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Original Article

Lateral flow immunoassay for rapid detection of carbapenemase-producing *E. coli* and *K. pneumoniae* at Cho Ray Hospital, Vietnam

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Abstract

Introduction: Carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* represent an escalating threat to global public health, particularly in Southeast Asia, where antimicrobial resistance (AMR) is highly prevalent. This study aimed to evaluate the diagnostic performance of the NG-Test Carba 5 lateral flow immunoassay for the rapid detection of carbapenemase-producing *E. coli* and *K. pneumoniae*.
Methodology: A cross-sectional study was conducted using 160 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates collected from Cho Ray Hospital in Vietnam. The NG-Test Carba 5 was compared with a composite reference method comprising the modified carbapenem inactivation method (mCIM) and Multiplex Real-time PCR (Entero DR Assay). Diagnostic accuracy was assessed using positive percent agreement (PPA) and negative percent agreement (NPA) with 95% confidence intervals (CIs).
Results: The assay showed excellent diagnostic performance, with a PPA of 98.7% (95% CI: 96.24%–99.56%) and an NPA of 100.0% (95% CI: 99.33%–100.0%). No false positives were observed. Three false negatives were identified in isolates co-harboring *bla*NDM and *bla*OXA-48. Importantly, no isolates tested positive by mCIM but negative by either the NG-Test Carba 5 or the Entero DR Assay.
Conclusions: The lateral flow immunoassay demonstrated high diagnostic accuracy in detecting carbapenemase-producing *E. coli* and *K. pneumoniae*. Its operational simplicity and excellent performance support its use as a reliable frontline diagnostic tool, with minimal risk of missing rare or emerging carbapenemase genotypes in this setting.

Keywords: bacterial drug resistance; carbapenemase; lateral flow assay; lateral flow immunochromatographic assay; rapid diagnostic tests.

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Introduction

Escherichia coli and *Klebsiella pneumoniae* are among the most clinically significant pathogens, frequently implicated in both community-acquired and healthcare-associated infections. These organisms can cause severe illnesses such as bloodstream infections and pneumonia, posing substantial risks to patient outcomes [1,2]. In Southeast Asia, where rates of antimicrobial resistance are alarmingly high, these infections significantly burden healthcare systems. The global rise of carbapenemase-producing *Enterobacterales* has rendered carbapenems, once the mainstay for treating multidrug-resistant Gram-negative infections, increasingly ineffective. As a result, treatment options are often limited to polymyxins, such as colistin, which are now considered the last therapeutic resort. However, polymyxins are associated with nephrotoxicity and neurotoxicity, and resistance to these agents is also emerging, further narrowing the therapeutic arsenal. This growing challenge underscores the critical need for rapid, reliable diagnostic tools to promptly detect carbapenemase-producing organisms and guide appropriate antimicrobial therapy [3,4].

Carbapenemase genes are often plasmid-mediated, facilitating horizontal gene transfer among bacterial populations and accelerating the spread of resistance both within hospitals and in the community [5,6]. The rapid proliferation of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* presents a significant challenge in clinical environments, as these pathogens exhibit high resistance to carbapenem antibiotics, often serving as the last line of defense against multidrug-resistant infections. As a result, these pathogens complicate treatment options due to the limited availability of effective antibiotics, making accurate identification essential for guiding therapy. This process ultimately prolongs treatment durations and increases overall costs [7].

Diagnosing carbapenemase-producing strains presents numerous challenges. Over the past decade, significant advancements have been made in developing phenotypic assays that enhance accuracy and enable faster, more streamlined molecular approaches. However, difficulties remain, including the inability to identify all carbapenemase variants, cumbersome and time-consuming workflows, and delays in obtaining results [8]. Therefore, it is essential

to address these limitations to improve diagnostic efficiency and ensure timely, effective treatment for infections caused by carbapenemase-producing strains.

Lateral flow immunoassay (LFIA) is an analytical technique designed to detect and quantify specific enzymes or biomolecules through immunological recognition and chromogenic (color-generating) reactions. The primary aim of this study was to assess the diagnostic accuracy of the NG-Test Carba 5 in identifying carbapenemase-producing *E. coli* and *K. pneumoniae*. Clinical isolates were collected from Cho Ray Hospital, and results were compared against a composite reference method combining multiplex real-time PCR and the modified carbapenem inactivation method (mCIM), known for their high sensitivity and specificity [9].

