Original articles

Molecular Characteristics and Antimicrobial Resistance of Carbapenem-Resistant *Klebsiella pneumoniae* Isolated at Can Tho University of Medicine and Pharmacy Hospital, 2024–2025

***Nguyen Thi Be Hai*[1,2], *Tran Dinh Binh*[1], *Ngo Viet Quynh Tram*[1*]**

[1]*Hue University of Medicine and Pharmacy, Hue University*

[2]*Can Tho University of Medicine and Pharmacy*

***Corresponding author:** Ngo Viet Quynh Tram; Email: nvqtram@huemed-univ.edu.vn

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Abstract

Background: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) poses a critical therapeutic challenge in hospitals of the Mekong Delta, Vietnam. Data on carbapenemase gene distribution and co-production of multiple genes at Can Tho remain limited.

Objectives: To describe the phenotypic antimicrobial resistance and the distribution of carbapenemase among CRKP isolates; and to determine the distribution of carbapenemase genes in mCIM-positive and indeterminate isolates by PCR, together with the relationship between carbapenemase genotype and resistance phenotype.

Methods: A cross-sectional descriptive study was conducted on 147 CRKP isolates at Can Tho University of Medicine and Pharmacy Hospital (November 2024 - July 2025). Susceptibility testing was performed per CLSI M100 2024 (colistin per EUCAST 2024). Carbapenemase detection followed a two-step approach: mCIM for phenotypic screening, D73C disk test for enzyme classification, and PCR confirmation for five genes ($bla_{\text{OXA-48-like}}$, bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}).

Results: All 147 isolates were multidrug-resistant (MDR, 100%), with extensively drug-resistant (XDR) at 56.5% and pandrug-resistant (PDR) at 0%. Colistin and tigecycline retained the highest susceptibility rates (98.6% and 98.0%, respectively). Carbapenemase production was detected in 57.1%: MBL 34.0%, OXA-48-like 19.0%, KPC 3.4%. Among 100 PCR-tested isolates, 84.0% carried at least one carbapenemase gene: bla_{NDM} predominated (60.0%), followed by $bla_{\text{OXA-48-like}}$ (47.0%), bla_{KPC} and bla_{VIM} (6.0% each); bla_{IMP} was not detected. Co-production of multiple genes was identified in 35.0%, predominantly the $bla_{\text{OXA-48-like}}$ + bla_{NDM} combination (82.9%). Agreement between D73C and PCR ranged from moderate to almost perfect: KPC (κ = 0.903), MBL (κ = 0.735), OXA-48-like (κ = 0.561), all $p < 0.001$.

Conclusion: bla_{NDM} and $bla_{\text{OXA-48-like}}$ were the predominant carbapenemase genes. The high co-production rate (35.0%) represents an important clinical warning. The sequential workflow of mCIM screening, D73C classification, and PCR confirmation demonstrated practical value for carbapenemase detection in clinical microbiology laboratories.

Keywords: *Klebsiella pneumoniae*; carbapenem resistance; carbapenemase genes; gene co-production; mCIM; D73C; PCR.

1. INTRODUCTION

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is currently a serious public health threat, classified by the World Health Organization (WHO) as a priority pathogen in 2024, with bloodstream infection–associated mortality ranging from 40 to 70% [1]. Of particular concern, the co-occurrence of multiple carbapenemase genes is increasingly documented; for example, a combined bla_{NDM} and $bla_{\text{OXA-48-like}}$ prevalence of up to 17.7% has been reported at Hue Central Hospital [2]. This co-occurrence simultaneously abrogates the activity of carbapenems and of novel β-lactam/β-lactamase inhibitor combinations, markedly narrowing therapeutic options.

Against this background, although carbapenem resistance and KPC/OXA-48-like genotypes have recently been reported at other hospitals in the region [3], molecular epidemiological data on CRKP at Can Tho University of Medicine and Pharmacy Hospital, a referral center serving patients from multiple provinces across the Mekong Delta, remain entirely lacking. Because enzymatic hydrolysis by carbapenemases is the predominant mechanism of carbapenem resistance in *K. pneumoniae*, this study focused on the genes encoding these

enzymes [4]. A stepwise carbapenemase detection workflow combining phenotypic screening by the modified Carbapenem Inactivation Method (mCIM), enzyme class differentiation by the disk-based carbapenemase detection combined test (D73C), and molecular confirmation by polymerase chain reaction (PCR) is well suited to clinical microbiology laboratories in Vietnam and directly informs therapeutic decision-making [4]. This study aimed:

