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ORIGINAL RESEARCH

Development and Evaluation of a Handmade Lateral Flow Immunoassay for the Rapid Detection of Carbapenem-producing Gram-Negative Bacteria in China

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Background: The global dissemination of carbapenemase-producing organisms (CPOs) poses a critical public health threat. Rapid, cost-effective diagnostic tools are urgently needed to guide antimicrobial therapy and mitigate resistance spread.

Objective: This study aimed to develop and evaluate a lateral flow immunoassay (LFIA)-based workflow for the rapid detection of carbapenemases (KPC, IMP, NDM, VIM, and OXA-48) in clinical settings.

Materials and Methods: The LFIA strips targeting carbapenemases were handmade in-house using monoclonal antibodies specific to five prevalent subtypes: KPC, IMP, NDM, VIM, and OXA-48. Performance was validated using 26 characterized carbapenemase-producing *Enterobacterales* (CPE) strains (10 KPC, 5 IMP, 7 NDM, 1 VIM, and 3 OXA-48 producers) and 105 clinical isolates. Among them, strains positive for carbapenemase by LFIA were further confirmed by Next-generation sequencing (NGS) and PCR, and their carbapenem susceptibility was determined by the Vitek2 Antibiotics Susceptibility Test (AST). Diagnostic metrics were further assessed through limit of detection (LoD), cross-reactivity, and interference studies. The overall workflow for the development and evaluation of the LFIA is presented in Figure 1.

Results: The LFIA demonstrated 100% sensitivity in detecting all 26 CPE strains. Among 105 clinical isolates, 44 were identified as CPO strains, with strong concordance to PCR/NGS results (discrepancies in 8 isolates observed between PCR and NGS). The assay exhibited high sensitivity (100%), specificity (100%), positive predictive value (PPV, 93.2%), negative predictive value (NPV, 100%), and accuracy (97.1%). The LoD for each carbapenemase type was established. No cross-reactivity with non-target bacteria or interference from clinical substances was observed. The workflow achieved results in 15 minutes at a lower cost than conventional methods.

Conclusion: This LFIA-based workflow provides a rapid, reliable, and cost-effective solution for detecting CPOs, particularly for critically ill patients or in resource-limited settings. Its implementation could enhance therapeutic decision-making and antimicrobial stewardship.

Keywords: carbapenemase-producing organisms, lateral flow immunoassay, carbapenem-resistance, rapid detection, cost-effective workflow

Introduction

Carbapenemase-producing organisms (CPOs) pose a significant threat to global public health due to their ability to hydrolyze a wide range of β-lactam antibiotics, including carbapenems, which are often considered last-resort treatments

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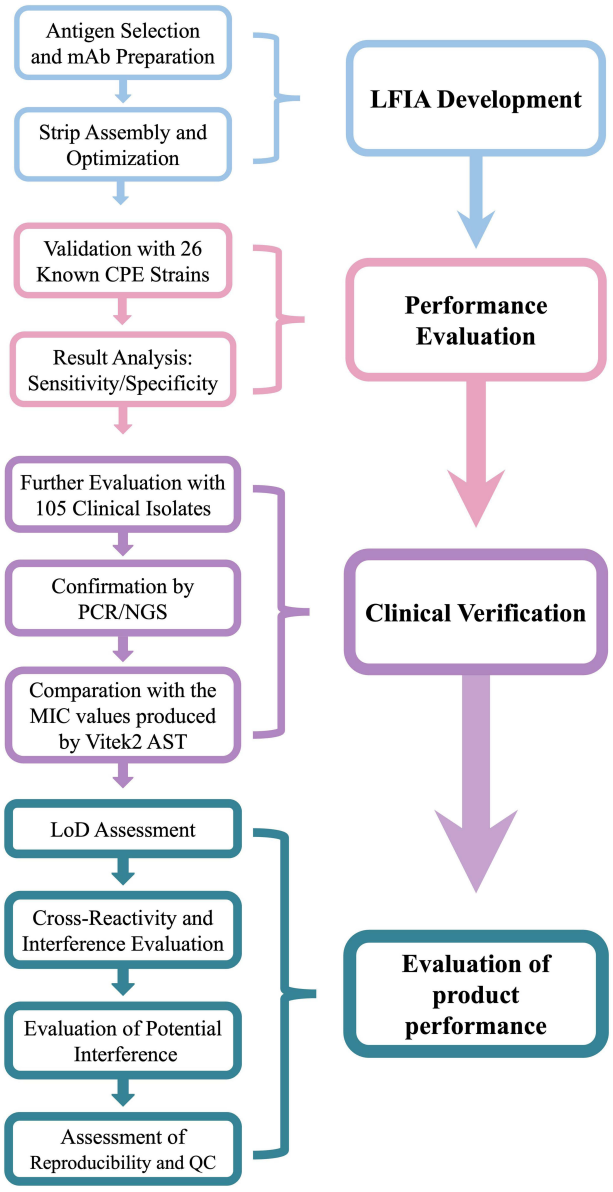

