Article published online: 2024-08-26

Prevalence and Molecular Characterization of Carbapenem and Colistin-Resistance Genes in Clinical Strains of *Pseudomonas aeruginosa* Isolated from Diabetic Patients

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J Health Allied Sci[NU]

Abstract

Introduction *Pseudomonas aeruginosa* is one of the important opportunistic nosocomial pathogens that are increasingly becoming resistant to many drugs including the drugs of last resort, carbapenems, and colistin.

Methods The study involved the examination of clinical isolates of *P. aeruginosa* recovered from diabetic patients for the presence of acquired genes implied in carbapenem and colistin resistance.

Results A total of 100 clinical isolates from diabetic patients' wound and sputum were investigated for their susceptibility to imipenem and colistin followed by phenotypic assay and resistance gene screening. The study revealed 27 sputum isolates being resistant to imipenem further confirmed by a modified Hodge test and 28 wound isolates were resistant to colistin. The resistant isolates of wound and sputum were found to possess bla_{OXA-48}, bla_{VIM}, *mcr-1*, and *mcr-5* genes. The strains had a coexistence of carbapenem- and colistin-resistant genes.

Conclusion Considering the fact that infection by such resistant isolates in diabetics could be frightening, clinical laboratories must employ rapid diagnostic methods to detect resistance which can aid in the treatment decision-making.

Keywords
- *Pseudomonas aeruginosa*
- diabetic patients
- resistance
- colistin
- carbapenem

Introduction

The emergence of new variants possessing acquired resistance genes is posing a threat to public health. These genes can be rapidly disseminated among the strains because they exist on the mobile genetic elements.[1] The continuous emergence of antibiotic resistance in bacteria is leading us into the postantibiotic era. The emergence of superbugs carrying extended-spectrum beta-lactamases, AmpC beta-lactamases, and metallo-beta-lactamases has reduced therapeutic choices. Antibiotic resistance in bacteria is a caution for implementing infection control.[2] *Pseudomonas aeruginosa* is an important opportunistic nosocomial pathogen that causes a wide spectrum of disease in the debilitated. The emergence of multidrug-resistant *P. aeruginosa* is of clinical importance as it leads to an increased morbidity with mortality rates ranging from 20 to 60% in hospital-acquired infections.[3] Due to the compromised defenses, diabetics are prone to microbial infections. Infections such as soft tissue infection, respiratory infection, urinary tract infection, and periodontitis frequently occur in patients with diabetes.[4] *Pseudomonas aeruginosa* is the most frequently

EPub Ahead of Print: 26 August 2024
Published: 23 April 2025
DOI https://doi.org/10.1055/s-0044-1789212.
ISSN 2582-4287.

This article was initially published as Online First by Thieme Medical & Scientific Publishers Pvt. Ltd., and later included in the Issue compiled by Scientific Scholar.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

isolated bacteria from diabetic foot ulcers and its presence can be correlated with poor wound healing.[5] A study has indicated that uncontrolled hyperglycemia in diabetics increases airway glucose levels, thereby creating an ideal environment for the growth of microorganisms.[6] Therefore, diabetic patients with cystic fibrosis and chronic pulmonary obstruction are at a risk of developing pulmonary-associated pneumonia, with *P. aeruginosa* being the most bothersome multidrug-resistant bacterium causing ventilator-associated pneumonia.[7] Infections by *P. aeruginosa* are challenging to treat due to its inherent resistance to several antipseudomonal drugs and its capacity to rapidly develop antibiotic resistance.[8] For these reasons, carbapenems and colistin have become the drugs of last resort for the clinical management of resistant *P. aeruginosa* infections. Although colistin was introduced for clinical use in the 1960s, due to its neurotoxic and nephrotoxic effects, in the 1970s, it was replaced with other antibiotics. Due to the rise in extremely drug-resistant (XDR) bacteria, colistin was reintroduced in the 1990s as an emergency drug. Similarly, for the treatment of drug-resistant *P. aeruginosa* infections, carbapenems have gained importance.[9] On account of the extensive use of carbapenems and colistin, there is an increase in the resistance to these antibiotics in the strains causing nosocomial infections, especially in immune-deficient patients.[10] The rise in the resistance to these antibiotics can be attributed to the evolution of divergent β-lactamases and also the acquisition of mobile colistin resistance (*mcr*) genes.[11] Owing to the multidrug-resistant nature of *P. aeruginosa*, it becomes imperative to have a deeper understanding of the acquired genes with carbapenem/colistin resistance. The paucity of studies in this area prompted us to undertake the current investigation to identify the acquired genes responsible for resistance to carbapenems and colistin in *P. aeruginosa*.

