

Methodological Evaluation of Carbapenemase Detection by Different Methods

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Abstract

The global proliferation of carbapenemase-producing bacteria (CPB) has garnered significant attention worldwide. Early diagnosis of CPB and accurate identification of carbapenemases are crucial for preventing the spread of CPB and ensuring targeted antibiotic therapy. Therefore, efficient and accurate identification of carbapenemases is paramount in clinically treating diseases associated with CPB. In this study, 58 CPB strains were collected and detected using the DNA endonuclease-targeted CRISPR trans reporter (DETECTR) method, a rapid detection platform based on CRISPR-Cas12a gene editing and isothermal amplification. Additionally, four conventional methods (the APB/EDTA method, PCR, NG-test Carba 5, and GeneXpert Carba-R) were employed and compared against whole genome sequencing (WGS) results, considered the gold standard, to evaluate their efficacy in detecting carbapenemases. Detection by the APB/EDTA method revealed that 29 strains were positive for Class A serine endopeptidases, while 29 strains were positive for Class B metalloenzymes. The classification of these zymotypes was consistent with the sequencing result. All target carbapenemases for KPC were identified with 100% sensitivity using NG-test Carba 5, PCR, DETECTR, and GeneXpert Carba-R. In the case of NDM, both Xpert Carba-R and DETECTR showed a sensitivity of 100%. In contrast, NG-test Carba 5 and PCR had a slightly lower sensitivity of 96.7%, each missing one target carbapenemase. In this study, the APB/EDTA method is capable of identifying the zymotype classification but not the specific resistant genes, while Xpert Carba-R and DETECTR are able to detect all target carbapenemases.

Key words: rapid detection; WSG, DETECTR, methodological evaluation

Introduction

Antibiotic resistance has imposed a significant strain on the global healthcare system. As per a predictive statistical model (Antimicrobial Resistance Collaborators 2022), it is projected that in 2019, there were approximately 4.95 million deaths linked to bacterial resistance, with 1.27 million attributed directly to bacterial resistance. Antimicrobial resistance (AMR) is a global scourge that threatens advancements in numerous medical domains. Carbapenem-resistant Enterobacterales (CRE), as a subset of multi-drug resistant organisms (MDRO), are especially concerning due to their limited therapeutic alternatives and potential for community transmission (Wang et al. 2022). In 2019, the Centers for Disease Control and Prevention (CDC) in the United States classified CRE as a critical antibiotic-resistant bacteria threat. The 2024 iteration of the World Health

Organization's "Priority Pathogens List" spotlights the principal threats to public health. With this revision, informed by evidence and expert deliberation, the list prioritizes the likes of carbapenem-resistant *Klebsiella pneumoniae*, third-generation cephalosporin-resistant *Escherichia coli*, carbapenem-resistant *Acinetobacter baumannii*, and rifampicin-resistant *Mycobacterium tuberculosis*, with a pronounced focus on the critical issue of CRE. The document advocates for pioneering strategies to counter the advancing menace of antibiotic resistance. CRE ranks among the top three pathogens requiring urgent development of new antibiotics.

Carbapenem-resistant drugs exhibit a broad antibacterial spectrum, potent antibacterial activity, and high stability against bacterial-produced β -lactamase enzymes. Therefore, carbapenem-resistant drugs have been extensively utilized for treating severe infections caused by multidrug-resistant strains, particularly for

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Gram-negative bacilli infections involving extended-spectrum β -lactamases (ESBLs) and sustained high production of Class C cephalosporinases (AmpCases). In clinical settings, these are crucial and potent tools for treating multi-drug-resistant Gram-negative bacilli and *Pseudomonas aeruginosa* infections, playing a vital role in anti-infective therapy. Nevertheless, the escalating utilization of these antibiotics in clinical settings has led to a yearly rise in drug-resistant strains. Before 2021, nearly 99.9% of global clinical *Enterobacteriaceae* strains were susceptible to carbapenem. The prevalence of CRE infections in European countries was 1.3 cases per 10,000 individuals during 2013–2014, while in China, it was 4 cases per 100,000 individuals in 2015. By 2020, the CRE infection rate in China had surged by over 60% compared to the rates recorded in 2015 (Zhang et al. 2018; Zong et al. 2021). These “super bacteria” are frequently challenging to treat and are associated with high mortality rates.

