Journal of Clinical Laboratory Analysis

WILEY

RESEARCH ARTICLE OPEN ACCESS

Phenotype Detection and Drug Resistance Analysis of Carbapenem-Resistant Gram-Negative Bacilli

Xiao-min Liu[1] | Ya-xian Wu[1] | Xiao-hui Li[1] | Jia-wen Shang[2] | Wen-na Xu[1] | Nan-song Xu[1] | Qiu-yao Zeng[1] | Xin Zheng[1]

[1]Department of Clinical Laboratory, State Key Laboratory of Oncology in South China, Guangdong Key Laboratory of Nasopharyngeal Carcinoma Diagnosis and Therapy, Guangdong Provincial Clinical Research Center for Cancer, Sun Yat-Sen University Cancer Center, Guangzhou, P. R. China | [2]College of Medical Technology, Guangdong Medical University, Dongguan, Guangdong, China

Correspondence: Xin Zheng (zhengxin_2022@163.com; zhengxin@sysucc.org.cn)

Received: 9 May 2024 | **Revised:** 15 January 2025 | **Accepted:** 14 February 2025

Funding: The authors received no specific funding for this work.

Keywords: carbapenem | clinical evaluation | enzyme-type experiment | gram-negative bacilli

ABSTRACT

Background: The increasing prevalence of infections caused by carbapenem-resistant organisms (CRO) represents a global health issue. Therefore, a rapid and accurate method for detecting these microbes in any clinical microbiology laboratory is crucial for the prevention and control of their transmission, as well as for clinical treatment. This study aimed to evaluate the phenotypic detection methods, PBA-EDTA, mCIM, PCR, and immunocolloidal gold kit for CRO.

Methods: We collected 99 samples from the inpatients in Sun Yat-Sen University Cancer Center (SYSUCC) from March 2019 to February 2022 and classified the drug resistance and genotype of various strains by various enzyme-type experiments.

Results: Out of 99 multidrug-resistant Gram-negative bacilli resistant to carbapenems, 58 (58.59%) were identified as carbapenemase-positive using the mCIM test. The carbapenemase genotypes included 19 NDM strains, 4 KPC strains, 1 IMP strain, and 5 OXA-23 strains. Enzyme detection revealed 21 strains positive for metallo-β-lactamase, 50 for serine-β-lactamase, and 2 positive for both, with a total positive rate of 73.74%. 26 strains were negative for enzyme detection, and mCIM showed limited effectiveness in detecting strains coproducing NDM and KPC. The immunocolloidal gold assay had a sensitivity of 96.9% and specificity of 98.5%.

Conclusion: This study used an immunocolloidal gold kit for carbapenemase detection, providing results within 15 min. This cost-effective method can quickly assist in identifying carbapenemase genotypes and holds potential as a new rapid detection and diagnostic tool for CRE in clinical and lab settings.

1 | Introduction

Gram-negative bacilli frequently contribute to hospital-acquired infections and are often isolated at high rates in clinical samples. Carbapenems, a class of β-lactam antibiotics, have broad-spectrum antibacterial properties and potent activity, making them the treatment of choice for combating multidrug-resistant Gram-negative bacilli [1]. However, it should be noted that the widespread use of carbapenems in recent years has, to a certain extent, caused the irrational application and even abuse of carbapenems in clinical practice [2]. The occurrence of the above clinical medication phenomenon directly leads to the increase of carbapenem-resistant organisms (CRO) year by year, which also brings challenges to the clinical realization of effective anti-infection treatment and reasonable control of nosocomial infection [3, 4]. Relevant studies [5, 6] have demonstrated that

Xiao-min Liu and Ya-xian Wu made equal contributions to the manuscript. They are co first authors.

the mechanisms underlying bacterial resistance to carbapenems encompass the reduction or absence of outer membrane porins, alongside the sustained production of high levels of β-lactamase [7]. This resistance can also arise from the production of carbapenemases, alterations in drug targets, and modifications to penicillin-binding proteins [8]. Carbapenemases can be divided into three categories according to the Ambler molecular classification method: A, B, and D. Class A is serine carbapenemase, mainly KPC type; Class B is metal β-lactamases, mainly NDM, IMP, and VIM. Class D is OXA-48 serine carbapenemases.

Bacterial infections with different enzyme types often require the use of different antimicrobial drugs. For instance, the novel β-lactam antibiotic/β-lactamase inhibitor combination of cefepime-avibactam is primarily used to treat infections caused by carbapenem-resistant bacteria producing KPC and OXA-48-like enzymes [9, 10]. In contrast, carbapenem-resistant bacteria that produce metalloenzymes can be treated with β-lactam antibiotic/β-lactamase inhibitor combinations like aztreonam-avibactam [11]. Therefore, there is an urgent clinical need for a method that can quickly and accurately detect carbapenemase types.

