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ORIGINAL ARTICLE

Diagnostic Accuracy of the Simplified Carbapenem Inactivation Method versus the Modified Hodge Test in Carbapenem-Resistant *Pseudomonas aeruginosa*: A Cross-Sectional Study

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ABSTRACT

Background: Carbapenem-resistant Pseudomonas aeruginosa (CRPA) is among the pathogens contributing most to the global burden of antimicrobial resistance, and its pooled prevalence in Indonesia reaches 43%. Carbapenem resistance and carbapenemase production are not equivalent, and only the enzymatic route is transmissible and therapeutically decisive. Molecular confirmation is unavailable in most Indonesian laboratories, so an accurate low-cost phenotypic assay is needed.

Objective: To compare the simplified carbapenem inactivation method (sCIM) with the Modified Hodge Test (MHT) for detecting carbapenemase production in carbapenem-resistant P. aeruginosa.

Methods: This cross-sectional diagnostic-accuracy study evaluated 30 meropenem- and imipenem-resistant clinical isolates archived at Dr. Soetomo General Academic Hospital, Surabaya, using PCR for blaIMP, blaGES, blaOXA, blaNDM, blaKPC and blaVIM as the reference standard.

Result: A carbapenemase gene was detected in 13/30 isolates (43.3%): blaIMP in 9, blaGES in 3 and blaOXA in 1. sCIM achieved 92.31% sensitivity (95% confidence interval 64.0-99.8), 100.00% specificity (80.5-100.0), 96.7% accuracy and near-perfect agreement with PCR (kappa 0.932). MHT achieved 46.15% sensitivity (19.2-74.9), 88.24% specificity (63.6-98.5) and fair agreement (kappa 0.360). The paired exact McNemar test confirmed the sensitivity advantage of sCIM ($p = 0.031$), and bootstrapping showed a 26.6-percentage-point difference in overall accuracy (95% confidence interval 10.0-43.3). MHT detected no blaGES-positive isolate and only 6/9 blaIMP-positive isolates; sCIM detected all of both, while neither assay detected the single poorly expressed blaOXA isolate.

Conclusion: sCIM is an accurate, rapid and inexpensive assay that should replace MHT in Indonesian laboratories and feed directly into stewardship and infection-control decisions.

1. Introduction

Antimicrobial resistance has become one of the leading causes of death worldwide. Bacterial antimicrobial resistance was directly responsible for an estimated 1.27 million deaths and associated with 4.95 million deaths in 2019, and modelling now forecasts approximately 1.91 million directly attributable deaths each year by 2050, with South and Southeast Asia carrying a disproportionate share of that burden[1]. *Pseudomonas aeruginosa* sits among the small group of pathogens responsible for most of this mortality. It is an opportunistic Gram-negative non-fermenting bacillus that accounts for roughly 5-10% of nosocomial infections, persists in moist hospital environments and in wastewater reservoirs, and causes bloodstream, urinary tract, respiratory, surgical-site and soft-tissue infections[2,3]. Multinational surveillance of World Health Organization priority pathogens places the Asia-Pacific region at the top of the global distribution for both carbapenem resistance and difficult-to-treat resistance, and intensive-care origin is among the strongest predictors of carbapenem non-susceptibility in this organism[4,5]. Pooled prevalence estimates place carbapenem resistance in *P. aeruginosa* at 32% across sub-Saharan Africa and at 43% (95% confidence interval 29-58) across Indonesia, the latter derived from 16 studies and 3,852 isolates, with East Java - the province in which the present study was conducted - contributing a pooled estimate of 40%[6,7]. Indonesian multicentre work has documented the national carbapenemase gene pool and the excess mortality that accompanies carbapenem non-susceptibility, a national systematic review has identified laboratory diagnostic capacity rather than clinical awareness as the binding constraint, and comparative carriage studies place Jakarta well above two European reference cities[8-11]. Within Surabaya itself, tertiary-centre data show multidrug-resistant Gram-negative organisms dominating neonatal sepsis[12], and national meta-analysis shows beta-lactamase prevalence rising steadily over sixteen years[13].

The clinical problem, however, is not carbapenem resistance as such but the mechanism that produces it. Carbapenem resistance in *P. aeruginosa* arises by two mechanistically distinct routes with entirely different consequences. The commoner route is chromosomal and non-enzymatic: loss or downregulation of the OprD porin, frequently combined with derepressed AmpC cephalosporinase and upregulated MexAB-OprM efflux[2]. This phenotype is not horizontally transmissible and often remains susceptible to the newer beta-lactam and beta-lactamase-inhibitor combinations. The less common route is acquisition of a mobile carbapenemase gene, usually carried on a class 1 integron within a plasmid or genomic island and concentrated in the international high-risk clones ST235, ST111 and ST175[14-16]. Carbapenemases are classified by the Ambler scheme into serine enzymes of class A (KPC, GES) and

class D (OXA-type) and the zinc-dependent class B metallo-beta-lactamases (IMP, VIM, NDM)[14]. The distinction is therapeutically decisive rather than taxonomic. In vitro surveys of carbapenem-resistant *P. aeruginosa* show that isolates producing a metallo-beta-lactamase remain non-susceptible to ceftolozane-tazobactam and ceftazidime-avibactam, while cefiderocol retains activity, whereas isolates whose resistance is porin-mediated frequently remain susceptible to those same agents[17,18]. The distinction also governs infection control, since only the enzymatic route can spread between species and drive outbreaks[18,19].

Treating a carbapenem-resistant susceptibility report as though it were evidence of carbapenemase production is therefore unsafe, and the size of the error is setting-specific. The prospective multinational POP cohort found that only a small minority of carbapenem-resistant *P. aeruginosa* isolates in the Americas carried an acquired carbapenemase, whereas the proportion is far higher across Asia[6,20]. Confirmatory testing is the only way to resolve this at the level of the individual isolate. Molecular assays and immunochromatographic lateral-flow devices achieve excellent accuracy and short turnaround, but they require target-matched panels, cold-chain logistics, capital equipment and per-test costs an order of magnitude above a disc-based method[21,22,23]. Scoping reviews of laboratory capacity in lower-middle-income countries repeatedly find that fewer than one third of laboratories perform any form of carbapenemase confirmation, and Indonesian point-prevalence data show that only a minority of hospital antibiotic prescriptions are culture-directed[10,23,24]. For most Indonesian hospitals the practical question is not whether molecular confirmation is superior, but which cheap phenotypic assay is accurate enough to be trusted.

Two candidates dominate that space. The Modified Hodge Test (MHT) was recommended by the Clinical and Laboratory Standards Institute as a confirmatory assay for Enterobacterales and remains in wide use in Indonesian laboratories because it requires nothing beyond agar, a carbapenem disc and a susceptible indicator strain. Its weaknesses were nevertheless documented early: it performs best against Ambler class A and class D serine enzymes and is unreliable for the class B metallo-beta-lactamases, and it generates false positives in isolates that hyperproduce AmpC or extended-spectrum beta-lactamases and thereby hydrolyse carbapenems weakly[25]. An Indonesian evaluation published this year makes the consequence concrete: in 21 meropenem-resistant *P. aeruginosa* isolates the Modified Hodge Test achieved a sensitivity of 0% with an area under the curve of 0.50, whereas mCIM reached 90.9% with an area under the curve of 0.71[26]. Those weaknesses drove the development of the carbapenem inactivation method family, which detects hydrolysis directly rather than inferentially. The standardised modified carbapenem inactivation method (mCIM) achieved sensitivity and specificity above 99% in Enterobacterales and entered CLSI M100, and a multicentre evaluation subsequently extended it to *P. aeruginosa* and *Acinetobacter baumannii*[27-29]. The simplified carbapenem inactivation method (sCIM) removes the broth-incubation step altogether by smearing the test organism directly onto an imipenem disc, and reported a sensitivity of 100% and a specificity of 99.6% in its derivation study across Gram-negative bacilli[25].

What is missing is evidence in the organism and the population that matter here. Performance in non-fermenters is not simply inherited from Enterobacterales, because *P. aeruginosa* secretes less enzyme into the surrounding agar and because its carbapenemase pool is dominated in Asia by class B metallo-enzymes rather than by the class A and class D enzymes on which MHT performs best[14,19,28]. To our knowledge, no Indonesian study has compared sCIM with MHT head to head in carbapenem-resistant *P. aeruginosa* against a molecular reference standard, and the international literature on this specific comparison in this specific organism remains thin. This study was designed with the combined expertise of the Master Program of Clinical Medicine at the Faculty of Medicine, Universitas Airlangga, the Faculty of Medicine of the University of Darussalam Gontor, and the Department of Medical Microbiology of the Faculty of Medicine, Universitas Airlangga - one of Indonesia's principal academic centres for clinical microbiology - working with isolates from the Clinical Microbiology Laboratory of Dr. Soetomo General Academic Hospital, Surabaya.

The objectives of this study were, first, to determine the diagnostic accuracy of sCIM and of MHT for the detection of carbapenemase production in carbapenem-resistant *P. aeruginosa*, using PCR detection of six carbapenemase genes as the reference standard; second, to compare the two assays directly in a paired analysis on the same isolates; third, to characterise how their performance varies by Ambler carbapenemase class; and fourth, to translate the findings into an implementable workflow for Indonesian clinical microbiology laboratories operating without molecular capability.

2. Methods

Study design and setting

This was an observational analytic study with a cross-sectional design, conducted as a laboratory diagnostic-accuracy evaluation of two index tests against a molecular reference standard. All work was performed at the Clinical Microbiology Laboratory of Dr. Soetomo General Academic Hospital, Surabaya, a national referral and teaching hospital affiliated with the Faculty of Medicine, Universitas Airlangga. The unit of analysis was the bacterial isolate. The flow of isolates from archive retrieval through eligibility screening, parallel index and reference testing, and statistical analysis is summarised in **Figure 1**.

