudomonas aeruginosa. Jundishapur Journal of Microbiology. 2014;7(11):e12289.
18. Sachdeva R, Sharma B, Sharma R. Evaluation of different phenotypic tests for detection of metallo-β-lactamases in imipenem-resistant Pseudomonas aeruginosa. Journal of Laboratory Physicians. 2017;9(4):249.
19. Sadeghi A, Rahimi B, Shojapour M. Molecular detection of blaVIM and blaIMP genes in Pseudomonas aeruginosa. Jundishapur J Microbiol. 2012;5(3):460-3.
20. Bunyan IA, Hadi OM, Al-Mansoori HA. Molecular detection of MBL-producing Pseudomonas aeruginosa. J Biol Agric Healthc. 2018;8(16):1-6.
21. Azimi A, Peymani A, Pour PK. Detection of MBL-producing Pseudomonas aeruginosa in burn patients. Rev Clin Med. 2018;5(2):52-6.
22. Kabir RB, Jobayer M, Farhana N, Shamsuzaman SM. Prevalence of MBL-producing Pseudomonas aeruginosa. Bangabandhu Sheikh Mujib Med Univ J. 2024;17(1):e65847.
23. Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev. 2009;22(4):582-610.