In this study, we focus on the clinical detection results and detection process of CRO commonly used enzyme-type experimental methods, and we used PCR targeting carbapenemase genes as the gold standard to evaluate the performance of the mCIM test, PBA-EDTA method, and immunocolloidal gold kit detection method in detecting common carbapenemase types in CRO.

2 | Materials and Methods

2.1 | Specimen Source

This experiment collected 99 specimens from a population of inpatients in Sun Yat-Sen University Cancer Center (SYSUCC) from March 2019 to February 2022. This included 38 sputum samples, 19 drainage fluid samples, 1 bile sample, 1 alveolar lavage fluid sample, 5 secretion samples, 1 celiac fluid sample, 3 ascites samples, 8 urine samples, 2 pus samples, 2 tracheal secretion samples, 2 incision secretion samples, 6 whole blood samples, 3 wound secretion samples, 1 external auditory canal secretion sample, 3 pleural effusion samples, 1 throat swab sample, and 1 vaginal swab sample. All the experimental samples obtained in this study included 11 strains of *Acinetobacter baumannii*, 3 strains of *Enterobacter aerobacter*, 3 strains of *Klebsiella acidophilus*, 21 strains of *Escherichia coli*, 13 strains of *Klebsiella pneumoniae*, 42 strains of *Pseudomonas aeruginosa*, 4 strains of *Enterobacter cloacae*, 1 strain of *Enterobacter cloacae complex*, and 1 strain of *Enterobacter cloacae subspecies*. Biochemical identification and drug sensitivity tests were performed on all strains, and their drug sensitivity to imipenem, meropenem, and ertapenem was tested. The results were judged according to CLSI 2023 guidelines.

2.2 | Main Reagents and Related Instruments and Equipment

Imipenem cilastatin sodium was purchased from Hangzhou MSD Pharmaceutical Co. LTD. Piperacillin tazobactam sodium was purchased from North China Pharmaceutical Co. LTD. Zinc sulfate heptahydrate, EDTA disodium salt, and phenol red were purchased from Shanghai Shenggong Bioengineering Co. LTD. Multiplex PCR Assay Kit and Premix Taq Version 2.0 Plus Dye were purchased from Bao Bioengineering Co. LTD. DL2000 DNA Marker was purchased from Tiangen Biological Co. Agarose was purchased from GENE Inc., et al. and all reagents were used within the expiry date. Meropenem disk is a British OXOID product with a drug content of 10 μg/tablet. The carbapenemase inhibitor kit was purchased from Zhuhai Deir Bioengineering Co. LTD. It contains EDTA and PBA. The dosage per time is 10 μL/tablet and the specification is 1 mL/bottle. The instruments and equipment involved in the experimental process of this study include an automatic microbial and drug sensitivity analysis system VITEK-2, Bio-Red electrophoresis apparatus, gel imager, 37°C incubator, and pH meter (Sartorius PB-10), etc. Immunocolloidal gold kit Company: Beijing Gold Mountainriver Tech Development Co. Ltd., and Shanghai FOSUN Diagnostics Co. Ltd. English name of the product: CARBAPEM-resistant KPC/NDM/IMP/VIM/OXA-38/OXA-23 Detection K-Set (Lateral Flow Assay).

2.3 | Methods

2.3.1 | mCIM Test and Detection Method

All tests and result interpretation were performed according to the requirements of CLSI M100-S33, 2023. Overnight cultured bacteria on a blood agar plate were collected with a 10 μL inoculation loop and mixed with 400 μL of sterile water. Meropenem, imipenem, and ertapenem disks (10 μg each) were added to the bacterial suspension. Enterobacterales were incubated at 35°C for 2 h, while nonfermentative bacteria were incubated at 35°C for 4 h. A suspension of *Escherichia coli* ATCC 25922 with 0.5 McKelley turbidity unit was spread evenly on M-H agar plates. The antibiotic disks were then removed from the suspension, blotted to remove excess liquid, and placed onto the Mueller-Hinton (M-H) agar plates. The center-to-center distance between each disk should exceed 24 mm, with a minimum of 15 mm from the plate edge. Each 9 cm M-H agar plate can accommodate six disks, and the results can be read after a 6-h incubation at 35°C or overnight. Interpretation of Results: Bacteria producing carbapenemase can hydrolyze the substrate on the disk, which is indicated by unrestricted growth of the ATCC25922 strain and a lack of inhibition zone (i.e., an inhibition zone diameter of 6 mm), resulting in a positive outcome. If the inhibition zone diameter exceeds 6 mm, the result is negative, regardless of the actual size of the zone. *Klebsiella pneumoniae* standard quality control strains ATCC BAA-1705 and ATCC BAA-1706 were used as positive and negative controls, respectively. Meropenem, imipenem, and ertapenem discs soaked in sterile water were used as blank controls.

