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Comparative Evaluation of Phenotypic and Molecular Methods for Early Detection of Carbapenemase-Producing Gram-Negative Bacteria: Implications for Antimicrobial Stewardship

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Abstract

Background: Carbapenem resistance among Gram-negative bacilli now sits at critical priority on the WHO pathogen list, yet head-to-head diagnostic-accuracy data comparing phenotypic and molecular assays remain sparse for resource-limited hospitals.[1] We set out to establish, prospectively, how the modified carbapenem inactivation method (mCIM) and its EDTA-based adjunct (eCIM) perform against polymerase chain reaction (PCR) for carbapenemase detection, and whether faster molecular results translate into measurable changes in prescribing.

Methods: Over a four-and-a-half-month period (7 July–15 November 2024) in the Department of Microbiology, Combined Military Hospital (CMH), Sialkot, Pakistan, 113 consecutive, non-duplicate Gram-negative isolates recovered from hospitalised adults underwent parallel mCIM, eCIM, and multiplex PCR testing for blaKPC, blaNDM, blaOXA-48, and blaVIM, following a STARD-compliant design with PCR as the reference standard. Sensitivity, specificity, predictive values, overall accuracy, and Cohen's kappa were computed for each phenotypic test, and multivariable logistic regression was used to identify independent predictors of carbapenem resistance and in-hospital death.

Results: Twenty-seven isolates (23.9%) were PCR-positive, blaKPC being the commonest determinant (37.0% of positives). mCIM reached 88.5% sensitivity and 96.5% specificity (94.6% accuracy; $\kappa = 0.850$); eCIM was less sensitive (63.0%) but perfectly specific (100.0%; $\kappa = 0.720$). PCR results returned a median of 19 hours faster than culture (23 vs 40 hours, $p<0.001$), and this speed advantage was associated with prescribing behaviour: 70.4% of PCR-positive patients had their empirical

regimen changed, versus none of the PCR-negative group ($p<0.001$). Chronic kidney disease (adjusted odds ratio [aOR] 4.44) and infection with Acinetobacter baumannii (aOR 4.73, versus E. coli) independently predicted carbapenem resistance, which in turn carried a substantially higher mortality burden (28.0% vs 4.5%, $p=0.002$).
Conclusion: mCIM offers dependable, low-cost carbapenemase screening for laboratories without molecular infrastructure, while PCR remains the indispensable tool for gene-level surveillance and was associated with a rapid, quantifiable stewardship benefit once results reached the ward. A tiered algorithm phenotypic screening backed by reflex molecular or eCIM confirmation appears well matched to the diagnostic and resource constraints of similar hospital settings.

Introduction

Few threats to hospital medicine have grown as quickly, or as consequentially, as resistance among Gram-negative bacilli. The current WHO Bacterial Priority Pathogens List keeps carbapenem-resistant Enterobacterales, carbapenem-resistant Acinetobacter baumannii, and carbapenem-resistant Pseudomonas aeruginosa at critical priority the category set aside for pathogens whose mortality, transmissibility, and shrinking treatment options together demand urgent action.[1] Behind that classification sits a sobering body of modelling work: the latest Global Burden of Disease estimates attribute 1.14 million deaths directly to bacterial AMR in 2021 alone, with a further 4.71 million deaths where resistance was a contributing factor, and the trajectory is expected to worsen through mid-century without meaningful intervention.[2] Earlier modelling had already placed the 2019 direct-death toll at 1.27 million, and in both analyses Gram-negative organisms carry a disproportionate share of the burden unsurprising, given their outer-membrane permeability barrier and their facility for shuttling resistance genes between species on mobile plasmids.[3]

This trend is not confined to mortality statistics alone. Data pooled through the WHO's Global Antimicrobial Resistance and Use Surveillance System (GLASS) show climbing resistance to Watch- and Reserve-category agents among Gram-negative bloodstream isolates, and a striking correlation between how much antibiotic a country consumes and how resistant its isolates become a reminder that stewardship and laboratory capacity are not separable problems but two faces of the same one.[4]

At the mechanistic level, carbapenem resistance in Gram-negative bacilli is driven overwhelmingly by carbapenemase enzymes: KPC (Ambler class A), the metallo-β-lactamases NDM and VIM (class B), and the OXA-48-family oxacillinases (class D). What makes these enzymes so difficult to contain is less their catalytic activity than their genetics the genes encoding them typically travel on conjugative plasmids, jumping between unrelated strains and species and, in the process, complicating any laboratory workflow built around a single detection target rather than the broader spectrum running from extended-spectrum β-lactamases through to true carbapenemases.[5] Pakistan illustrates the scale of the problem locally: tertiary-hospital surveys have repeatedly found carbapenem non-susceptibility above 40% among Gram-negative clinical isolates, with blaNDM the most frequently implicated determinant in Enterobacterales.[6,7] The reservoir is not confined to hospital wards, either sampling of Islamabad's sewage system has independently tracked a rising trend in meropenem-resistant E. coli over time, evidence of a One Health dimension to the problem that ward-based surveillance alone would miss entirely.[8]

Why does the distinction matter clinically, beyond the laboratory bench? Because treatment options for carbapenemase producers are narrow and enzyme-specific. Of the handful of newer β-lactam–β-lactamase inhibitor combinations available ceftazidime–avibactam, meropenem–vaborbactam, imipenem–relebactam, ceftolozane–tazobactam none covers the full carbapenemase spectrum, so prescribing rationally requires knowing not just that an isolate is carbapenem-resistant but which enzyme class is responsible.[9] The price of getting this wrong, or getting it late, has

been measured directly: 30-day mortality following carbapenem-resistant K. pneumoniae bacteraemia has ranged from 28% to 38% across large Asian cohorts, with renal impairment, prior antimicrobial exposure, and overall comorbidity burden emerging repeatedly as independent predictors of both acquiring the infection and dying from it.[10,11,12]

Given these stakes, laboratory turnaround time is not a peripheral operational detail but a variable with direct bearing on survival. Culture-based identification and disk-diffusion susceptibility testing, still the mainstay in most resource-limited settings, typically take 24–72 hours to yield an actionable result a window during which a patient may continue receiving a carbapenem that a hidden carbapenemase has already defeated.[13] The CLSI-endorsed modified carbapenem inactivation method (mCIM) was developed precisely to shorten this gap: a simple, low-cost phenotypic screen that can be run in any laboratory equipped for standard disk diffusion. Its companion assay, the EDTA-modified carbapenem inactivation method (eCIM), adds EDTA to chelate the divalent cations that metallo-β-lactamases require, allowing carbapenemase-producing isolates to be further split into metallo- and serine-carbapenemase producers. Validation work from South and Southeast Asian laboratories has generally reported mCIM sensitivities above 90% against a PCR reference standard, supporting its use as a practical first-line screen.[14,15]