The primary mechanism of carbon CRE resistance lies in the production of carbapenemases (Reygaert 2018), mainly Ambler Classes A, B, and D enzymes (metallo- β -lactamases (MBLs)). Class A enzymes possess a serine structure in their active center, making them susceptible to inhibition by boronic acid compounds but not by EDTA; this class mainly includes KPC enzymes. On the other hand, Class B enzymes are metalloenzymes that can be inhibited by EDTA but not by boronic acid compounds; notable examples include NDM, IMP, and VIM enzymes. Class D enzymes primarily comprise OXA carbapenemases, such as OXA-23 and OXA-48 (Ma et al. 2023). Following the initial detection of *A. baumannii* harboring *bla*_{NDM} in 2011 (Chen et al. 2011), subsequent detections of *bla*_{NDM} have occurred in clinical strains of *K. pneumoniae*, *Klebsiella oxytoca*, *E. coli*, *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Citrobacter*, distributed across various cities or regions in China. The types of resistant genes carried by CRE strains vary significantly from one region to another, with IMP, VIM, and OXA-48 being detected at a lower frequency in China. *bla*_{KPC-2} and *bla*_{NDM} are the pivotal carbapenemase genes responsible for driving the emergence of carbapenem-resistant phenotypes in Chinese CRE strains. In China, clinical CRE carrying *bla*_{NDM} and *bla*_{KPC-2} genes undergo horizontal transmission, involving the transfer of the core structure of the mobile genetic element (Zhang et al. 2017). These genes exhibit distinct transcriptional responses to various carbapenems, leading to varying resistance levels (Diene and Rolain 2014; Ma et al. 2023).

The prompt and precise identification of carbapenemase enzymes is paramount for the adept stewardship of clinical isolates from carbapenemase-producing *Enterobacteriaceae* (CPE), and for bolstering hospital efforts in infection prevention and control. Despite the

scarcity of therapeutic options for CPE, the pharmaceutical landscape has been invigorated by the advent of novel drugs that leverage the properties of inhibitors, exemplified by ceftazidime/avibactam and aztreonam/avibactam. When synergistically combined, these agents have demonstrated their potency in a cohort of 47 NDM and 5 VIM-producing strains, effectively neutralizing the threat posed by NDM and VIM-type metallo- β -lactamases (Falcone et al. 2021). The clinical and microbiological synergy observed with the concurrent administration of ceftazidime/avibactam (CZA) and aztreonam (AT) in treating KP-NDM infections underscores a safe and efficacious therapeutic strategy. This dual-drug approach presents a beacon of hope for managing CPE infections, offering a compelling alternative in an otherwise limited therapeutic arsenal (Guzek et al. 2024). It is noteworthy that while ceftazidime/avibactam effectively counters KPC-type serine β -lactamases, its impact on strains producing metallo- β -lactamases is considerably less pronounced. In addition, meropenem-vaborbactam has shown robust antimicrobial efficacy against strains harboring KPC enzymes, yet it falls short in its activity against MBL and OXA-48 carbapenemase-producing strains (Yahav et al. 2020). Therefore, rapid and specific detection of carbapenemases is crucial for guiding treatment decisions and improving clinical outcomes. This aids in therapeutic efficacy, reducing mortality rates but also helps alleviate the significant economic burden associated with managing such infections (Katsiari et al. 2015; Zhen et al. 2020).

Several methods have been devised to achieve accurate and swift detection of carbapenemases. These include the Nitro Speed-Carba NP test, which relies on biochemical analysis, the lateral flow immunochromatographic method NG-test Carba 5, and the qRT-PCR-based Xpert Carba-R test. In this study, 58 CPB strains were collected and detected using the DNA endonuclease-targeted CRISPR trans reporter (DETECTR) method, a rapid detection platform based on CRISPR-Cas12a gene editing and isothermal amplification (Xu et al. 2022). Additionally, four conventional methods (the APB/EDTA method, PCR, NG-test Carba 5, and GeneXpert Carba-R) were employed and compared against whole genome sequencing (WGS) results, considered the gold standard, to evaluate their efficacy in detecting carbapenemases.

Experimental

Materials and Methods

Clinical strains. A total of 58 CPB strains were isolated from sputum, urine, abdominal fluid, and cerebrospinal fluid samples obtained by the Hefei Public

Table I
Oligonucleotide sequences used in this study.

Primer name	Sequence (5'–3')	Size of product (bp)	References
KPC-F	ATGTCACTGTATCGCCGTCT	918	This study
KPC-R	TTACTGCCCCGTTGACGC		
NDM-F	ATGGAATTGCCCAATATTATGC	813	This study
NDM-R	TCAGCGCAGCTTGTCGG		

Health Clinical Centre between January 2021 and December 2022. These strains included 9 *Escherichia coli* strains and 49 *K. pneumoniae* strains. The strains were inoculated onto Columbia blood agar (bioMérieux, France) and incubated overnight at 35°C in an environment containing 5% CO₂. Species identification was performed using MALDI-TOF/MS (Bruker, Germany), and WGS was performed using Illumina® (Illumina, Inc., USA) and Nanopore sequencing technologies (Oxford Nanopore Technologies plc., UK) to identify species and detect resistance genes accurately. *E. coli* ATCC® 25922™ was included in the assay to assess and ensure quality control.

Antibacterial test. The drug sensitivity spectra of all strains were determined using the Clinical and Laboratory Standards Institute (CLSI) Minimum Inhibitory Concentration (MIC) method (CLSI 2020). The MIC values of the 58 strains were measured using the BD Phoenix™ M50 automated microbiological system (Becton, Dickinson and Company, USA).