2.3.2 | PBA-EDTA Experiment Procedure

All tests and result interpretation were performed according to the requirements of CLSI M100-S33, 2023. The tested bacteria were adjusted to 0.5 McKelley turbidity suspension and evenly spread on MH AGAR plates of disk susceptibility test. Each plate was coated with four pieces of meropenem

with different components, which were respectively meropenem (MER, Bacterial resistance control), meropenem plus PBA (10 μL), meropenem plus EDTA (10 μL), and meropenem plus PBA plus EDTA. The cells were incubated at 35°C ± 2°C for 16–18 h in air. The interpretation criteria were as follows: 9 (1). If the diameter of the inhibition zone of the meropenem disc with added PBA solution is ≥ 5 mm greater than that of the single-drug disc, the test strain can be judged to produce Class A carbapenemase; (2). If the diameter of the inhibition zone of the imipenem disc with added EDTA solution is ≥ 5 mm greater than that of the single-drug disc, the test strain can be judged to produce Class B metallo-enzyme; (3). If only the diameter of the inhibition zone of the imipenem disc with both PBA and EDTA added is ≥ 5 mm greater than that of the single-drug disc, the test strain can be judged to produce both Class A carbapenemase and Class B metallo-enzyme simultaneously; (4). If the diameter of the inhibition zone of the imipenem disc with enzyme inhibitor is < 5 mm greater than that of the single-drug disc, the strain can be judged to not produce Class A or B metallo-enzyme, and its resistance mechanism may be due to the production of OXA-48 type carbapenemase, or the production of ESBL enzyme and/or AmpC enzyme combined with the downregulation or absence of porin protein.

2.3.3 | Multiplex PCR Detection Procedure

Total bacterial DNA was extracted by the boiling lysis method as the template, and primers (Table 1) were divided into three groups according to the different sizes of gene fragments for the Multiplex PCR reaction. Reaction system: Multiplex PCR Mix 12.5 μL, Primer Mix appropriate amount, DNA template 1 μL, dH2O supplemented to 25 μL, PCR reaction conditions: Predenaturation, 94°C for 10 min; 36 cycles of denaturation, annealing, and extension were repeated at 94°C for 30 s, 52°C for 40 s, and 72°C for 50 s. The samples were stored at 72°C for 5 min and 4°C. PCR products were electrophoresed on a 2% agarose gel at 100 V for 30 min. The results were observed and photographed under a UV gel electrophoresis imager. ATCC25922 (*Escherichia coli*) was used as a negative control. The primer sequence is shown in Table 1.

2.3.4 | Immunocolloidal Gold Kit Detection Method

The description of the kit operation can be completed according to the kit instruction. Detection steps: The operation steps of the immune colloidal gold kit are as follows: Drop 10 drops of sample treatment solution into the sterile EP tube, dip the disposable inoculum ring into the overnight culture of bacterial colonies, insert it into the sterile EP tube with the sample treatment solution, stir thoroughly to mix it with the solution, take 50 μL of the treated sample and add it to the colloid gold detection box sample adding hole, and read the results after 15 min. If two red bands appear in the detection area of the detection card, the result is positive. Four types of carbapenemases, including OXA-23, IMP, NDM, and KPC, could be recognized by this method.

3 | Results

3.1 | Strains and Drug Resistance

The in vitro susceptibility test results of 99 strains of multidrug-resistant Gram-negative bacilli to carbapenems were as follows: the resistance rate to imipenem was 78.79% (78/99), the intermediary rate was 11.11% (11/99), and the sensitivity rate was 10.10% (10/99). The experimental results showed that the resistance rate of 99 strains of multidrug-resistant Gram-negative bacilli to meropenem was 67.68% (67/99), the intermediary rate was 21.21% (21/99), and the sensitivity rate was 11.11% (11/99). The experimental results showed that the drug resistance rate of 99 strains of multidrug-resistant Gram-negative bacilli to ertapenem was 95.96% (95/99), the intermediary rate was 4.04% (4/99), and the drug resistance rate was 0. 52 strains were resistant to all three drugs. See Table 2 for details.