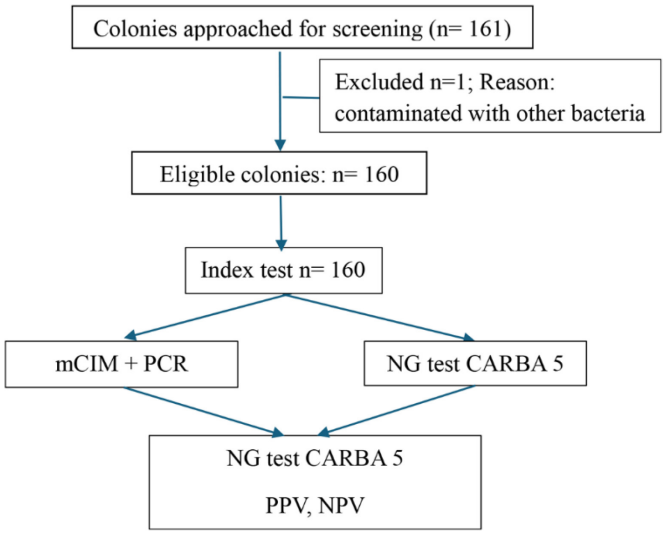
Methodology

Bacterial Isolates

A cross-sectional study was conducted at Cho Ray Hospital in Vietnam from October 2023 to January 2024. The study included 161 unique, consecutive *isolates of E. coli* and *K. pneumoniae* that exhibited resistance to at least one carbapenem agent collected from various clinical sources (Figure 1). However, one bacterial strain was excluded due to contamination, resulting in 160 eligible strains for analysis.

Bacterial strains were identified using the Vitek MS System (BioMérieux, France), while carbapenem-resistant strains were detected with the Vitek-2 Compact System (BioMérieux, France). After evaluation, each isolate was preserved in Tryptic Soy Broth (TSB) supplemented with 15% glycerol for long-term storage. Further laboratory experiments on the stored isolates were conducted between January 2024 and December 2024.

Figure 1. Flow chart of participants.



The results were meticulously documented and analyzed. All isolates underwent subculturing twice before testing. Those recovered from frozen stocks were streaked onto blood agar and incubated at 35 ± 2°C overnight.

Method Used

The Lateral Flow Immunoassay Method: NG-Test Carba 5

Following the manufacturer's guidelines, overnight bacterial cultures grown on blood agar were assessed using the NG-Test Carba 5 assay (NG Biotech, Guipry, France). A 1-µl loop was utilized to collect three colonies, which were then transferred to a 1.5-ml microcentrifuge tube containing five drops of extraction buffer. The mixture was vortexed for 3 to 5 seconds to ensure thorough homogenization. For mucoid (sticky) colonies, vortexing was extended to 10–15 seconds to achieve complete dispersion. Subsequently, 100 µl of the suspension was transferred into the NG-Test Carba 5 cassette sample well using the pipette provided with the kit. After 15 minutes, the results were visually examined for the presence or absence of control and test lines.

Quality control was performed daily, which included: a Negative Control with *Klebsiella pneumoniae* ATCC BAA-1706, and Positive Controls consisting of one strain for each carbapenemase target, including KPC-producing *K. pneumoniae* (ATCC BAA-1705), OXA-48-producing *K. pneumoniae* (NCTC 13442), VIM-producing *K. pneumoniae* (NCTC 13439), IMP-producing *Escherichia coli* (NCTC 13476), and NDM-producing *K. pneumoniae* (ATCC BAA-2146).

The Multiplex Real-time PCR(Entero DR Assay) + mCIM arcording CLSI M100 S34

The results of the NG-Test Carba 5 were compared with a composite reference method consisting of: (1) phenotypic detection using the modified carbapenem inactivation method (mCIM) according to CLSI M100S34, and (2) genotypic detection via the Allplex™ Entero-DR Assay (Seegene Inc., Seoul, South Korea), targeting $bla_{\text{OXA-48}}$, bla_{NDM}, bla_{KPC}, bla_{VIM}, and bla_{IMP}. This composite method was selected because NG-Test Carba 5 directly detects enzymes, whereas mCIM assesses enzymatic activity, and the Entero DR Assay detects the presence of carbapenemase genes. All primers and probes are pre-validated and provided by the manufacturer. The procedure was carried out strictly following the

manufacturer's instructions.

Strains with discordant results between the NG-Test Carba 5 and the reference method were further analyzed using targeted PCR and sequencing to identify specific carbapenemase gene variants.

All tests were performed on blood agar, with positive and negative controls run daily for the Entero DR Assay, mCIM, following the manufacturer's instructions and according to CLSI M100S34 guidelines.

Statistical analysis

Negative percent agreement (NPA) and positive percent agreement (PPA) were calculated from data entered into and analyzed in Microsoft Excel software. Upper- and lower-bound 95% confidence intervals (CIs) were shown in the results.