Methods

Revival of the Isolates

A total of 100 clinical isolates (wound: 64; sputum: 36) recovered from diabetes patients' wound and sputum samples and stored as glycerol stocks in −80°C were revived. The initial recovery of the isolates from the patients' sample was done by taking clearance from the Central Ethics Committee of the Nitte University (NU/CEC/2019/0229) and was conducted as per the ethical guidelines. Pus/wound swab and sputum samples from diabetic patients with respiratory infection were collected. The samples from diabetic patients older than 18 years and irrespective of sex with a history of diabetes (of more than 5 years) and with a blood glucose concentration more than 150 mg/dL were included in the study. The isolates were enriched in asparagine broth and streaked onto cetrimide agar to get a single colony. Further, the single colony was picked and subcultured on nutrient agar medium.

Antibiotic Susceptibility Testing

Pseudomonas aeruginosa strains were checked for their susceptibility to carbapenem using the Kirby–Bauer disc diffusion method following the Clinical and Laboratory Standards Institute guidelines (CLSI 2020). The control strain used for antibiotic susceptibility testing was *P. aeruginosa* ATCC 27853. A 100 µL of 0.6 OD culture grown in Mueller–Hinton broth (MHB) was swabbed over a sterile Mueller–Hinton agar (MHA) plate. A 10-µg imipenem disc (HiMedia Laboratories Pvt. Ltd., India) was placed on the swabbed MHA plate and incubated at 37°C for 16 to 18 hours. The zone of inhibition (mm) around the disc was compared with the interpretive chart of CLSI, and the strains were designated as sensitive, intermediate, or resistant. Susceptibility to colistin was performed by microbroth dilution method as described in the CLSI guidelines. In brief, a serial twofold dilution of colistin (HiMedia Laboratories Pvt. Ltd.) was carried out in cation-adjusted Mueller–Hinton II broth (CA-MHBII) (500 µL) ranging from 0.125 to 256 µg/mL. A 100 µL of 10^5 CFU/mL culture was inoculated to the medium with different dilutions of the antibiotic and incubated for 24 hours at 37°C. The isolates were classified as per the CLSI colistin breakpoints, as susceptible (2 µg/mL) or resistant (4 µg/mL) based on the turbidity.

Modified Hodge Test

Modified Hodge assay was performed as described by Amjad et al (2011).[12] A 100 µL of 10^6 culture of *Escherichia coli* ATCC 25922 was inoculated to 5 mL of MHB and incubated at 37°C for 16 to 24 hours. After the incubation, 0.5 mL of the 10^6 culture was added to another 4.5 mL of MHB to create a 1:10 dilution. A 100 µL of diluted culture was used to prepare a lawn on an MHA plate. Meropenem disk (10 µg) was placed at the center of the plate, and *P. aeruginosa* was streaked from the edge of the disc to the edge of the plate in a straight line. Four isolates were tested on a single plate. The plate was incubated for 16 to 24 hours at 37°C.

Molecular Detection of Antibiotic-Resistant Genes

The method of Ausubel et al (1992)[13] with slight modifications was employed for the extraction of genomic DNA from the strains of *P. aeruginosa* and the reference strain PAO1. Precipitation of DNA was carried out by using isopropanol and 100 µL of 1× Tris-EDTA (pH 8.0) buffer was used to resuspend it. The extracted DNA was checked for its concentration and purity using the spectrophotometer at 260 and 280 nm (BioSpectrometer, Eppendorf, Germany). Specific primers for antibiotic-resistant genes were used to amplify the isolated DNA (►**Table 1**). Polymerase chain reaction (PCR) was carried out in a 25-µL reaction mixture with 40 ng of DNA, 40 pmol of primer, 200 µM of deoxynucleotide triphosphates, 1.0 U of Taq DNA polymerase (HiMedia Laboratories Pvt. Ltd.), and 3 µL of 10X buffer. The PCR programming was with an initial denaturation of 5 minutes at 94°C, followed by 35 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 55°C, and extension for 1 minute at 72°C (Bio-Rad T100, United States). A 1.5% agarose gel was used for electrophoresis and documented using a gel documentation system.