3.2 | Results of mCIM Test

In this study, the mCIM test identified 58 strains positive, accounting for 58.59% (58/99), and 41 strains were negative, accounting for 41.41% (41/99). The results of carbapenemase genotyping showed 19 NDM strains (2 strains of *Enterobacter*

TABLE 1 | Sequence of the PCR primers.

Name of primer	Sequence of primers	bp
KPC	F:CATTCAAGGGCTTTCTTGCTGC	538
	R:ACGACGGCATAGTCATTTGC	
IMP	F:TTGACACTCCATTTACAG	139
	R:GATCGAGAATTAAGCCACCCT	
VIM	F:GATGGTGTTTGGTCGCATA	390
	R:CGAATGCGCAGCACCAG	
NDM-1	F:GGTTTGGCGATCTGGTTTTC	621
	R:CGGAATGGCTCATCACGATC	
OXA-48	F:GCTTGATCGCCCTCGATT	281
	R:GATTTGCTCCGTGGCCGAAA	

TABLE 2 | Sensitivity of each strain to each drug.

The name of the strain	Imipenem resistance rate	Meropenem resistance rate	Ertapenem resistance rate
Acinetobacter baumann ($n=11$)	10 (90.91)	11 (100.00)	11 (100.00)
Enterobacter aerogens ($n=3$)	3 (100.00)	3 (100.00)	2 (66.67)
Klebsiella acidophilus ($n=3$)	0 (0.00)	2 (66.67)	3 (100.00)
Escherichia coli ($n=21$)	15 (71.43)	19 (90.48)	20 (95.24)
Klebsiella pneumoniae ($n=13$)	8 (61.54)	9 (69.23)	10 (76.92)
Pseudomonas aeruginosa ($n=42$)	39 (92.86)	17 (40.48)	42 (100.00)
Enterobacter cloacae ($n=4$)	3 (75.00)	4 (100.00)	4 (100.00)
Enterobacter cloacae complex ($n=1$)	0 (0.00)	1 (100.00)	1 (100.00)
Subspecies of Enterobacter cloacae ($n=1$)	1 (100.00)	1 (100.00)	1 (100.00)

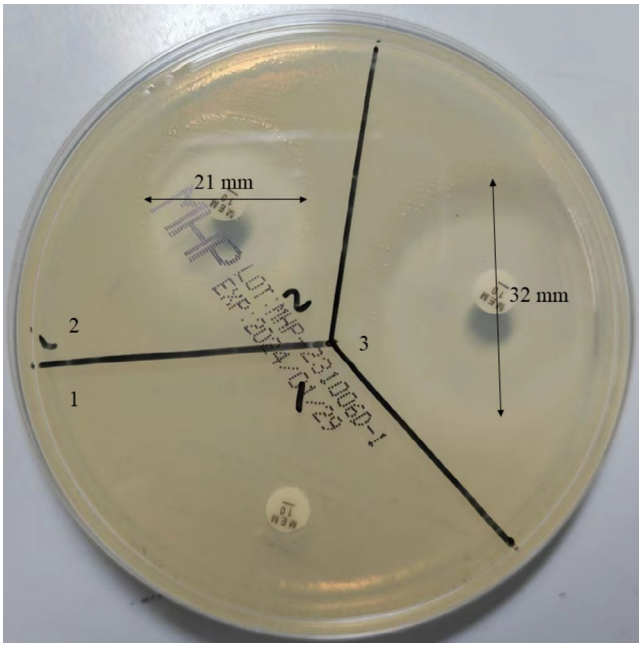

FIGURE 1 | Positive and negative results of mCIM test. 1, The mCIM test is positive, indicating that the isolate produces carbapenemase; 2 and 3, The mCIM test is negative, indicating that the isolate does not produce carbapenemase.

cloacae, 16 strains of *Escherichia coli*, and 1 strain of *Klebsiella pneumoniae*); 4 strains of KPC type (all *Klebsiella pneumoniae*); 1 strain of the IMP-1 type (*Klebsiella acidophilus*), and 5 strains with the OXA-23 genotypes (4 strains of *Acinetobacter baumannii* and 1 strain of *Klebsiella pneumoniae*) were carried. See Figure 1 for details.

3.3 | EDTA Synergistic Test for Enzyme Detection

The results of enzyme detection showed that 21 strains were metallo-β-lactamase (MBL) positive, 50 strains were serinase positive, and 2 strains were metalase + serinase positive, with a total positive rate of 73.74%. The results of enzyme detection showed that 26 strains were negative. mCIM could not effectively detect the strains producing both NDM and KPC carbapenemases. See Figure 2 for details.