Ethical considerations

The study collected frozen bacterial strains that met the selection criteria without patient intervention. The Ethics Committee of the University of Medicine and Pharmacy in Ho Chi Minh City approved this research under contract number 142 /HDDD-DHYD.

Results

Characteristics of the study strains

In Table 1, out of the 160 bacterial isolates obtained from various specimens in this study, the majority were derived from phlegm (30%) and body fluids (28.1%). Among these isolates, *K. pneumoniae* was more prevalent than *E. coli*, with proportions of 80.6% and 19.4%, respectively. Notably, most of the bacterial strains exhibited resistance to all three tested carbapenems, totaling 93.8%.

The lateral flow immunoassay versus Multiplex Real-time PCR + mCIM

Table 2 shows the pre-discrepant analysis; 160 bacterial isolates were tested using the NG-Test Carba 5 and a composite reference method comprising multiplex real-time PCR and mCIM. Each isolate was analyzed for the presence of five major carbapenemase genes (KPC, NDM, OXA-48-like, VIM, and IMP), resulting in a total of 800 gene-level assessments. Out of these, 230 gene-level results were positive, and 570 were negative according to the composite reference method. The LFIA correctly identified 227 of the 230 gene-level positives, yielding a Positive Percent Agreement (PPA) of 98.7% (95% CI: 96.24% – 99.56%). It also accurately detected all 570 gene-level negatives, resulting in a Negative Percent Agreement (NPA) of 100.0% (95% CI: 99.33 –100.0%).

Table 1. Characteristics of the study strains.

Characteristics	n	%
Specimens		
Blood	31	19.4
Urine	23	14.4
Phlegm	48	30.0
Pus	13	8.1
Other Body fluids	45	28.1
Bacteria		
Klebsiella pneumoniae	129	80.6
Escherichia coli	31	19.4
Resistance to carbapenems		
Ertapenem; Imipenem; Meropenem;	152	94.4
Ertapenem	3	1.9
Imipenem, Meropenem	4	2.5
Ertapenem, Imipenem	1	0.6
Ertapenem, Meropenem	1	0.6
Total	160	100

No false-positive results were observed. However, three false-negative results were recorded, all from *Klebsiella pneumoniae* isolates that co-harbored bla_{NDM} (Class B) and bla_{OXA-48}(Class D). In these cases, the LFIA only detected OXA-48, while failing to detect the NDM band.

Summary of Discrepant Results in Lateral Flow Immunoassay

In Table 3, three false-negative results were observed, all involving *Klebsiella pneumoniae* isolates co-harboring *bla*NDM and *bla*OXA-48. In these cases, NG-Test Carba 5 successfully detected OXA-48 but failed to identify the NDM band. Sequencing confirmed

Table 2. Pre-discrepant analysis result interpretation of the lateral flow immunoassay compared to the composite reference method.

Composite reference method result		LFIA result[β]	Pre-discrepant LFIA interpretation	No of results by pre-discrepant analysis by genes
Realtime PCR	mCIM[α]			
+	+	+	True positive	180
+	-	+	True positive (less expression)	47
-	-	+	False positive	0
-	+	+	false positive	0
+	+	-	False-negative	3
-	-	-	True negative	570
-	+	-	True negative (off-target carbapenemase)	0
+	-	-	True negative (lack of or less expression)	0

[α]mCIM: modified carbapenem inactivation method. [β]LFIA result: Lateral Flow Immunoassay. [γ] Of the 3 false-negative, both were *K. pneumoniae* and showed positive PCR results with NDM and OXA-48, but only OXA-48 by LFIA.

Table 3. The lateral flow immunoassay's discrepant results.

Strains	AST results				Realtime PCR	LFIA[α]	PCR + sequencing
	ETP	IMP	MEM	CAZ/AVI			
17,92,131 *(K. pneumoniae)*	R	R	R	S	*bla*OXA-48 + *bla*NDM	OXA-48	*bla*OXA-48 + *bla*NDM

[α]These results were confirmed by repeat testing using colonies grown on blood agar (BA), tryptic soy agar (TSA), and chocolate agar (CBA), all of which yielded consistent findings.

the presence of *bla*NDM in all three isolates. Repeat LFIA testing on blood agar, Columbia agar, and tryptic soy agar (TSA) produced consistent findings.