The study is reported in accordance with the Standards for Reporting of Diagnostic Accuracy Studies (STARD 2015). A completed STARD checklist is provided as supplementary material. The study was not prospectively registered, which is stated here in accordance with that checklist. Isolates were retrieved consecutively from the archive; the number of archived isolates screened before the 30 analysed were obtained was not recorded in the source laboratory log and is therefore not reported, which is acknowledged as a reporting limitation in section 4.8.

Bacterial isolates

The study sample consisted of clinical isolates of *P. aeruginosa* recovered from urine, blood, pus, tissue and sputum specimens submitted for routine diagnostic culture. Isolates were eligible if they were resistant to both meropenem and imipenem as determined by the BD Phoenix semi-automated system, if a viable subculture could be recovered from the −80°C archive, and if the accompanying specimen record was complete. Isolates that failed to grow on subculture, that proved to be mixed on purity plating, or that lacked specimen documentation were excluded. All isolates originated from a preceding institutional programme of work on carbapenem resistance and had been stored at the Clinical Microbiology Laboratory of Dr. Soetomo General Academic Hospital[8]. Thirty consecutive eligible isolates were included; one isolate per patient was analysed, so no patient contributed more than one isolate.

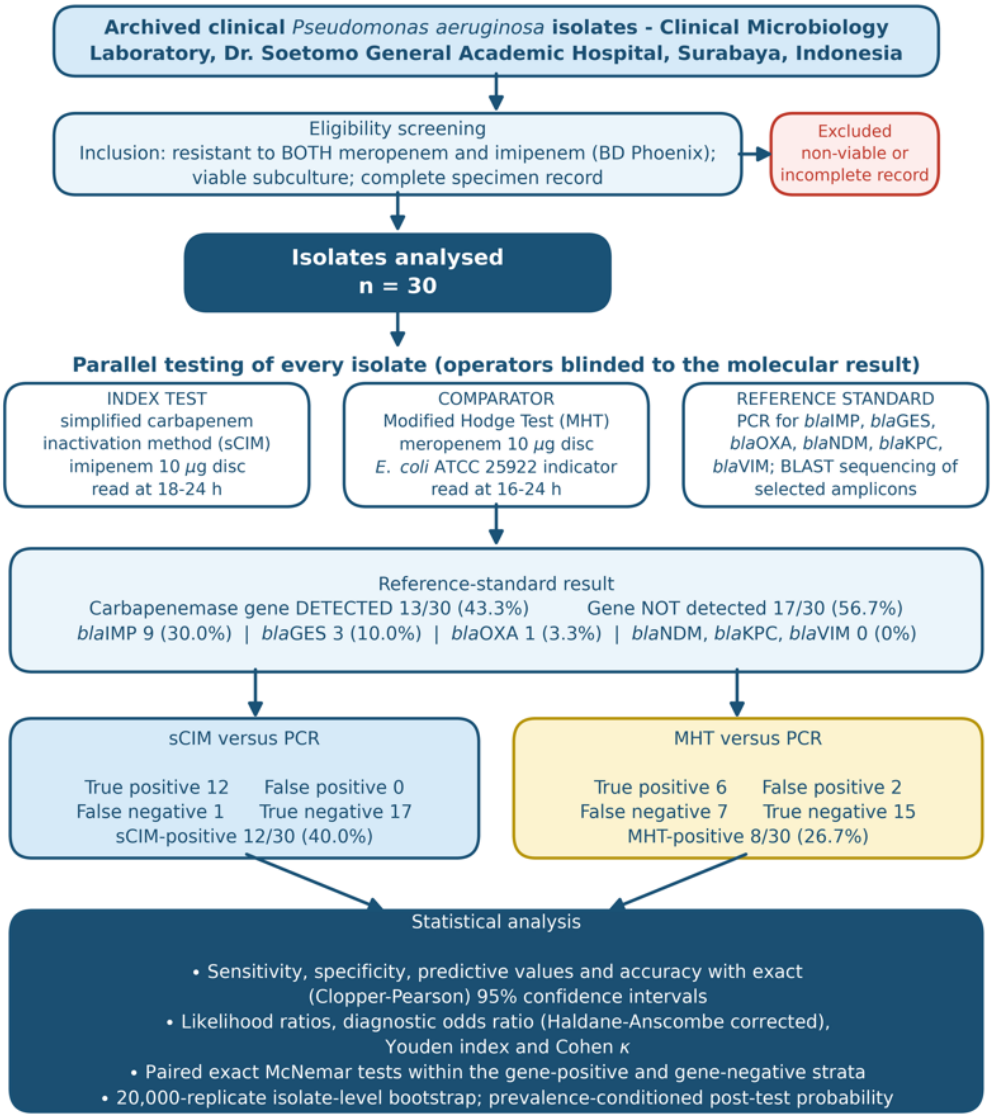

Figure 1. Flow of isolates through eligibility screening, parallel index and reference-standard testing, and statistical analysis.

No formal a priori sample-size calculation was performed, because the study was constrained by the number of eligible archived carbapenem-resistant isolates available in the collection. The achievable precision was nevertheless examined post hoc. With 13 gene-positive and 17 gene-negative isolates, a sensitivity of 92% is estimated with an exact 95% confidence interval approximately 36 percentage points wide and a specificity of 100% with an interval approximately 20 percentage points wide. This precision is sufficient to separate an assay performing near the upper bound of acceptability from one performing near chance, which is the comparison at issue here, but it is not sufficient to estimate either accuracy measure to within the ten-percentage-point margin that would be required to establish equivalence between closely matched assays. The sample size is stated as a limitation in section 4.8 and is reflected throughout in the reporting of interval rather than point estimates.

Isolation and identification

Each archived isolate was thawed from −80°C storage and streaked onto MacConkey agar (Oxoid, Hampshire, United Kingdom) using a sterile loop under aseptic conditions, working through four quadrants to obtain single colonies. MacConkey agar was used to suppress Gram-positive bacteria and fungi. Plates were incubated at 35-37°C for 18-24 hours. Colonies showing the characteristic non-lactose-fermenting morphology, grape-like odour and pigment production of *P. aeruginosa* were subcultured onto Mueller-Hinton agar (Oxoid) and confirmed by conventional biochemical testing before use. Only pure, freshly subcultured growth was used for the index tests.

Index test: simplified carbapenem inactivation method (sCIM)

sCIM was performed according to the originally described procedure[25]. A suspension of *Escherichia coli* ATCC 25922 adjusted to a 0.5 McFarland standard was diluted 1:10 in sterile saline and inoculated onto Mueller-Hinton agar plates by the routine disc-diffusion lawn method. Plates were allowed to dry for 3-10 minutes. One to three overnight colonies of the test organism grown on blood agar were then smeared directly onto one side of an imipenem 10 microgram disc (Oxoid), so that the organism covered the disc surface without contaminating the opposite face. The disc was applied, smeared side downward, to the inoculated Mueller-Hinton agar plate. Plates were incubated at 35°C for 18-24 hours in ambient air. Abolition or marked reduction of the inhibition zone around the disc, relative to the carbapenem-susceptible control, was read as a positive result indicating carbapenemase-mediated hydrolysis of imipenem.

Readings were made by comparing the diameter of the inhibition zone around the smeared disc with that around an unsmeared imipenem disc applied to the same plate and with that produced by a carbapenem-susceptible *P. aeruginosa* control. Complete abolition of the zone, or a zone reduced to a diameter at which the indicator strain grew up to the disc edge on the smeared side, was recorded as positive; an unaltered zone was recorded as negative. Readings that could not be assigned confidently on first inspection were re-read independently by a second operator as described in section 2.8. The absolute zone diameters were not retained in the laboratory record, so a threshold expressed in millimetres cannot be reported retrospectively; this is stated as a limitation in section 4.8 and should be recorded prospectively in any future evaluation.

Comparator: Modified Hodge Test (MHT)

MHT was performed following the Clinical and Laboratory Standards Institute procedure in force at the time of testing[26,29]. A colony of *E. coli* ATCC 25922 was suspended in broth or saline to a 0.5 McFarland standard and further diluted 1:10, giving a bacterial density of approximately 1 x 10^7 to 2 x 10^7 colony-forming units per millilitre. This suspension was used to inoculate Mueller-Hinton agar plates with a sterile cotton swab, swabbing the entire surface three times and rotating the plate 60 degrees between passes. After the surface had dried, a meropenem 10 microgram disc was placed at the centre of the plate. The test isolate was streaked in a straight line from the edge of the disc outward to the periphery of the plate. Plates were incubated at 35°C for 16-24 hours. A clover-leaf-shaped indentation of the indicator-strain inhibition zone along the streak of the test organism was read as a positive result.

Procedural control: modified carbapenem inactivation method (mCIM)

mCIM was performed in parallel as an internal procedural control on the enzymatic-hydrolysis principle, following the CLSI-endorsed method[27,29]. A 10 µL loopful of *P. aeruginosa* growth from blood agar was emulsified in 2 mL of tryptic soy broth, a meropenem 10 microgram disc was immersed in the suspension, and the tube was incubated for at least four hours at 35°C. A 0.5 McFarland suspension of *E. coli* ATCC 25922 was prepared by direct colony suspension in saline and used to inoculate Mueller-Hinton agar plates. The meropenem disc was then removed from the broth and applied to the inoculated plate, which was incubated at 35°C for 18-24 hours. mCIM results were used to verify the integrity of the hydrolysis-based workflow and are not reported as an index test in the accuracy analysis.