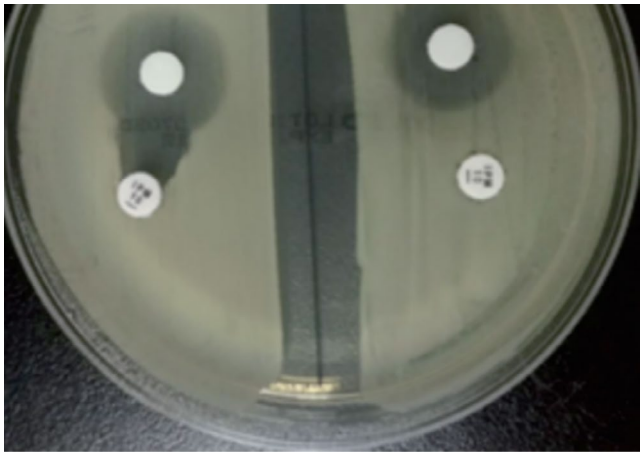

FIGURE 2 | Positive and negative results of EDTA synergy test (left is positive, right is negative).

3.4 | PCR Test Results

In this study, PCR detection showed that 18 strains of NDM (16 strains of *Escherichia coli*, 1 strain of *Klebsiella pneumoniae*, 1 strain of *Enterobacter cloacae*), 3 strains of KPC (all *Klebsiella pneumoniae*), 10 strains of OXA-23 genotype (8 strains of *Acinetobacter baumannii*, 1 strain of *Enterobacter cloacae*, 1 strain of *Klebsiella pneumoniae*), and the other genotypes were not detected. These results suggested that most *Escherichia coli* were NDM type, most *Klebsiella pneumoniae* were KPC type, and most *Acinetobacter baumannii* carried OXA-23 genotype. See Figures 3 and 4 for PCR-related electrophoretic graph.

3.5 | Detection Results of Immune Colloidal Gold Kit

Immune colloidal gold kit showed that 18 strains of NDM (16 strains of *Escherichia coli*, 1 strain of *Klebsiella pneumoniae*, 1 strain of *Enterobacter cloacae*), 4 strains of KPC (all *Klebsiella*

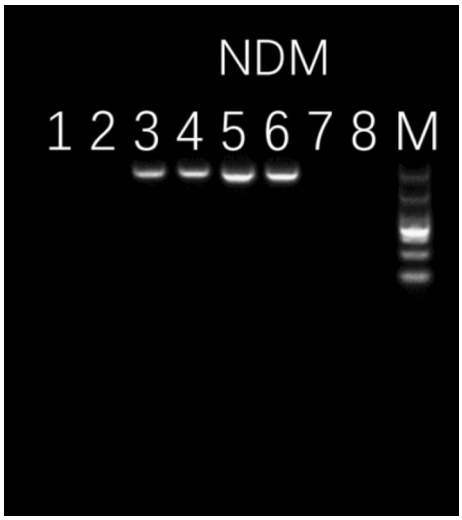

FIGURE 3 | Agarose electrophoretic pattern of some NDM-positive strains. Lane 1-lane 8 are the running results of different patients; 1, 2, and 8 are NDM-negative, and 3–6 are NDM-positive.

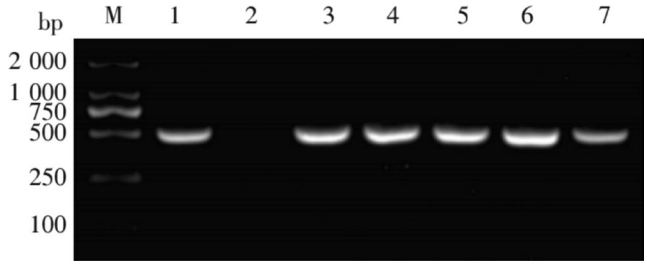

FIGURE 4 | Agarose electrophoretic pattern of some KPC-positive strains. Lane 1–lane 7 are the running results of different patients; 2 is KPC-negative, and the remaining patients are KPC-positive.

pneumoniae), 10 strains of OXA-23 genotype (8 strains of *Acinetobacter baumannii*, 1 strain of *Enterobacter cloacae*, 1 strain of *Klebsiella pneumoniae*), and the other genotypes were not detected (Figure 5). Using PCR as the gold standard, the overall sensitivity and specificity of the immunocolloidal gold kit were 96.9% and 98.5%, the positive predictive value (PPV) was 96.9%, the negative predictive value (NPV) was 98.5%. The sensitivity and specificity of the NDM enzyme kit were 100%. The sensitivity and specificity of the VIM enzyme kit were 100%.

