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Comparison Of *Modified Hodge Test* (Mht), Modified Carbapenem Inactivation Method (Mcim), And *Vitek-2 System* For Carbapenemase Detection In Clinical Isolates Of Carbapenem-Resistant *Pseudomonas aeruginosa*

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ABSTRACT

Introduction: *Pseudomonas aeruginosa* is a resilient nosocomial pathogen with intrinsic and acquired resistance to multiple antibiotics. A small percentage (2–3%) of carbapenem-resistant isolates carry mobile genetic elements encoding carbapenemases, making detection essential for therapy, infection control, and epidemiological surveillance. This study aimed to compare three phenotypic methods for detecting carbapenemase production in meropenem-resistant *P. aeruginosa* isolates: the *Modified Hodge Test* (MHT), *Modified Carbapenem Inactivation Method* (mCIM), and the *VITEK-2* automated system.

Methods: The mCIM followed standard protocols. *Escherichia coli* ATCC 25922 was cultured on Mueller-Hinton agar (MHA) according to CLSI 2024 guidelines. MHT steps followed the Clinical Microbiology Procedures Handbook (2016). Results from both tests were compared to those from the VITEK-2 system. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were assessed. Diagnostic accuracy was further analysed using ROC curves and AUC.

Results: Of 21 meropenem-resistant *P. aeruginosa* isolates, 11 (52.4%) were identified as carbapenemase producers by *VITEK-2 compact*. MHT failed to detect any of these, showing a sensitivity of 0% and specificity of 100%. mCIM detected 10 of 11 carbapenemase producers (90.9% sensitivity) but had 50% specificity. The positive predictive value and negative predictive value were 0% and 47.6% for MHT, respectively, and 66.67% and 83.33% for mCIM, respectively. AUC values were 0.5 for MHT and 0.71 for mCIM, indicating mCIM performed more closely to VITEK-2 for carbapenemase detection.

Conclusion: 52,4% of the meropenem-resistant *P. aeruginosa* isolates were carbapenemase producers based on VITEK-2 compact. Among phenotypic methods, mCIM demonstrated greater precision than MHT, compared with Vitek-2 compact as the standard, making it a useful screening tool. The selection of the carbapenemase detection method usage depends on each laboratory's capabilities.

Keywords: *Pseudomonas aeruginosa*, carbapenemase, MHT, mCIM, VITEK-2.
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INTRODUCTION

Pseudomonas aeruginosa is a resilient Gram-negative bacterium with good resistance and adaptability in a variety of environments, including clinical settings. Known to cause nosocomial infections, *P. aeruginosa* presents a significant challenge because it has an intrinsic resistance mechanism and can acquire resistance.[1] According to SINAR PAMKI 2024, the Indonesian Association of Clinical Microbiology Specialists, carbapenem-resistant *P. aeruginosa* was detected in 24% of all specimens from hospitals across Indonesia.[2] In the Asia-Pacific region, based on the ATLAS 2015-2019 program across 14 countries, 18.9% of *P.aeruginosa* were found to be carbapenem-resistant, with 32.8% harboring at least one carbapenemase gene.[3]

Studies show that early notification of carbapenemase helps to target empirical therapy, leading to more effective treatment and less selective pressure for the emergence of antimicrobial resistance.[4] Genotypic testing is the gold standard for detecting carbapenemase genes, with good sensitivity and specificity, but it requires specialised equipment, expertise, and high cost.[5] Phenotypic detection is easier and more convenient to implement than genotyping.[6]

The method for the phenotypic identification of carbapenemases in *P. aeruginosa* and *Enterobacterales* was performed using CarbaNP and mCIM (Modified Carbapenem Inactivation Methods).[7] The Modified Hodge Test