Figure 1 Workflow for the development and evaluation of the LFIA.
Abbreviations: LFIA, Lateral flow immunoassay; mAb, Monoclonal antibodies; CPE, Carbapenemase-producing Enterobacterales; PCR, Polymerase reaction; NGS, Next generation sequencing; MIC, Minimum inhibitory concentration; AST, Antimicrobial susceptibility testing; LoD, Limit of detection; QC, Quality control.

for severe infections.[1] The globally prevalent types of carbapenemases include Klebsiella pneumoniae carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), oxacillinase-48 (OXA-48), Verona integron-encoded metallo-β-lactamase (VIM), and Imipenemase (IMP), all of which contribute to the carbapenem resistance development of gram-negative bacteria.[2]

To date, a total of 96 subtypes of KPC carbapenase genes have been identified. KPC-2 and KPC-3 are the most common globally, with KPC-2 accounting for over 70% of carbapenem-resistant Klebsiella pneumoniae (CRKP) isolates in China.[3] For IMP-type enzymes, blaIMP-1 is the dominant variant, while blaIMP-4 is primarily endemic to China.[4] Among the 12 reported NDM subtypes, NDM-1 and NDM-5 remain the major globally circulating genotypes.[5] Similarly, VIM-1 and VIM-2 are the predominant VIM subtypes.[6] OXA-type enzymes, particularly OXA-48, have disseminated extensively across *Enterobacterales* in Europe, Asia, North Africa, and the Middle East since their discovery.[7]

The rapid global proliferation of these carbapenemase-producing pathogens has escalated morbidity, mortality, and healthcare expenditures.[8] Rapid and accurate detection is therefore critical to guiding appropriate antimicrobial therapy and implementing effective infection control measures to curb the dissemination of resistance.

Traditional diagnostic techniques, such as PCR and NGS are widely used for the carbapenemases detection due to their high specificity and reliability.[9] However, these methods are time-consuming, require specialized equipment and technical expertise., and often impractical in resource-limited settings.[10] These limitations underscore the need for simpler, faster, and more accessible diagnostic alternatives.

LFIA is a rapid and simple diagnostic technique based on the antigen-antibody reaction. It works by detecting specific antigens (such as carbapenemases) in a sample, with the results visible on a membrane as colored lines. LFIA offers several advantages: it is low-cost, easy to use, and does not require specialized equipment, making it ideal for use in municipal hospitals or resource-limited settings.[11] By incorporating monoclonal antibodies that specifically bind to the target carbapenemases, LFIA provides a quick and reliable method for pathogen detection, which can significantly improve clinical decision-making and infection control.[12] In this study, we report the development of a handmade lateral flow immunoassay (LFIA), which represents a new assay design rather than an adaptation of existing commercial LFIA platforms. The LFIA was specifically developed to target the most prevalent carbapenemases: KPC, IMP, NDM, VIM, and OXA-48. This assay offers a streamlined, efficient, and practical solution for the rapid identification of carbapenemase-producing Enterobacterales (CPE), thereby supporting effective infection control and antibiotic stewardship.[13]

Furthermore, we conducted a comprehensive evaluation of the accuracy and reliability of this novel LFIA against a collection of clinically relevant bacterial isolates using gold-standard molecular and microbiological methods as references. By providing a rapid and cost-effective diagnostic tool, this study aims to improve detection capabilities in municipal hospitals, particularly for critically ill patients requiring immediate pathogen identification. Its implementation promises to optimize clinical outcomes and effective management of antimicrobial resistance.