1. To describtermine the phenotypic antimicrobial resistance and the distribution of carbapenemaseprevalence and distribution of carbapenemase , determined bygenes among mCIM-positive and D73C, amongindeterminate CRKP isolates at Can Tho University of Medicine and Pharmacy Hospital in 2024 - 2025 using a phenotypic screening and molecular confirmation workflow;

2. To determine the distribution of carbapenemase genes among mCIM-positivecharacterize antimicrobial susceptibility profiles, and indeterminate CRKP isolates by PCR, and to to analyze the relationshipassociations between carbapenemase genotype and resistance phenotype, including the phenomenon of multi-gene co-carriage.

2. MATERIALS AND METHODS

2.1. Study isolates

Carbapenem-resistant *K. pneumoniae* isolates recovered from clinical specimens collected at Can Tho University of Medicine and Pharmacy Hospital between November 2024 and July 2025 were included.

Inclusion criteria: Isolates identified to species level by the MicroScan Walk Away system with a meropenem minimum inhibitory concentration (MIC) > 8 µg/mL or classified as resistant (R) to at least one carbapenem per CLSI M100, 34th edition, 2024; one isolate per patient (first isolate) to ensure statistical independence; isolates stored at −80°C in 20% glycerol with complete clinical records.

Exclusion criteria: Isolates meeting inclusion criteria but failing quality control for analysis due to non-recovery, contamination, or inability to perform PCR.

2.2. Methods

Study design and sample:

A cross-sectional descriptive study with analytic components.

Sample size was calculated using the single-proportion estimation formula: $n = Z^2_{1-\alpha/2} \times p(1-p)/d^2$, where $Z_{1-\alpha/2} = 1.96$ (95% confidence level); p = 0.90, the proportion of carbapenemase producers among CRKP isolates reported in a previous study at Can Tho General Hospital [5], selected because that setting shares the same regional patient population and laboratory context as the present study; d = 0.05. The minimum required sample size was 139; all 147 CRKP isolates that met the inclusion criteria during the study period were enrolled.

All 147 isolates were assessed for the first objective (antimicrobial susceptibility and mCIM/D73C phenotypic characterization), whereas the second objective (molecular confirmation of carbapenemase genes by PCR) was performed on the mCIM-positive and indeterminate isolates (n = 100).

Study variables:

The study variables fell into three groups. Demographic characteristics were age and sex. Clinical characteristics were specimen types, wards of origin, infection origin (hospital- vs. community-acquired infection), length of stay before specimen collection, and underlying conditions. Microbiological characteristics were antimicrobial susceptibility and MIC results, ESBL production, mCIM and D73C results, detected carbapenemase genes, multi-gene co-carriage, and resistance phenotype, classified per Magiorakos et al. (2012) [8] as MDR (non-susceptibility to ≥ 1 agent in ≥ 3 antimicrobial categories), XDR (non-susceptibility to all but one or two categories), or PDR (non-susceptibility to all categories tested).

Bacterial identification and antimicrobial susceptibility testing:

Species identification and MIC determination were performed simultaneously on the MicroScan Walk Away automated system (Beckman Coulter, USA) and interpreted per CLSI M100, 34th edition, 2024 [6]; colistin MICs were interpreted per EUCAST 2024 [7]. Resistance categories (MDR, XDR, and PDR) were classified according to international expert criteria [8].

Phenotypic carbapenemase detection:

The mCIM was performed on all isolates per CLSI M100. A zone diameter of 6 - 15 mm was interpreted as positive, 16 - 18 mm as indeterminate, and ≥ 19 mm as negative [6]. mCIM-negative isolates were classified as non-carbapenemase-producing K. pneumoniae (non-CPKP). Isolates with positive or indeterminate mCIM results were further tested with the MASTDISCS Combi Carba Plus (D73C) disk panel (Mast Group Ltd., UK) to differentiate the main carbapenemase classes: metallo-β-lactamase (MBL: NDM, VIM, IMP), KPC, and OXA-48-like, including

isolates with co-production of multiple mechanisms [9]. Agreement between D73C and PCR was assessed using Cohen's kappa coefficient (κ).