Where infrastructure allows, cartridge-based molecular platforms push performance further still. Xpert Carba-R and similar automated PCR assays have reported sensitivity and specificity in the 95–98% range, working directly from isolates or even positive blood-culture broth, with results available in under an hour though the capital and consumable costs involved keep such platforms out of reach for many low- and middle-income laboratories.[16,17] Whichever platform delivers the answer, what matters clinically is how quickly that answer reaches the prescriber: early, appropriate diagnostics paired with an active stewardship response have consistently been linked to faster initiation of effective therapy and better survival in MDR Gram-negative infection.[18]

The value of rapid carbapenemase detection extends past the individual patient's treatment plan and into infection control. A systematic review of containment strategies for carbapenem-resistant Enterobacterales concluded that active surveillance culturing, prompt patient placement, and contact-precaution bundles meaningfully cut nosocomial spread provided they are triggered quickly enough, which again depends on how fast the laboratory can deliver a result.[19]

What is largely missing from the South Asian literature, Pakistan included, is a study that puts mCIM, eCIM, and PCR side by side and then follows the diagnostic result all the way to the prescribing decision it produced. Existing reports have tended to describe carbapenemase gene prevalence as a standalone epidemiological finding, without tracking turnaround time, phenotypic–molecular agreement, and documented prescribing changes for the same patients.[6,7] That gap makes it difficult for laboratories to design cost-effective, tiered testing algorithms suited to their own resource constraints. This study was undertaken to close part of that gap: to compare mCIM and eCIM against PCR for carbapenemase detection in a cohort of hospitalised patients, to map the local distribution of carbapenemase genes and antimicrobial susceptibility, to identify independent risk factors for carbapenem resistance, and to quantify how diagnostic turnaround time related to antimicrobial stewardship action and clinical outcome at a tertiary-care hospital in Pakistan.

Materials and Methods

Study Design, Setting, and Population

We conducted a prospective, hospital-based diagnostic accuracy study in the Department of Microbiology, Combined Military Hospital (CMH), Sialkot, Pakistan, between 7 July 2024 and 15 November 2024. Reporting followed the STARD

framework throughout, with PCR treated as the reference standard against which both mCIM and eCIM were judged. Adults aged 18 years or older who were hospitalised with clinically suspected bacterial infection and who yielded a culture-confirmed Gram-negative isolate were eligible; where a patient contributed more than one isolate during the study window, only the first was retained for analysis, to keep each patient's data independent of the others.

A total of 156 patients were initially assessed for eligibility. Forty-three were excluded prior to enrolment: 21 did not meet the inclusion criteria, 12 had received fewer than 48 hours of antibiotic therapy before specimen collection, 6 had polymicrobial infections, and 4 yielded an inadequate or contaminated specimen. The remaining 113 eligible patients had their isolates collected and processed for parallel mCIM, eCIM, and PCR testing, and form the analytic cohort reported throughout this manuscript (Figure 1).

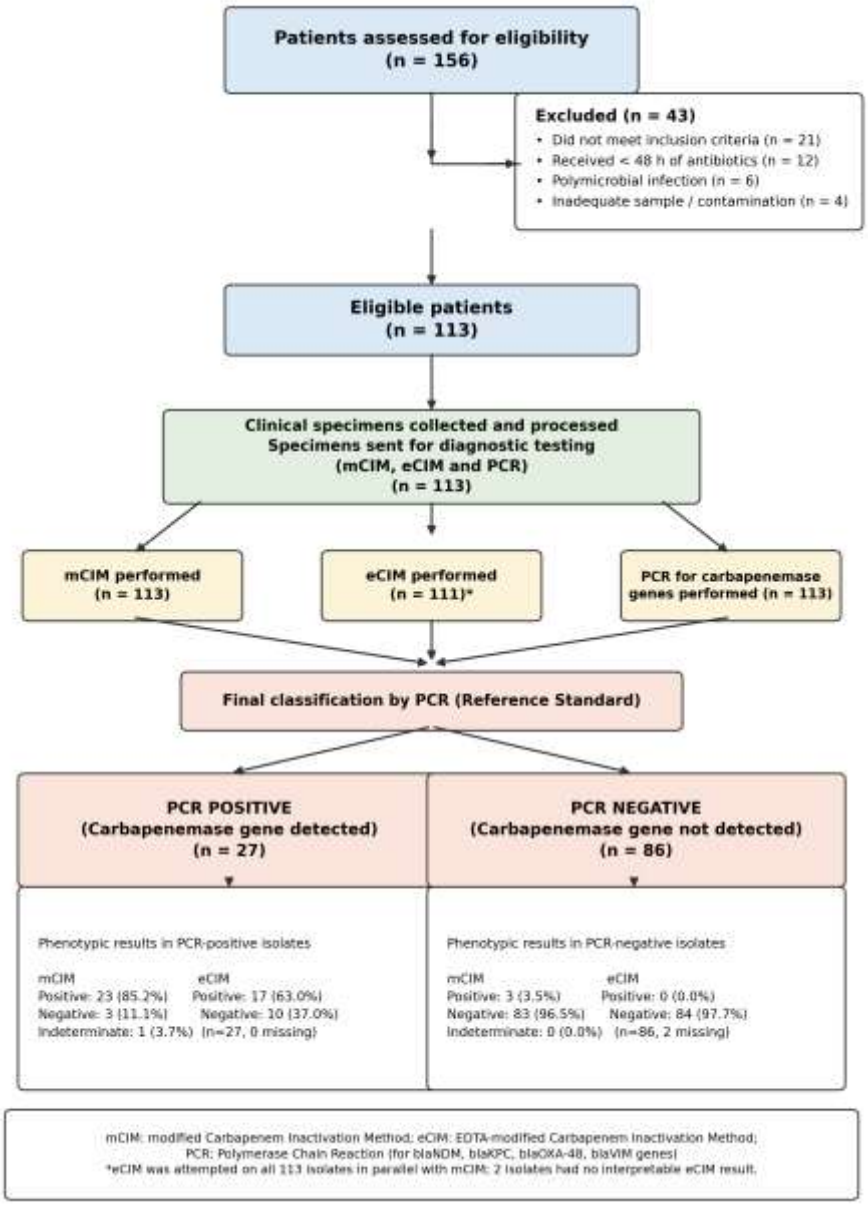

Figure 1. STARD flow diagram of patient screening, eligibility, exclusion, and allocation to phenotypic (mCIM, eCIM) and molecular (PCR) testing, with final classification against the PCR reference standard.