Testing method. Isolates of CRKP and CRE were selected for WGS. The APB/EDTA method was employed as one phenotyping technique. The drug-resistant zymotype of the 58 strains was repeatedly assessed using PCR, NG-test Carba 5, GeneXpert Carba-R, and DETECTR methods.

APB/EDTA method. The paper diffusion method recommended by CLSI was performed as follows: the bacteria to be tested were adjusted to a 0.5 McFarland bacterial suspension and evenly spread on Muller-Hinton agar (MHA). Combination disc tests were carried out using imipenem alone, imipenem + 300 mg of APB or 292 mg of EDTA, and imipenem + 300 mg of APB + 292 mg of EDTA. The diameter of the inhibition zones on the paper discs was measured after overnight incubation. The results were interpreted as follows: (1) if the difference between the diameter of the inhibition zone of the imipenem paper with APB solution and that of the single-drug paper was ≥ 5 mm, it could be concluded that the tested strain produced Class A carbapenemases; (2) if the difference between the diameter of the inhibition zone of the imipenem paper with EDTA solution and that of the single-drug paper was ≥ 5 mm, it could be inferred that the tested strain produced Class B carbapenemases; and (3) if

the difference between the diameter of the inhibition zone of the imipenem paper with APB and EDTA solution and that of the single-drug paper was ≥ 5 mm, it could be inferred that the tested strain produced both Class A carbapenemases and Class B β-lactamases (Doi et al. 2008).

Detection by PCR. The KPC and NDM genes were retrieved from the GenBank NCBI database and employed as references to design the most appropriate primers for PCR amplification. Primer design was carried out using the Primer Explorer V5 website for KPC gene primers and Primer 5.0 for NDM gene primers. According to the reaction system (19 µl of ddH₂O, 2 µl of Tap PCR Master Mix, 2 µl of PrimeF/R (10 µmol/l), and 2 µl of DAN template), the samples were added to carry out the reaction program (pre-denaturation at 94°C × 4 min, 1 cycle; deformation at 94°C × 30 s; annealing at 55°C × 30 s; and extension at 72°C × 1 min, with a total of 30 cycles). Following amplification, the PCR products were subjected to agarose gel electrophoresis (Table I).

NG-test Carba 5. CPB bacteria were detected using the NG-test Carba 5 method (NG Biotech, France). Briefly, 1 ml of the bacterial loop was mixed with five drops of Carba-5 extraction buffer. Subsequently, 100 ml of the resulting mixture was spun and transferred to a Carba-5 box, where it was incubated for 15 min to evaluate the results.

GeneXpert Carba-R. The qRT-PCR-based GeneXpert Carba-R detection (Cepheid Inc., USA) method was used for the rapid detection of five key carbapenemases (KPC, IMP, NDM, VIM, and OXA-48-like). A 0.5 McFarland suspension was prepared using a pure culture colony, and 10 µl of this suspension was mixed with 5 ml of the sample and spun for 10 s. Subsequently, 1.7 ml of the resulting mixture was added to the sample wells of the Xpert Carba-R detection kit and processed on the GeneXpert system.

DETECTR. The DETECTR is a rapid detection platform based on CRISPR-Cas12a gene editing and isothermal amplification (Xu et al. 2022). Exponential nucleic acid amplification was achieved under a constant temperature of 37°C, facilitated by the involvement of various enzymes and single-strand binding (SSB) proteins. In this process, recombinase polymerase

amplification (RPA) was utilized to amplify the carbapenemase-resistant gene. Subsequently, the homologous target sequences and the single-stranded DNA (ssDNA) reporter gene were simultaneously cleaved by CRISPR-Cas12a. The ssDNA probes labeled with FAM fluorescein and biotin were captured by immunochromatographic strips, leading to visible signals that could be observed with the naked eye on the test strips. The results could be interpreted in various ways. In addition to reading the signals on lateral flow strips (LFS), signal peaks observed after fluorescence quantitative amplification could also be utilized for analysis. Briefly, 1 ml of the bacteria to be tested was heated (100°C, 5 min) and centrifuged for 10 min at 12,000×g. The resulting supernatant was used as a template. Samples were added according to the reaction system (4 µl of ddH₂O, 29.5 µl of BufferA, 2.4 µl of PrimeF/R (10 µmol/l), and 9.2 µl of DAN template). The template was added in the last step. After mixing thoroughly, 2.5 µl of 280 mM magnesium acetate (MgOAc) was added to the lid of the reaction tube of each sample. The reaction was carried out at 37°C for 15 min. The amplification products were added to the reaction system with a pre-mixed mixture of crRNA and Cas12a proteins. Following fluorescence quantitative proliferation, the signal peak displayed an S-shaped curve, indicating successful gene proliferation (Table II).

