Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

Evaluation of Five Phenotypic Methods of Carbapenemase Enzyme Detection, In Identification of Carbapenem-resistant Pseudomonas aeruginosa.

P.Samatha, C.H.Suryamani, Sangeetha Panigrahy, Mudde Sravanthi, P.B.C.Shekar

1 Professor & Head, Department of Microbiology, Great Eastern Medical School & Hospital, Srikakulam

2 Assistant Professor, Department of Microbiology, Government Medical College, Vizianagaram.

3,4 Assistant Professor, Department of Microbiology, Great Eastern Medical School, Srikakulam

Corresponding Author

Dr.P.Samatha,

Professor & Head,

Department of Microbiology,

Great Eastern Medical School & Hospital, Srikakulam

Abstract

Introduction: Pseudomonas aeruginosa is one of the most frequently isolated pathogens in patients with healthcare-associated infections.Carbapenems are widely used in the treatment of infections with MDR *P. aeruginosa.* Carbapenemase-producing P. aeruginosa strains are resistant to almost all β-lactams and the rising prevalence of MDR *P. aeruginosa* have become a major concern of public health practitioners.

Materials and methods: This study was conducted at Great Eastern Medical School and Hospital, in the department of microbiology from July 2021 to December 2022. Pseudomonas isolates exhibiting resistance to meropenem and/or imipenem by disk diffusion method were subjected to five phenotypic methods such as Combined Disc Test (CDT), EDTA-Disk Synergy (EDS) Test**,** modified Hodge test (MHT), modified carbapenem inactivation method (mCIM), and Growth on KPC CHROMagar for carbapenemase detection.

Results: Of 1638 Pseudomonas aeruginosa isolates, 236 exhibited resistance to carbapenem by disk diffusion method. 157 (66.52%) isolates of the 236 were positive by Double-Disk Synergy Test(DDST). 194 (82.22%) isolates were positive by Combined disk test, 204 (86.44%) isolates were positive by Modified Hodge test (MHT). 196(83.05%) isolates were found positive for carbapenemases by Modified Carbapenem Inactivation Method (mCIM). Overall, 231 (97.88%) Meropenem resistant isolates were found positive for carbapenemase by Growth on KPC CHROMagar.

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

Conclusion: KPC CHROMagar, exhibited higher sensitivity and specificity than the other four phenotypic methods performed in our study. Regular monitoring and documenting carbapenem resistance in Pseudomonas aeruginosa, help us in judiciously using antibiotics and developing the strategies to control its spread in hospital settings.

Key words:
Carbapenems, Meropenem, Carbapenemases, Pseudomonas aeruginosa.

Introduction

Pseudomonas aeruginosa is an opportunistic pathogen, most frequently isolated in healthcare-associated infections, that can be particularly severe in immunocompromised patients. These pathogens are common causative agents of pneumonia, bacteremia, urinary tract, skin and soft tissue infections.[1,2] Carbapenems are widely used in the treatment of infections with MDR *P. aeruginosa*, and colistin is used as an antibiotic of last resort for the past 10 years. However, there has been a significant growth of carbapenem-resistant organisms that cause severe damage to public health.[3,4] Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) is noted as an organism posing a severe threat by the Centers for Disease Control and Prevention.[5]

P. aeruginosa is recognized for its intrinsically advanced mechanisms of resistance to many antimicrobial classes, which may limit the choice of effective antibiotic therapy.[2] Carbapenem resistance in *P. aeruginosa* has shown to be multifactorial, including production of carbapenemase, overexpression/overproduction of efflux pump, and porin loss. Carbapenemases in *P. aeruginosa* belong to three molecular classes, for example, class A (^{bla}KPC), class B (^{bla}IMP, ^{bla}VIM, ^{bla}NDM, ^{bla}SIM, ^{bla}GIM, and ^{bla}SPM), and class D (^{bla}OXA-48) genes.[4] The commonly producing carbapenemases include Ambler class B or metallo-beta-lactamases (MBLs), mainly VIM-, IMP-, and NDM-types.(Z.Bousla) Ethylene-diamine-tetra-acetic acid (EDTA) and sodium mercapto-acetic acid (SMA) inhibit MβLs, whereas β-lactase inhibitors such clavulanic acid, sulbactam, and tazobactam had no effect on MLs.[6]

The early detection of carbapenemases is important for the early initiation of antibiotic therapy and to implement infection control procedures. Choosing an exact technique to detect carbapenemase production in *P. aeruginosa* is not recommended by CLSI to date. The aim of the study was to determine the frequency of carbapenem resistance *Pseudomonas aeruginosa* and detect the presence of carbapenemase enzymes in carbapenem-resistant *P. aeruginosa* (CRPA) isolates by different phenotypic methods.