Results and Discussion

A surge in the use of carbapenems and colistin to treat infections with *P. aeruginosa* is due to the increase in

Table 1 Primers used for the detection of antibiotic-resistant genes

Gene	Primer sequence	Product size (bp)	Reference
bla_{NDM}	F: GGTTTGGCGATCTGGTTTTC R: GGAATGGCTCATCACGATC	621	14
bla_{OXA-48}	F: AAGTATTGGGGCTTGTGCTG R: CCCCTCTGCGCTCTACATAC	599	15
bla_{VIM}	F: GATGGTGTTTGGTCGCAT R: CGAATGCGCAGCACCAG	390	16
mcr-1	F: CGGTCAGTCCGTTTGTTC R: CTTGGTCGGTCTGTAGGG	375	This study
mcr-5	F: ATGTTGCCAGAAGGTCCAAC R: GATCAAGGTGCCGATGATCT	650	This study

its resistance to the commonly used antibiotics.[17] The escalated use of carbapenems and colistin has in turn led to a rise in the resistance to these antibiotics making the treatment difficult. As studies related to carbapenem and colistin resistance are limited, they fail to address the enormity of the major problem confronting our country's health sector. This instigated us to take up the current investigation to detect the carbapenem- and colistin-resistant genes in isolates of *P. aeruginosa* recovered from diabetic patients.

Antibiotic Susceptibility Test

Antibiotic susceptibility testing of 64-wound and 36-sputum isolates against imipenem revealed greater resistance in sputum isolates (75%) followed by wound isolates (43.7%). More number of wound isolates (51%) was found to be sensitive to imipenem. Studies in the past have also observed resistance of *P. aeruginosa* strains to imipenem. Premanadham et al (2016)[18] reported a resistance of 25.8%, Kaur et al. (2016)[6] with 17.8%, Ahmed (2016)[19] with 25%, and Lakum et al (2016)[20] with 21.6% resistance to imipenem in *P. aeruginosa* isolates. In comparison to previous studies, our study found a substantial increase in resistance in *P. aeruginosa* against imipenem. This could be attributed to the overuse and misuse of imipenem in treating infections with *P. aeruginosa* or ineffective infection prevention and control which has led to an increase in resistance to this drug. The transfer of resistance genes could be vertical, where the daughter cells inherit the genes, or horizontal, when the genetic material is shared between different species.[21] The presence of more number of sputum isolates being resistant to imipenem could be attributed to the source of the sample from which it was isolated. For instance, carbapenem resistance is higher in isolates from respiratory infection than other sites of infection.[22] Eighty-six percent of the sputum and 82% wound isolates were resistant to colistin. The number of isolates resistant to colistin was more as compared with imipenem. The resistance observed against colistin in our study was significantly greater than the studies of the past. In studies by Tahmasebi et al (2020)[23] and Abd El-Baky et al (2020),[24] *P. aeruginosa* isolates have shown resistance of 3.5 and 21.3%, respectively.

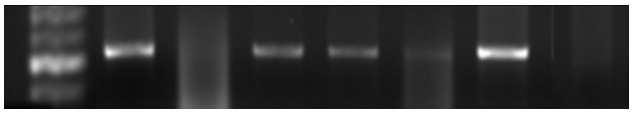
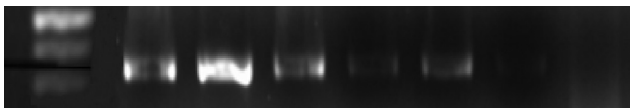
Modified Hodge Test

All the isolates that showed resistance to imipenem by disc diffusion method were further confirmed by Hodge test. The increased growth of the indicator strain toward the carbapenem disk and the clover leaf-like indentation at the spot where the isolate and the indicator strain intersected were signs of a positive result. Of the 55 isolates (wound = 28 and sputum = 27) subjected to the test, all the isolates were positive indicating the elaboration of the carbapenemase enzyme conferring them with resistance to the antibiotic (►**Supplementary Fig. S1**). In the present investigation, all the 55 isolates were positive for the test, whereas studies by Jayalakshmi and Pandurangan (2016)[25] and Datta et al (2017)[26] observed a positive result in nearly 50% of the isolates. The increase in the number of isolates producing carbapenemase enzyme as observed in the present study might be correlated with the rise in the horizontal transfer of plasmid between the isolates carrying genes encoding the enzyme. Moreover, horizontal gene transfer has been shown to play an important role in the spread of multidrug resistance in gram-negative bacteria.[27]