Specimen Processing and Susceptibility Testing

Urine, blood, sputum, endotracheal aspirate, and pus/wound specimens arrived through routine clinical channels and were handled under standard biosafety

precautions throughout. Primary culture used blood and MacConkey agar incubated aerobically at 35–37°C for 18–24 hours, with organism identity confirmed by colony morphology, Gram stain, and the laboratory's standard biochemical panel. Susceptibility to meropenem (10 µg), imipenem (10 µg), ceftazidime (30 µg), and ciprofloxacin (5 µg) was read by Kirby–Bauer disk diffusion and interpreted against the CLSI M100 breakpoints current at the time of testing.[20]

An isolate was classed as multidrug-resistant (MDR) if it showed acquired non-susceptibility (intermediate or resistant) to at least one agent in each of three or more antimicrobial classes; because only carbapenems (meropenem, imipenem), a third-generation cephalosporin (ceftazidime), and a fluoroquinolone (ciprofloxacin) were tested, MDR classification under this scheme could only be met by isolates non-susceptible across all three of these classes, including carbapenem non-susceptibility. This overlap is addressed explicitly in Section 2.5 and in the Discussion. Carbapenem resistance (CarbR) was defined as resistance (R) to meropenem and/or imipenem by CLSI breakpoints, or intermediate susceptibility to both agents.

ESBL production was confirmed phenotypically by the combination disk method, performed independently of the routine ceftazidime susceptibility result. Disks containing ceftazidime (30 µg) alone and ceftazidime plus clavulanic acid (30/10 µg) were placed on the same Mueller–Hinton plate for each isolate, and the zone diameters were compared after overnight incubation. An increase in zone diameter of ≥5 mm around the ceftazidime–clavulanate disk relative to ceftazidime alone was interpreted as confirmatory of ESBL production, per CLSI criteria.

Phenotypic and Molecular Detection of Carbapenemase Production

Every isolate was screened by mCIM, following the CLSI protocol: a meropenem disk was incubated in a standardised bacterial suspension for four hours before being transferred onto a Mueller–Hinton plate seeded with the carbapenem-susceptible control strain E. coli ATCC 25922, and the resulting inhibition zone was read after overnight incubation. eCIM was performed in parallel with mCIM on the same isolate set, using a EDTA-supplemented inactivation step compared against the standard mCIM zone; a disproportionately larger zone in the EDTA arm indicated a metallo-β-lactamase producer, distinguishing it from a serine-carbapenemase producer. eCIM produced an interpretable result for 111 of 113 isolates (98.2%); the remaining 2 isolates were excluded from eCIM accuracy calculations for want of an interpretable zone. Independently of the phenotypic workflow, genomic DNA extracted from overnight culture was subjected to multiplex PCR targeting blaKPC, blaNDM, blaOXA-48, and blaVIM using previously published primer sets under optimised cycling conditions; amplification products were visualised by agarose gel electrophoresis under UV light. The PCR result, not either phenotypic assay, served as the reference standard for every diagnostic-accuracy calculation reported here.

Data Collected and Study Outcomes

A structured case record form captured, for every patient, demographic details, ward of admission, Charlson Comorbidity Index, SOFA score at enrolment, diabetes and chronic kidney disease status, antibiotic exposure in the preceding 90 days, specimen type, organism identity, full susceptibility and resistance profile, mCIM/eCIM/PCR results and carbapenemase gene identity where applicable, time from specimen collection to result for each diagnostic method, whether the empirical antimicrobial regimen was subsequently changed, length of hospital stay, and vital status at discharge. The pre-specified primary outcome was the diagnostic accuracy of mCIM and eCIM relative to PCR; secondary outcomes covered the distribution of isolates and carbapenemase genes, phenotypic–molecular concordance, comparative turnaround time, the association between diagnostic speed and regimen modification,

independent predictors of carbapenem resistance, and clinical outcome stratified by resistance status.

Statistical Methods

Analyses were performed in IBM SPSS Statistics, version 27.0, and cross-checked independently in Python. The Shapiro–Wilk test was used to check each continuous variable for normality; because none met that assumption, continuous variables are summarised as median (interquartile range) throughout and compared between groups using the Mann–Whitney U test, while categorical variables are given as n (%) and compared using the Chi-square or, where expected cell counts were small, Fisher's exact test. For mCIM and eCIM, sensitivity, specificity, positive and negative predictive values, and overall accuracy were each calculated with 95% confidence intervals against the PCR reference standard, and concordance between the two phenotypic methods and PCR was quantified using Cohen's kappa.

Independent predictors of carbapenem resistance and of in-hospital death were identified through multivariable logistic regression, reported as adjusted odds ratios with 95% confidence intervals. Because MDR status, as defined in Section 2.2, structurally overlaps with carbapenem non-susceptibility in a 3-antibiotic-class panel, MDR was excluded a priori from the carbapenem-resistance model to avoid circular inference; the retained predictors for that model age, chronic kidney disease, prior antibiotic exposure, and organism identity were specified before analysis on clinical grounds. MDR was retained as a candidate predictor of in-hospital mortality, an outcome to which its definition bears no structural relationship. A two-tailed p-value below 0.05 was taken as the threshold for statistical significance.

Ethical Considerations

Institutional ethical clearance was obtained before enrolment began. Every participant, or a legally authorised representative acting on their behalf, gave written consent to take part, and all clinical and laboratory records were anonymised before analysis to protect patient identity.

Results

Patient and Clinical Characteristics

The final cohort comprised 113 patients with a culture-confirmed Gram-negative infection, enrolled prospectively over the study period (median age 54 years, IQR 34–73; 57 male [50.4%]). Admissions spanned five wards, led by Medicine (30.1%), Surgery (22.1%), and Pulmonology (19.5%), with the intensive care unit (ICU) accounting for a further 17.7%. This was, on the whole, a sicker-than-average population: median SOFA score at enrolment was 5 (IQR 2–7) and median Charlson Comorbidity Index was 4 (IQR 2–6), with diabetes present in 37.2%, chronic kidney disease (CKD) in 16.8%, and antibiotic exposure in the preceding 90 days in 40.7%. Median hospital stay ran to 15 days (IQR 8–21), and 11 of the 113 patients (9.7%) died before discharge. Shapiro–Wilk testing confirmed that age, SOFA score, Charlson index, and length of stay all departed significantly from a normal distribution ($p<0.05$); accordingly, each is summarised as a median throughout and compared with non-parametric methods (Table 1).

Table 1. Demographic and Clinical Characteristics of the Study Cohort (N = 113)

Characteristic	Value
Age, years, median (IQR)	54 (34–73)
Male sex, n (%)	57 (50.4)
Ward of admission, n (%)	

Medicine	34 (30.1)
Surgery	25 (22.1)
Pulmonology	22 (19.5)
Intensive care unit	20 (17.7)
Nephrology	12 (10.6)
SOFA score, median (IQR)	5 (2–7)
Charlson Comorbidity Index, median (IQR)	4 (2–6)
Diabetes mellitus, n (%)	42 (37.2)
Chronic kidney disease, n (%)	19 (16.8)
Prior antibiotic exposure, n (%)	46 (40.7)
Length of hospital stay, days, median (IQR)	15 (8–21)
In-hospital mortality, n (%)	11 (9.7)

IQR, interquartile range; SOFA, Sequential Organ Failure Assessment.