Materials and methods:

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

This study was conducted at Great Eastern Medical School and Hospital, in the department of microbiology from July 2021 to December 2022. All consecutive nonduplicate isolates of *P. aeruginosa* from different clinical samples (blood, respiratory sample, pus, urine, sterile body fluids, and other samples) resistant to meropenem and/or imipenem by disk diffusion method were included in the study. Antimicrobial susceptibility testing was performed for all isolates using the Kirby-Bauer disk diffusion method according to the CLSI 2021 guidelines.[7] Phenotypic methods for carbapenemase detection were performed using the Combined Disc Test (CDT), EDTA-Disk Synergy (EDS) Test**,** modified Hodge test (MHT), modified carbapenem inactivation method (mCIM), and Growth on KPC CHROMagar**.**

Combined Disc Test (CDT): The test isolates along with standard control strains are adjusted to 0.5 McFarland opacity standard, were lawn cultured on MHA plate as recommended by CLSI. After drying, two 10 µg meropenem discs were placed on the lawn culture with 20 mm distance from centre to centre of the discs. A 10 µl of 0.5 M EDTA (Himedia) was added to one of the meropenem discs and incubated overnight. Isolates showing ≥7 mm increase in the inhibition zone size of meropenem-EDTA disc than the meropenem disc alone were considered as MBL producers.[4]

EDTA-Disk Synergy (EDS) Test: For the EDTA-disk synergy test an overnight broth culture of the test strain, (opacity adjusted to 0.5 McFarland opacity standard, bacterial inoculum was lawn cultured on a Muller Hinton Agar plate. After drying, a 10 µg meropenem disc and 6 mm disk of Whatmann filter were placed 10mm apart from edge to edge, 10 µl of 0.5 M EDTA (Himedia) solution was then applied to the blank disc, which resulted in approximately 1.5 mg/disc. The plates were incubated overnight at 37°C and an enhanced zone of inhibition was interpreted as EDS positive.[8]

Modified Carbapenem Inactivation Test Method (mCIM): Following overnight incubation, 1 µL loopful of test bacteria was transferred to a tube containing 2 mL of trypticase soy broth (TSB) and the suspension was vortexed. A 10 µg meropenem disk was added to the suspension. The TSB-disk suspension was incubated for 4 h at 37°C. A 0.5 McFarland dilution of *E. coli* ATCC 25922 was cultured on an MHA plate. The meropenem disk was removed from the TSB suspension and placed on an MHA plate inoculated with *E. coli* ATCC 25922. The plates were incubated overnight at 37°C. The presence of a zone diameter of 6–15 mm was considered positive (Figure 3) and a zone diameter of ≥19 mm was considered negative.[9]

Modified Hodge Test (MHT): Modified-Hodge test was carried out on Mueller-Hinton agar. An inoculum of overnight culture suspension of Klebsiella pneumoniae (ATCC700603) (Opacity of the tube was adjusted by comparing with a 1:10 dilution of 0.5 McFarland opacity standard, dipped in cotton swab was inoculated onto the plate as lawn culture. After brief drying, 10 µg meropenem disc was placed at

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

the center of the plate and the test strains(pseudomonas aeruginosa) were streaked from the edge of the disc to the periphery of the plate and the plates were incubated over-night at 37°C and organisms producing a 'cloverleaf shaped' zone of inhibition were identified as carbapenemase producers and interpreted as MHT positive. *Klebsiella pneumoniae* (ATCC BAA-1705) and *Klebsiella pneumoniae* (ATCC BAA-1706) were used as positive and negative controls respectively.[10]

Growth on KPC CHROMagar: The media was prepared with dehydrated powder of CHROMagar KPC (Himedia), which is supplemented with agents that inhibit the growth of gram-positive/gram-negative carbapenem-sensitive bacteria, according to the manufacturer's instructions. The suspected carbapenemase producing strains were inoculated and incubated for 24 hours at 37°C. Isolates that showed growth on CHROMagar KPC were considered as carbapenemase producers. The carbapenemase producing *Pseudomonas* isolates take green colours.[8]

Results:

A total of 7302 gram-negative bacilli were isolated from the processed clinical samples, of which 2284 (31.27%) were NFGNB The NFGNB includes *Pseudomonas aeruginosa* (1638), *Acinetobacter spp* (603), *Stenotrophomonas maltophilia* (36) and *Burkholderia cepacia* (7).