Reference standard: PCR detection of carbapenemase genes

The reference standard was PCR detection of carbapenemase-encoding genes. Primers targeting *bla*KPC, *bla*IMP, *bla*VIM, *bla*NDM, *bla*OXA-48-like and *bla*OXA-23-like, together with *bla*GES, were used as previously described[14,25], and amplification followed established protocols[14,23]. Each reaction contained 25 µL of PCR master mix (CWBio, Beijing, China), 4 µL of each primer, nuclease-free water to a final volume of 45 µL, and 5 µL of crude lysate from the test isolate. Cycling comprised initial denaturation at 94°C for 5 minutes; 35 cycles of 94°C for 10 seconds, 56°C for 30 seconds and 72°C for 90 seconds; and a final extension at 72°C for 2 minutes. Amplicons were resolved by agarose gel electrophoresis, and selected products were sequenced and confirmed by BLAST alignment. An isolate was classified as reference-standard positive if any one of the six target genes was detected.

Quality control and blinding

E. coli ATCC 25922 was used as the carbapenem-susceptible indicator strain throughout, and *Klebsiella pneumoniae* ATCC BAA-1705 and ATCC BAA-1706 were used as the carbapenemase-positive and carbapenemase-negative controls respectively for each batch of phenotypic testing. Media, discs and control strains were verified before use, and any batch in which a control strain gave an unexpected result was repeated in full. The two index tests were performed and read by operators who were unaware of the molecular result, and the molecular testing was performed independently of the phenotypic readings; index-test results were recorded on separate worksheets before the reference-standard result was disclosed. Phenotypic readings that were equivocal on first reading were re-read independently by a second operator, and the consensus reading was recorded.

Statistical analysis

Isolate characteristics were summarised as frequencies and percentages. Each index test was cross-classified against the reference standard in a conventional two-by-two table whose cells are the number of true positives (TP), false positives (FP), false negatives (FN) and true negatives (TN), from which the accuracy measures were derived as set out below. Because the sample was small and several cells were sparse, all proportions are reported with exact (Clopper-Pearson) 95% confidence intervals rather than with normal-approximation intervals. Positive and negative likelihood ratios, the Youden index J, and the diagnostic odds ratio were derived from the same tables; where a zero cell was present the Haldane-Anscombe continuity correction of 0.5 was applied before computing the diagnostic odds ratio and its confidence interval. Agreement between each index test and the reference standard, and between the two index tests, was quantified with Cohen's kappa, with kappa values above 0.5 regarded as acceptable for practical use and values above 0.8 as near-perfect agreement.

Sensitivity = TP / (TP + FN) × 100%

Specificity = TN / (TN + FP) × 100%

Positive predictive value = TP / (TP + FP) × 100%

Negative predictive value = TN / (TN + FN) × 100%

Accuracy = (TP + TN) / (TP + FP + FN + TN) × 100%

LR^+ = Sensitivity / (1 − Specificity); LR^- = (1 − Sensitivity) / Specificity

Diagnostic odds ratio = (TP × TN) / (FP × FN); Youden index J = Sensitivity + Specificity − 1

$\kappa = (p_o - p_e) / (1 - p_e)$

Because both index tests were applied to the same isolates, the comparison between them was treated as paired. Exact binomial McNemar tests were computed separately within the reference-standard-positive stratum, which compares sensitivities, and within the reference-standard-negative stratum, which compares specificities; the pooled McNemar test across all isolates is also reported for completeness, although it conflates the two error types. Uncertainty in the difference between the two assays was quantified by non-parametric bootstrap resampling of isolates with replacement over 20,000 replicates, from which the mean difference in overall accuracy, its percentile 95% confidence interval, and the proportion of replicates favouring sCIM were derived. The distribution of carbapenemase genes among gene-positive isolates was tested against a uniform expectation by a chi-square goodness-of-fit test, and the observed sex ratio and carbapenemase prevalence were tested against reference values by exact binomial tests. Finally, because predictive values are prevalence-dependent, positive and negative predictive values were recomputed across a range of plausible pre-test carbapenemase prevalences from 5% to 80% using Bayes' theorem, so that the results can be transported to settings whose carbapenemase epidemiology differs from that observed

here. All analyses were performed in Python 3 using NumPy; two-sided p values below 0.05 were regarded as statistically significant, and exact p values are reported to three decimal places.

Three further points about the analysis should be stated explicitly. First, the analysis is estimative rather than confirmatory: no adjustment was made for multiple comparisons, and the p values reported should be read descriptively alongside the interval estimates rather than as decision rules. The goodness-of-fit and sex-ratio tests in particular are descriptive and are peripheral to the diagnostic-accuracy question. Second, the bootstrap was paired at the level of the isolate: each resampled isolate carried its reference-standard result and both index-test results together, so the correlation between the two assays induced by testing them on the same organisms is preserved; intervals are simple percentile intervals, which may be anticonservative near the accuracy ceiling, and replicates containing no reference-positive or no reference-negative isolate were discarded. Third, analyses were performed in Python 3.11 with NumPy 2.x using a fixed random seed, and the analysis script together with the isolate-level dataset is available from the corresponding author so that every figure in this report can be reproduced exactly.

3. Results

Characteristics of the isolates

Thirty carbapenem-resistant *P. aeruginosa* isolates met the inclusion criteria and were analysed, as traced in **Figure 1**. Eighteen (60.0%) were recovered from male patients and 12 (40.0%) from female patients; although this male predominance is consistent with the 55.6% reported in a large Saudi tertiary-centre series, it did not differ significantly from an equal sex distribution in the present sample (exact binomial p = 0.362), and is therefore described rather than interpreted[30]. Patients spanned the full age range from under 10 to 89 years, with the largest single groups in the fifth and sixth decades. Urine was by far the commonest source, contributing 14 isolates (46.7%), followed by pus with 7 (23.3%); the predominance of urinary isolates matches regional descriptive series and systematic review data identifying the urinary tract as the leading source of resistant Gram-negative organisms in Asia[31]. The full demographic and specimen distribution is set out in **Table 1**.

Table 1. Demographic and specimen characteristics of the 30 carbapenem-resistant Pseudomonas aeruginosa isolates.

Characteristic	Total n (%)	Male n (%)	Female n (%)
Age group (years)			
< 10	3 (10.0)	2 (6.7)	1 (3.3)
10-19	4 (13.3)	1 (3.3)	3 (10.0)
20-29	2 (6.7)	2 (6.7)	0 (0.0)
30-39	3 (10.0)	3 (10.0)	0 (0.0)
40-49	5 (16.7)	1 (3.3)	4 (13.3)
50-59	5 (16.7)	3 (10.0)	2 (6.7)
60-69	4 (13.3)	3 (10.0)	1 (3.3)
70-79	2 (6.7)	1 (3.3)	1 (3.3)
80-89	2 (6.7)	2 (6.7)	0 (0.0)
Specimen type			
Urine	14 (46.7)	-	-
Pus	7 (23.3)	-	-
Sputum	4 (13.3)	-	-
Blood	3 (10.0)	-	-
Tissue	2 (6.7)	-	-
Total	**30 (100.0)**	**18 (60.0)**	**12 (40.0)**

Percentages are calculated on the total of 30 isolates. The observed sex ratio did not differ significantly from 1:1 (exact binomial p = 0.362).

Distribution of carbapenemase genes

All 30 isolates whose characteristics are given in **Table 1** were tested for the six target genes. A carbapenemase gene was detected in 13 isolates (43.3%, exact 95% confidence interval 25.5-62.6), while 17 (56.7%) carried no detectable carbapenemase gene and are therefore presumed to owe their carbapenem resistance to OprD porin loss, AmpC derepression or efflux. Each gene-positive isolate carried a single gene. *bla*IMP was overwhelmingly dominant, found in 9 isolates (69.2% of gene-positive isolates), followed by *bla*GES in 3 (23.1%) and *bla*OXA in 1 (7.7%); *bla*NDM, *bla*KPC and *bla*VIM were not detected. This distribution departs significantly from a uniform one (chi-square = 8.00, df = 2, p = 0.018), confirming that the carbapenemase pool in this population is genuinely metallo-beta-lactamase-dominated rather than evenly mixed: class B enzymes accounted for 69.2% of all gene-positive isolates. The observed carbapenemase prevalence of 43.3% is not to be confused with the pooled national estimate of 43% for carbapenem resistance among all Indonesian *P. aeruginosa* isolates, which has a different denominator[7]; it is far above the 2-3% recorded among carbapenem-resistant *P. aeruginosa* in the Americas in the prospective POP cohort (exact binomial p <0.001), which quantifies how strongly the value of confirmatory testing depends on local epidemiology[20]. Gene distribution by specimen type is shown in **Table 2**; the two resistance routes and the classification of the detected enzymes are illustrated in **Figure 2**.

Table 2. Distribution of carbapenemase genes detected by PCR, overall and by specimen type.

Carbapenemase gene	Ambler class	n (%) of all isolates	% of gene-positive isolates	Specimen distribution
*bla*IMP	B (metallo)	9 (30.0)	69.2	Urine 8; sputum 2; tissue 1*
*bla*GES	A (serine)	3 (10.0)	23.1	Pus 3
*bla*OXA	D (serine)	1 (3.3)	7.7	Urine 1
*bla*NDM	B (metallo)	0 (0.0)	0.0	-
*bla*KPC	A (serine)	0 (0.0)	0.0	-
*bla*VIM	B (metallo)	0 (0.0)	0.0	-
Any carbapenemase gene	-	**13 (43.3)**	**100.0**	**Urine 8; pus 3; sputum 2; tissue 1**
No gene detected	-	17 (56.7)	-	Urine 6; pus 4; sputum 2; blood 3; tissue 1
Total	-	**30 (100.0)**	-	**30**

One blaIMP-positive isolate was recovered from each of the tissue specimens; totals by specimen sum to the 13 gene-positive isolates. The distribution of the three detected genes departs significantly from uniform (chi-square = 8.00, df = 2, p = 0.018). Exact 95% confidence interval for the overall carbapenemase prevalence of 43.3%: 25.5-62.6%.