Table 4 shows the distribution of mCIM results by gene group, including the subset of isolates that tested positive by both LFIA and PCR but negative by mCIM. A total of 155 isolates were stratified by carbapenemase genotype to evaluate mCIM performance. Among 65 isolates carrying both OXA-48 and NDM**,** 18 isolates (27.7%) had negative mCIM results. Of the 4 isolates co-harboring OXA and KPC**,** 1 isolate (25.0%) was mCIM-negative. All 4 isolates (100%) carrying the triple combination OXA-48 + NDM + KPC were mCIM-positive. Among isolates carrying a single carbapenemase gene**,** 8 of 28 isolates (28.6%) with OXA-48 alone tested mCIM-negative. For NDM-only isolates, 1 of 31 isolates (3.2%) was negative. All 23 isolates carrying KPC alone tested positive by mCIM (0% negative).

The lateral flow immunoassay post-discrepant assay analysis by isolation

In Table 5, the post-discrepant analysis evaluated LFIA performance by carbapenemase genotype. Among 62 OXA-48 + NDM isolates, LFIA detected 59, with a PPA of 95.4% and NPA of 100%. All isolates carrying OXA-48 + KPC (n = 4), OXA-48 + NDM + KPC (n = 4), OXA-48 alone (n = 28), NDM alone (n = 31), and KPC alone (n = 21) were correctly identified, yielding 100% PPA and NPA in each group. Confidence intervals varied due to sample size. No false positives were observed. Three false-negative NDM results were noted in isolates co-harboring *bla*NDM and *bla*OXA-48, with susceptibility to ceftazidime-avibactam and no detectable NDM band despite repeat testing, were noted in Table 3.

Table 4. Discordant mCIM Results Among PCR and LFIA Positive Isolates by Genotype.

Gene type	n	mCIM (–) n (%)
OXA + NDM	65	18 (27.7%)
OXA + KPC	4	1 (25.0%)
OXA + NDM + KPC	4	0 (0.0%)
OXA	28	8 (28.6%)
NDM	31	1 (3.2%)
KPC	23	0 (0.0%)

Discussion

Accurate and timely detection of carbapenemase genes is crucial for managing infections caused by carbapenem-resistant Enterobacterales (CRE). These enzymes-KPC, NDM, OXA-48, IMP, and VIM-make carbapenems ineffective, significantly restricting therapeutic options. Early identification enables targeted therapy and effective infection control, particularly in high-burden regions like Vietnam. This study highlighted a high prevalence of NDM (97/160) and OXA-48 (96/160) among isolates from a tertiary hospital in Vietnam, with co-occurrence observed in 66 samples. These findings underscore a complex resistance landscape where the co-production of multiple enzymes poses diagnostic and therapeutic challenges. NDM enzyme can hydrolyze nearly all β-lactams, while OXA-48 mainly affects carbapenems but spares extended-spectrum cephalosporins. Their co-expression frequently results in treatment failures and limited therapeutic options.

Similar patterns have been observed in Vietnam and other Southeast Asian nations, where NDM and OXA-48 are more prevalent than KPC [3,10,11]. In contrast, developed countries such as the United States,

Table 5. Post-discrepant analysis result interpretation of the lateral flow immunoassay compared to the composite reference method by isolation.

The lateral flow immunoassay Target	No of results				PPA	95% CI		NPA	95% CI	
	TP	FP	FN	TN		Low	High		Low	High
OXA-48 + NDM	62	0	3*	95	95.4	87.7	98.2	100.0	96.1	100.0
OXA-48 + KPC	4	0	0	156	100.0	51.0	100	100.0	97.6	100.0
OXA48 + NDM + KPC	4	0	0	156	100.0	68.9	100.0	100.0	97.6	100.0
OXA-48	28	0	0	132	100	88.0	100.0	100.0	97.2	100.0
NDM	31	0	0	129	100	89.1	100.0	100.0	96.9	100.0
KPC	21	0	0	139	100.0	84.7	100.0	100.0	97.3	100.0

TP: true positive; FP: false positive; TN: true negative; TN: true negative; 95% CI: 95% confidence interval; PPA: Positive Percent Agreement; NPA: Negative Percent Agreement. * Three false-negative results for NDM detection correspond to isolates harboring *bla-NDM-1* and *bla-OXA-48*, with confirmed susceptibility to ceftazidime-avibactam (CAZ/AVI) and no NDM band on NG-Test Carba 5 even after repeated testing on multiple media.

Colombia, Argentina, Greece, and Italy primarily report KPC as the most common carbapenemase. However, global studies indicate that other regions exhibit NDM, OXA-48, VIM, and IMP endemicity, sometimes at rates exceeding KPC [10]. This epidemiological variability underscores the necessity of tailoring diagnostic approaches to local resistance profiles.