4 | Discussion

Since carbapenem-resistant *Klebsiella pneumoniae* was first reported in the United States in 2001, CRE has spread rapidly worldwide. CRE infections often occur in patients with severe underlying diseases, immune deficiencies, and/or long-term repeated use of broad-spectrum antibiotics, and have a poor prognosis. Especially for patients with CRE bloodstream infections, the mortality rate can be as high as 50% or more. The types of carbapenemase produced vary among different countries, regions, hospitals, populations, and bacteria. The carbapenemases produced by CRE strains isolated clinically in China are mainly of the KPC and NDM types, with a minority of strains producing OXA-48, IMP, and VIM type carbapenemases. KPC-2 is the predominant

FIGURE 5 | Results of immune colloidal gold kit. Colloidal gold assay for OXA-23 carbapenemases positive, carbapenemases negative, and KPC carbapenemases positive, respectively. PCR was used as the gold standard; the sensitivity, specificity, PPV, and NPV statistics of each assay are shown in Table 3. EDTA synergistic test had poor performance in terms of specificity (26.5%) and NPV (34.6%) for carbapenemase detection. This low PPV was associated with the high number of false-positive results: 48 of the nine strains that yielded false-positive results produced serinase. Similarly, mCIM scored low in specificity (53.0%) and PPV (45.8%) for carbapenemase detection. The mCIM is used to detect carbapenemase in Enterobacteriaceae and *Pseudomonas aeruginosa*. Immune colloidal gold kit had the best performance in terms of sensitivity (96.9%), specificity (98.5%), PPV (96.9%), and NPV (34.6%) for carbapenemase detection.

TABLE 3 | Performance of these three methods in comparison with PCR tests.

	mCIM test	EDTA synergistic test	Immune colloidal gold kit
Sensitivity	87.1%	87.1%	96.9%
Specificity	53.0%	26.5%	98.5%
PPV	45.8%	34.6%	96.9%
NPV	90.0%	81.8%	98.5%

subtype of KPC type carbapenemase, while NDM-1 and NDM-5 are the most common subtypes of NDM type metallo-enzyme. OXA-181 and OXA-232 are the most common subtypes of OXA-48 type carbapenemase. Given the varying in vitro antimicrobial activity of different types of antimicrobials against different carbapenem-producing strains, accurate and rapid detection and

typing of carbapenem-producing CRE is of significant value for precision antimicrobial therapy to improve the cure rates in clinical infection treatment and hospital infection prevention.

Currently, methods for detecting carbapenemases in the laboratory can be divided into phenotypic detection and genotypic detection [12]. Phenotypic detection methods include the mCIM and eCIM, carbapenemase inhibitor enhancement tests, and time-of-flight mass spectrometry, among others [13–17]. Genotypic detection methods include PCR, enzyme immunoassays, and other molecular detection techniques. Each method has distinct advantages and limitations. In this study, we focused on the clinical detection results and performance evaluation of commonly used enzyme-type experimental methods, including the mCIM test, the PBA-EDTA synergy test, and the immunocolloidal gold kit, using PCR-based carbapenemase gene detection as the gold standard. Our aim was to assist laboratories at different levels in selecting appropriate detection methods based on their actual circumstances.

The mCIM test, as a widely used phenotypic detection method, is simple to operate, low in cost, and does not require specialized equipment. Its high sensitivity and specificity make it suitable for routine carbapenemase detection in clinical microbiology laboratories at all levels. However, it has limitations. The mCIM test and related enhancement tests, such as the eCIM, require a long incubation time (24–28 h) [16]. Moreover, the eCIM test may yield false-negative results when both the KPC (a serine-β-lactamase) and NDM (a metallo-β-lactamase) genes are co-carried, as it can only detect the presence of KPC in such cases [18, 19]. Despite these challenges, the mCIM test remains a robust and accessible phenotypic method. Previous studies have demonstrated its reliability in distinguishing KPC from NDM carbapenemases when they occur independently, highlighting its utility in clinical microbiology.

The EDTA synergy test is another phenotypic detection method used for identifying metallo-β-lactamase activity. It enhances the detection of metalloenzymes such as NDM by inhibiting their activity with EDTA, a chelating agent for zinc ions. This method has been widely used in studies for its simplicity and effectiveness. However, its performance may be affected by factors such as the concentration of EDTA and the choice of carbapenem used in the test. Future research comparing EDTA synergy test results across different settings and strains could provide further insights into its variability and optimization.

As an efficient amplification technology, PCR is still the gold standard for the detection of drug resistance genotypes and a powerful weapon for the discovery of new molecular phenotypes and drug resistance mechanisms [20]. However, PCR technology also has its limitations. It cannot be generally carried out in daily work due to the need for specialized technology [17], specialized equipment, and high cost. In addition, false positives or false negatives may also occur due to changes in environmental conditions or sample contamination [21]. Despite these drawbacks, PCR serves as an indispensable tool in the molecular epidemiology of CRE.