Molecular confirmation by PCR:

PCR was performed on all mCIM-positive and indeterminate isolates. Total DNA was extracted by the ethanol precipitation method (Vazyme, China) and quality-assessed by NanoDrop spectrophotometry (Thermo Fisher Scientific, USA). Five carbapenemase genes were independently amplified by PCR; primer sequences and amplicon sizes are presented in Table 1 (primers synthesized by ZESBIO Biotechnology Co., Ltd., Vietnam). Amplicons were resolved by electrophoresis on 2% agarose gel and visualized on a Gel Doc XR system (Bio-Rad, USA) using a 100-bp DNA ladder (Bio-Helix, Taiwan). Isolates positive for two or more genes were confirmed as carrying multiple carbapenemase genes (co-carriage).

Table 1. Primer sequences and PCR parameters for carbapenemase gene detection [10, 11, 12]

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Amplicon size (bp)	Annealing Tm
bla_{KPC}	TGTCACTGTATCGCCGTC	CTCAGTGCTCTACAGAAAACC	798	58°C
$bla_{OXA-48-like}$	GCGTGGTTAAGGATGAACAC	CATCAAGTTCAACCCAACCG	438	56°C
bla_{IMP}	GAAGGCGTTTATTGTTCATAC	GTACGTTTCAAGAGTGATGC	587	55°C
bla_{NDM}	GCAGCTTGTCGGCCATGCGGGC	GGTCGCGAAGCTGAGCACCGCAT	621	58°C
bla_{VIM}	TTATGGAGCAGCAACCGATGT	CAAAAGTCCCGCTCCAACGA	920	56°C

2.3. Statistical analysis

Data were analyzed using SPSS 22.0 (IBM Corp., USA). Proportions were compared by chi-square test or Fisher's exact test; MIC distributions were compared by the Kruskal-Wallis test. Inter-method agreement was assessed using Cohen's kappa (κ) interpreted per Landis and Koch criteria. Statistical significance was set at $p < 0.05$.

2.4. Ethical approval

The study was approved by the Institutional Review Board of Hue University of Medicine and Pharmacy under Decision No. H2024/601, dated 12 November 2024.

3. RESULTS

3.1. General characteristics of the study population

A total of 147 CRKP isolates meeting the inclusion criteria were collected between November 2024 and July 2025. Isolates were predominantly recovered from elderly patients (median age 66 years). The sex distribution was balanced (male 49.7%, female 50.3%). Sputum was the most common specimen type (78.9%), followed by urine (10.2%) and body fluids (4.1%). More than half of the isolates originated from patients in the intensive care unit (ICU, 53.1%). Hospital-acquired infections accounted for 59.2% versus 40.8% community-acquired. The median pre-isolation length of stay was 8 days. The most frequent underlying conditions were chronic obstructive pulmonary disease (3.4%), diabetes mellitus (2.7%), and malignancy (1.4%).

3.2. Antimicrobial resistance and phenotypic carbapenemase distribution

Table 2. Antimicrobial resistance rates among 147 CRKP isolates

Antimicrobial class	Agent/Parameter	No. resistant (n)	Rate (%)
Cephalosporins	Cefazolin	130	88.4
	Cefoxitin	117	79.6
	Cefepime	117	79.6
	Ceftazidime	122	83.0
Carbapenems	Meropenem	111	75.5
	Imipenem	123	83.7
	Doripenem	107	72.8
Polymyxins	Colistin	2	1.4
Glycylcyclines	Tigecycline	3	2.0

Aminoglycosides	Amikacin	76	51.7
	Gentamicin	104	70.7
	Tobramycin	109	74.1
Fluoroquinolones	Ciprofloxacin	119	81.0
	Levofloxacin	120	81.6
Monobactams	Aztreonam	126	85.7
Sulfonamides/Trimethoprim	Trimethoprim/ Sulfamethoxazole	117	79.6
β-lactam/β-lactamase inhibitor combinations	Ampicillin/sulbactam	144	98.0
	Ticarcillin/clavulanic acid	115	78.2
	Piperacillin/tazobactam	109	74.1
	Ceftazidime/avibactam	71	48.3
Resistance phenotype	ESBL production	88	59.9
	MDR	147	100
	XDR	83	56.5

Among the 147 CRKP isolates, resistance rates to cephalosporins and aztreonam were very high (79.6 - 88.4%), followed by carbapenems (72.8 - 83.7%), fluoroquinolones (81.0–81.6%), and aminoglycosides (51.7 - 74.1%). Colistin and tigecycline retained the highest susceptibility rates (98.6% and 98.0%, respectively). Resistance phenotypes: MDR 100% and XDR 56.5%. ESBL production was detected in 59.9% of isolates. Ampicillin/sulbactam showed the highest resistance rate (98.0%), whereas ceftazidime/avibactam resistance was lower (48.3%).