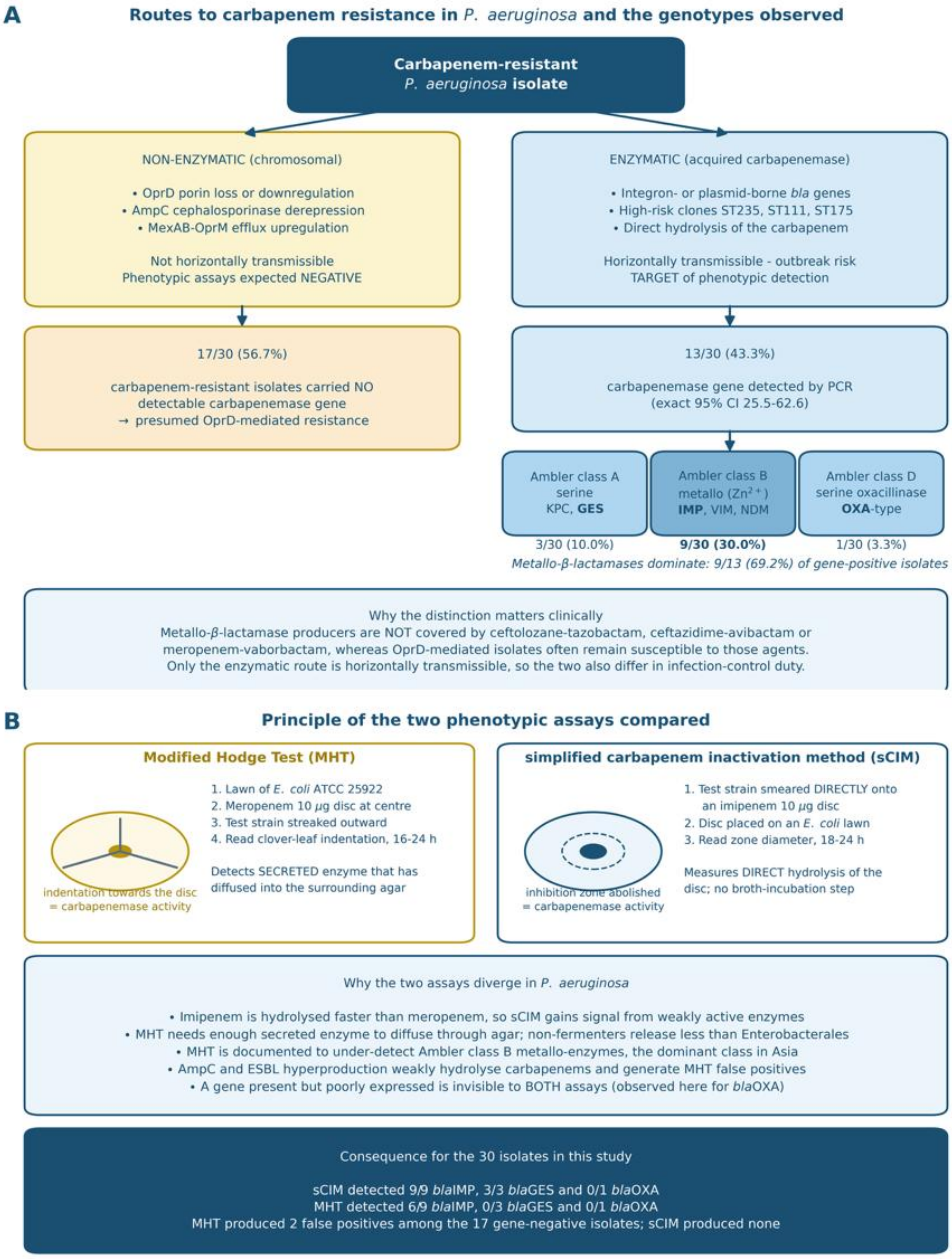

Figure 2. (A) The two routes to carbapenem resistance in Pseudomonas aeruginosa and the carbapenemase genes detected in this study. (B) Principle of the Modified Hodge Test and of the simplified carbapenem inactivation method, and the mechanistic reasons for their divergence in this organism.

Performance of the Modified Hodge Test

MHT was positive in 8 of the 30 isolates (26.7%) and negative in 22 (73.3%). Cross-classification against PCR gave 6 true positives, 2 false positives, 7 false negatives and 15 true negatives, as shown in **Table 3**. The sensitivity of MHT was therefore 46.15% (19.2-74.9) and its specificity 88.24% (63.6-98.5), with a positive predictive value of 75.0% (34.9-96.8), a negative predictive value of 68.2% (45.1-86.1) and an overall accuracy of 70.0% (50.6-85.3). The Youden index was 0.344, the positive likelihood ratio 3.92 and the negative likelihood ratio 0.610, giving a diagnostic odds ratio of 6.4 (1.0-40.3) whose lower confidence bound lies at the boundary of no discrimination. Raw agreement with the reference standard was 70.0%, and chance-corrected agreement was only fair (kappa 0.360, 95% confidence interval 0.010-0.710), well below the value of 0.5 conventionally regarded as the minimum for practical reliance. In substantive terms, MHT missed 7 of the 13 carbapenemase-producing isolates and misclassified 2 of the 17 gene-negative isolates as producers.

Performance of the simplified carbapenem inactivation method

sCIM was positive in 12 isolates (40.0%) and negative in 18 (60.0%). Cross-classification against PCR gave 12 true positives, 0 false positives, 1 false negative and 17 true negatives, also presented in **Table 3**. Sensitivity was 92.31% (64.0-99.8) and specificity 100.00% (80.5-100.0); the positive predictive value was 100.0% (73.5-100.0), the negative predictive value 94.4% (72.7-99.9) and overall accuracy 96.7% (82.8-99.9). The Youden index was 0.923. Because no false positive occurred, the positive likelihood ratio is formally infinity; the negative likelihood ratio was 0.077, and the continuity-corrected diagnostic odds ratio was 291.7 (11.0-7765.8). Agreement with PCR was near-perfect, with raw agreement of 96.7% (kappa 0.932, 95% confidence interval 0.800-1.000). The single false-negative result occurred in the isolate carrying *bla*OXA, in which the gene was present at the genotypic level but evidently not expressed at a level sufficient to hydrolyse the imipenem disc. The complete accuracy profile of both assays, with exact confidence intervals, is given in **Table 4** and displayed in **Figure 3**.

Table 3. Cross-classification of the two phenotypic assays against PCR as the reference standard.

Index test result	PCR positive (n = 13)	PCR negative (n = 17)	Total
Modified Hodge Test			
MHT-positive	6 (true positive)	2 (false positive)	8
MHT-negative	7 (false negative)	15 (true negative)	22
simplified carbapenem inactivation method			
sCIM-positive	12 (true positive)	0 (false positive)	12
sCIM-negative	1 (false negative)	17 (true negative)	18
Total isolates	**13**	**17**	**30**

Both index tests were applied to all 30 isolates. Cohen kappa against PCR: MHT 0.360 (95% CI 0.010-0.710); sCIM 0.932 (95% CI 0.800-1.000).

Table 4. Diagnostic accuracy of the Modified Hodge Test and the simplified carbapenem inactivation method against PCR (n = 30 isolates).

Measure	Modified Hodge Test (95% CI)	Simplified carbapenem inactivation method (95% CI)
Sensitivity, %	46.15 (19.2-74.9)	92.31 (64.0-99.8)
Specificity, %	88.24 (63.6-98.5)	100.00 (80.5-100.0)
Positive predictive value, %	75.0 (34.9-96.8)	100.0 (73.5-100.0)
Negative predictive value, %	68.2 (45.1-86.1)	94.4 (72.7-99.9)
Overall accuracy, %	70.0 (50.6-85.3)	96.7 (82.8-99.9)
Youden index J	0.344	0.923
Positive likelihood ratio	3.92	infinity*
Negative likelihood ratio	0.610	0.077
Diagnostic odds ratio	6.4 (1.0-40.3)	291.7 (11.0-7765.8)+
Cohen kappa versus PCR	0.360 (0.010-0.710)	0.932 (0.800-1.000)

*Confidence intervals are exact (Clopper-Pearson) intervals. *The positive likelihood ratio is undefined (formally infinite) because no false positive occurred. +Haldane-Anscombe continuity correction of 0.5 applied to all cells before computing the diagnostic odds ratio and its interval.*