Of 1638 Pseudomonas aeruginosa isolates, 236 exhibited resistance to carbapenem by disk diffusion method. Carbapenem resistant Pseudomonas aeruginosa in different clinical specimens. Highest number of pseudomonas aeruginosa was isolated from Pus & Wound swabs 74 (31.35%) followed by endotracheal secretions 63(26.69%), urine 47(19.91%), blood 24(10.16%), sputum26(11.01%) and BAL 3(1.27%) (Table:1).

All the 236 isolates were subjected to four different phenotypic tests for carbapenemase detection, which included Combined Disc Test (CDT), EDTA-Disk Synergy (EDS) Test**,** modified Hodge test (MHT), modified carbapenem inactivation method (mCIM), and Growth on KPC CHROMagar (Table-2).

Of the 236 Meropenem resistant P.aeruginosa isolates 149 (63.13%) isolated from males and 87(36.86%) were isolated from females. All the 236 isolates were tested for carbapenemases by different phenotypic tests, of the 236 subjected to Double-Disk Synergy Test(DDST), 157 (66.52%) isolates were positive. By Combined disk test, 194 (82.22%) Meropenem P.aeruginosa were positive. 204 (86.44%) isolates were positive by Modified Hodge test (MHT). 196(83.05%) Pseudomonas aeruginosa were found positive for carbapenemases by Modified Carbapenem Inactivation Method (mCIM). Overall 231 (97.88%) Meropenem resistant isolates were found positive for carbapenemase by Growth on KPC

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

CHROMagar, which is higher than the other four phenotypic methods performed in our study.

Table 1: Distribution of Pseudomonas aeruginosa in different clinical specimens.

S.No	Organism	No. (%)
1	Pus & Wound swabs	74 (31.35%)
2	Urine	47 (19.91%)
3	Blood	24 (10.16%)
4	Endo tracheal aspirate	63(26.69%)
5	Sputum	26(11.01%)
6	BAL	2(0.84%)
Total		**236**

Table:2:- Comparison of Phenotypic methods in detection of carbapenemase production *(n=236)*.

S.No	Phenotypic tests	*Pseudomonas aeruginosa(n=236)*
1	Combined Disk Test (CDT)	194 (82.22%)
2	EDTA-Disk Synergy (EDS) Test	157 (66.52%)
3	Modified-Hodge Test (MHT)	204 (86.44%)
4	Modified Carbapenem Inactivation Method (mCIM)	196(83.05%)
5	Growth on CHROMagar KPC	231 (97.88%)

Discussion:

Due to its intrinsic and acquired resistance to a wide range of antibiotics, limited number of effective antimicrobial agent remain a choice for treatment. Carbapenem drugs are the most valuable drugs for treating multi-drug resistant Pseudomonas, which are producing extended-spectrum β-lactamases. Resistance to carbapenems is progressively being observed, especially in healthcare settings, which are difficult to treat, pose a huge economic burden and are allied with improved mortality and morbidity.[4]

In the present study, out of 2284 NFGNB, the most common organism isolated was *Pseudomonas aeruginosa* 1638(71.7%), *Acinetobacter spp* 603(26.4%), similar to a study reported by R.P.R. Suyambu Meenakshi et al,[11] 87% Pseudomonas aeruginosa, followed by 13% Acinetobacter baumanii. Almost similar findings were

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

observed by Gokhale and Metgud et al.,[12] with an isolation rate of Pseudomonas aeruginosa 82.3% followed by 16% of Acinetobacter baumanii.