Materials and Methods

Methods

Development Of Lateral Flow Immunoassays

The LFIA was developed to specifically detect the presence of five key carbapenemases. Based on the amino acid sequence alignment of common variants of five carbapenemases: KPC, IMP, NDM, VIM, and OXA-48. The development began with the amino acid sequence alignment of common variants of these carbapenemases, from which conserved epitope sequences were identified and used to prepare immunogens. Briefly, the immunogen preparation strategy was determined by comparing the sequences of the most commonly epidemic variants. Expression plasmids for the respective carbapenemase antigens were constructed and used to prepare the immunogens.

After immunizing mice, highly specific monoclonal antibodies were generated through hybridoma technology. The monoclonal antibodies were screened using multi-platform methods, including indirect ELISA, Western blot, and immunochromatography, to ensure strong affinity and broad reactivity to multiple carbapenemase variants. The most promising monoclonal antibodies were then conjugated to colloidal gold nanoparticles. The conjugation process involved adsorbing the antibodies onto gold nanoparticles at pH 8.5, followed by stabilization using bovine serum albumin (BSA) to prevent nonspecific binding. These antibody-gold conjugates were then integrated into the LFIA strips, which consist of a sample pad, a conjugate pad, a nitrocellulose membrane with immobilized capture antibodies, and an absorbent pad. The strips were assembled and optimized to ensure rapid and accurate detection of carbapenemase enzymes.

All animal experiments conducted for antibody generation were approved by the Institutional Animal Care and Use Committee of Nanjing University of Chinese Medicine and performed in strict accordance with the national guidelines for the care and use of laboratory animals issued by the Ministry of Science and Technology of China.

Bacterial Strains and Clinical Isolates

A total of 26 CPE strains from the clinical specimen biobank of the Second Hospital of Nanjing were used to evaluate the LFIA workflow. These strains comprised 10 KPC, 5 IMP, 7 NDM, 1 VIM, and 3 OXA-48 producers. Additionally, 105 clinical isolates obtained from the same source were tested to assess the assay's effectiveness in real-world conditions.

The use of clinical samples in this study complied with the ethical principles of the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of the Second Hospital of Nanjing (Approval No.: 2023-LS-ky-039), and informed consent was obtained from all patients or their legal guardians prior to sample collection.

Validation of Carbapenemase Production

The 105 clinical isolates were firstly subjected to the LFIA for initial carbapenemase detection. For the strains positive for carbapenemase, next generation sequencing (NGS) and polymerase chain reaction (PCR) were further employed to confirm the presence of carbapenemase (The primers for PCR were listed in Table S1).[14] Antimicrobial susceptibility testing (AST) was performed using the Vitek2 system to determine the strains' resistance to imipenem and meropenem. Results from the LFIA were compared to those from PCR and NGS to evaluate the diagnostic accuracy of the LFIA.

Evaluation of Product Performance

Limit of Detection (LoD) Assessment

Purified preparations of KPC, IMP, NDM, VIM, and OXA-48 carbapenemases were diluted in a suitable buffer (50mM Tris, 2% Triton-X 100, 1%TWEEN20, PH7.4) to create serial concentrations for LoD testing.[15] Initial concentrations covered both below and above the expected LoD for each carbapenemase type: KPC type (≤600 pg/mL), IMP type (≤200 pg/mL), NDM type (≤150 pg/mL), VIM type (≤300 pg/mL), and OXA-48 type (≤300 pg/mL). Each concentration was tested in triplicate to ensure statistical reliability. The LoD for each carbapenemase type was determined as the lowest concentration at which at least 95% of the true-positive replicates tested positive.

Evaluation of Cross Reactivity and Potential Interference

The following bacterial strains were selected to assess the cross-reactivity and potential interference of the LFIA: *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa, Acinetobacter baumannii*, and *Staphylococcus aureus*. Both carbapenemase-producing and non-carbapenemase-producing strains were included.[16] Samples were spiked with bacterial cultures at appropriate concentrations to evaluate cross-reactivity. The test was conducted according to the manufacturer's protocol, with all tests carried out in triplicate to ensure consistency and reliability of the results. The concentrations of bacterial strains used were as follows: KPC-producing strains (10^6 CFU/mL), IMP (10^6 CFU/mL), and other strains based on preliminary testing.