The colloidal gold-based immunoassay was also used to detect carbapenemase in this study. This method usually only requires a few simple steps, without the need for complex equipment and advanced technical operations. The results can be viewed within 15 min, making it suitable for urgent clinical diagnoses [14, 22]. In this study, the overall sensitivity and specificity of the immune colloidal gold kit were 96.9% and 98.5%, the PPV was 96.9%, the NPV was 98.5%, and the total coincidence rate was 98.9%. These results underscore its potential for rapid identification of carbapenemase genotypes at a relatively low cost. However, its applicability is limited by its design, as specific antibody detection strips must be developed for each type of carbapenemase. This constraint prevents the simultaneous detection of all enzyme types and may result in false negatives if the target gene differs from the designed antigen–antibody system. Additionally, false positives may arise from nonspecific antigen interference, while false negatives can occur due to low bacterial enzyme production, excessively high antigen concentration leading to a hook effect, or postreaction issues.

Rapid tests, including immunochromatographic assays and lateral flow tests, have gained prominence due to their speed and simplicity. A review of studies on these methods highlights their growing role in clinical microbiology, particularly in low-resource settings. Comparing the performance of these methods with traditional phenotypic and genotypic techniques can guide their optimal use in CRE detection [16, 23, 24].

In conclusion, the immune colloidal gold kit was used to detect carbapenemase, which is simpler to operate, and the results can be viewed within 15 min. This method can be used to rapidly assist in the identification of carbapenemase genotypes, and its relative cost is greatly reduced. It is also expected to become a new method for the rapid detection and diagnosis of CRE in clinical and laboratory settings. However, due to the influence of the region and the types of diseases affecting the patients, the diversity of pathogens in this study is relatively limited. This study is a single-center study, and future research should include patients from various regions to enhance the accuracy and comprehensiveness of the findings.

Acknowledgments

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The simulation experiment data used to support the findings of this study are available from the corresponding author upon request.

References

1. A. Adler, N. D. Friedman, and D. Marchaim, "Multidrug-Resistant Gram-Negative Bacilli: Infection Control Implications," *Infectious Disease Clinics of North America* 30, no. 4 (2016): 967–997.

2. P. Nordmann and L. Poirel, "Epidemiology and Diagnostics of Carbapenem Resistance in Gram-Negative Bacteria," *Clinical Infectious Diseases* 69, no. Suppl 7 (2019): S521–S528.

3. L. K. Logan and R. A. Weinstein, "The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menace," *Journal of Infectious Diseases* 215, no. suppl_1 (2017): S28–S36.

4. P. D. Tamma, S. L. Aitken, R. A. Bonomo, A. J. Mathers, D. van Duin, and C. J. Clancy, "Infectious Diseases Society of America Guidance on the Treatment of AmpC β-Lactamase-Producing Enterobacterales, Carbapenem-Resistant Acinetobacter Baumannii, and *Stenotrophomonas maltophilia* Infections," *Clinical Infectious Diseases* 74, no. 12 (2022): 2089–2114.

5. J. Iredell, J. Brown, and K. Tagg, "Antibiotic Resistance in Enterobacteriaceae: Mechanisms and Clinical Implications," *British Medical Journal* 352 (2016): h6420.

6. P. D. Tamma and P. J. Simner, "Phenotypic Detection of Carbapenemase-Producing Organisms From Clinical Isolates," *Journal of Clinical Microbiology* 56, no. 11 (2018): e01140-18.

7. S. Nasrollahian, J. P. Graham, and M. Halaji, "A Review of the Mechanisms That Confer Antibiotic Resistance in Pathotypes of *E. coli*," *Frontiers in Cellular and Infection Microbiology* 14 (2024): 1387497.

8. P. Nordmann, L. Dortet, and L. Poirel, "Carbapenem Resistance in Enterobacteriaceae: Here Is the Storm!," *Trends in Molecular Medicine* 18, no. 5 (2012): 263–272.

9. Y. Wang, J. Wang, R. Wang, and Y. Cai, "Resistance to Ceftazidime-Avibactam and Underlying Mechanisms," *Journal of Global Antimicrobial Resistance* 22 (2020): 18–27.

10. S. Cavallini, I. Unali, A. Bertoncelli, R. Cecchetto, and A. Mazzariol, "Ceftazidime/Avibactam Resistance Is Associated With Different Mechanisms in KPC-Producing *Klebsiella pneumoniae* Strains," *Acta Microbiologica et Immunologica Hungarica* 68, no. 4 (2021): 2021.01626.