Molecular Detection of Antibiotic-Resistant Genes

All the isolates resistant to imipenem and colistin were screened for the presence of carbapenem- (bla_{VIM}, bla_{NDM}, bla_{OXA-48}) and colistin- (*mcr-1*, *mcr-5*) resistant genes. The representative gel images and prevalence of resistance genes are given in ►**Table 2** and ►**Fig. 1**. All the resistant isolates of wound and sputum were found to possess bla_{OXA-48} gene. None of the sputum and wound isolates were found to harbor bla_{NDM}. Of the 28 resistant wound isolates, bla_{OXA-48} was observed in 20 isolates, and 8 isolates were shown to be with both bla_{OXA-48} and bla_{VIM}. None of the wound isolates was found to be with only bla_{VIM}. The same trend was seen in sputum isolates also. Of the 27 sputum isolates, bla_{OXA-48} was found in 20 of the strains, and bla_{OXA-48} and bla_{VIM} was seen in 5 isolates. In contrast to our findings, Datta et al (2017)[26] observed the presence of 74 and 34% of isolates with bla_{VIM} and bla_{OXA-48}, respectively. The higher prevalence of OXA-48 and VIM as seen in the current investigation is following a study by Suwantarat and Carroll (2016)[28] who has shown these genes as the predominant carbapenemases occurring in Southeast Asia.

Table 2 Representative agarose gel electrophoresis image of PCR amplification of antibiotic-resistant genes

Gene	M L1 L2 L3 L4 L5 L6 L7	Product size (bp)
bla$_{VIM}$		390
bla$_{OXA}$		599
mcr-5		650
mcr-1		375

Abbreviations: L1–L6, bacterial isolates; L7, negative control; M, 100 bp marker; PCR, polymerase chain reaction.

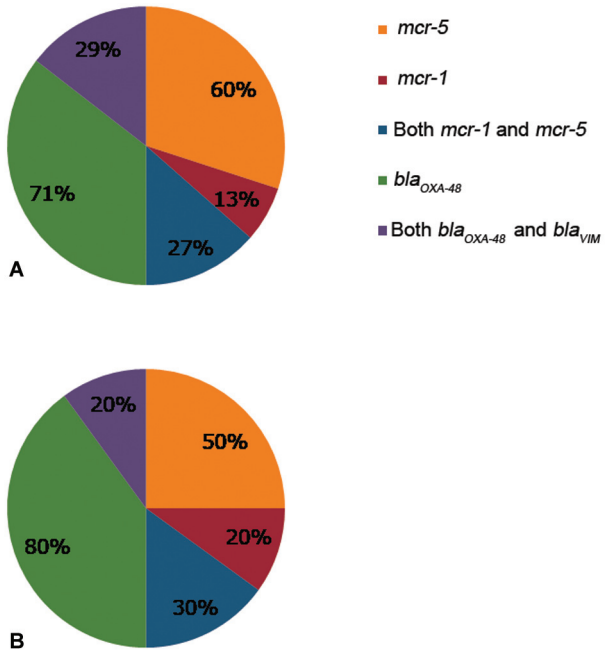


Fig. 1 Prevalence of carbapenem- and colistin-resistant genes in *Pseudomonas aeruginosa* (A) wound and (B) sputum isolates.

Of the 53 resistant wound isolates, *mcr* genes *(mcr-1* and *mcr-5*) were found in 30 isolates. In 18 isolates, only *mcr-5* gene was found and in 4 isolates, only *mcr-1* gene was observed. In eight isolates, both *mcr-1* and *mcr-5* were found. Of the 27 resistant sputum isolates, *mcr-5* was noticed in 10 strains and 4 isolates were with *mcr-1*. Both *mcr-1* and *mcr-5* were detected in six isolates. The number of isolates with *mcr-5* gene exceeded that of *mcr-1*. The *mcr* genes widely reported in Enterobacteriaceae are very rare in *P. aeruginosa*.[29] Although, *P. aeruginosa* strains have been recognized to be with carbapenemase genes, resistance to polymyxins and the detection of *mcr* genes is not frequent. As the study has indicated the presence of *mcr* genes in a large number of isolates, their detection becomes very important to improve the treatment of infected cases with MDR and XDR *P. aeruginosa* strains. The lower prevalence of *mcr-1* gene in the isolates could be possibly a mechanism to reduce changes in the membrane permeability that occurs due to phosphoethanolamine transferase encoded by *mcr-1* gene that alters the structure of lipopolysaccharide making it susceptible to hydrophobic antibiotics.[30] In both wound and sputum isolates, there was a coexistence of colistin and carbapenemase genes in 16 and 31% of the strains, respectively. Similar results have been observed in a study by Chen et al (2022) [31] where the isolates coharbored *bla*$_{NDM-1}$ and *mcr-1* genes.