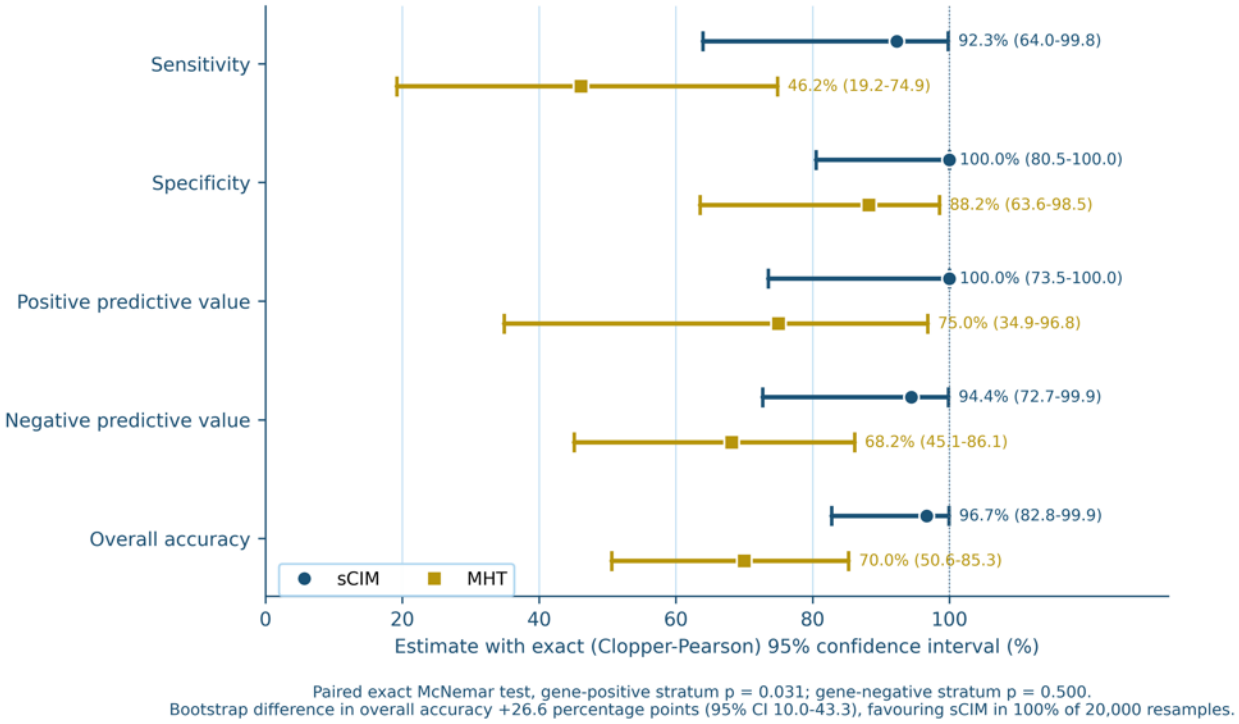

Figure 3. Diagnostic accuracy of the simplified carbapenem inactivation method and the Modified Hodge Test against PCR, with exact (Clopper-Pearson) 95% confidence intervals.

Head-to-head comparison of the two assays

Because both assays were applied to every isolate, they were compared in a paired analysis. Of the 12 sCIM-positive isolates, 6 were also MHT-positive and 6 were MHT-negative; of the 18 sCIM-negative isolates, 2 were MHT-positive and 16 were MHT-negative, giving raw agreement between the two assays of 73.3% and only moderate concordance (kappa 0.412, 95% confidence interval 0.063-0.761), as detailed in **Table 5**. The informative comparison, however, is stratified. Within the 13 reference-positive isolates, sCIM detected 6 that MHT missed while MHT detected none that sCIM missed, and the exact McNemar test confirms that this asymmetry is unlikely to be due to chance (p = 0.031). Within the 17 reference-negative isolates, MHT produced 2 false positives and sCIM none, a difference that is in the same direction but not statistically significant in a sample of this size (p = 0.500). The pooled McNemar test across all isolates, which mixes the two error types and is reported only for completeness, was not significant (p = 0.289). Bootstrap resampling gave a mean difference in overall accuracy of 26.6 percentage points in favour of sCIM (95% confidence interval 10.0-43.3), with sCIM superior in 100.0% of 20,000 replicates; the corresponding difference in Youden index was 0.578 (0.267-0.902). Expressed in practical terms, replacing MHT with sCIM corrected the classification of 26.7 isolates per 100 tested, so that approximately 3.8 isolates need to be tested by sCIM instead of MHT to gain one additional correct carbapenemase call.

Table 5. Paired comparison of the simplified carbapenem inactivation method and the Modified Hodge Test on the same 30 isolates.

Comparison	sCIM better	MHT better	Concordant	Exact McNemar p
Reference-standard positive isolates (n = 13) - sensitivity	6	0	7	0.031
Reference-standard negative isolates (n = 17) - specificity	2	0	15	0.500
All isolates (n = 30) - pooled*	6	2	22	0.289
Cross-classification of the two assays				
sCIM-positive	MHT-positive 6	MHT-negative 6	Total 12	
sCIM-negative	MHT-positive 2	MHT-negative 16	Total 18	
Raw agreement between assays	73.3%			
Cohen kappa between assays	0.412 (95% CI 0.063-0.761)			
Bootstrap difference in overall accuracy	**+26.6 percentage points**	**95% CI 10.0-43.3**	**sCIM superior in 100.0% of replicates**	

**The pooled test mixes false-negative and false-positive discordance and is reported only for completeness; the stratified tests are the informative comparisons. Bootstrap: 20,000 isolate-level resamples with replacement; difference in Youden index 0.578 (95% CI 0.267-0.902).*

Detection stratified by carbapenemase class

The aggregate accuracy figures conceal a sharp and clinically important class effect, set out in **Table 6** and displayed in panel A of **Figure 4**. Among the 9 isolates carrying the class B metallo-enzyme gene *bla*IMP, sCIM detected 9 (100.0%) whereas MHT detected 6 (66.7%). Among the 3 isolates carrying the class A gene *bla*GES, sCIM detected all 3 while MHT detected none. The single isolate carrying the class D gene *bla*OXA was missed by both assays. Every isolate missed by MHT but detected by sCIM therefore carried either *bla*IMP or *bla*GES, and the entire sensitivity advantage of sCIM in this population is attributable to those two gene groups.

Table 6. Phenotypic detection stratified by carbapenemase gene and Ambler class.

Gene (Ambler class)	Isolates, n	Detected by sCIM, n (%)	Detected by MHT, n (%)	Interpretation
*bla*GES (class A, serine)	3	3 (100.0)	0 (0.0)	MHT failed on every class A isolate; GES variants hydrolyse carbapenems weakly
*bla*IMP (class B, metallo)	9	9 (100.0)	6 (66.7)	MHT missed one third of metallo-beta-lactamase producers, its documented blind spot
*bla*OXA (class D, serine)	1	0 (0.0)	0 (0.0)	Gene present but not phenotypically expressed; invisible to any activity-based assay
All gene-positive isolates	**13**	**12 (92.3)**	**6 (46.2)**	**Entire sCIM advantage arises from blaIMP and blaGES**
Gene-negative isolates	17	0 (0.0) false positive	2 (11.8) false positive	MHT false positives attributable to AmpC or ESBL hyperproduction

Percentages are calculated within each gene stratum.

Transportability across settings

Because predictive values depend on pre-test probability, both assays were re-evaluated across a range of plausible carbapenemase prevalences among carbapenem-resistant *P. aeruginosa*, from the very low values reported in the Americas to the high values reported across Asia. The results are tabulated in **Table 7** and plotted in panel B of **Figure 4**. Because sCIM produced no false positive, its positive predictive value remains at 100.0% across the whole range, while its negative predictive value falls only gradually, from 99.2% at 10% prevalence to 89.7% at 60%. MHT behaves very differently: at a prevalence of 10% its positive predictive value is only 30.4%, meaning that roughly two of every three MHT-positive results would be false, and even at the 43.3% prevalence observed here its positive predictive value reaches only 75.0%. Its negative predictive value falls below 70% once prevalence exceeds about 40%, so a negative MHT result carries little reassurance in a high-prevalence Asian setting.

Table 7. Post-test probabilities of the two assays across plausible pre-test carbapenemase prevalences among carbapenem-resistant Pseudomonas aeruginosa.

Pre-test prevalence, %	sCIM PPV, %	sCIM NPV, %	MHT PPV, %	MHT NPV, %	Setting to which this prevalence corresponds
5	100.0	99.6	17.1	96.9	Low-prevalence high-income setting
10	100.0	99.2	30.4	93.6	Screening context; POP cohort reported 2-3% in the Americas
20	100.0	98.1	49.5	86.8	Mixed European tertiary centres
30	100.0	96.8	62.7	79.3	Regional Asian referral hospitals
43.3	**100.0**	**94.5**	**75.0**	**68.2**	**Observed in the present study**
60	100.0	89.7	85.5	52.2	High-endemicity intensive-care units
80	100.0	76.5	94.0	29.1	Outbreak investigation

PPV: positive predictive value; NPV: negative predictive value. Values computed by Bayes' theorem from the sensitivity and specificity in Table 4, holding those estimates fixed.

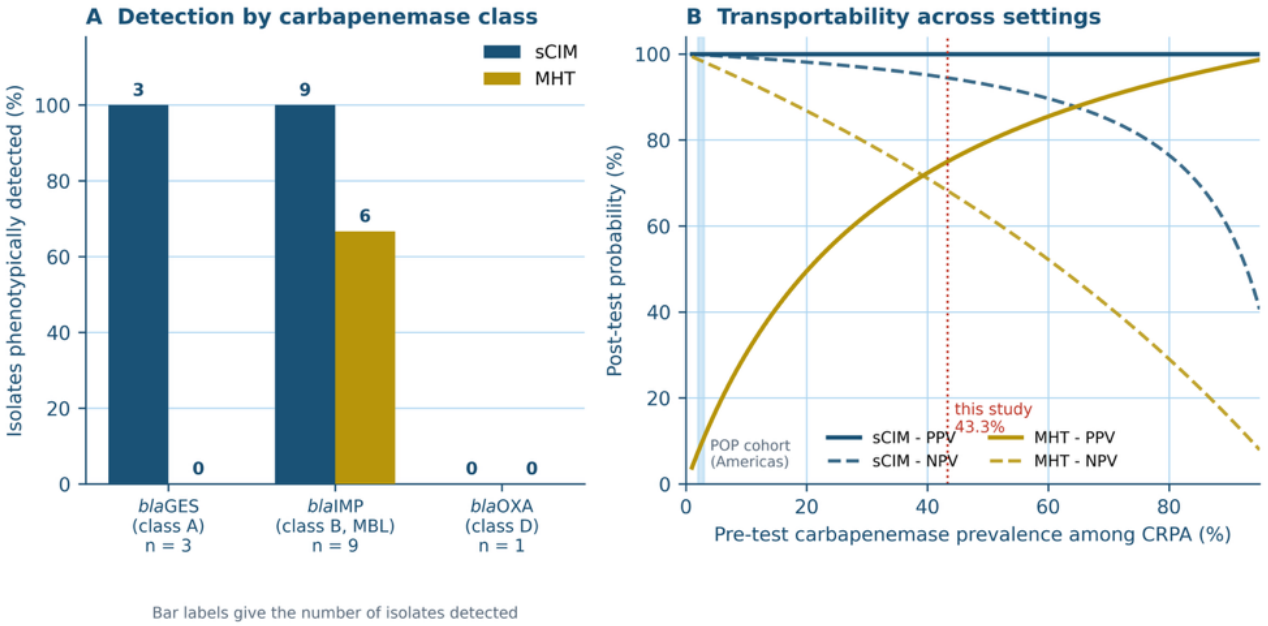

Figure 4. (A) Phenotypic detection stratified by carbapenemase gene and Ambler class. (B) Positive and negative predictive values of both assays across the range of plausible pre-test carbapenemase prevalences.