Evaluation of Potential Interference Effects on LFIA

We evaluated the potential interference of endogenous substances naturally present in clinical samples and drugs that may be introduced during treatment on carbapenemase detection. To this end, representative compounds were selected and tested at defined concentrations, including hemoglobin (0.5 g/dL), bilirubin (0.2 mg/dL), and lipids (1.5 g/dL) as common blood components, as well as ampicillin (10 μg/mL), meropenem (5 μg/mL), and ciprofloxacin (10 μg/mL) as representative antibiotics. Each compound was assessed individually to determine its potential impact on LFIA performance. All tests were conducted in triplicate to ensure accuracy, reproducibility, and reliability of the results.

Performance Verification and Quality Control Evaluation

To assess the reproducibility and quality control of the LFIA, three different product lots were tested on 8 bacterial strains over a 20-day period. This was done to evaluate inter-lot reproducibility and ensure the consistency of the LFIA.

Data Analysis

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy of the LFIA were calculated by comparing the results with those obtained from PCR and NGS. For each key metric, 95% confidence intervals (CIs) were calculated using the Wilson score method to estimate the variability and precision. To compare categorical results between LFIA and reference methods (PCR/NGS), chi-square tests were performed. When expected frequencies were <5, Fisher's exact test was applied to ensure robust comparisons. A p-value < 0.05 was considered statistically significant. All analyses were performed using SPSS (Version 25.0).

The study included 105 clinical isolates, which were selected based on availability during the study period. Although the sample size was not determined through a formal power calculation due to logistical constraints, it is comparable to that used in similar diagnostic validation studies and provides a reasonable basis for preliminary evaluation. We acknowledge that the relatively small number of isolates producing certain enzyme types (eg, VIM, OXA-48) represents a limitation of this study, and this has been explicitly stated in the Results and Discussion sections.

Results

Performance Evaluation of the LFIA

The LFIA developed in this study exhibited comprehensive detection capability for all targeted carbapenemases (KPC, IMP, NDM, VIM, and OXA-48) across a panel of 26 CPE strains. The assay demonstrated 100% sensitivity, accurately identifying all targeted variants, including 10 KPC-, 5 IMP-, 7 NDM-, 1 VIM-, and 3 OXA-48-producing strains (Table 1). These results highlight the assay's robustness in detecting clinically significant carbapenemases with high precision.

The LFIA workflow was validated using 26 CPEs and further assessed against 105 clinical isolates. Results were cross-referenced with PCR and NGS to evaluate accuracy, reliability, and practicality.

Validation with Clinical Isolates

Among 105 clinical isolates analyzed (Table 2), 44 were identified as CPOs via the LFIA (Table 3). The distribution included 24 KPC-positive, 17 NDM-positive, 2 KPC/NDM co-positive, and 1 NDM/OXA-48 co-positive strains. The LFIA results showed strong concordance with next-generation sequencing (NGS) and PCR in identifying KPC- and NDM-producing strains within diverse bacterial species. However, eight instances of genotype discrepancies between PCR and NGS were observed (Table 4). The Vitek2 AST confirmed a high rate of resistance rates among the isolates, consistent with carbapenemase detection outcomes.

Table 1 The 26 Carbapenemase -Producing Organism Used in Training Set

Species	Carbapenemase					Sum
	KPC	IMP	NDM	VIM	OXA-48	
Klebsiella pneumoniae (*K. pneumoniae*)	-	2	1	1	1	5
Pseudomonas aeruginosa (*P. aeruginosa*)	2	3	-	-	-	5
Enterobacter cloacae (E. cloacae)	1	-	2	-	-	3
Acinetobacter baumannii (*A. baumannii*)	7	-	4	-	2	13

Abbreviations: KPC, *Klebsiella pneumoniae* carbapenemase; IMP, Imipenemase; NDM, New Delhi metallo-β-lactamase; VIM, Verona integron-encoded metallo-β-lactamase; OXA-48, oxacillinase-48.