(MHT) is recommended in CLSI M100-2017 as a phenotypic cloverleaf test.[8] It is still recommended as a phenotypic test for detecting carbapenemase.[9] Carvalhaes et al. 's research showed that AES™ Vitek(R)-2 accurately reported the carbapenemase phenotype in 93.7% of isolates, with a sensitivity of 96.4% and a specificity of 91.7% compared to Whole Genome Sequencing (WGS) in *Enterobacterales.*[10] Zhou et al.[11] compared the phenotypic methods of MHT, CarbaNP, and mCIM in *Enterobacteriaceae*, but this has not been done in *Pseudomonas aeruginosa*. The study by Verma *et al.*[12] compared the combination of the phenotypic methods mCIM-eCIM with Vitek-2 to identify CRPA and Carbapenem-Producing *Enterobacterales.* Still, it did not compare the *Modified Hodge Test* method.

METHODS

This study was conducted to compare phenotypic methods (MHT and mCIM) with the VITEK-2 System for the identification of carbapenemase in carbapenem-resistant clinical isolates of *Pseudomonas aeruginosa*. The design of this study is a categorical descriptive, cross-sectional design. A bacterial isolate of *P. aeruginosa* resistant to carbapenem (meropenem) was obtained from the Clinical Microbiology Laboratory of Dr Saiful Anwar Hospital in February–April 2024 from blood, urine, body fluids, sputum, and pus/swab specimens. The specimen had previously been used by other researchers and stored in a cryotube containing glycerol at -20 °C until this study was carried out in February-March 2025. Identification of carbapenem-resistant *P.aeruginosa* using VITEK-2, followed by phenotypic identification with the AES™ system, and consistent results were obtained. We utilised all available eligible carbapenem-resistant *P. aeruginosa* isolates stored during the specified period (total sampling).

This study applied strict inclusion and exclusion criteria in the selection process for bacterial isolates. The inclusion criteria included *Pseudomonas aeruginosa* isolates identified as resistant to carbapenems (meropenem), which were obtained from the Clinical Microbiology Laboratory at Dr Saiful Anwar Regional General Hospital

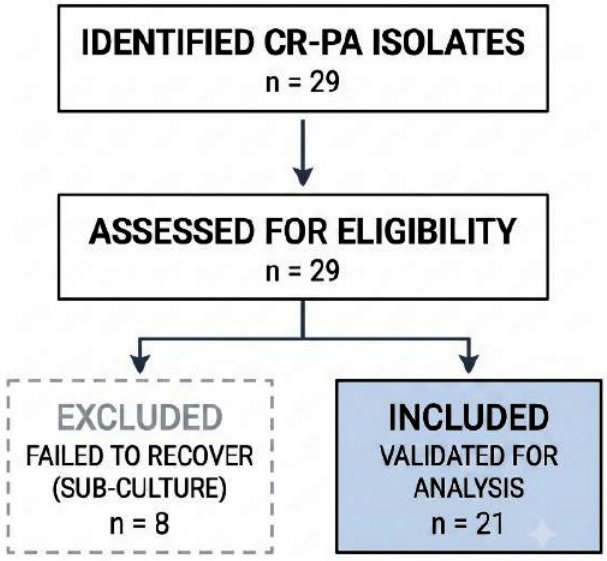


Figure 1. Schematic representation of the study selection and inclusion criteria for phenotypic carbapenemase detection.