Results

Antibacterial test. A single carbapenemase gene was identified in all 58 strains, containing two carbapenemase-coding genes: *bla*_{KPC-2} and NDM-1. Species identification and drug sensitivity analyses of the 58 strains were performed using MALDI-TOF and the BD Phoenix™ M50 automated microbiological system. As indicated, 49 (84.4%) strains were carbapenem-resistant *K. pneumoniae* (CRKP), and nine (15.6%) strains were carbapenem-resistant *E. coli* (CRE). All strains were resistant to ertapenem and cefepime, and 57 strains (98.2%) were resistant to imipenem and meropenem. Some strains were resistant to amikacin (22/58, 37.9%), cefazolin (57/58, 98.2%), ceftazidime/avibactam (38/58, 65.5%), aztreonam (46/58, 79.3%), amoxicillin-clavulanic acid (57/58, 98.2%), colistin (1/58, 1.8%), levofloxacin (47/58, 81.0%), and tigecycline (7/58, 12.0%). Next-generation sequencing of these strains indicated that KPC-2 and NDM-1 accounted for 50% (29/58) of the cases (Table III).

Five methods for detecting differences in carbapenemase-resistant zymotype. *Detection by the APB/EDTA method.* When compared to imipenem paper used alone, strains exhibiting a 5 mm or more increase in the diameter of the inhibitory zone when APB and/or EDTA were added were considered carbapenemase-positive (Fig. 1).

Table II
Oligonucleotide sequences used in this study.

Primer name	Sequence (5'–3')	Size of product (bp)	References
KPC-F	ATCTCGGAAAAATATCTGACAACAGGCATGACGGTG	309	[14]
KPC-R	CGGTCGTGTTTCCCTTTAGCCAATCAACAAAAGTGCT		
NDM-F	TCGCACCGAATGTCTGGCAGCACACTTCCTAT	278	[14]
NDM-R	GTTCGACAACGCATTGGCATAAGTCGCAATCC		

Table III
Carbapenemase-resistant spectra of the 58 strains.

Antimicrobial	S	I	R
Amikacin	36	–	22
Ertapenem	–	–	58
Imipenem	1	–	57
Meropenem	–	1	57
Cefazolin	–	1	57
Ceftazidime and avibactam	20	–	38
Cefepime	–	–	58
Aztreonam	12	–	46
Amoxicillin and clavulanate potassium	–	1	57
Polymyxin	57	–	1
Levofloxacin	2	9	47
Tigecycline	50	1	7