Table 2 The 105 Clinical Isolates Used as Validation Set in Our Study

Validation Set Species	Number of Isolates
A. baumannii	21
P. aeruginosa	7
E. coli	15
E. cloasace	18
K. pneumoniae	44
Total	105

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Table 3 Detailed Results Comparison by Different Test Methods

Species	Number	LFIA						NGS/PCR Results						Vitek2 AST Meropenem/ Imipenem/Ertapenem	
		KPC	NDM	OXA	KPC/NDM	NDM/OXA	neg	KPC	NDM	OXA	KPC/NDM	NDM/OXA	neg	All Resistant	All Susceptible
A. baumannii	5	3	2	0	0	0	0	2	2	0	0	0	1	4	1
P. aeruginosa	2	2	0	0	0	0	0	2	0	0	0	0	0	2	0
E. coli	8	1	5	0	2	0	0	1	5	0	1	0	1	7	1
E. cloasace	10	1	9	0	0	0	0	1	9	0	0	0	0	10	0
K. pneumoniae	19	17	1	0	0	1	0	16	2	0	0	0	1	17	2
Total	44	24	17	0	2	1	0	22	18	0	1	0	3	40	4
Sum		44					0	41					3	44	

Abbreviations: LFIA, Lateral flow immunoassay; NGS, Next generation sequencing; PCR, Polymerase reaction; AST, antimicrobial susceptibility testing; neg, negative; KPC, *Klebsiella pneumoniae* carbapenemase; IMP, Imipenemase; NDM, New Delhi metallo-β-lactamase; VIM, Verona integron-encoded metallo-β-lactamase; OXA, oxacillinase.

Table 4 Isolates with Discrepancy NGS and Simplex PCR Results

Isolates	LFIA	NGS	PCR	NGS/PCR Combined
A. baumannii	KPC	OXA-66	KPC	KPC
A. baumannii	NDM	OXA-366, OXA-66	NDM	NDM
A. baumannii	NDM	OXA-421	NDM	NDM
P. aeruginosa	KPC	OXA-50	KPC	KPC
P. aeruginosa	KPC	OXA-50	KPC	KPC
E. coli	NDM	CTX-M-27, OXA-358	NDM	NDM
E. coli	KPC/NDM	NDM-5	KPC/NDM	KPC/NDM
E. coli	KPC/NDM	KPC-2, CTX-M-14	neg	KPC

Abbreviations: LFIA, Lateral flow immunoassay; NGS, Next generation sequencing; PCR, Polymerase reaction; neg, negative; KPC, *Klebsiella pneumoniae* carbapenemase; IMP, Imipenemase; NDM, New Delhi metallo-β-lactamase; VIM, Verona integron-encoded metallo-β-lactamase; OXA, oxacillinase; CTX-M, Cefotaximase.

Diagnostic Accuracy

The assay exhibited exceptional diagnostic performance across all metrics, with sensitivity (100%; 95% CI: 91.4–100%), specificity (95.3%; 95% CI:86.9–99%), PPV (93.2%; 95% CI: 81.3–98.6%), NPV (100%; 95% CI: 94.1–100%), and overall accuracy (97.1%; 95% CI: 91.9–99.4) (Table 5). Statistical comparison of LFIA with PCR/NGS using chi-square and Fisher's exact tests showed no significant discrepancies ($p > 0.05$), supporting the robustness of the LFIA results.

Limit of Detection (LoD)

The LoD values were determined as follows: KPC (≤600 pg/mL), IMP (≤200 pg/mL), NDM (≤150 pg/mL), VIM (≤300 pg/mL), and OXA-48 (≤300 pg/mL). These thresholds confirm the assay's excellent capacity to detect carbapenemases at clinically relevant concentrations.

Table 5 LFIA Results Compared to NGS and PCR Results

	Value	Percentage (95% CI)
Positive Agreement	41/41	100% (91.4–100%)
Negative Agreement	61/64	95.3% (86.9–99%)
Positive Predict Value	41/44	93.2% (81.3–98.6%)
Negative Predict Value	61/61	100% (94.1–100%)
Accuracy	102/105	97.1% (91.9–99.4%)

Abbreviations: LFIA, lateral flow immunoassay; NGS, Next generation sequencing; PCR, Polymerase reaction; neg, negative.

Table 6 Cross-Reactivity with the Tested Non-Carbapenemase-Producing Bacterial Strains at the Evaluated Concentrations

Potential Cross-Reactant	Concentration Tested	Cross-Reactivity (Yes/No)
E. coli	1.0×10^6 CFU/mL	No
K. pneumoniae	1.0×10^6 CFU/mL	No
E. cloacae	1.0×10^6 CFU/mL	No
P. aeruginosa	1.0×10^6 CFU/mL	No
A. baumannii	1.0×10^6 CFU/mL	No
Staphylococcus aureus (S. aureus)	1.0×10^2 CFU/mL	No

Abbreviation: CFU, Colony forming unit.