Distribution of Clinical Specimens and Bacterial Isolates

Urine specimens contributed the largest share of isolates (40.7%), ahead of pus/wound swabs (20.4%), blood (15.9%), sputum (11.5%), and endotracheal aspirate (11.5%). Across the whole cohort, Escherichia coli was the leading organism (42.5%), followed at some distance by Klebsiella pneumoniae (28.3%), Pseudomonas aeruginosa (15.0%), and Acinetobacter baumannii (14.2%). The organism mix tracked specimen source closely: nearly seven in ten urine isolates (69.6%) were E. coli, while A. baumannii and P. aeruginosa clustered instead in respiratory specimens (Table 2).

Table 2. Distribution of Bacterial Isolates by Clinical Specimen Type

Specimen	A. baumannii	E. coli	K. pneumoniae	P. aeruginosa	Total
Blood	3	4	6	5	18
ETA	4	1	1	7	13
Pus/wound	5	5	10	3	23
Sputum	3	6	3	1	13
Urine	1	32	12	1	46
Total	**16**	**48**	**32**	**17**	**113**

ETA, endotracheal aspirate.

Antimicrobial Susceptibility Profile

In-vitro activity was best preserved for the two carbapenems tested meropenem (54.9% fully susceptible) and imipenem (50.4%) and eroded most for ciprofloxacin, where susceptible and resistant isolates were almost evenly split (34.5% vs 32.7%). Ceftazidime resistance was likewise substantial at 26.5% (Table 3, Figure 2).

Table 3. Antimicrobial Susceptibility Profile of All Isolates (N = 113)

Antibiotic	Susceptible, n (%)	Intermediate, n (%)	Resistant, n (%)
Meropenem	62 (54.9)	38 (33.6)	13 (11.5)

Imipenem	57 (50.4)	37 (32.7)	19 (16.8)
Ceftazidime	47 (41.6)	36 (31.9)	30 (26.5)
Ciprofloxacin	39 (34.5)	37 (32.7)	37 (32.7)

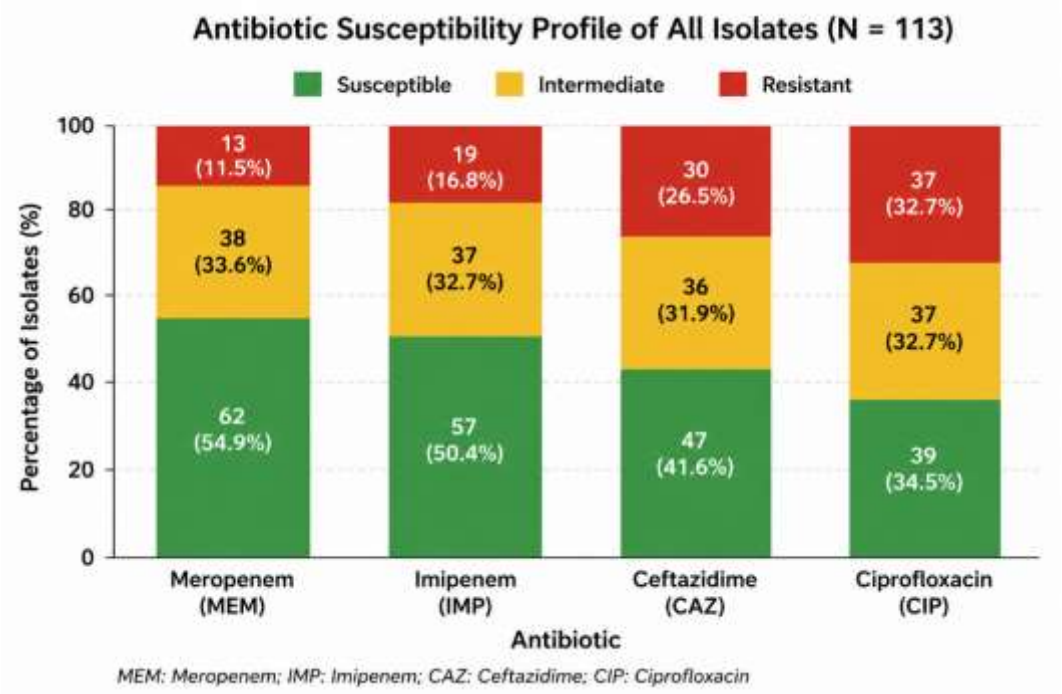

Figure 2. Antimicrobial susceptibility profile of Gram-negative isolates (N = 113), showing the descending gradient of in-vitro susceptibility from meropenem to ciprofloxacin.

Prevalence of Multidrug Resistance, ESBL Production, and Carbapenem Resistance

Because only four agents spanning three antimicrobial classes (carbapenems, a third-generation cephalosporin, and a fluoroquinolone) were tested, multidrug resistance (MDR) as defined in Section 2.2 required non-susceptibility across all three tested classes; applying this rule strictly, 38.1% of isolates (43/113) met MDR criteria. ESBL production, determined independently of the ceftazidime disk result (Section 2.2), was present in 31.0% of isolates, and 22.1% (25/113) were phenotypically carbapenem-resistant. MDR prevalence ranged from 27.3% to 60.0% across wards, highest in the ICU, but this variation did not reach statistical significance (χ^2=5.44, df=4, p=0.245). Carbapenem resistance did differ significantly by organism (χ^2=15.56, df=3, p=0.001), reaching 56.3% in A. baumannii against just 6.3% in K. pneumoniae, while ESBL positivity varied significantly by specimen source (χ^2=9.92, df=4, p=0.042) and was most frequent among urine isolates (41.3%). On univariable comparison, diabetes showed no association with carbapenem resistance (p=0.300), whereas CKD did (p=0.033), foreshadowing its role as an independent predictor in the multivariable model reported in Section 3.8.

Molecular Characterization of Carbapenemase Genes

Multiplex PCR identified at least one carbapenemase gene in 27 of the 113 isolates (23.9%). blaKPC was the single most frequent finding, present in 37.0% of PCR-positive isolates (8.8% of the whole cohort), ahead of blaOXA-48 (29.6% of positives; 7.1% overall), blaNDM (22.2%; 5.3% overall), and blaVIM (11.1%; 2.7% overall) (Table 4, Figure 3).