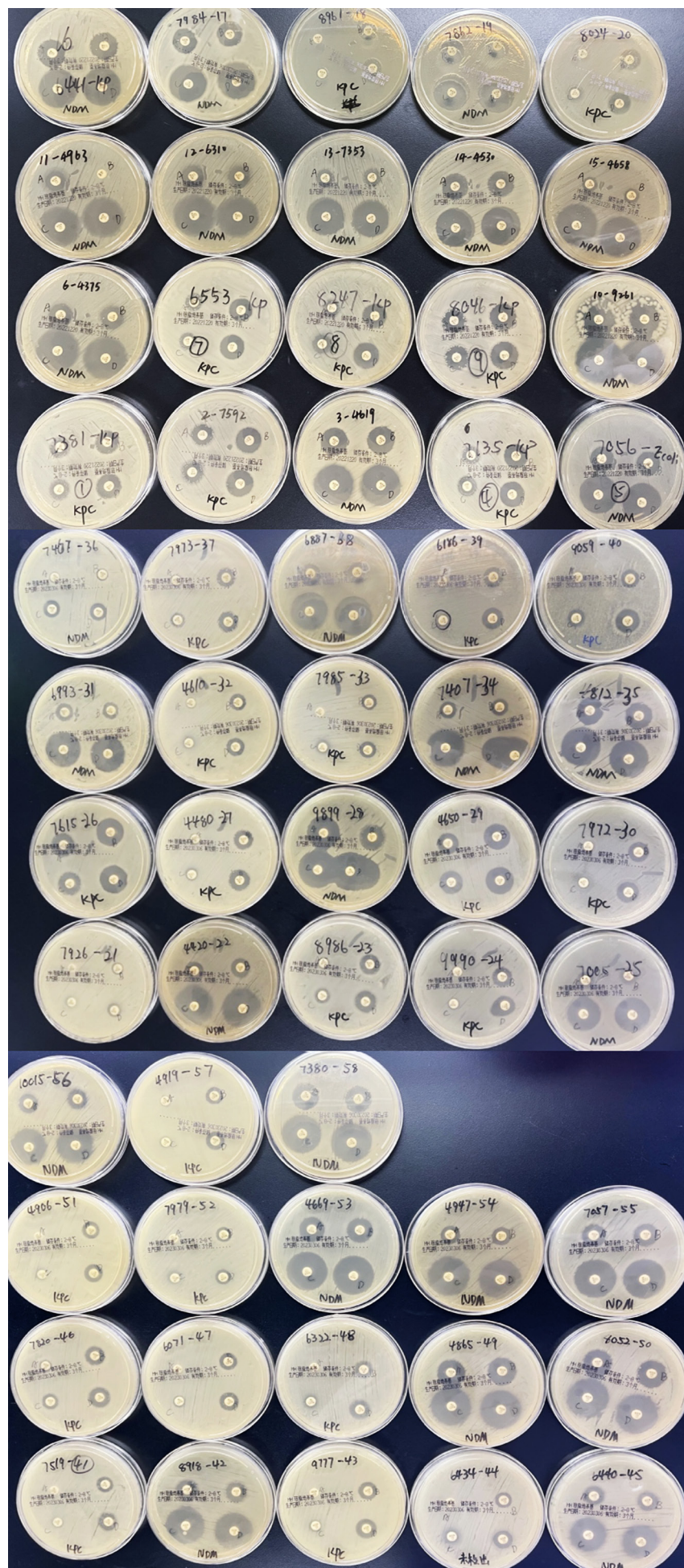


Fig. 1. Apb/EDTA method for the detection of carbapenemase

Herein, 29 strains produced Class A serine endopeptidases and 29 strains produced Class B metalloenzymes. The classification and sequencing results of the zymotype were consistent (Fig. 1).

Detection by PCR. PCR is commonly employed in general laboratories for pathogen detection but is seldom utilized for zymotype detection. Specific primers targeting KPC and NDM genes were designed, and subsequently, the PCR-amplified products were subjected to agarose gel electrophoresis. The electrophore-

sis results revealed successful amplification of 29 KPC and 28 NDM genes. Notably, NDM was not detected in sample 36 (Fig. 2).

Detection by NG-test Carba 5. It is the quickest colony-based detection method utilizing antigen-antibody immunochromatography technology. Currently, the test strip can detect up to five carbapenemases simultaneously. The outcomes from this method agreed with the PCR results; detecting 29 strains of KPC, 28 strains of NDM, and NDM was absent in sample 36 (Fig. 3).

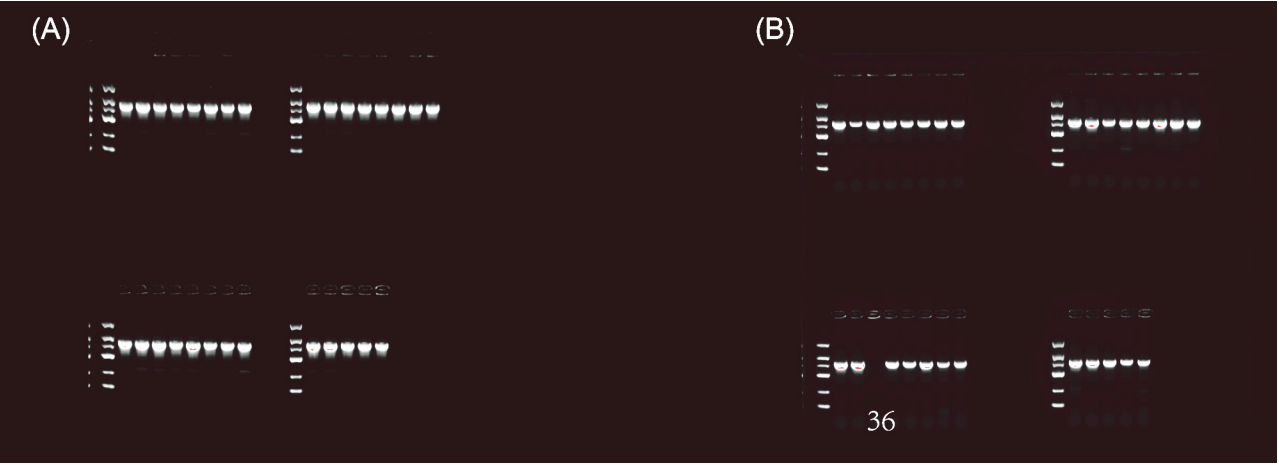


Fig. 2. A) Electrophoretic bands showed positive KPC; B) electrophoretic bands showed positive NDM