Table 7 Cross-Reactivity with Any of the Tested Potential Interfering Substances at the Specified Concentrations

Potential Interfering Substance	Concentration	Cross-Reactivity (Yes/No)
Culture medium	100mg/mL	No
Whole blood	50μg/mL	No
Saliva	50μg/mL	No
Urine	50μg/mL	No
Meropenem	10μg/mL	No
imipenem	10μg/mL	No
doripenem	10μg/mL	No
ertapenem	10μg/mL	No
ceftriaxone	500μg/mL	No

Cross-Reactivity Assessment

The LFIA demonstrated no cross-reactivity with non-carbapenemase-producing strains or potential interfering substances at evaluated concentrations (Tables 6 and 7), affirming its high specificity.

Time and Cost Efficiency

The LFIA workflow was completed within 15 minutes, significantly outperforming traditional NGS and PCR methods in turnaround time. Furthermore, its cost-effectiveness, and the less requirements of resource and specialized equipment make it particularly suitable for resource-limited healthcare settings.

Clinical Application

The LFIA's user-friendly design, rapid results, and diagnostic accuracy render it an ideal tool for clinical microbiology laboratories. By enabling timely identification of carbapenemase-producing pathogens, the assay facilitates prompt initiation of targeted antimicrobial therapy, thereby improving patient outcomes and supporting antimicrobial stewardship efforts.

Discussion

The rapid and accurate detection of CPOs is paramount in clinical settings to inform timely therapeutic interventions and mitigate the global threat of antimicrobial resistance.[17] While conventional methods such as AST and PCR remain reliable, their time-consuming protocols and resource-intensive requirements often hinder their utility in urgent and resource-limited clinical scenarios.[18,19] Recent studies have evaluated commercial LFIAs for carbapenemase detection and reported high sensitivity and specificity (often above 95%),[20] yet these assays are usually expensive, not handmade, and may not be optimized for local epidemiological variants. Our study evaluates a novel handmade LFIA tailored for prevalent carbapenemase types, thereby addressing both accessibility and regional relevance.

Our findings highlight the outstanding diagnostic performance of the LFIA, which demonstrated 100% sensitivity across a comprehensive panel of 26 CPEs, including clinically prevalent carbapenemases (KPC, IMP, NDM, VIM, and

OXA-48). This performance is consistent with prior evaluations of commercial LFIAs,[20] but extends the evidence by validating the assay with local isolates and demonstrating feasibility in a hospital-based setting. Compared with molecular diagnostics or phenotypic assays that require specialized equipment, extended processing times, and technical expertise,[21] our LFIA offers distinct advantages in simplicity, rapidity (15 minutes), and cost-efficiency, aligning with findings reported by Bianco G et al for the RESIST-5 O.K.N.V.I assay, while emphasizing its adaptability for resource-limited contexts.[22]

Validation with 105 clinical isolates provides compelling evidence of diagnostic reliability. The identification of 44 CPO-positive strains (24 KPC, 18 NDM, 2 KPC/NDM, and 1 NDM/OXA-48 co-producers) underscores the assay's robust capability. Similar performance has been observed in multicenter validations of other LFIA platforms,[23] suggesting that such assays can serve as rapid front-line diagnostic tools. The eight observed discrepancies between PCR and NGS highlight known limitations of molecular reference methods, including potential primer mismatches or sequencing depth issues.[24] Further investigation into these cases could provide valuable insights into the genetic diversity of carbapenemase-producing strains and opportunities for refining diagnostic tools.

The alignment between LFIA results and Vitek2 AST further validates accuracy, reinforcing the value of combining phenotypic and genotypic methods for comprehensive resistance profiling. In addition, high resistance rates observed in isolates emphasize the clinical significance of CPOs in this cohort. This concordance highlights the importance of integrating phenotypic AST results with genotypic carbapenemase detection for a comprehensive understanding of resistance profiles. The ability of the LFIA to accurately detect KPC and NDM carbapenemases, as well as co-production of carbapenemase types (eg, NDM/OXA), has significant implications for clinical decision-making. Rapid identification of CPOs allows for timely optimization of antimicrobial regimens, reducing the risk of treatment failure and curbing the spread of resistant pathogens.[25] Additionally, the assay's ability to detect co-production of carbapenemases emphasizes its critical role in increasingly complex resistance patterns.