Table 4. Distribution of Carbapenemase Genes Among PCR-Positive Isolates (n = 27)

Gene	n	% of PCR-positive isolates	% of total cohort (N=113)
blaKPC	10	37.0	8.8
blaOXA-48	8	29.6	7.1
blaNDM	6	22.2	5.3
blaVIM	3	11.1	2.7
Total	**27**	**100.0**	**23.9**

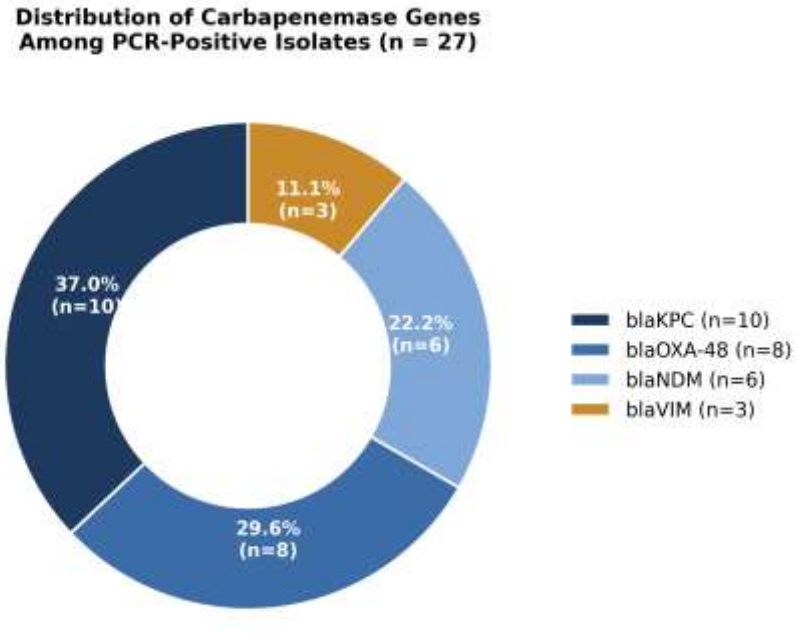

Figure 3. Proportional distribution of carbapenemase genes among the 27 PCR-positive isolates.

Two isolates one blaVIM-positive E. coli and one blaOXA-48-positive K. pneumoniae carried a carbapenemase gene but remained phenotypically susceptible to both meropenem and imipenem, a genotype–phenotype discordance consistent with the well-documented variable expression of these two determinants. Conversely, PCR was negative in all 86 isolates that were also carbapenem-susceptible, so that all 25 phenotypically carbapenem-resistant isolates were PCR-positive; the reverse was not quite true, giving an overall genotype–phenotype concordance of 111/113 isolates (98.2%).

Diagnostic Performance of Phenotypic Tests Against PCR

Benchmarked against PCR, mCIM classified 106 of 112 evaluable isolates correctly (one indeterminate result was excluded), for an overall accuracy of 94.6%, sensitivity of 88.5%, and specificity of 96.5%. eCIM, performed in parallel with mCIM on 111 of the 113 isolates, was less sensitive (63.0%) but never returned a false positive across the evaluable set specificity and positive predictive value both reached 100.0% giving an overall accuracy of 91.0%. Concordance with PCR sat at the "almost perfect" threshold for mCIM (κ=0.850) and "substantial" for eCIM (κ=0.720). In practical terms, mCIM was the stronger stand-alone screen by virtue of its sensitivity, while eCIM's flawless specificity is consistent with its established role as a confirmatory test for metallo-β-lactamase activity (Table 5, Figure 4).

Table 5. Diagnostic Performance and Agreement of mCIM and eCIM Versus PCR

Test	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)	Cohen's κ	Agreement
mCIM	88.5% (71.0–96.0)	96.5% (90.2–98.8)	88.5% (71.0–96.0)	96.5% (90.2–98.8)	94.6% (88.8–97.5)	0.850	Almost perfect
eCIM	63.0% (44.2–78.5)	100.0% (95.6–100.0)	100.0% (81.6–100.0)	89.4% (81.5–94.1)	91.0% (84.2–95.0)	0.720	Substantial

PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval. Reference standard: PCR. eCIM evaluable n=111 (2 isolates lacked an interpretable result).

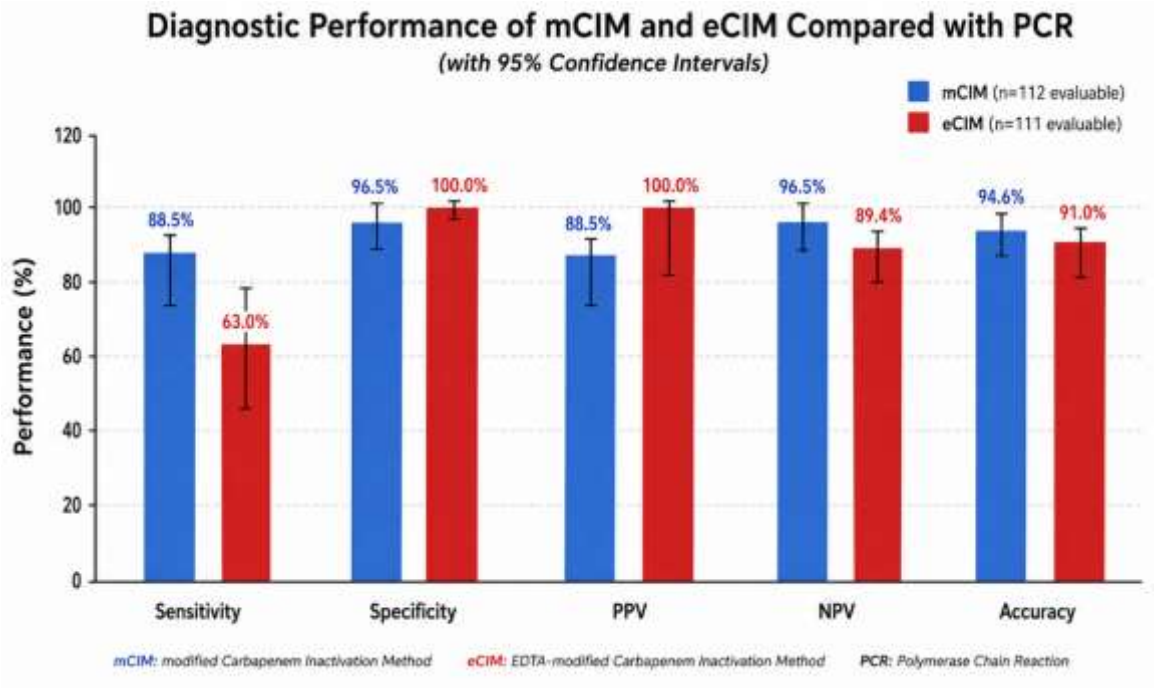

Figure 4. Diagnostic performance of mCIM and eCIM against the PCR reference standard, with 95% confidence intervals.

Turnaround Time and Impact on Antimicrobial Stewardship

PCR beat conventional culture on speed by a wide margin: a median of 23 hours (IQR 14–30) versus 40 hours (IQR 32–50), a 19-hour advantage that was highly significant (Wilcoxon signed-rank $p<0.001$). Empirical regimens were revised in 70.4% of PCR-positive patients but in none of the PCR-negative group (Fisher's exact $p<0.001$), and a near-identical pattern held for phenotypic carbapenem resistance (72.0% revised among resistant patients vs 1.1% among susceptible patients, $p<0.001$). Among the 25 patients whose isolate proved carbapenem-resistant, 36.0% had started on empirical meropenem that their resistance profile subsequently rendered ineffective (Table 6, Figure 6).