Conclusion

Injudicious use of drugs is one of the main contributors to the emerging resistance of *P. aeruginosa* to antimicrobial therapy. The study has clearly indicated that it is not only carbapenem resistance that is higher in a nonfermenter such as *P. aeruginosa* but also colistin resistance which is significantly increasing. The investigation has revealed the presence of a large number of colistin-resistant *P. aeruginosa* isolates harboring the resistant genes found in diabetic patients with respiratory and wound infections. An increase in resistance to these drugs indicates their excessive use in hospitals, ineffective infection control, and inadequate availability of rapid diagnostic tests to enable early treatment in patients who are colonized by carbapenem- and colistin-resistant pathogens. Infection by such resistant pathogens in patients with diabetes is quite alarming. As carbapenem- and colistin-resistant *P. aeruginosa* is a threat to public health care, it is important for clinical laboratories to employ rapid diagnostic tests to detect carbapenem and colistin resistance which can facilitate therapeutic decision-making. And also, characterization of the epidemiological trends in high-risk *P.*

aeruginosa isolates is required to optimize the use of these last-resort antibiotics. Furthermore, effective infection control measures are promptly needed to prevent further carbapenem- and colistin-resistant transmission.

Authors' Contribution

S.S. contributed to the investigation, data curation, writing—original draft, and formal analysis. G.S. contributed to the investigation, formal analysis, and writing—original draft. A.N. and B.J.N. contributed to the formal analysis and writing—original draft. R.P. contributed to the conceptualization, funding acquisition, supervision, and writing—review and editing.

Ethical Approval

Central Ethics Committee of the NITTE University (-NU/CEC/2019/0229)—2019.

Funding

The work was supported by the NITTE University Research Grant with the grant number (N/RG/NUSR2/NUCSER/2021).

Conflict of Interest

None declared.

Acknowledgment

We thank NITTE (Deemed to be University) for all the facilities provided.