Because the sensitivity and specificity used in these calculations are themselves point estimates from a small sample, the predictive values in Table 7 were recomputed at the lower exact confidence bounds of both measures as a sensitivity analysis. Holding sCIM at a sensitivity of 64.0% and a specificity of 80.5%, its positive predictive value at a pre-test prevalence of 10% falls from 100.0% to 26.7% and its negative predictive value from 99.2% to 95.3%; at the observed prevalence of 43.3% the corresponding values are 71.5% and 74.5%. The same treatment applied to MHT gives a positive predictive value of 5.5% at a prevalence of 10%. The column of positive predictive values of 100.0% for sCIM in Table 7 is therefore a property of a point estimate derived from 17 reference-negative isolates, and should not be read as a property of the assay.

4. Discussion

Principal findings

In 30 carbapenem-resistant *P. aeruginosa* isolates from an Indonesian national referral hospital, sCIM identified carbapenemase production with a sensitivity of 92.31% and a specificity of 100.00%, achieving near-perfect agreement with PCR, as summarised in **Table 4** and displayed in **Figure 3**, whereas MHT achieved a sensitivity of only 46.15% and a specificity of 88.24%, with agreement that was merely fair. The paired analysis in **Table 5** locates the difference precisely: sCIM's advantage lies overwhelmingly in sensitivity, is statistically robust in the stratum where it matters ($p = 0.031$), and is entirely explained by the detection of *bla*IMP- and *bla*GES-positive isolates that MHT failed to detect. A second, quieter finding deserves equal emphasis. More than half of these carbapenem-resistant isolates - 17 of 30 (56.7%) - carried no detectable carbapenemase gene at all. Carbapenem resistance in *P. aeruginosa* is therefore not a proxy for carbapenemase production even in a high-prevalence setting, and a laboratory that reports the two as equivalent will misclassify a majority of its isolates.

Why the two assays diverge mechanistically

The divergence is not arbitrary; it follows from what each assay actually measures, as set out schematically in **Figure 2** and quantified by class in **Table 6**. MHT is an indirect, diffusion-dependent assay: it requires the test organism to secrete enough active enzyme into the surrounding agar to protect a susceptible indicator strain growing towards a meropenem disc. Three properties of the isolates studied here work against that requirement. First, non-fermenting organisms release less periplasmic enzyme into the medium than Enterobacterales, for which the test was originally validated[19,28]. Second, meropenem is hydrolysed more slowly than imipenem by most carbapenemases, so a meropenem-based readout is intrinsically less sensitive to weakly active enzymes[14,25]. Third, and decisively for this population, the Modified Hodge Test performs worst against the Ambler class B metallo-beta-lactamases; the only other Indonesian head-to-head evaluation in this organism found a Modified Hodge Test sensitivity of 0% against a comparator standard[26]. Since class B enzymes accounted for 69.2% of the gene-positive isolates in this study, MHT was being applied to precisely the enzyme class on which it is known to be least reliable. sCIM, by contrast, measures hydrolysis of the disc itself. The enzyme need not diffuse anywhere; it need only inactivate the imipenem in the disc on which the organism has been smeared. That is why sCIM recovered every *bla*IMP-positive isolate. The two false positives generated by MHT are equally explicable: AmpC cephalosporinase and extended-spectrum beta-lactamase hyperproduction weakly hydrolyse carbapenems and are a recognised source of Hodge-test false positivity[2,25].

Reconciling the class A result with the published literature

One result in this study appears at first sight to contradict the established literature and deserves to be addressed directly rather than glossed over. MHT is conventionally described as highly sensitive for Ambler class A carbapenemases - detecting KPC producers in approximately 98% of cases - and for class D OXA-48-like enzymes, and unreliable only for class B enzymes[14,26]. In the present sample, MHT detected none of the three class A *bla*GES-positive isolates. Three considerations reconcile the discrepancy. First, the published class A performance figures derive almost

entirely from KPC in Enterobacterales, not from GES in *P. aeruginosa*; GES-type enzymes are a heterogeneous family in which only certain variants have appreciable carbapenemase activity, and their carbapenem-hydrolysing efficiency is typically far lower than that of KPC[14]. A weakly hydrolysing class A enzyme in an organism that secretes little enzyme is close to the worst case for a diffusion-dependent assay. Second, the number of class A isolates here is three, so the point estimate of 0/3 carries very wide uncertainty and should be read as consistent with poor rather than absent detection. Third, the direction of the finding nonetheless agrees with the broader message of the multicentre evaluation that extended mCIM to non-fermenters: assay performance established in Enterobacterales cannot be assumed to transfer to *P. aeruginosa*[28]. The honest conclusion is that MHT's reputation for class A sensitivity is organism-specific and enzyme-specific, and should not be extended to GES-producing *P. aeruginosa* on present evidence.

A second apparent tension concerns specificity. The derivation study for sCIM reported a specificity of 99.6% but also cautioned that extended-spectrum beta-lactamases and AmpC enzymes in Enterobacterales can hydrolyse imipenem weakly enough to generate occasional false positives[25]. No false positive occurred here, which is a more favourable result than that caution would predict. Two explanations are plausible and cannot be separated in this dataset. The first is genuine: the imipenem disc carries a relatively high drug load, and weak hydrolysis by a non-carbapenemase enzyme may be insufficient to abolish the zone. The second is statistical: with only 17 reference-negative isolates, the exact 95% confidence interval around a specificity of 100% still extends down to 80.5-100.0%, which is fully compatible with a true false-positive rate of up to roughly one in five. The specificity claim made here is therefore that sCIM is at least as specific as MHT, not that it is infallible.

The single discordant isolate carrying *bla*OXA, missed by both assays, illustrates the fundamental and irreducible limitation of all phenotypic testing. Detection of a gene by PCR establishes only that the gene is present; phenotypic assays detect enzymatic activity, and a gene that is transcriptionally silent, poorly expressed, or truncated will be invisible to any activity-based method[2,23]. In such an isolate the operative resistance mechanism is likely to be OprD loss rather than the carbapenemase gene it happens to carry. This is a genuine discordance between genotype and phenotype rather than an assay failure, and it is one reason why a negative phenotypic result should not override a strong epidemiological suspicion of transmission.

Comparison with the wider evidence base

The accuracy achieved by sCIM here is consistent with, though slightly below, the sensitivity of 100% and specificity of 99.6% reported in its derivation study across Gram-negative bacilli[25], and with the performance of the closely related mCIM, which exceeded 99% for both measures in Enterobacterales and remained the best-performing phenotypic assay when extended to *P. aeruginosa* and *A. baumannii*, outperforming colorimetric Carba NP for metallo-enzymes[27,28]. The slight shortfall in sensitivity observed here is attributable entirely to the single non-expressing *bla*OXA isolate, and the point estimate would be 100% if that isolate were excluded. The performance of MHT in this study, conversely, sits at the pessimistic end of the published range but is, if anything, more favourable than the only directly comparable Indonesian evaluation. Working with 21 meropenem-resistant *P. aeruginosa* isolates and the VITEK-2 system as comparator, that study recorded a Modified Hodge Test sensitivity of 0% and a specificity of 100%, against 90.9% and 50% respectively for mCIM, with areas under the curve of 0.50 and 0.71[26]. Our sensitivity of 46.15% therefore sits between that value and the figures reported from Enterobacterales, which is what a class-dependence explanation predicts. Evaluations of the wider carbapenem inactivation family point the same way: mCIM was positive in 96% of 250 carbapenem-resistant Enterobacterales and *P. aeruginosa* isolates in a recent series, of which 97.5% were also eCIM positive and therefore metallo-enzyme producers, and simplified hydrolysis-based readouts that dispense with the broth step have reproduced molecular detection closely[32,33]. Taken together with the withdrawal of MHT from the routine CLSI recommendation in favour of mCIM and eCIM, the direction of the evidence is now unambiguous[29]. Cross-sectional work in Nigerian tertiary laboratories has likewise combined phenotypic screening with molecular confirmation and reported substantial carbapenemase carriage, demonstrating that the workflow is deliverable outside well-resourced settings[34,35]. Two caveats from the wider literature nonetheless apply to any member of this assay family. Work on *Acinetobacter baumannii* has shown that carbapenem inactivation methods require organism-specific optimisation of inoculum and indicator-strain density rather than direct transfer between species, so the parameters validated here should not be assumed to hold for other non-fermenters[36]. Isolates carrying more than one carbapenemase are also increasingly reported; an activity-based assay registers such an isolate as positive but cannot resolve the combination, and the combination determines which of the newer agents retain activity[37].