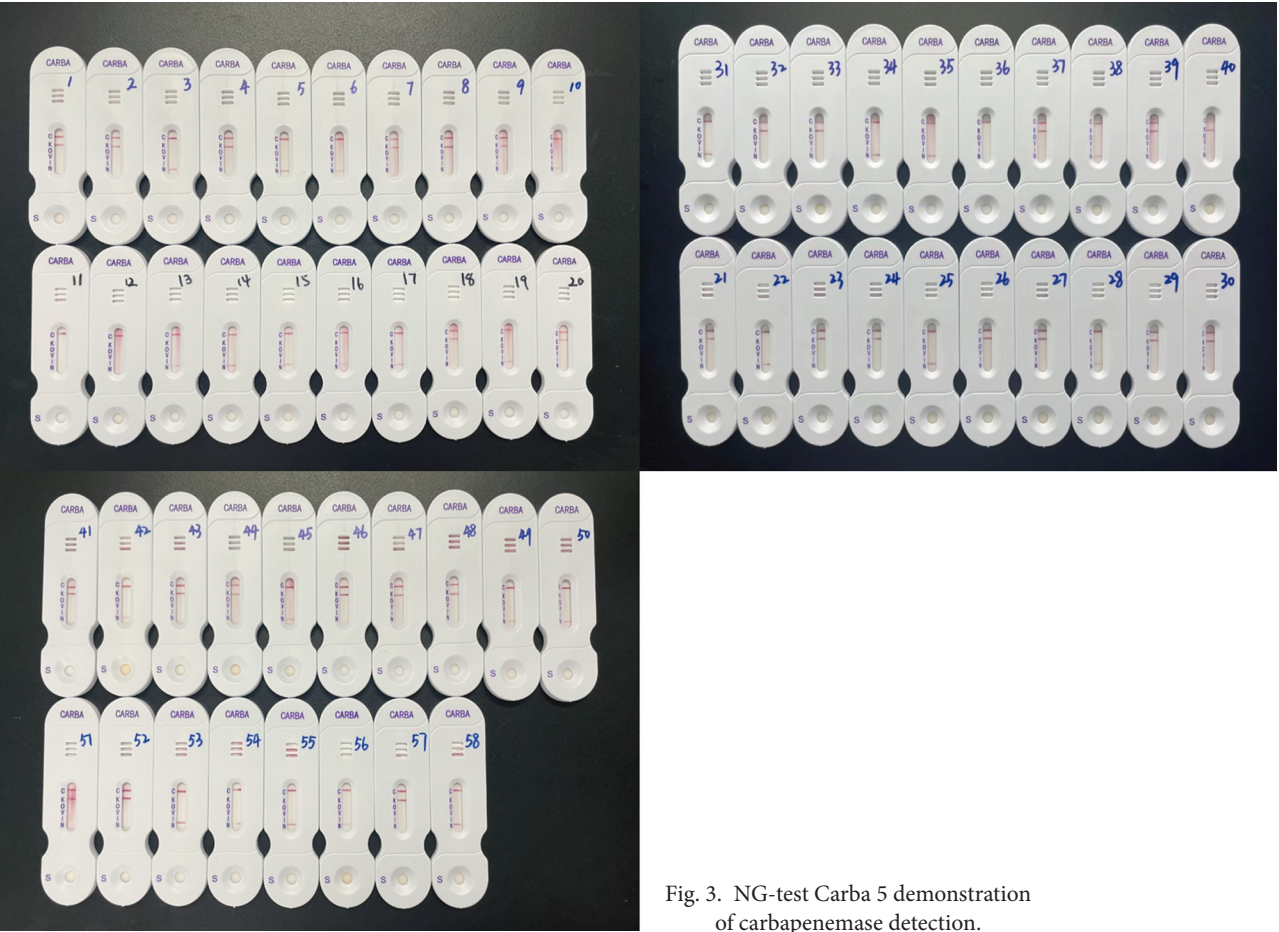


Fig. 3. NG-test Carba 5 demonstration of carbapenemase detection.

Table IV
Xpert Carba-R assay results by target carbapenemase genes.

Xpert Carba-R assay results	Specimens (n = 58)
KPC	29
IMP	0
NDM	29
OXA-48	0
VIM	0

Detection by GeneXpert Carba-R. Similar to traditional molecular diagnostic methods, GeneXpert Carba-R utilized fluorescence quantitative PCR to detect the five key carbapenemase genes. Our results showed that all genes, including NDM, were successfully detected in sample 36 (Table IV).

Detection by DETECTR. Sample DNA was extracted for RT-LAMP amplification, and the results (A) represented the signal peak of the corresponding KPC and NDM genes after fluorescence quantitative proliferation. The presence of an S-shaped curve indicated successful amplification of the genes, while negative samples did not exhibit any signal peak. The results indicated complete detection of all genes, with 29 strains testing positive for KPC and 29 strains testing positive for NDM, aligning with the findings from the GeneXpert Carba-R assay (Fig. 4).

All four methods (NG-test Carba 5, PCR, DETECTR, and GeneXpert Carba-R) accurately detected all target

carbapenemases for KPC. However, for NDM, both NG-test Carba 5 and PCR missed the detection of one target carbapenemase in specimen No. 36 (Fig. 5). This occurrence could be attributed to the impact of bacterial mucus during sample extraction, leading to false-negative results. Based on the WGS results, the APB/EDTA method, DETECTR, and GeneXpert Carba-R exhibited 100% accuracy and specificity. On the other hand, the NG-test Carba 5 and PCR showed an accuracy rate of 98%. The APB/EDTA method is proficient in identifying the zymotype classification but does not pinpoint specific resistant genes (Table V).