Table 6. Turnaround Time and Antimicrobial Regimen Modification by Test Result

Metric	Value
Culture turnaround time, hours, median (IQR)	40 (32–50)

PCR turnaround time, hours, median (IQR)	23 (14–30)
Median time saved with PCR vs culture, hours (p-value)	19 (p<0.001)
Regimen changed, PCR-positive patients, n (%)	19/27 (70.4)
Regimen changed, PCR-negative patients, n (%)	0/86 (0.0)
Regimen changed, CarbR-positive patients, n (%)	18/25 (72.0)
Regimen changed, CarbR-negative patients, n (%)	1/88 (1.1)

CarbR, phenotypic carbapenem resistance; IQR, interquartile range.

Risk Factors Associated with Carbapenem Resistance

Patients carrying a carbapenem-resistant isolate were numerically older on simple comparison (median 59 vs 52 years), though this gap did not reach significance (p=0.472). Because MDR status is structurally entangled with carbapenem non-susceptibility in this dataset (Section 2.5), it was excluded a priori from this model; the pre-specified predictor set instead comprised age, CKD, prior antibiotic exposure, and organism identity (E. coli as reference). After adjustment, two variables remained independently associated with carbapenem resistance: chronic kidney disease (aOR 4.44, 95% CI 1.29–15.30, p=0.018) and infection with A. baumannii relative to E. coli (aOR 4.73, 95% CI 1.34–16.73, p=0.016). Age, prior antibiotic exposure, and infection with K. pneumoniae or P. aeruginosa were not independently associated with carbapenem resistance after adjustment (Table 7, Figure 5).

Table 7. Multivariable Logistic Regression: Independent Predictors of Carbapenem Resistance and In-Hospital Mortality

Predictor of carbapenem resistance	Adjusted OR (95% CI)	p-value
Age (per year)	1.01 (0.99–1.04)	0.369
Chronic kidney disease	**4.44 (1.29–15.30)**	**0.018**
Prior antibiotic exposure	0.71 (0.25–1.99)	0.513
A. baumannii (vs E. coli)	**4.73 (1.34–16.73)**	**0.016**
K. pneumoniae (vs E. coli)	0.21 (0.04–1.10)	0.064
P. aeruginosa (vs E. coli)	1.21 (0.30–4.93)	0.787
Predictor of in-hospital mortality	**Adjusted OR (95% CI)**	**p-value**
Age (per year)	**1.04 (1.01–1.08)**	**0.018**
MDR status	**6.67 (1.56–28.50)**	**0.010**

OR, odds ratio; CI, confidence interval; MDR, multidrug resistance (retained in the mortality model only, as this outcome bears no structural relationship to the MDR definition; see Section 2.5). The mortality model should be interpreted cautiously given only 11 deaths.

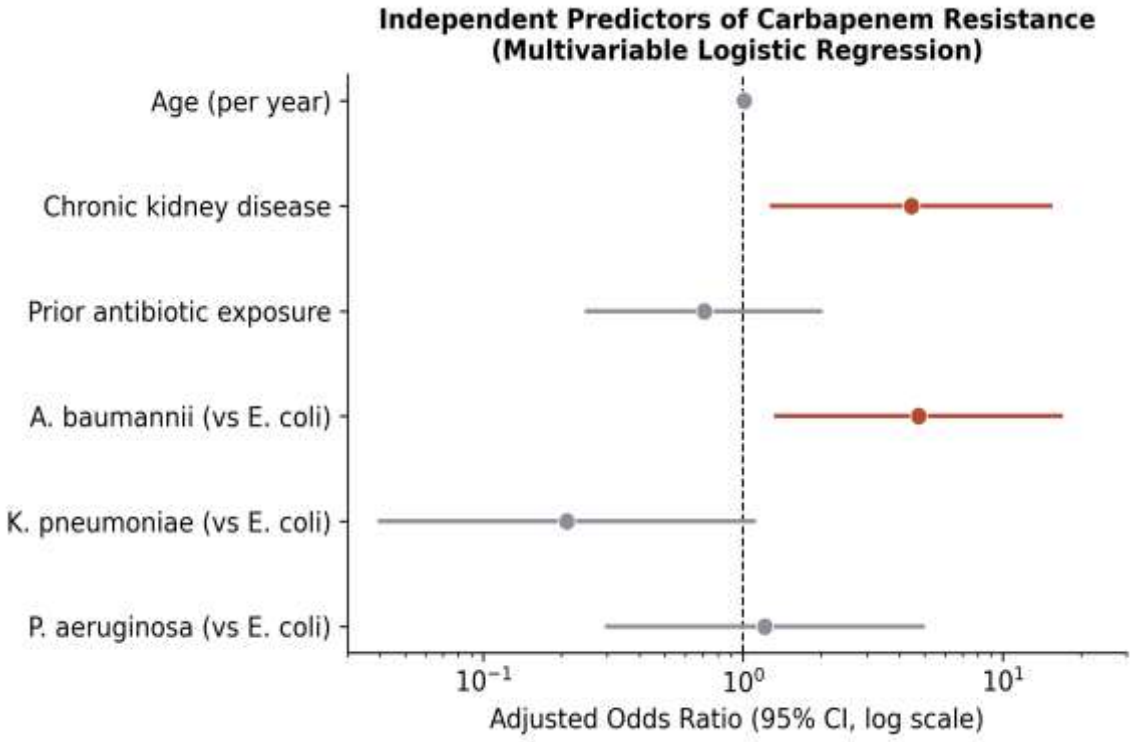

Figure 5. Forest plot of adjusted odds ratios for independent predictors of carbapenem resistance. Chronic kidney disease and A. baumannii (versus E. coli) were the only predictors whose 95% CI excluded 1 (shown in red).

Clinical Outcomes According to Resistance Status

Illness severity at enrolment did not distinguish survivors from non-survivors: SOFA scores were statistically indistinguishable between the two groups (p=0.428). Resistance status did, however: mortality reached 28.0% among patients with a carbapenem-resistant isolate versus 4.5% among those without (p=0.002), and 18.6% among patients with an MDR isolate versus 4.3% among those without (p=0.020). Length of stay told a different story it did not differ meaningfully by carbapenem-resistance status (median 16 vs 14 days, p=0.777) pointing to mortality, rather than duration of admission, as the outcome most tightly bound to resistance in this cohort (Table 8, Figure 6B).