Phenotypic carbapenemase detection by mCIM and D73C

Table 3. Carbapenemase detection and classification by mCIM and D73C (n = 147)

mCIM		**D73C**	
Classification	**N (%)**	**Carbapenemase phenotype**	**N (%)**
CPKP (Carbapenemase-producing *K. pneumoniae*) mCIM-positive	**84 (57.1)**	MBL (NDM/VIM/IMP)	50 (34.0)
		OXA-48-like	28 (19.0)
		KPC	5 (3.4)
		Unclassified	1 (0.7)
Non-CPKP (Non-carbapenemase-producing *K. pneumoniae*)	**63 (42.9)**	**Negative**	**63 (42.9)**
mCIM-negative	47 (32.0)	Negative	47 (32.0)
Presumed non-enzymatic mechanism	16 (10.9)	Negative	16 (10.9)
Total	**147 (100)**	**Total**	**147 (100)**

Based on mCIM screening of all 147 isolates and D73C confirmation, 84 isolates (57.1%) were confirmed as CPKP and 63 (42.9%) were classified as non-CPKP. Among CPKP, MBL (NDM/VIM/IMP) was the predominant class (50 isolates, 34.0%), followed by OXA-48-like (28 isolates, 19.0%) and KPC (5 isolates, 3.4%); one isolate (0.7%) could not be classified by D73C. Among non-CPKP, 47 isolates (32.0%) were classified directly on the basis of a negative mCIM result (inhibition zone ≥19 mm), and 16 isolates (10.9%) had a negative D73C result despite a positive or indeterminate mCIM, suggestive of non-enzymatic carbapenem resistance. Because confirmatory tests for these mechanisms were not performed, the underlying basis which may include porin loss (OmpK35/36), ESBL or AmpC β-lactamase overproduction, or other mechanisms could not be determined.

3.3. Carbapenemase gene distribution and genotype–phenotype relationship

Table 4. Distribution of carbapenemase genes by PCR (n = 100 isolates)

Gene / Parameter	No. of isolates (n)	Prevalence (%)
$bla_{\text{OXA-48-like}}$	47	47.0
bla_{NDM}	60	60.0
bla_{KPC}	6	6.0
bla_{IMP}	0	0.0
bla_{VIM}	6	6.0
PCR result		
Carried ≥1 carbapenemase gene	84	84.0
No gene detected	16	16.0

Note: Prevalences are calculated among 100 PCR-tested isolates (including mCIM-positive and indeterminate isolates).

Among 100 PCR-tested isolates, 35 (35.0%) were positive for ≥2 carbapenemase genes (co-carriage). The most frequent combination was $bla_{\text{OXA-48-like}}$ + bla_{NDM} (29 isolates, 82.9% of co-carriers), followed by bla_{NDM} + bla_{VIM} (5 isolates, 14.3%) and bla_{NDM} + bla_{KPC} (1 isolate, 2.9%) (Tables 4 and 5). Overall, bla_{NDM} was the most prevalent gene (60 isolates, 60.0%), followed by $bla_{\text{OXA-48-like}}$ (47 isolates, 47.0%); bla_{KPC} and bla_{VIM} were each detected in 6 isolates (6.0%); bla_{IMP} was not detected. Amplicon sizes on agarose gel electrophoresis were consistent with target gene sizes: $bla_{\text{OXA-48-like}}$ ~438 bp, bla_{KPC} ~798 bp, bla_{NDM} ~621 bp, bla_{IMP} ~587 bp, bla_{VIM} ~920 bp (Figure 1).

Table 5. Co-carriage patterns of carbapenemase genes (n = 35 isolates)

Gene combination	No. of isolates (n)	Prevalence* (%)
$bla_{\text{OXA-48-like}}$ + bla_{NDM}	29	82.9
bla_{NDM} + bla_{KPC}	1	2.9
bla_{NDM} + bla_{VIM}	5	14.3
Total co-carrying isolates	**35**	**100.0**

*Prevalence calculated among the 35 co-carrying isolates. Genes detected by PCR.