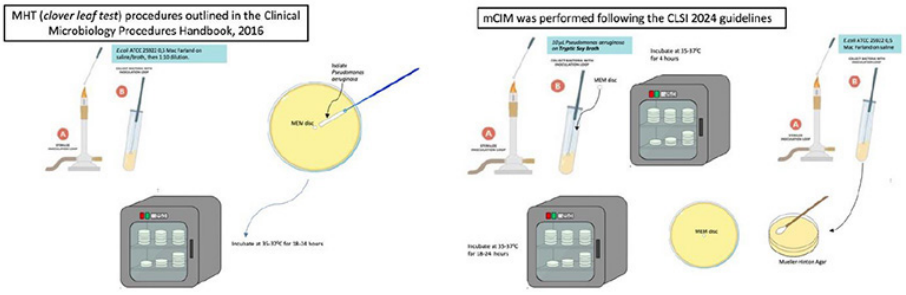
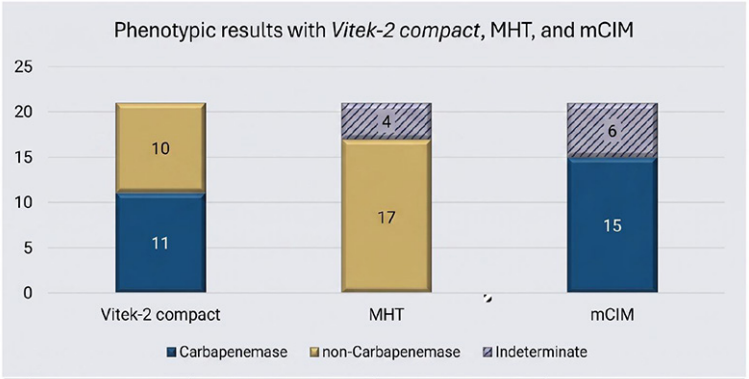


Figure 2. Procedure Modified Hodge Test (MHT) and modified Carbapenemase Inactivation Method (mCIM)



	Vitek-2 compact	MHT	mCIM
Indeterminate	0	4	6
Non-Carbapenemase	10	17	0
Carbapenemase	11	0	15
Total	21	21	21

Figure 3. *The comparison results of the carbapenemase phenotypic test with the Vitek-2 compact, the Modified Hodge Test (MHT), and the Modified Carbapenem Inactivation Method (mCIM).*

between February and April 2024. These isolates were sourced from various clinical specimens, including blood, urine, body fluids, sputum, and pus or swabs, which had previously been stored in cryotubes containing glycerol at -20 °C. Conversely, the exclusion criteria established in this study included all bacterial isolates that could not be re-cultured at the time of the study.

In this study, to minimise bias, standard protocols were used for all tests (MHT and mCIM), and the results were interpreted by two different researchers (where applicable) or by strictly following the CLSI/Procedures Handbook guidelines to ensure objectivity.

Modified Hodge Test (MHT)

The MHT procedure involves preparing *E. coli* (ATCC 25922) to a 0.5 McFarland standard in broth or saline, followed by a 1:10 dilution. The MHA plate is inoculated evenly with *E. coli* ATCC 25922 using a sterile swab. A meropenem disc is placed in the centre of the plate. Then, using a 10-µL loop, 3-5 *P. aeruginosa* overnight colonies from blood agar are streaked in a straight line from the edge of the disc to the plate edge. The plate is incubated at 35°C in ambient air for 16-24 hours.[13]

Modified Carbapenem Inactivation Method (mCIM)

The mCIM procedure involves emulsifying a 10-µL loopful of overnight-grown *P. aeruginosa* into 2 mL of *Tryptic Soy Broth*. Mix with a vortex for 10–15 seconds, then submerge a 10-µg meropenem disc in the suspension and incubate at 35°C for 4 hours. Meanwhile, prepare a 0.5 McFarland E. *coli* ATCC 25922 suspension in NaCl and inoculate an MHA plate with *E. coli* as per standard disc diffusion. After the 4-hour incubation, place the meropenem disc from the broth onto the inoculated MHA plate. Invert and incubate the plate at 35°C in ambient air for 18–24 hours, then measure the inhibition zone.[7]

AES™ system Vitek-2 Compact

Using *a VITEK-2 system* machine with *AES™* and GN ID cards and AST-GN93

Statistical analysis

Comparison with the values of sensitivity, specificity, positive predictive value, and negative predictive value. Analyse the data with SPSS to obtain the ROC (Receiver Operating Characteristic) curve and AUC (Area Under Curve) values to get the diagnostic precision of a test. Statistical analysis was conducted using descriptive and comparative methods for the entire sample, and no subgroup analysis was performed.