Meropenem disk diffusion test was used in Screening test for carbapenem resistance according to CLSI guidelines. In the present study 14.40% of the isolates of Pseudomonas aeruginosa showed Meropenem resistance. Varying rates of carbapenem resistance were reported by other studies, Manoharan et al.,[13] reported 31.46% resistance to Meropenem, 44% by Adesola Olalekan et al.; 56% by Sonika Agarwal et al,[14] 51% by Raghdaa A.Ramadan et al.,[15] and 72.7% by Santosh Kumar Yadav et al.,[17]

Carbapenemase producing isolates were more commonly isolated from males 63.13% compared to females 36.86%, in this study. Similar findings were observed in a study by Ruchita Mahajan et al.,[18] and Elbadawi, H.S et al.,[19] who reported 59.79% from males; 40.20% from females and 53.4 % in Males and 46.6 % isolated in females respectively.

Meropenem resistant strains were screened for MBL production by CDT, IMP-EDTA disc synergy test, modified hodge test and Growth on KPC CHROMagar. The present study detected 194 (82.22%) and 157 (66.52%) MBL producers by CDT and DDST among Meropenem resistant Pseudomonas aeruginosa respectively, this is similar to the study reported by Jane Esther[8] observed 88.46% and 84.61%. R.P.R Suyambu et al.,[11] reported 59.8% and 50% MBL producers by CDT and DDST among Meropenem resistant NFGNB respectively. In contrast several researchers from various parts of the world observed increased sensitivity to DDST compared to CDT. A study by Vamsi et al.,[4] detected highest results (91.2% MBL producers) through DDST method in GNB.

In our study carbapenemases were detected in (86.44%) isolates by MHT, whereas a study by Jane Esther et al.,[8] has proven to be less sensitive as only 55.77% of the isolates were positive for MHT and Lee K et al.,[12] 66.66%. We observed highest efficacy with growth on CHROMagar KPC 231 (97.88%) has shown positivity for carbapenemase, which is higher than other phenotypic methods (MHT, DDST and CDT) performed in our study, similar findings were observed by Jane Esther et al.,[8] reported 94.23% positivity. Colistin and tigecycline can be considered as a therapeutic option for severe infections by Carbapenem resistant *A. baumannii* and *P. aeruginosa*.

Conclusion:

Carbapenemases do not always produce resistant breakpoints for carbapenems tested by routine susceptibility testing methods. The isolate may thus be reported as sensitive, while still harbouring carbapenemase enzyme, which results in potential treatment failure and dissemination of the resistant isolates. The infection control team and microbiologist needs to work together to determine the risk carried by multi drug resistant Pseudomonas aeruginosa infections. Hence, regular monitoring and documenting carbapenem resistance in Pseudomonas aeruginosa, help us in

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

judiciously using antibiotics and developing the strategies to control its spread in hospital settings.

Conflicts: No Conflicts

References:

1. Meletis G, Exindari M, Vavatsi N, Sofianou D, Diza E. Mechanisms responsible for the emergence of carbapenem resistance in Pseudomonas aeruginosa. Hippokratia. 2012 Oct;16(4):303-7. PMID: 23935307; PMCID: PMC3738602.

2. Z. Bouslah. Carba NP test for the detection of carbapenemase-producing Pseudomonas aeruginosa. Médecine et maladies infectieuses 50 (2020) 466–479.

3. Adesola Olalekan, Baris Kai Bader, Bamidele Iwalokun, Sophia Wolf, Albert Lalremruata, Adobi Dike, Morounke Mannie-Udoh, Libera Lo Presti, Jan Liese, Julia Guther, Paul D'alvise, Silke Peter, High incidence of carbapenemase-producing *Pseudomonas aeruginosa* clinical isolates from Lagos, Nigeria, *JAC-Antimicrobial Resistance*, Volume 5, Issue 2, April 2023, dlad038,

4. Vamsi SK, Moorthy RS, Hemiliamma MN, Chandra Reddy RB, Chanderakant DJ, Sirikonda S. Phenotypic and genotypic detection of carbapenemase production among gram negative bacteria isolated from hospital acquired infections. Saudi Med J. 2022 Mar;43(3):236-243.

5. Verma N, Prahraj AK, Mishra B, Behera B, Gupta K. Detection of carbapenemase-producing *Pseudomonas aeruginosa* by phenotypic and genotypic methods in a tertiary care hospital of East India. J Lab Physicians. 2019 Oct-Dec;11(4):287-291.