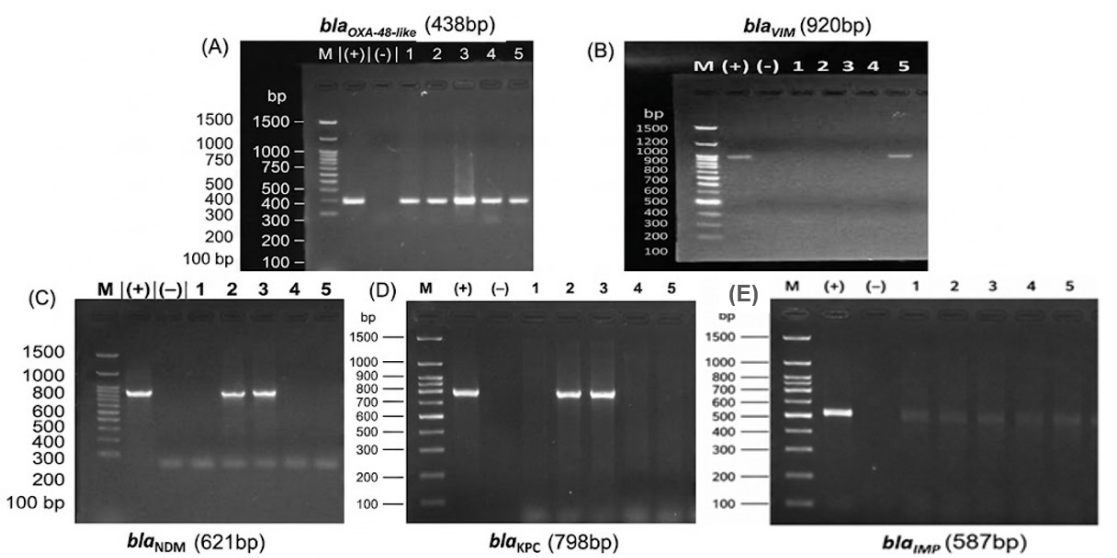

Figure 1. Representative agarose gel electrophoresis of PCR products for each carbapenemase gene

Figure 1 legend: M: 100-bp DNA ladder; (+): positive control; (−): negative control; panels A, B, C, D, and E represent the $bla_{\text{OXA-48-like}}$, bla_{VIM}, bla_{NDM}, bla_{KPC}, and bla_{IMP} genes, respectively.

Association between carbapenemase genotype and resistance phenotype

Table 6. Agreement between D73C disk test and PCR

Carbapenemase class	Comparator	Kappa (κ)	95% CI	p
MBL (NDM/VIM/IMP)	D73C vs PCR positive	0.735	0.586 - 0.884	< 0.001
OXA-48-like	D73C vs PCR positive	0.561	0.406 - 0.716	< 0.001
KPC	D73C vs PCR positive	0.903	0.715 - 1.000	< 0.001

κ: Cohen's kappa coefficient. Interpreted per Landis & Koch (1977): 0.41–0.60: moderate; 0.61–0.80: substantial; 0.81 - 1.00: almost perfect agreement.

Cohen's kappa coefficients demonstrated agreement between D73C and PCR ranging from moderate to almost perfect across the three carbapenemase classes: KPC (κ = 0.903, almost perfect), MBL (κ = 0.735, substantial), and OXA-48-like (κ = 0.561, moderate), all p <0.001 (Table 6). Kruskal-Wallis testing revealed statistically significant differences in meropenem MIC distributions across carbapenemase classes (p < 0.001); all groups exhibited a median MIC > 16 µg/mL (Table 7).

Table 7. Meropenem MIC by carbapenemase class

Carbapenemase class	Meropenem MIC, median (IQR) (µg/mL)
MBL (NDM/VIM/IMP)	> 16
NDM + OXA-48-like	> 16
OXA-48-like alone	> 16 (4 –> 16)
KPC	> 16

MIC: minimum inhibitory concentration, expressed as median (IQR: interquartile range).