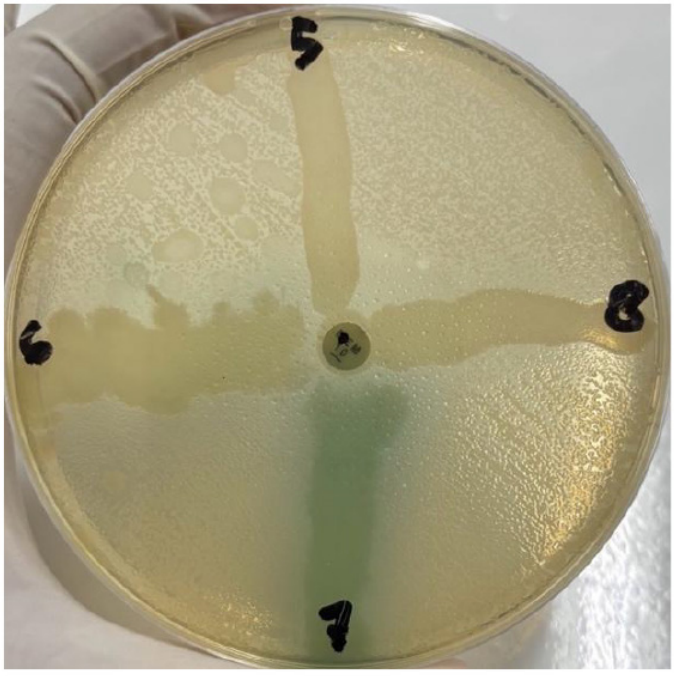


Figure 4. *The MHT test results for isolate number 5 were negative for carbapenemase; i.e., No. Clover-like morphology was observed, whereas isolates numbers 6, 7, and 8 showed indeterminate results. The blue-green pigment is seen in isolate number 7.*

Table 1. Carbapenemase phenotypic tests MHT and Vitek-2 compact

MHT results	Vitek-2	
	Carbapenemase	Non-Carbapenemase
Positive MHT (Carbapenemase)	0	0
Negative MHT (Non-Carbapenemase)	11	10

RESULTS

Carbapenem-resistant *Pseudomonas aeruginosa* isolates obtained from the Microbiology Laboratory of Dr Saiful Anwar Malang Hospital from February to April 2024 met the inclusion criteria. There were 29 isolates, but eight could not be grown and were excluded from the study, leaving 21. This study continued previous research on carbapenemases in *P. aeruginosa* and was carried out with the same isolate, stored in a cryotube containing glycerol at -20°C.

In total, 21 carbapenem-resistant P. aeruginosa isolates were evaluated. MHT identified no carbapenemase producers, 17 non-producers (81%), and four indeterminate results (19%). mCIM identified 15 (71.4%) carbapenemase-producing isolates and 6 (28.6%) *indeterminate* results. VITEK-2 compact identified 11 (52.4%) carbapenemase producers and 10 (47.6%) nonproducers. Indeterminate results for both MHT[14] and mCIM[15] were classified as negative. For MHT, sensitivity was 0%, specificity was 100%, PPV was 0%, and NPV was 47.6%.

mCIM results were compared to VITEK-2, with indeterminate results classified as negative.[15] mCIM demonstrated 90.9% sensitivity, 50% specificity, 66.67% PPV, and 83.33% NPV. ROC analysis showed diagnostic precision

values of 0.5 for MHT and 0.71 for mCIM, indicating mCIM's superior accuracy.