One further caveat governs how far Table 7 can be transported. Sensitivity and specificity are conventionally treated as properties of an assay that are invariant to prevalence, but the class-stratified results reported here show that they are not: performance depends on which Ambler class is present, and the class distribution differs systematically between regions. A setting with a carbapenemase prevalence of 10% is likely to be one in which class A KPC predominates, and MHT performs considerably better against KPC than against the metallo-enzymes that dominated this sample. Applying the sensitivity of 46.15% measured here to a KPC-dominated population would therefore understate MHT, just as applying a KPC-derived sensitivity to an Indonesian population would overstate it. The predictive values in Table 7 should be read as conditional on a metallo-beta-lactamase-dominated enzyme distribution similar to the one observed here.

It is also worth being explicit about what this study does not show. It does not show that sCIM can replace molecular testing. Immunochromatographic lateral-flow assays detect the five commonest carbapenemase families within fifteen minutes with accuracy approaching that of PCR, and molecular platforms additionally assign the enzyme class that determines the regimen[21,22]. What sCIM offers is not equivalence with those platforms but availability: it detects enzymatic activity irrespective of which gene is responsible, including genes absent from any commercial panel, and it does so at a cost and infrastructure requirement that a district hospital laboratory can meet today. In a laboratory that already has molecular capability, sCIM is a screening step; in a laboratory that does not, it is the only confirmatory step available.

Interpretation in the Indonesian context

Three features of the Indonesian setting make this comparison more consequential than it would be in a high-income laboratory. The first is epidemiological. The carbapenemase pool documented here and set out in **Table 2** - *bla*IMP-dominant, with *bla*GES present and *bla*KPC absent - matches the wider Asian pattern in which integron-borne *bla*IMP and *bla*VIM predominate, and matches earlier Indonesian-Japanese collaborative work identifying *bla*IMP

and *bla*NDM as the principal Indonesian determinants[8,14]. It differs sharply from the VIM-dominant landscape reported in European tertiary centres[38], which is one reason why carbapenemase-detection policy cannot simply be imported. Because MHT's blind spot is precisely the class B metallo-enzymes, the assay that Indonesian laboratories are most likely to have in place is the assay least suited to the enzymes they are most likely to encounter. The mismatch is structural rather than incidental.

The second feature is capacity. Fewer than one third of laboratories in lower- and middle-income settings perform any carbapenemase confirmation, and Indonesian intensive-care and point-prevalence data show both endemic carbapenemase circulation and a low proportion of culture-directed prescribing[8,10,23,24]. sCIM is unusually well matched to this constraint. It requires an imipenem disc, Mueller-Hinton agar and a stock indicator strain - materials already present in any laboratory performing disc-diffusion susceptibility testing - and it needs no thermocycler, no cold chain, no proprietary cartridge and no molecular training. It also removes the four-hour broth-incubation step of mCIM, which matters where technologist time rather than reagent cost is the binding constraint. The operational comparison of the available methods, and their realistic placement across the tiers of the Indonesian laboratory network, is summarised in **Table 8**.

Table 8. Operational comparison of carbapenemase detection methods and their applicability across the tiers of the Indonesian laboratory network.

Method	Principle	Turnaround	Equipment required	Relative cost per test	Realistic Indonesian tier
Modified Hodge Test	Indirect: growth of an indicator strain towards a carbapenem disc	16-24 h	Incubator; agar; disc; indicator strain	Very low	Widely available, but should be discontinued for *P. aeruginosa*
sCIM	Direct: hydrolysis of an imipenem disc smeared with the test organism	18-24 h	Incubator; agar; disc; indicator strain	Very low	District hospital (RSUD) and above
mCIM	Direct: hydrolysis of a meropenem disc in broth	22-28 h (includes 4 h broth step)	Incubator; broth; agar; disc	Low	District hospital and above where technologist time permits
eCIM (with mCIM)	mCIM plus EDTA to distinguish metallo- from serine enzymes	22-28 h	As mCIM plus EDTA	Low	Referral and academic centres
Colorimetric (Carba NP)	Acidimetric detection of carbapenem hydrolysis	2-4 h	Spectrophotometer or visual reading; reagents	Moderate	Referral centres; reduced sensitivity for metallo-enzymes
Immunochromatographic lateral flow	Antibody capture of five carbapenemase families	15-30 min	None beyond the cassette; cold chain for storage	High	Referral and academic centres
PCR / multiplex PCR	Detection of carbapenemase genes	4-6 h plus batching	Thermocycler; electrophoresis; molecular training	High (plus capital cost)	Academic and national reference laboratories
Whole-genome sequencing	Complete resistome and clonal assignment	Days	Sequencing platform; bioinformatics capacity	Very high	National reference and research centres

Turnaround times are from availability of a pure subculture. Cost bands are indicative orders of magnitude for the Indonesian market rather than measured unit costs, and should be verified locally before procurement decisions.

The third feature is policy. Indonesian hospitals are required to operate antimicrobial stewardship programmes through hospital antimicrobial resistance control committees, yet a multicentre point-prevalence survey found that only a minority of prescriptions were culture-directed, and the national systematic review identifies laboratory capacity as the limiting factor[10,24]. Those committees cannot act on mechanism-level information their laboratories are not equipped to generate. A confirmed carbapenemase result changes three decisions simultaneously: it argues against relying empirically on ceftolozane-tazobactam or ceftazidime-avibactam, which are inactive against metallo-beta-lactamase producers, and carbapenemase carriage is itself a strong predictor of ceftolozane-tazobactam non-susceptibility[17]; it triggers contact precautions and outbreak investigation, because only the enzymatic mechanism is horizontally transmissible, and a systematic review of CRPA outbreak investigations shows that mechanism confirmation is what converts a cluster of resistant isolates into an actionable transmission hypothesis[19,39,40]; and, when negative, it supports carbapenem-sparing and novel-agent-sparing decisions rather than reflexive escalation[18]. Because environmental and carriage reservoirs feed clinical isolates, a laboratory that can confirm carbapenemase production cheaply can also participate in the source-tracking studies that infection-control programmes increasingly require[3,41]. Given that carbapenem non-susceptibility carries substantial excess mortality in Indonesian hospitals, an assay that delivers this information overnight at negligible marginal cost has a plausible route to patient benefit[9].

Implications for practice

On the evidence presented here, MHT should no longer be used as the confirmatory carbapenemase assay for *P. aeruginosa* in Indonesian laboratories. Its sensitivity of 46.15% means that more than half of carbapenemase producers would be reported as non-producers, and its positive predictive value at realistic prevalences is too low for a positive result to be acted upon without confirmation, as

Table 7 makes plain across the range of prevalences an Indonesian laboratory might plausibly face. We propose instead the tiered workflow set out in **Figure 5**: sCIM performed on every carbapenem-resistant *P. aeruginosa* isolate at district-hospital level and above, with positive results triggering contact precautions and infection-control notification and with referral to a reference centre for class assignment by eCIM or PCR, and with negative results interpreted as presumptive OprD-mediated resistance while retaining the caveat that poorly expressed genes remain assay-negative. Every result, positive or negative, should be reported to the hospital PPRA committee and submitted to national surveillance. The specific recommendations that follow from each finding, with the strength of evidence supporting each, are collected in **Table 9**, and the position of sCIM among the alternative platforms is set out in **Table 8**.

The certainty labels in Table 9 are applied on a simple, explicitly stated basis rather than through a formal grading system: a finding is labelled high certainty when it follows deterministically from the data or is concordant with multinational evidence, moderate when it rests on the present sample with an interval that excludes the null but remains wide, and low when it rests on fewer than five isolates. Readers should treat these labels as a transparent summary of the preceding argument rather than as GRADE ratings, which would not be appropriate for a single primary study.