4. DISCUSSION

4.1. General characteristics of the study population

The 147 CRKP isolates were recovered predominantly from elderly patients (median age 66 years), consistent with the greater burden of comorbidity, prior antibiotic exposure, and healthcare contact in this age group. Sputum was the most common specimen (78.9%), and more than half of the isolates originated from the intensive care unit (53.1%), reflecting the concentration of invasive procedures, mechanical ventilation, and broad-spectrum antibiotic use in critical care settings that favor the selection and transmission of carbapenem-resistant organisms [23]. Hospital-acquired infections accounted for the majority of cases (59.2%), and the median pre-isolation length of stay of 8 days is in keeping with the nosocomial acquisition of CRKP at a tertiary referral center serving the Mekong Delta.

4.2. Antimicrobial resistance and phenotypic carbapenemase distribution

All 147 CRKP isolates were MDR (100%), with XDR accounting for 56.5% and PDR for 0%, reflecting intense antimicrobial selection pressure at this tertiary referral center. These proportions are consistent with reports from Military Hospital 103, where XDR ranged from 57.80 to 71.22% and PDR from 5.05 to 24.27% [13]. The widespread dissemination of XDR strains represents a major challenge for empiric antibiotic selection and underscores the need for hospital-wide antimicrobial stewardship and rigorous epidemiological surveillance.

Colistin and tigecycline remained the most active agents, with susceptibility rates of 98.6% and 98.0%, respectively, consistent with data from Ho Chi Minh City [14]. Colistin resistance in *K. pneumoniae* remains uncommon and is most often mediated by chromosomal mgrB inactivation [15].

ESBL production was detected in 59.9% of isolates, a higher rate than previously reported in comparable studies [2]. This is expected given that the study population was pre-selected for carbapenem resistance; CRKP strains commonly harbor multiple co-existing resistance mechanisms, with ESBL production as a common background preceding further evolution toward carbapenemase acquisition [16].

Diagnostic value of the mCIM assay for carbapenemase screening

The mCIM identified 87 positive isolates (59.2%) and 13 indeterminate isolates (8.8%), totaling 100 isolates (68.0%) requiring further confirmation. The mCIM is endorsed by both the CDC and CLSI as a simple, low-cost phenotypic assay requiring no specialized equipment, well suited to clinical microbiology laboratories in Vietnam [17]. Pierce

et al. (2017) demonstrated that the mCIM achieves 97% sensitivity and 100% specificity compared with molecular reference methods [18].

In this study, 47 mCIM-negative isolates (32.0%) were classified as non-CPKP-carbapenem-resistant but not through an enzymatic mechanism. These isolates may achieve resistance via non-enzymatic mechanisms; because such mechanisms were not directly tested in this study, their basis cannot be confirmed. Possible contributors recognized in Vietnam and Southeast Asia include OmpK35/36 porin loss and ESBL or AmpC β-lactamase expression, among others [2]. Further characterization of this subgroup is warranted.

Indeterminate mCIM results (8.8%) reflect the biological reality that carbapenemases such as OXA-48-like possess weak carbapenem-hydrolyzing activity insufficient to reduce the meropenem zone diameter below the positive threshold [19]. This is the rationale for forwarding all indeterminate cases to D73C and PCR confirmation to avoid missing CPKP isolates with low-level enzymatic activity.

4.3. Carbapenemase gene distribution and genotype–phenotype relationship

PCR among 100 isolates revealed 84 (84.0%) carrying at least one carbapenemase gene. bla_{NDM} was the most prevalent (60.0%), followed by $bla_{OXA\text{-}48\text{-}like}$ (47.0%), while bla_{KPC} and bla_{VIM} each accounted for 6.0%; bla_{IMP} was not detected.

The predominance of bla_{NDM} is consistent with regional trends in Southeast Asia and recent reports from southern Vietnam [20]. The high prevalence of $bla_{OXA\text{-}48\text{-}like}$ (47.0%) reflects co-circulation of two major carbapenemase classes, posing distinct treatment challenges given their differential activity profiles against novel β-lactam/β-lactamase inhibitor combinations, particularly ceftazidime/avibactam. The lower bla_{KPC} prevalence (6.0%) contrasts with some reports from major hospitals in southern Vietnam, suggesting that carbapenemase gene distribution may vary according to local epidemiological factors, as also observed at other regional centers in Vietnam [3, 21].

The 16.0% of isolates with positive mCIM but undetected carbapenemase genes by PCR suggests a role for non-enzymatic resistance mechanisms such as porin loss combined with ESBL overexpression, or carbapenemase genes outside the scope of the five targets tested [2].