Diagnostic metrics revealed outstanding performance, with high sensitivity, specificity, PPV, NPV, and overall accuracy, consistent with and in some cases exceeding the performance reported for existing LFIA platforms,[20] indicating the assay's excellent capacity to identify carbapenemase-producing strains and exclude non-producers, solidifying its role in clinical diagnostics. The LoD studies revealed that the lowest detectable concentration for each carbapenemase type, highlighting the assay's high sensitivity,[26] ensuring it can detect even low-burden infections, which is crucial for early accurate diagnosis.

Furthermore, the results of cross-reactivity analysis highlight the assay's specificity and robustness. The absence of cross-reactivity with non-carbapenemase-producing strains underscores the assay's ability to precisely differentiate between carbapenemase producers and other bacteria, minimizing false-positive results.[27] This is critical for accurate diagnosis and treatment planning, as false positives could lead to unnecessary use of last-resort antibiotics or inappropriate infection control measures. Moreover, the lack of cross-reactivity with potential interfering substances, including biological matrices such as whole blood, saliva, and urine, as well as with various culture media and commonly used antibiotics, further validates its reliability in diverse clinical scenarios. The ability to perform consistently in the presence of these substances ensures that the test results remain accurate, even under real-world conditions where samples may contain complex mixtures. Since the absence of interference from antibiotics like meropenem, imipenem, doripenem, ertapenem, and ceftriaxone is particularly significant, as these drugs are frequently encountered in patients undergoing treatment for bacterial infections. This feature not only enhances the assay's applicability in clinical microbiology but also ensures that ongoing antimicrobial therapy does not compromise the test's performance. Thus, the demonstrated specificity and resistance to interference make the LFIA a reliable tool for rapid and accurate identification of CPOs. This has important implications for infection control, antimicrobial stewardship, and the optimization of treatment regimens in healthcare settings.

Notably, A key distinguishing feature of the LFIA is its rapid workflow, enabling test completion within 15 minutes. This represents a significant advancement over traditional methods such as PCR and AST, which typically require several hours to days. Furthermore, The LFIA is proved to be cost-efficiency, demanding fewer resources and minimal specialized equipment compared to conventional molecular techniques. These attributes of the LFIA make it particularly valuable for municipal hospitals and community healthcare settings with limited infrastructure. The assay's simplicity, combined with its high diagnostic accuracy and rapid turnaround time, positions it as an ideal tool for clinical microbiology laboratories. Its ability to swiftly identify CPOs facilitates initiation of targeted antimicrobial therapy for

critically ill patients. This reliable and efficient diagnostic method supports the goal of enhancing patient management and reducing the spread of resistant organisms in healthcare facilities.

Nonetheless, limitations remain. The study focused on a defined set of carbapenemase genotypes, with relatively small numbers for certain variants (eg, VIM, OXA-48), which may affect generalizability. Geographic restriction to a single hospital cohort further narrows extrapolation. These constraints parallel those noted in earlier single-center LFIA evaluations and underscore the need for multicenter studies incorporating diverse epidemiological backgrounds. Future research should expand validation to larger, geographically varied cohorts and include emerging resistance variants.

Conclusion

This study demonstrates that the handmade LFIA provides a rapid, precise, and practical tool for detecting carbapenemase-producing organisms, with strong concordance to PCR/NGS and potential value for antimicrobial stewardship and infection control. While its validation was limited to isolates from a single hospital and a small number of certain enzyme types, future multicenter studies including diverse and emerging variants are warranted. For broader implementation, practical considerations such as cost-effectiveness, quality control, staff training, and integration into existing diagnostic workflows should be addressed to maximize its clinical and global impact.

Ethical Approval

All animal experiments conducted for antibody generation were approved by the Institutional Animal Care and Use Committee of Nanjing University of Chinese Medicine and performed in strict accordance with the national guidelines for the care and use of laboratory animals issued by the Ministry of Science and Technology of China. The 26 CPE strains and 105 clinical isolates utilized in this study were sourced from the clinical specimen biobank of the Second Hospital of Nanjing. This study was conducted in accordance with the principles of the Declaration of Helsinki. The research protocol, involving the use of these clinical strains, was reviewed and approved by the Institutional Review Board of the Second Hospital of Nanjing (Approval No.: 2023-LS-ky-039). Written informed consent was obtained from all participants prior to their inclusion in the study.

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Disclosure

The authors declare no conflict of interest.

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