6. Saedii, A., Ahmed, A., Mohamed, O. Carbapenem resistant strains of pseudomonas species at Minia university hospitals. *Minia Journal of Medical Research*, 2022; 33(4): 160-165.

7. Wayne P. Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial disk diffusion susceptibility tests 19th ed. approved standard. CLSI document M100-S19. 2009; 29(2011):M100-S21.

8. Esther J, Edwin D, Uma. Prevalence of Carbapenem Resistant Non-Fermenting Gram Negative Bacterial Infection and Identification of Carbapenemase Producing NFGNB Isolates by Simple Phenotypic Tests. J Clin Diagn Res. 2017 Mar;11(3):DC10-DC13.

9. Khare AP, Gopinathan A, Leela KV, Naik S. Comparison of Three Phenotypic Methods of Carbapenemase Enzyme Detection to Identify Carbapenem-resistant Enterobacterales. *J Pure Appl Microbiol*. 2022;16(4):2679-2687.

10. Othman HB, Halim RMA, Abdul-Wahab HEE, Atta HA, Shaaban O. Pseudomonas aeruginosa - Modified Hodge Test (PAE-MHT) and ChromID Carba Agar for Detection of Carbapenemase Producing Pseudomonas Aeruginosa Recovered from Clinical Specimens. Open Access Maced J Med Sci. 2018 Dec 15;6(12):2283-2289.

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

11. R.P.R. Suyambu Meenakshi, S. Gowri, G. Sucilathangam and C. Revathy. Detection of Carbapenamase Resistance in Non-Fermenter Gram Negative Bacilli by Phenotypic Methods in A Tertiary Care Hospital. Int.J.Curr.Microbiol.App.Sci (2018) 7(3): 212-217.
12. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-beta-lactamase-producing isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol. 2003 Oct;41(10):4623-9.
13. Manoharan, A., S. chatterji *et al.,* detection and characterization of metallobetalactamse producing *Pseudomonas aeruginosa*. Indian Journal of Medical Microbiology. 2010; (28) (3): 241-244.
14. Adesola Olalekan, Baris Kai Bader, Bamidele Iwalokun, Sophia Wolf, Albert Lalremruata, Adobi Dike, Morounke Mannie-Udoh, Libera Lo Presti, Jan Liese, Julia Guther, Paul D'alvise, Silke Peter, High incidence of carbapenemase-producing *Pseudomonas aeruginosa* clinical isolates from Lagos, Nigeria, *JAC-Antimicrobial Resistance*, Volume 5, Issue 2, April 2023, dlad038,
15. Sonika Agarwal, Barnali Kakati, Sushant Khanduri, Shalini Gupta. Emergence of Carbapenem Resistant Non-Fermenting Gram-Negative Bacilli Isolated in an ICU of a Tertiary Care Hospital. Journal of Clinical and Diagnostic Research. 2017 Jan, Vol-11(1): DC04-DC07.
16. Raghdaa A Ramadan, Manar G Gebriel, Heba M Kadry, Ahmed Mosallem. Carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: characterization of carbapenemase genes and E-test evaluation of colistin-based combinations. Infection and Drug Resistance. 2018:11 1261–1269.
17. Santosh Kumar Yadav, Rajshree Bhujel, Pradip Hamal, Shyam Kumar Mishra, Sangita Sharma, Jeevan Bahadur Sherchand. Burden of Multidrug-Resistant *Acinetobacter baumannii* Infection in Hospitalized Patients in a Tertiary Care Hospital of Nepal. Infection and Drug Resistance, 03 Mar 2020, 13:725-732.
18. Ruchitha Mahajan, Neeraj, Sarika, Bella Mahajan. Isolation and Identification of Non Fermenting Gram Negative Bacilli: A Prospective Study. *Innovations in Microbiology and Biotechnology Vol. 4*, 12 March 2022 , Page 131-137.
19. Elbadawi, H.S., Elhag, K.M., Mahgoub, E. *et al.* Detection and characterization of carbapenem resistant Gram-negative bacilli isolates recovered from hospitalized patients at Soba University Hospital, Sudan. *BMC Microbiol* 21, 136 (2021).