The differing distribution of carbapenemase genes across regions also impacts the cost-effectiveness of genotyping methods, emphasizing the importance of customizing diagnostic strategies to align with local epidemiological contexts to optimize resource allocation, enhance diagnostic accuracy, and effectively combat antimicrobial resistance (AMR) [4]. In this study, LFIA demonstrated exceptional diagnostic accuracy, achieving an overall PPA of 98.7% and NPA of 100%. These findings reflect excellent concordance with the composite reference method. Notably, the assay exhibited perfect specificity across all carbapenemase types and reliably detected rare combinations such as OXA + NDM + KPC.

Three false-negative cases were identified, all involving *K. pneumoniae* strains that co-harbored *bla*NDM and *bla*OXA-48. In these isolates, the NG-Test Carba 5 successfully detected OXA-48 but failed to identify NDM. Sequencing confirmed the presence of intact *bla*NDM genes. Notably, all three isolates also exhibited susceptibility to ceftazidime-avibactam (CAZ/AVI), suggesting low or absent *bla*NDM expression. Moreover, the co-expression of multiple carbapenemases, such as OXA-48 and NDM, may contribute to epitope competition during detection, potentially hindering the recognition of NDM when OXA-48 is predominant [12,13]. Low or non-functional expression of *bla*NDM may be attributed to several molecular mechanisms, including regulatory mutations, reduced plasmid copy number, or transcriptional suppression [14,15]. While the overall diagnostic performance of the NG-Test Carba 5 remained excellent, these findings suggest a potential limitation in detecting NDM in co-expressing isolates. Similar detection challenges have been reported in previous studies [16,17], reinforcing the need for careful interpretation in such complex genetic backgrounds. In our study, all discordant cases were resolved through sequencing, confirming the presence of both genes despite incomplete immunoassay detection.

Importantly, all isolates that tested positive by mCIM were also detected by either the NG-Test Carba 5 or the Entero DR Assay. This confirms that the LFIA did not miss any carbapenemase activity attributable to uncommon or novel enzymes beyond the five targets of the assay. Such concordance reinforces its clinical utility as a reliable frontline diagnostic tool, with minimal risk of overlooking rare or emerging carbapenemase genotypes in our setting.

Our results align with international studies, including those by Huang et al. (2022) and Dortet et al. (2018), which reported similarly high performance for the NG-Test Carba 5 [9,16]. Unlike molecular methods like Xpert CARBA-R, lateral flow assays provide comparable accuracy while offering greater speed and ease of use [17]. LFIA presents a practical solution for swift detection and effective infection control in resource-limited settings [18].

Study Limitations and Future Directions

While our study offers strong evidence for the effectiveness of LFIA, certain limitations must be acknowledged. Firstly, the sample size for less prevalent carbapenemase combinations was limited, resulting in wider confidence intervals for these targets. Secondly, the research was conducted at a single tertiary hospital, which may restrict the generalizability of the findings to other settings or geographic areas. Moreover, incorporating molecular typing methods, such as multilocus sequence typing (MLST) or whole-genome sequencing (WGS), would provide a more comprehensive assessment of strain diversity and help rule out clonal spread, particularly in single-center studies.

Further research is necessary to confirm and validate the performance of the lateral assay method across varied clinical contexts and larger sample sizes, especially for rare carbapenemase variants. Additionally, investigating its cost-effectiveness relative to molecular methods and exploring its integration into standard diagnostic workflows would yield valuable insights for its wider adoption.

Conclusions

The lateral flow immunoassay showed high sensitivity and specificity in detecting carbapenemase-producing *E. coli* and *K. pneumoniae*. Its speed, ease of use, and broad detection range support its clinical utility. Importantly, it missed no mCIM-positive isolates, indicating that no carbapenemase genes outside the five targeted by the assay were present in this study. This highlights its reliability as a front-line diagnostic tool. The findings align with global data and support its role in combating antimicrobial resistance.

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Authors' Contributions
Conceptualization: Tran Bich Ngoc, Truong Thien Phu. Data curation: Tran Bich Ngoc, Truong Thien Phu. Formal analysis: Tran Bich Ngoc, Truong Thien Phu. Project administration: Tran Thi Hue Van, Ngo Quoc Dat. Methodology: Tran Bich Ngoc, Truong Thien Phu, Tran Thi Hue Van, Ngo Quoc Dat. Supervision: Truong Thien Phu, Ngo Quoc Dat. Validation: Tran Thi Hue Van. Writing – original draft: Tran Bich Ngoc, Truong Thien Phu. Writing – review and editing: Tran Bich Ngoc, Truong Thien Phu.

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Conflict of interest
No conflict of interest is declared.

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