Table 8. In-Hospital Mortality and Length of Stay According to Resistance Status

Resistance status	Expired, n (%)	Recovered, n (%)	LOS, days, median (IQR)	p-value
CarbR-negative (n=88)	4 (4.5)	84 (95.5)	14 (9–21)	0.002*
CarbR-positive (n=25)	7 (28.0)	18 (72.0)	16 (8–22)	
MDR-negative (n=70)	3 (4.3)	67 (95.7)	15 (10–20)	0.020*
MDR-positive (n=43)	8 (18.6)	35 (81.4)	15 (7–23)	

**Fisher's exact test comparing mortality between resistance groups. CarbR, phenotypic carbapenem resistance; LOS, length of stay; IQR, interquartile range.*

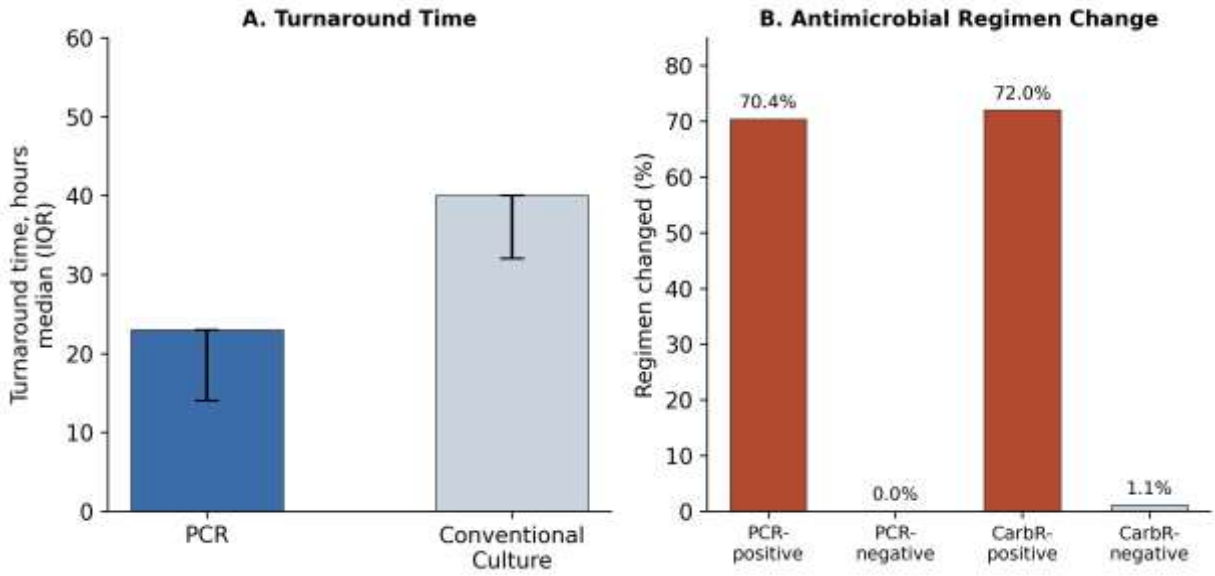

Figure 6. (A) Median turnaround time for PCR versus conventional culture. (B) Proportion of patients with a modified antimicrobial regimen, stratified by PCR and phenotypic carbapenem-resistance (CarbR) status.

Discussion

This prospective, STARD-compliant study of 113 hospitalised patients set out to test how well two phenotypic assays, mCIM and eCIM, stand up against molecular PCR as tools for detecting carbapenemase-producing Gram-negative bacteria, and whether the speed of that detection actually changes what clinicians prescribe. PCR confirmed a carbapenemase gene in roughly one isolate in four, blaKPC being the commonest finding. Against that molecular benchmark, mCIM performed as a strong, sensitive screen; eCIM, while less sensitive, was essentially flawless on specificity exactly what its role as a confirmatory adjunct requires. Faster molecular results were associated with changed prescribing on the wards, and carbapenem resistance itself carried an independent association with chronic kidney disease and with A. baumannii infection, alongside a markedly higher risk of death, placing this hospital's experience squarely within the wider regional and global AMR picture described at the outset.[1,2]

How mCIM and eCIM Performed

The sensitivity and specificity we recorded for mCIM — 88.5% and 96.5% sit comfortably inside the range reported by recent South and Southeast Asian validation studies, most of which have put mCIM sensitivity above 90% with specificity close to 100%.[14,15] Our figure came in slightly lower, and a plausible explanation lies in the isolate mix: unlike cohorts restricted to Enterobacterales, ours included a meaningful share of non-fermenters (P. aeruginosa, A. baumannii), against which mCIM is known to behave somewhat less predictably, and the modest size of our PCR-positive subgroup (n=27) widens every confidence interval built on it. For context, cartridge-based molecular assays such as Xpert Carba-R have reported 95–98% sensitivity and specificity with results in under an hour a performance ceiling that phenotypic testing does not match, but which most laboratories in settings like ours simply cannot afford to chase.[16,17] eCIM's lower sensitivity (63.0%) is not really a weakness so much as a reflection of what it is built to do: distinguish metallo- from serine-carbapenemase activity, not screen independently for carbapenemase production in the first place. Its perfect specificity and positive predictive value in our data are exactly what that narrower job should produce. Read together, these findings support running both tests in parallel on carbapenem-non-susceptible isolates mCIM

as the more sensitive general screen, eCIM as the specificity-anchored adjunct for metallo-β-lactamase discrimination an approach well suited to laboratories without routine molecular capacity.

What the Gene Distribution Tells Us

blaKPC accounted for 37.0% of our PCR-positive isolates, which is a departure from the pattern usually reported in Pakistan, where blaNDM has tended to dominate sometimes accounting for 40–75% of carbapenem-resistant Enterobacterales and E. coli in other tertiary-hospital series.[6,7] It is worth noting that a comparable shift toward KPC has been documented elsewhere: multi-year Chinese surveillance recorded a significant year-on-year climb in KPC prevalence until it overtook other mechanisms as the leading cause of carbapenem resistance in K. pneumoniae, which suggests that KPC expansion is not locked to regions with a long KPC history and can instead reflect a clonal introduction event followed by local amplification.[21] Several explanations could account for our own KPC-heavy result institution-specific clonal spread, a case-mix skewed toward non-fermenters, or simply a genuine shift in circulating plasmids since earlier Pakistani reports were published but distinguishing between them would require whole-genome or plasmid-level sequencing that fell outside this study's scope. Two isolates in our cohort carried a carbapenemase gene (blaVIM, blaOXA-48) without a resistant phenotype, a reminder that genotype and phenotype are related but not interchangeable measures, and that gene detection alone cannot substitute for phenotypic susceptibility testing in guiding therapy.

Susceptibility Patterns and the Wider Resistance Burden

The susceptibility gradient we observed carbapenems holding up best, ciprofloxacin faring worst fits a pattern seen across South Asian hospitals and echoed in WHO GLASS data, where fluoroquinolone and cephalosporin consumption correlates measurably with rising resistance among bloodstream Enterobacterales, and it is consistent with the continued critical-priority status the WHO assigns to carbapenem-resistant Enterobacterales and A. baumannii.[1,4] A little over a third of isolates in our cohort met a strict, three-class MDR definition; prevalence was numerically highest in the ICU (60.0%) but this did not reach statistical significance across wards ($p=0.245$), so we are cautious about reading a strong ward-level clustering effect into this cohort. A larger, multicentre sample would be needed to establish whether ICU-associated MDR clustering is a real phenomenon here or simply this study's small ICU subgroup (n=20).