DISCUSSION

The Modified Hodge Test method, which is easy to perform, has a sensitivity of 0% compared to phenotypic identification of carbapenemases using *AES Vitek-2 compact* AST-GN93. This is similar to the results of a study by Coseriu *et al*, which obtained a low MHT sensitivity to *P. aeruginosa* isolates of only 6.0%, compared to the PCR method.[16] Another study on MHT sensitivity found it to be only 8.23% against non-lactose-fermenting *Enterobacteriaceae* and non-lactose-fermenting Gram-negative bacilli, compared to PCR.[17]

The low sensitivity of MHT may be due to the test's inability to detect *Metallo β-lactamases* (MBLs)[18] and NDM (*New Delhi metallo β-lactamase*).[8] These results are contrary to the literature: MHT can detect type B β-lactamases, namely *Metallo-β-lactamases.*[19] Different results were obtained in the study by Alkhudairy and Rasool, which reported an MHT sensitivity of 80% compared to the Vitek-2 system.[20] The study differed in the MHT procedure, namely, without dilution of 1:10 E. *coli ATCC* 0.5 McFarland. In this study, the MHT examination had a specificity of 100%. This result is estimated to be due to a large number of negative results. The Positive Predictive Value (PPV) is 0%, and the Negative Predictive Value (NPV) of MHT is 47.6%. This result is slightly different from the study by Coseriu et al., which reported a high MHT specificity of 94.44% and a PPV of 75.0% for MHT compared to PCR genotypics, with an NPV of 26.56%.[16]

There were four indeterminate MHT results because there was an area of decreased growth of *E.coli* ATCC 25922 in the area around the streaking *P. aeruginosa* isolate. Indeterminate results in MHT are due to the possible secretion of substances such as *colicin*, *a bacteriocin-peptide* secreted by Gram-negative bacilli bacteria that may be able to inhibit the growth of indicator strains and interfere with test results.[14] In this study, indeterminate MHT was observed in one isolate of *P. aeruginosa* that produces green-blue pigments (pyoverdine and pyocyanin),

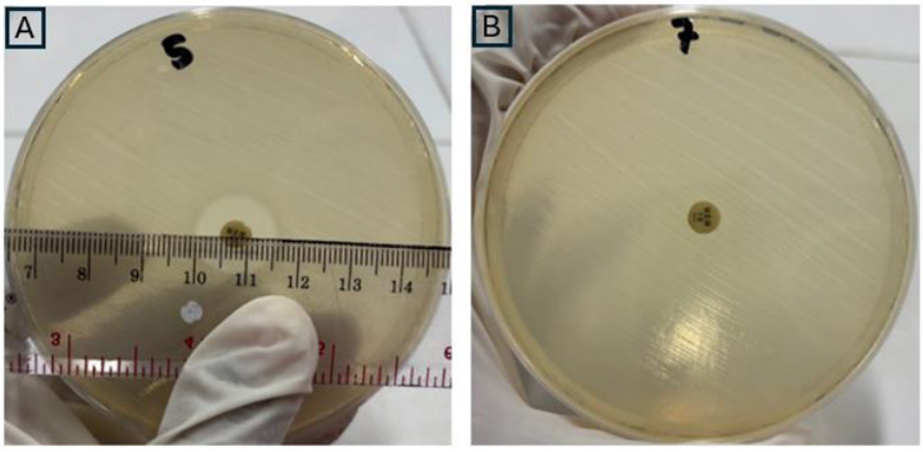


Figure 5. The mCIM test results for isolate 5 (A) and 7 (B). (A) Shows 16mm inhibition, indicating indeterminate. (B) Shows no inhibition zone, indicating a positive carbapenemase.

Table 2. ***Carbapenemase phenotypic tests mCIM and Vitek-2 compact***

mCIM results	Vitek-2	
	Carbapenemase	Non-Carbapenemase
Positive mCIM (Carbapenemase)	10	5
Negative mCIM (non-Carbapenemase)	1	5