Discussion

The global proliferation of CPB has garnered significant attention worldwide. Early diagnosis of CPB and accurate identification of carbapenemases are crucial for preventing the spread of CPB and ensuring targeted antibiotic therapy (Khalifa et al. 2019). Therefore, early diagnosis and a precise antibiotic treatment regimen are essential for the rapid and accurate detection of CPB. This study assessed the effectiveness of five primary CRE detection techniques: a phenotypic approach (the APB/EDTA method), PCR, NG-test Carba 5, GeneXpert Carba-R, and the DETECTR detection method. This report represents the first assessment of DETECTR's ability to detect carbapenemases, analyzing five detection techniques. Significantly, it compares

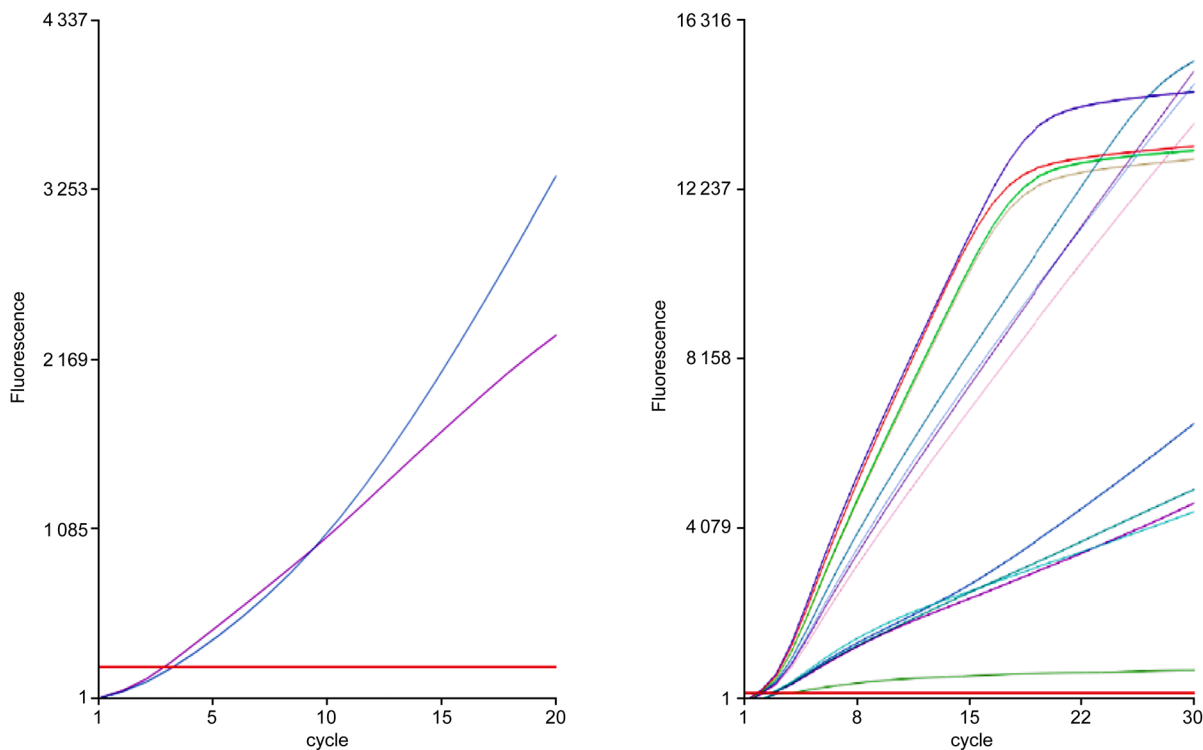


Fig. 4. A represents the fluorescence diagram of DETECTR for pre-experimental detection of KPC gene NDM gene amplification, and B represents the partial fluorescence display diagram of carbapenemase detection with DETECTR.