What This Means for Antimicrobial Stewardship

If there is one finding from this study with immediate clinical relevance, it is the scale of the prescribing response associated with molecular positivity: over 70% of PCR-positive patients had their regimen changed, against essentially none of the PCR-negative group, and the 19-hour speed advantage PCR held over culture coincided with earlier decisions at the bedside. We want to be precise about what this design can and cannot show: because PCR positivity itself carries clinically actionable information (which carbapenemase gene, if any, is present) independent of how quickly that information arrived, our data demonstrate an association between rapid PCR availability and regimen modification, not a proven causal effect of speed alone that would require a randomised comparison of rapid versus standard-turnaround diagnostics, which this study did not perform. With that caveat, the pattern is consistent with the broader literature on early appropriate diagnostics, which links faster diagnostic availability paired with an active stewardship response to shorter time to effective therapy in MDR Gram-negative infection.[18] It also has a direct bearing on how the newer β-lactam–β-lactamase inhibitor combinations should be used: ceftazidime–avibactam and meropenem–vaborbactam do not cover metallo-β-

lactamase producers, and none of the currently available agents handles blaNDM reliably without adding aztreonam, so knowing the carbapenemase class not just that an isolate is carbapenem-resistant is a precondition for using these drugs sensibly.[9] More than a third of our carbapenem-resistant patients had started on empirical meropenem before their resistance profile came back, a concrete measure of what delayed carbapenemase detection costs in this setting.

Risk Factors and What Happened to Patients

Chronic kidney disease and infection with A. baumannii were the only variables that survived multivariable adjustment as independent predictors of carbapenem resistance. We deliberately excluded MDR status from this model: because only three antimicrobial classes were tested, MDR classification in this dataset cannot occur without carbapenem non-susceptibility already being present, so including it as a predictor of carbapenem resistance would have been close to circular reasoning rather than a genuine test of an independent risk factor. With MDR removed, CKD remained a robust predictor consistent with a large multicentre case-control and cohort study of carbapenem-resistant K. pneumoniae, which found renal impairment independently predicted both infection and death, and with ICU-based case-control data pointing to comorbidity burden as a consistent acquisition risk.[10,12] The mechanistic explanation is not hard to construct: patients with impaired renal function tend to have more healthcare contact, more antibiotic courses over time, and dosing complications from altered drug clearance, all of which can tilt the balance toward resistant organisms. The independent association with A. baumannii infection matches this organism's well-documented propensity for intrinsic and acquired carbapenem resistance relative to Enterobacterales, and is corroborated by our own descriptive data, where 56.3% of A. baumannii isolates were carbapenem-resistant against just 6.3% of K. pneumoniae isolates. MDR remained a legitimate predictor in the separate mortality model, where its definition bears no structural relationship to the outcome being predicted: mortality reached 18.6% among MDR-positive patients versus 4.3% among MDR-negative patients (aOR 6.67, 95% CI 1.56–28.50, p=0.010), a magnitude broadly consistent with the 28–38% mortality range reported for carbapenem-resistant K. pneumoniae bacteraemia in comparable Asian cohorts.[11,12] Set against global attributable-mortality modelling, these findings underscore that resistance itself not just illness severity at presentation carries independent prognostic weight in this population.[2,3]

What This Study Can and Cannot Tell Us

The study's design gives it some real strengths: it was prospective, followed STARD reporting standards, used an unambiguous molecular reference standard, and tracked the link between a diagnostic result and the prescribing decision it produced for the same patient. That said, several limitations temper how far these findings can be extended. It was conducted at a single centre, so hospitals with a different case-mix, antibiotic policy, or resistance ecology may not see the same pattern. The PCR-positive (n=27) and carbapenem-resistant (n=25) subgroups were both modest, which widens the confidence intervals around several accuracy and regression estimates, and the mortality model built on only 11 deaths should be read as hypothesis-generating rather than conclusive. Our multiplex PCR panel covered four carbapenemase gene families (blaKPC, blaNDM, blaOXA-48, blaVIM) but not rarer determinants such as blaIMP, so the true molecular positivity rate may be somewhat higher than what we report. Our susceptibility panel was limited to four agents across three classes, which, as discussed above, constrained how MDR could legitimately be used analytically; a broader panel would allow a less structurally entangled MDR definition in future work. And without whole-genome sequencing or minimum inhibitory concentration testing against the newer β-lactam–β-lactamase inhibitor combinations, we cannot

speak to clonal relationships between isolates or to how they would actually respond to these agents.

Where This Leaves Practice, and What Should Come Next

Taken as a whole, these results point toward a practical, tiered diagnostic pathway for hospitals like ours: mCIM as routine first-line screening for any carbapenem-non-susceptible Gram-negative isolate, eCIM run in parallel for MBL discrimination, and confirmatory PCR wherever it can be arranged prioritised for isolates coming from patients with CKD or A. baumannii infection, given their independent association with resistance in this cohort.[9,19] The natural next steps are multicentre studies with a broader antimicrobial panel that would allow MDR to be defined independently of carbapenem susceptibility, whole-genome sequencing to clarify the drivers of the KPC-predominant gene distribution observed here, and prospective designs ideally with a randomised or historically controlled comparison of rapid versus standard-turnaround diagnostics that could establish a causal, rather than associative, link between diagnostic speed and stewardship outcomes.

Conclusion

mCIM performed as a sensitive, specific, and genuinely low-cost phenotypic surrogate for carbapenemase detection in this cohort, and run in parallel with eCIM as a confirmatory adjunct, the two together offer a workable screening pathway for laboratories without routine access to molecular platforms. Molecular PCR nonetheless remains the tool of choice for gene-level surveillance, and its faster turnaround was associated with a clear, measurable difference in prescribing behaviour. Chronic kidney disease and A. baumannii infection independently predicted carbapenem resistance once the structurally circular MDR variable was removed from that model, and carbapenem resistance in turn carried a substantially higher risk of death, consistent with both regional and global patterns of AMR-associated mortality. A diagnostic strategy that pairs rapid phenotypic screening with reflex molecular confirmation, targeted toward higher-risk patients, may offer a practical way to strengthen both diagnostic accuracy and antimicrobial stewardship in comparable resource-constrained hospitals.

Declarations

Ethics Approval and Consent to Participate

This study received clearance from the study hospital's Institutional Ethical Review Committee before any patient was enrolled. Every participant or, where necessary, a legally authorised representative acting on their behalf gave written consent in line with the principles of the Declaration of Helsinki.

Consent for Publication

Not applicable; the manuscript contains no identifying patient information or images.

Availability of Data and Materials

The dataset underlying these analyses can be obtained from the corresponding author on reasonable request.

Competing Interests

The authors have no competing interests to declare.

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Author Contributions

All authors participated in study conception and design, specimen collection, phenotypic and molecular laboratory testing, data analysis and interpretation, and drafting and critical revision of the manuscript. All authors read and approved the final version submitted for publication.

Declaration of Generative AI and AI-Assisted Technologies

During preparation of this manuscript, the authors used an AI-based language-editing tool solely to improve clarity, grammar, and readability of the text. No AI tool was used to generate, analyse, or interpret study data, or to draw scientific conclusions. All AI-assisted edits were reviewed by the authors, who take full responsibility for the accuracy and integrity of the final content.

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