References

1 Zavascki AP, Barth AL, Gonçalves AL, et al. The influence of metallo-β-lactamase production on mortality in nosocomial *Pseudomonas aeruginosa* infections. J Antimicrob Chemother 2006;58(02):387–392
2 Shindo Y, Hasegawa Y. Regional differences in antibiotic-resistant pathogens in patients with pneumonia: implications for clinicians. Respirology 2017;22(08):1536–1546
3 Gholami S, Tabatabaei M, Sohrabi N. Comparison of biofilm formation and antibiotic resistance pattern of *Pseudomonas aeruginosa* in human and environmental isolates. Microb Pathog 2017;109:94–98
4 Toniolo A, Cassani G, Puggioni A, et al. The diabetes pandemic and associated infections: suggestions for clinical microbiology. Rev Med Microbiol 2019;30(01):1–17
5 Goldufsky J, Wood SJ, Jayaraman V, et al. *Pseudomonas aeruginosa* uses T3SS to inhibit diabetic wound healing. Wound Repair Regen 2015;23(04):557–564
6 Kaur A, Singh S, Kaur A, Kaur N. Prevalence and antimicrobial susceptibity pattern of *Pseudomonas aeruginosa* isolated from various clinical samples in a tertiary care hospital, Bathinda. Indian J Basic Appl Med Res 2016;5:777–784
7 Huber P, Basso P, Reboud E, Attrée I. *Pseudomonas aeruginosa* renews its virulence factors. Environ Microbiol Rep 2016;8(05):564–571
8 Çopur Çiçek A, Ertürk A, Ejder N, et al. Screening of antimicrobial resistance genes and epidemiological features in hospital and community-associated carbapenem-resistant *Pseudomonas aeruginosa* infections. Infect Drug Resist 2021;14:1517–1526
9 Poirel L, Lambert T, Türkoglü S, Ronco E, Gaillard J, Nordmann P. Characterization of Class 1 integrons from *Pseudomonas aeruginosa* that contain the bla(VIM-2) carbapenem-hydrolyzing β-lactamase gene and of two novel aminoglycoside resistance gene cassettes. Antimicrob Agents Chemother 2001;45(02):546–552
10 Hakemi Vala M, Hallajzadeh M, Hashemi A, et al. Detection of Ambler class A, B and D ß-lactamases among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* clinical isolates from burn patients. Ann Burns Fire Disasters 2014;27(01):8–13
11 Knopp M, Babina AM, Gudmundsdóttir JS, Douglass MV, Trent MS, Andersson DI. A novel type of colistin resistance genes selected from random sequence space. PLoS Genet 2021;17(01):e1009227
12 Amjad A, Mirza Ia, Abbasi S, Farwa U, Malik N, Zia F. Modified Hodge test: a simple and effective test for detection of carbapenemase production. Iran J Microbiol 2011;3(04):189–193
13 Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, et al. Short Protocols in Molecular Biology. Vol. 275. New York:1992:28764–28773
14 Shetty V, Divyashree M, Kumar DV, Shetty A, Karunasagar I. Emergence of New Delhi metallo-β-lactamase (NDM-1)-encoding gene among gram-negative bacteria isolated from hospital effluents. Open Forum Infect Dis 2015;2:1789
15 Woodford N, Ellington MJ, Coelho JM, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int J Antimicrob Agents 2006;27(04):351–353
16 Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-β-lactamases. J Antimicrob Chemother 2007;59(02):321–322
17 Vinodkumar CS, Hiresave S, Kandagal Giriyapal B, Bandekar N. Metallo beta lactamase producing *Pseudomonas aeruginosa* and its association with diabetic foot. Indian J Surg 2011;73(04):291–294
18 Premanadham N, Jitendra K, Siva Prasad Reddi M, Ramya,Kumar C. Antibiotic resistance pattern of *Pseudomonas aeruginosa* strains isolated from blood cultures-Batec/Alert3D in a Tertiary Care Centre Narayana Hospital & Medical College Nellore AP, India. Int J Curr Microbiol Appl Sci 2016;5:263–268
19 Ahmed OB. Incidence and antibiotic susceptibility pattern of *Pseudomonas aeruginosa* isolated from inpatients in two Tertiary Hospitals. Clin Microbiol 2016;5:248–252
20 Lakum S, Pandya H, Shah K, Lakhani SJ. Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* at the tertiary care center, Dhiraj Hospital, Piparia, Gujarat. Int Arch Integr Med 2016;3:133–137
21 Tao S, Chen H, Li N, Wang T, Liang W. The spread of antibiotic resistance genes in vivo model. Can J Infect Dis Med Microbiol 2022;2022:3348695
22 Cai B, Echols R, Magee G, et al. Prevalence of carbapenem-resistant Gram-negative infections in the United States predominated by *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Open Forum Infect Dis 2017;4(03):ofx176
23 Tahmasebi H, Dehbashi S, Arabestani MR. Prevalence and molecular typing of colistin-resistant *Pseudomonas aeruginosa* (CRPA) among β-lactamase-producing isolates: a study based on high-resolution melting curve analysis method. Infect Drug Resist 2020;13:2943–2955
24 Abd El-Baky RM, Masoud SM, Mohamed DS, et al. Prevalence and some possible mechanisms of colistin resistance among multidrug-resistant and extensively drug-resistant *Pseudomonas aeruginosa*. Infect Drug Resist 2020;13:323–332
25 Jayalakshmi S, Pandurangan S. Performance of modified Hodge test for detection of carbapenemase producing clinical isolates of *Pseudomonas aeruginosa*. Int J Curr Microbiol Appl Sci 2016;5:127–132
26 Datta S, Dey R, Dey JB, Ghosh S. A comparative study of modified Hodge test and Carba NP test for detecting carbapenemase production in Gram-negative bacteria. . Med. j. Dr. D Y Patil Univ 2017;10:365–369
27 Hardiman CA, Weingarten RA, Conlan S, et al. Horizontal transfer of carbapenemase-encoding plasmids and comparison with hospital epidemiology data. Antimicrob Agents Chemother 2016;60(08):4910–4919

28 Suwantarat N, Carroll KC. Epidemiology and molecular characterization of multidrug-resistant Gram-negative bacteria in Southeast Asia. Antimicrob Resist Infect Control 2016;5:15
29 Snesrud E, Maybank R, Kwak YI, Jones AR, Hinkle MK, McGann P. Chromosomally encoded *mcr-5* in colistin-nonsusceptible *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2018;62 (08):e00679–e18
30 Wei Y, Zhao X, Sun J, Liu H. Fast repetition rate fluorometry (FRRF) derived phytoplankton primary productivity in the Bay of Bengal. Front Microbiol 2019;10:1164
31 Chen H, Mai H, Lopes B, Wen F, Patil S. Novel *Pseudomonas aeruginosa* strains co-harbouring *bla* $_{\text{NDM-1}}$ metallo β-lactamase and *mcr-1* isolated from immunocompromised paediatric patients. Infect Drug Resist 2022;15:2929–2936