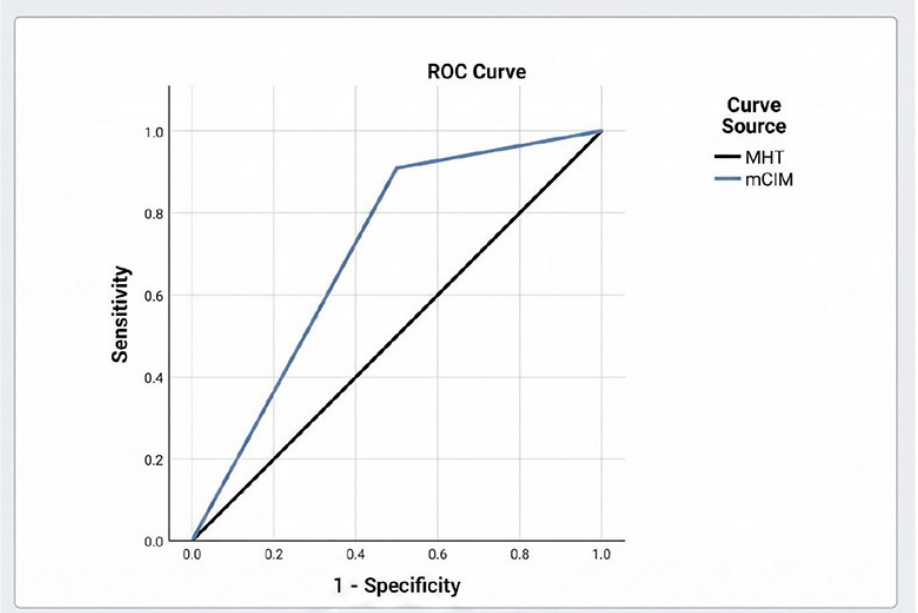


Figure 6. *The ROC curves were obtained for the MHT and mCIM tests, with AUC values, and compared with the carbapenemase results from the Vitek-2 compact system.*

as shown in **Figure 3**. The indeterminate results of the MHT test may also occur due to the absence of positive and negative control bacterial isolates as comparators, which is a weakness of this study.[16]

The AUC ROC curve is used to obtain a picture of the diagnostic precision of a test.[21] In Figure 5, the ROC curve for MHT yields an AUC of 0.5. In contrast to the results of Zhong et al.'s meta-analysis, the AUC of the ROC curve for MHT was 0.97 in *Enterobacteriaceae* using genotypic standards.[8] There is a difference in the results because the carbapenemase in *Enterobacteriaceae* is included in the Ambler classification class A (subgroup 2f carbapenemase according to the Bush-Jacoby classification), and class D (subgroup 2df carbapenemase), while *Pseudomonas aeruginosa* that produces carbapenemase is Ambler class D (Bush-Jacoby classification 2de ESBL) and class B (group 3 *Metallo carbapenemase*).[19] The low sensitivity of MHT and AUC MHT in *P. aeruginosa* may be due to MHT's strong ability to identify carbapenemases in Ambler classes A and D, but its poor ability to identify carbapenemases in Ambler class B (group 3: *Metallo-β-lactamases*). Unfortunately, PCR was not performed in this study.

The mCIM test was performed to detect carbapenemase in *Enterobacteriaceae* and *Pseudomonas aeruginosa* according to CLSI M100 from 2017 to 2024.[8] This study achieved a high mCIM sensitivity of 90.9%, similar to the study by Alkhudairy and Rasool, with mCIM sensitivity in *P. aeruginosa* reaching 94.3% when compared to the Vitek(R)-2 compact system.[20] The specificity of the mCIM test in this study was 50%. These results differ from the same study, which reported a 96.5% mCIM specificity in *P. Aeruginosa*.[20] Some false-positive and false-negative results from mCIM reduced the overall specificity percentage. The positive predictive value (PPV), or true-positive rate, for mCIM was 66.67% compared with phenotypic identification using Vitek-2 compact, and the negative predictive value (NPV) was 83.33%.