Multi-gene carbapenemase co-carriage

This study identified 35 of 100 isolates (35.0%) carrying two or more carbapenemase genes, substantially exceeding rates reported from other Vietnamese studies (5 - 15%) [2, 20]. The $bla_{OXA\text{-}48\text{-}like}$ + bla_{NDM} combination was predominant (82.9%), followed by bla_{NDM} + bla_{VIM} (14.3%) and bla_{NDM} + bla_{KPC} (2.9%).

Mechanistically, co-carriage arises from the co-existence of multiple plasmids within a single bacterial cell. The simultaneous presence of serine carbapenemases (OXA-48-like, KPC) and metallo-β-lactamases (NDM, VIM) simultaneously inactivates carbapenems and avibactam-containing β-lactam/β-lactamase inhibitor combinations, critically narrowing therapeutic options [22].

Prolonged antibiotic exposure may drive the accumulation of multiple resistance genes [23], underscoring the importance of rational antimicrobial stewardship and infection prevention and control programs.

Agreement between D73C and PCR

Cohen's kappa between D73C and PCR indicated moderate to almost perfect agreement: KPC (κ = 0.903), MBL (κ = 0.735), OXA-48-like (κ = 0.561), all $p < 0.001$. These results are comparable to those reported by Nordmann et al. (2012), who documented sensitivities of 90–100% and specificities of 95–100% [24].

KPC showed almost perfect agreement, reflecting its high enzymatic activity and clear phenotypic expression. MBL agreement was substantial. OXA-48-like had the lowest κ (0.561), corresponding to only moderate agreement per Landis & Koch criteria [25], likely because of its weak hydrolytic activity leading to ambiguous D73C results.

Notably, 16 isolates (10.9%) were mCIM-positive or indeterminate but D73C-negative and were likewise negative for all five carbapenemase genes by PCR, consistent with their classification as presumed non-enzymatic resistance (Tables 3 and 4). D73C nevertheless showed its weakest agreement for OXA-48-like (κ = 0.561), whose low hydrolytic activity can yield ambiguous results, underscoring the value of PCR confirmation for indeterminate cases [22]. The stepwise workflow of mCIM screening, followed by D73C classification and PCR confirmation, is therefore both rational and necessary to balance turnaround time with diagnostic accuracy.

Meropenem MIC by carbapenemase genotype

All carbapenemase classes exhibited a median meropenem MIC > 16 μg/mL, consistent with high-level carbapenem resistance. These findings are consistent with the comparatively weak carbapenem-hydrolyzing activity of OXA-48-like β-lactamases relative to metallo-β-lactamases such as NDM [19]. This difference carries clinical implications:

isolates carrying OXA-48-like with relatively lower MICs may benefit from prolonged meropenem infusion strategies to optimize pharmacokinetic/pharmacodynamic (PK/PD) target attainment [26].

Limitations

The single-center design limits generalizability of findings to the broader Mekong Delta region. Isolates with positive mCIM but no detectable carbapenemase gene by PCR were not further characterized for non-enzymatic resistance mechanisms.

5. CONCLUSION

All 147 study isolates were MDR (100%), with XDR and PDR rates of 56.5% and 0%, respectively. Colistin and tigecycline were the only agents retaining high susceptibility (>98%). Carbapenemase production (CPKP) was detected in 57.1% of isolates, with MBL predominating (34.0%), followed by OXA-48-like (19.0%) and KPC (3.4%). Among 100 PCR-tested isolates, 84.0% carried at least one carbapenemase gene. bla_{NDM} was the most prevalent (60.0%), followed by $bla_{OXA\text{-}48\text{-}like}$ (47.0%), with bla_{KPC} and bla_{VIM} each at 6.0%; bla_{IMP} was not detected. Multi-gene co-carriage was identified in 35.0% of isolates, predominantly the $bla_{OXA\text{-}48\text{-}like}$ + bla_{NDM} combination (82.9%). Agreement between D73C and PCR ranged from moderate to almost perfect across the three enzyme classes: KPC (κ = 0.903), MBL (κ = 0.735), and OXA-48-like (κ = 0.561), all p <0.001. All carbapenemase-producing groups exhibited high-level meropenem resistance with a median MIC >16 µg/mL.

Conflict of interest statement: The authors declare no conflicts of interest regarding this research, authorship, or publication.

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