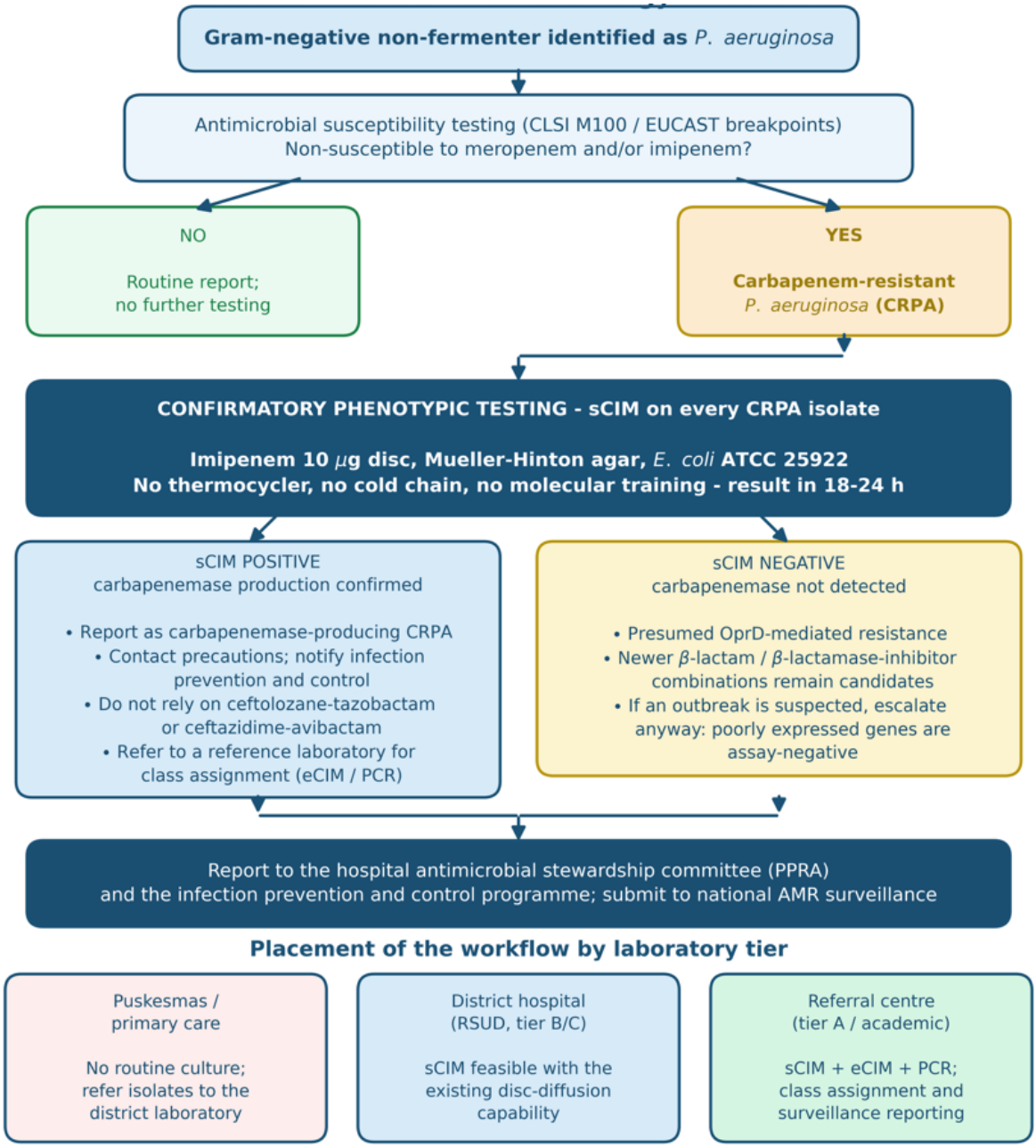

Figure 5. Proposed tiered workflow for carbapenemase confirmation in Indonesian clinical microbiology laboratories.

Table 9. Principal findings, strength of the supporting evidence, and recommendations for Indonesian clinical practice.

Finding	Evidence in this study	Certainty	Recommendation for Indonesian practice
sCIM detects carbapenemase production accurately in carbapenem-resistant *P. aeruginosa*	Sensitivity 92.31%, specificity 100.00%, kappa 0.932 versus PCR	Moderate (single centre, n = 30, wide intervals)	Adopt sCIM as the routine confirmatory assay at district-hospital level and above
MHT substantially under-detects carbapenemase producers	Sensitivity 46.15%; 7/13 producers missed; kappa 0.360	Moderate to high (consistent with the international literature)	Discontinue MHT for *P. aeruginosa*; retain it only where no alternative exists, and report negative results as uninformative
The advantage of sCIM is concentrated in class B and class A enzymes	sCIM 9/9 *bla*IMP and 3/3 *bla*GES versus MHT 6/9 and 0/3	Moderate for class B; low for class A (n = 3)	Where MHT remains in use, treat a negative result in a metallo-beta-lactamase-endemic setting as non-informative
Carbapenem resistance is not equivalent to carbapenemase production	17/30 (56.7%) carbapenem-resistant isolates carried no carbapenemase gene	High (concordant with multinational cohort data)	Never report carbapenem resistance as carbapenemase production; confirm every isolate before infection-control escalation
A carbapenemase gene may be present without phenotypic expression	One *bla*OXA-positive isolate negative on both assays	Low (single isolate)	Do not use a negative phenotypic result to overrule strong epidemiological suspicion of transmission
Predictive values are strongly prevalence-dependent	MHT PPV falls to 30.4% at 10% prevalence; sCIM PPV remains 100.0%	High (deterministic from Bayes' theorem)	Interpret confirmatory results against local carbapenemase prevalence, and audit that prevalence periodically
Confirmatory results are actionable for stewardship and infection control	Class assignment determines activity of ceftolozane-tazobactam and ceftazidime-avibactam	High (guideline-based)	Route every result to the hospital antimicrobial stewardship (PPRA) committee and the control programme, and onward to national AMR surveillance

Certainty reflects the precision of the estimate in this study together with its consistency with the external evidence base cited in the Discussion.

Strengths

The principal strength of this study is that both index tests were applied to every isolate and compared against a molecular reference standard covering six carbapenemase gene families, with sequencing confirmation of selected amplicons, which permits a properly paired analysis rather than a comparison of separately reported accuracy figures. The analysis reports exact rather than approximate confidence intervals throughout, which is the appropriate treatment for sparse two-by-two tables; it separates the sensitivity and specificity comparisons into their own McNemar strata rather than conflating them; and it quantifies the uncertainty in the difference between assays by bootstrap resampling. Reporting predictive values across a range of pre-test prevalences, rather than only at the prevalence observed, makes the findings transportable to settings with different carbapenemase epidemiology. Finally, the class-stratified analysis converts an aggregate accuracy difference into a mechanistic explanation, which is what allows the result to be generalised beyond this sample.

Limitations

Several limitations qualify these findings. The sample of 30 isolates from a single national referral hospital is small, and the confidence intervals reported throughout are correspondingly wide; the specificity of 100.00% in particular has an exact lower bound of 80.5-100.0%, and the class-stratified results rest on as few as one isolate for class D. Isolates were archived rather than collected prospectively, which introduces the possibility of selection towards isolates of interest and precludes any analysis of clinical outcome; no patient-level clinical data, treatment or mortality information was available, so the downstream benefit of correct classification is argued from the literature rather than demonstrated here. The molecular panel covered six gene families and did not include less common determinants such as *bla*SPM, *bla*GIM or *bla*AIM, so an isolate carrying one of these would have been misclassified as reference-negative; this would, if anything, bias specificity estimates downward rather than inflate them. Non-carbapenemase resistance mechanisms were inferred rather than measured, since OprD sequencing, efflux-pump expression assays and AmpC quantification were not performed, so the attribution of the 17 gene-negative isolates to porin loss is presumptive. Reproducibility was not formally quantified by repeat testing across operators or days, and although readings were made without knowledge of the molecular result and equivocal readings were resolved by a second reader, no kappa for inter-observer agreement can be reported. Finally, the cross-sectional design captures a single point in time and cannot describe the evolution of carbapenemase epidemiology in this institution.

Three reporting limitations should also be recorded. Absolute inhibition-zone diameters were not retained in the laboratory record, so the sCIM reading criterion cannot be expressed retrospectively as a numerical threshold and readers cannot apply an alternative cut-off to these data; prospective recording of zone diameters should be standard in any future evaluation. The number of archived isolates screened to obtain the 30 analysed was not recorded, so the flow diagram cannot report a screening denominator. And the study was not prospectively registered. None of these affects the internal validity of the accuracy estimates, but each limits the completeness with which the study can be reported against STARD 2015 and each is straightforward to remedy prospectively.

Future research

Four priorities follow. First, a prospective multicentre Indonesian evaluation of sCIM across hospitals of differing tiers, with consecutive rather than archived isolates and with formal inter-observer reproducibility testing, is required before the assay can be recommended nationally rather than institutionally. Second, the reference standard should be broadened from a six-gene panel to whole-genome sequencing, which would resolve both the undetected-determinant problem and the expression question raised by the discordant *bla*OXA isolate. Third, the class A finding reported here - failure of MHT to detect GES-producing *P. aeruginosa* - should be tested in a purpose-designed series with adequate numbers of class A isolates, since it contradicts the conventional description of MHT and, if confirmed, has

implications well beyond Indonesia. Fourth, an implementation study linking sCIM results to prescribing decisions, infection-control actions and patient outcomes would establish whether the diagnostic gain demonstrated here translates into clinical benefit, which is the question that ultimately justifies the change in practice.

5. Conclusion

In carbapenem-resistant *P. aeruginosa* isolates from an Indonesian national referral hospital, the simplified carbapenem inactivation method detected carbapenemase production with a sensitivity of 92.31% and a specificity of 100.00% and agreed almost perfectly with PCR, whereas the Modified Hodge Test achieved a sensitivity of only 46.15% and agreement that was merely fair. The advantage of sCIM was concentrated in the metallo-beta-lactamase and class A GES isolates that dominate the Asian carbapenemase pool and that MHT is mechanistically least able to detect, and it was statistically robust in a paired analysis. More than half of the carbapenem-resistant isolates carried no carbapenemase gene, confirming that carbapenem resistance must not be reported as though it were carbapenemase production. As set out in **Table 9** and **Figure 5**, sCIM requires no equipment beyond that already present in any laboratory performing disc-diffusion testing and returns a result overnight, which makes it implementable across the Indonesian hospital network today. We therefore recommend that sCIM replace the Modified Hodge Test as the routine confirmatory assay for carbapenem-resistant *P. aeruginosa* in Indonesian clinical microbiology laboratories, with results fed directly to hospital antimicrobial stewardship and infection prevention and control programmes and onward to national resistance surveillance. The benefit to patients that would follow from this change is inferred from guideline-level evidence linking mechanism-level diagnosis to antimicrobial selection and to time to appropriate therapy; it was not measured in this study and remains to be demonstrated prospectively in Indonesian practice.

Ethical clearance

The research protocol was reviewed and approved by the Health Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (approval number 1158/KEPK/V/2019). The study used archived bacterial isolates together with anonymised specimen records; no identifiable patient data were accessed and no additional clinical specimens were collected for the purpose of this study.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contribution

Experimental design: FNF. Materials preparation: FNF. Research implementation: FNF. Research supervision: EBW, K. Statistical analysis: FNF, KBR. Manuscript writing: FNF, KBR, BNL. Manuscript editing and critical revision: KBR, BNL, EBW, K. All authors read and approved the final manuscript.

Data availability

The isolate-level dataset supporting the findings of this study, comprising the specimen type, carbapenemase genotype and both phenotypic assay results for each of the 30 isolates, is available from the corresponding author on reasonable request.

Generative artificial intelligence use

All authors reviewed and approved the final content and take full responsibility for the accuracy, integrity and originality of the work.

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