The ROC and AUC curves on mCIM, compared to Vitek-2 as the standard, showed an AUC of 0.71. This result differs slightly from that of Rizvi et al., who reported an AUC of 1 for mCIM in bacteria isolates carrying the OXA-48 resistance gene in *K. pneumoniae* and the NDM-1 resistance gene in *E. Coli*.[5] Five false-positive mCIM results in this study matched those reported by Simner *et al.*, in which one false-positive occurred in isolates harbouring multiple β-lactamases, including PAO, OXA-2, and OXA-5023.[22] Howard et al. found that indeterminate mCIM results, followed by PCR testing, yielded the VIM gene.[15] The presence of false-negative mCIM results aligns with Zhou et al., who observed false negatives in NDM-1-producing *Enterobacteriaceae*.[11]

According to Harrington Emerson, management analysis requires five factors: man, material, method, machines, and money. A human factor was needed because both tests were done manually, while a machine performs the Vitek-2, but still requires a human factor in preparation.[23] The material factors required in mCIM are almost the same as MHT, with the addition of TSB (Tryptic Soy Broth) and a ruler for the measurement of the inhibitory zone. The materials required for Vitek-2 implementation include a *P. aeruginosa* isolate at 0.5 McFarland, an ID-GN card, an AST-GN93 card, and tubes and racks for suspension placement. The MHT method was similar to the Kirby-Bauer AST, with the addition of streaking *P. aeruginosa* colonies stacked with *E. coli* ATCC 25922. The mCIM procedure is more complicated than MHT. Namely, initial preparation involves immersing 10-µg meropenem discs in a suspension of *P. aeruginosa* for 4 hours in an aerobic incubator, then placing the discs in MHA streaked with E. *coli* ATCC 25922. The Vitek-2 compact is handy for AES™, which is performed by a closed-system machine. Factor machines for MHT and mCIM are vortex and aerobic incubators.

The money factor for MHT compared to mCIM, as determined in research conducted at the Microbiology Laboratory, Faculty of Medicine, University of Brawijaya, Malang, was not significantly different. Regarding the MHT and mCIM costs per isolate, both are in the range of 25,000–35,000 IDR. This is also shown by Zhong et al, who found that the average cost for the MHT test and mCIM is 1 USD per isolate.[8] The operational costs for the isolation identification card and antibiotic sensitivity test with Vitek-2 compact at Dr Saiful Anwar Malang Hospital are 250,000 IDR. This concludes that the mCIM method is more advantageous and has higher sensitivity than MHT, compared with Vitek-2.

The limitations of this study include the fact that genotyping tests (such as PCR), which serve as the gold standard for confirming the presence of carbapenemase-encoding genes, were not performed. Therefore, comparisons were based solely on results from the Vitek-2 Compact system. Additionally, this study was cross-sectional in nature, providing an overview of resistance characteristics during a specific time period; therefore, future longitudinal studies could be conducted to monitor trends in the dynamic shift of *P. aeruginosa* resistance profiles in the hospital setting.

CONCLUSION

This study confirms that early detection of carbapenemase in carbapenem-resistant *P. aeruginosa* isolates is a crucial step in therapeutic management and the control of nosocomial infections. Among the phenotypic methods tested, mCIM proved to be a far superior and more precise screening tool compared to MHT, which consistently failed to detect carbapenemase production in this study. Although the Vitek-2 Compact system remains the primary standard in clinical laboratory settings, mCIM can be recommended as a more reliable alternative method to enhance detection accuracy in laboratories with limited access to molecular techniques. Therefore, the selection of the appropriate detection method depends heavily on clinical considerations and the availability of resources in each laboratory to ensure the administration of targeted empirical therapy and to curb the spread of antimicrobial resistance in the future.

DISCLOSURES

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This is self-funding research.

Conflict of Interest

The authors have no conflict of interest to declare

Author Contribution

All authors were involved in the conception, design, and supervision of the manuscript. The first author conducted the study and analysed the data. All authors prepare the manuscript and agree to this final version of the manuscript to be submitted to this journal.

Ethical Clearance

Ethical clearance was obtained from the Health Research Ethics Committee of the Faculty of Medicine, Brawijaya University, No. 36 / EC / KEPK–PPDS / 02 / 2025.

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