11. H. Zou, S.-J. Xiong, Q.-X. Lin, M.-L. Wu, S.-Q. Niu, and S.-F. Huang, "CP-CRE/Non-CP-CRE Stratification and CRE Resistance Mechanism Determination Help in Better Managing CRE Bacteremia Using Ceftazidime-Avibactam and Aztreonam-Avibactam," *Infection and Drug Resistance* 12 (2019): 3017–3027.

12. S. Nasrollahian, M. Halaji, A. Hosseini, et al., "Genetic Diversity, Carbapenem Resistance Genes, and Biofilm Formation in UPEC Isolated From Patients With Catheter-Associated Urinary Tract Infection in North of Iran," *International Journal of Clinical Practice* 2022 (2022): 9520362.

13. X. Hu, C. Wu, B. Situ, et al., "EDTA-K(2) Improves the Detection Sensitivity of SARS-CoV-2 IgM and IgG Antibodies by Chelating Colloidal Gold in the Immunochromatographic Assay," *International Journal of Nanomedicine* 16 (2021): 715–724.

14. P. Wang, X. Xu, L. Liu, et al., "A Colloidal Gold Immunochromatography for the Detection of Flumioxazin Residues in Fruits," *Journal of Food Science* 87, no. 10 (2022): 4538–4547.

15. C. M. Gill, M. J. Lasko, T. E. Asempa, and D. P. Nicolau, "Evaluation of the EDTA-Modified Carbapenem Inactivation Method for Detecting Metallo-β-Lactamase-Producing *Pseudomonas aeruginosa*," *Journal of Clinical Microbiology* 58, no. 6 (2020): e02015-19.

16. Y. M. Tsai, S. Wang, H. C. Chiu, C. Y. Kao, and L. L. Wen, "Combination of Modified Carbapenem Inactivation Method (mCIM) and EDTA-CIM (eCIM) for Phenotypic Detection of Carbapenemase-Producing Enterobacteriaceae," *BMC Microbiology* 20, no. 1 (2020): 315.

17. Z. Zhang, D. Wang, Y. Li, Y. Liu, and X. Qin, "Comparison of the Performance of Phenotypic Methods for the Detection of Carbapenem-Resistant Enterobacteriaceae (CRE) in Clinical Practice," *Frontiers in Cellular and Infection Microbiology* 12 (2022): 849564.

18. A. Crowe, L. Brenton, M. Kingston, D. Jardine, and M. J. Waters, "Comparison of the Carbapenem Inactivation Method (CIM) and Modified Carbapenem Inactivation Method (mCIM) for the Detection of Carbapenemase-Producing Organisms," *Pathology* 50, no. 7 (2018): 764–766.

19. J. C. Howard, J. Creighton, R. Ikram, and A. M. Werno, "Comparison of the Performance of Three Variations of the Carbapenem Inactivation Method (CIM, Modified CIM [mCIM] and In-House Method (iCIM)) for the Detection of Carbapenemase-Producing Enterobacterales and Non-Fermenters," *Journal of Global Antimicrobial Resistance* 21 (2020): 78–82.

20. S. Dutka-Malen, S. Evers, and P. Courvalin, "Detection of Glycopeptide Resistance Genotypes and Identification to the Species Level of Clinically Relevant Enterococci by PCR," *Journal of Clinical Microbiology* 33, no. 1 (1995): 24–27.

21. X. Chen, J. He, J. Liu, Y. You, L. Yuan, and Y. Wen, "Nested PCR and the TaqMan SNP Genotyping Assay Enhanced the Sensitivity of Drug Resistance Testing of *Mycobacterium leprae* Using Clinical Specimens of Leprosy Patients," *PLoS Neglected Tropical Diseases* 13, no. 12 (2019): e0007946.

22. B. Ang, X. Xu, L. Xu, L. Liu, H. Kuang, and C. Xu, "Development of a Colloidal Gold Strip Assay for the Detection of Total Homocysteine in Serum Samples," *Analyst* 147, no. 19 (2022): 4348–4355.

23. Q. Liao, Y. Xie, C. Wang, et al., "Development and Evaluation of the Method for Detecting Metallo-Carbapenemases Among Carbapenemase-Producing Enterobacteriaceae," *Journal of Microbiological Methods* 163 (2019): 105652.

24. S. Liu, X. Wang, J. Ge, et al., "Analysis of Carbapenemase-Resistant Genotypes of Highly Virulent *Klebsiella pneumoniae* and Clinical Infection Characteristics of Different MLST Types," *Evidence-Based Complementary and Alternative Medicine* 2021 (2021): 3455121.