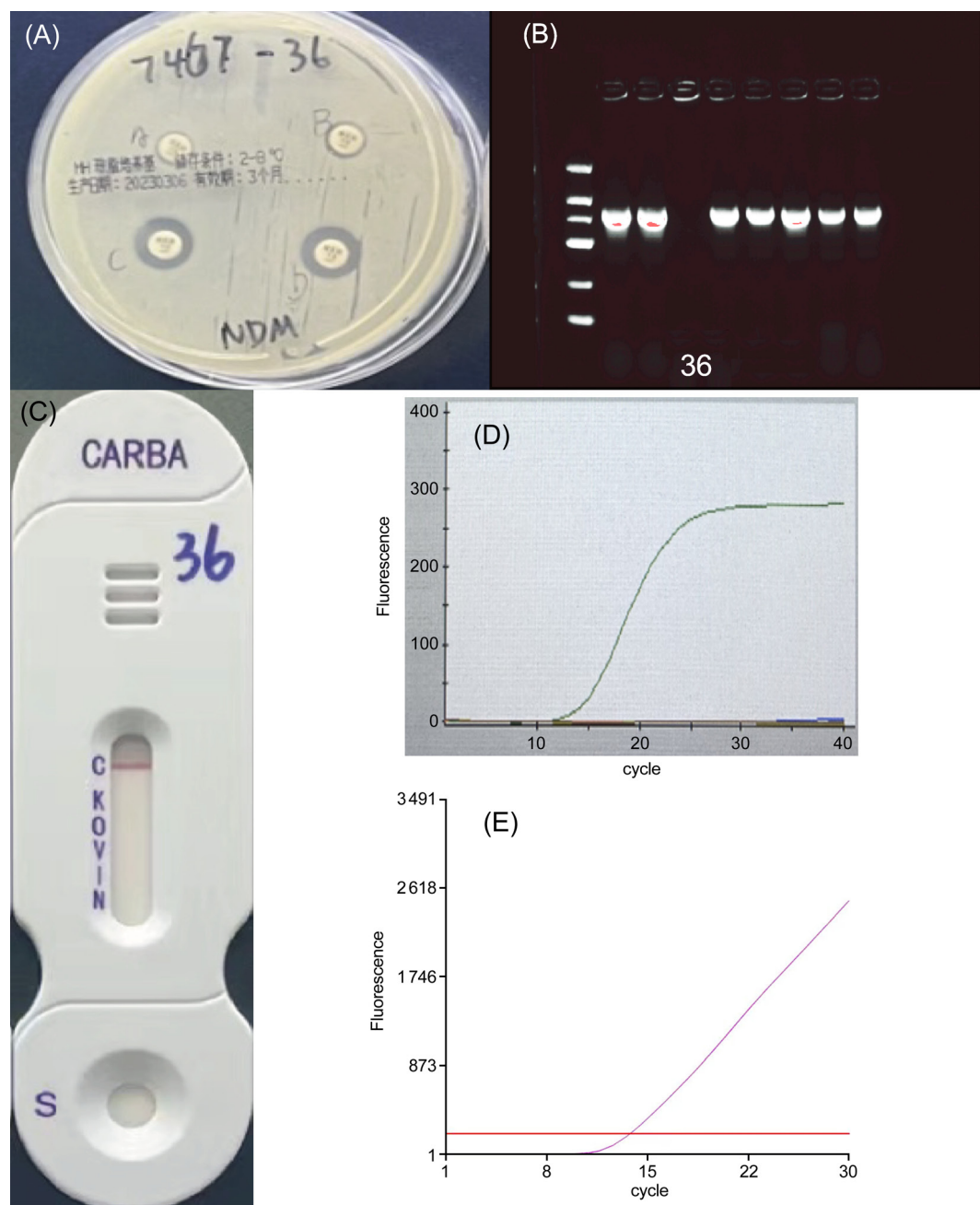


Fig. 5. Characterization of five carbapenemase methods for detecting specimen No. 36.

A) mAPB/EDTA method (panel A) ertapenem (10 mg), 6 mm; (panel B) ertapenem plus APB (300 mg), 6 mm; (panel C) ertapenem plus EDTA (292 mg), ≥ 11 mm; (panel D) ertapenem plus APB and EDTA, ≥ 11 mm; judged as carbapenemase category B positive. B) PCR: NDM negative. C) NG-test Carba 5: NDM negative. D) GeneXpert Carba-R: NDM positive. E) DETECTR: NDM positive.

DETECTR and the APB/EDTA method alongside PCR, NG-test Carba 5, and GeneXpert Carba-R, marking a new milestone in evaluation methodologies.

The APB/EDTA method, GeneXpert Carba-R, and DETECTR have widely employed techniques for zymotype detection in clinical settings. The underlying principle of the APB/EDTA method is that APB and EDTA can inhibit the activities of Class A serine carbapenemases and Class B β-lactamases, respectively. Although this approach relies on straightforward reagents, its intricate procedure and extended processing time hinder its practical use in clinical laboratories. The phenotypic

method, exemplified by the APB/EDTA approach, is capable of initially determining the classification of the resistant gene but cannot detect the specific zymotype (Gu et al. 2023). It has been demonstrated that NG-test Carba 5 and GeneXpert Carba-R can reduce the detection time to less than 2 h. NG-test Carba 5 is a widely utilized method for zymotype detection in laboratory settings, and this aligns with the findings of a previous study (Oueslati et al. 2020), which reported a sensitivity of 100% for NG-test Carba 5 in detecting KPC enzymes with carbapenemase activity. In this study, the NG-test Carba 5 failed to detect an NDM carbapenemase. This

Table V
Results of carbapenemase detection using five assays.

No.	WGS	APB/EDTA	NG-test Carba 5	PCR	DETECTR	gene- Xpert
1-7381-kp	KPC-2	category A	KPC	KPC	KPC	KPC
2-7592-kp	KPC-2	category A	KPC	KPC	KPC	KPC
3-4619-kp	NDM-1	category B	NDM	NDM	NDM	NDM
4-7135-kp	KPC-2	category A	KPC	KPC	KPC	KPC
5-7056- <i>E. coli</i>	NDM-1	category B	NDM	NDM	NDM	NDM
6-4375-kp	NDM-1	category B	NDM	NDM	NDM	NDM
7-6553-kp	KPC-2	category A	KPC	KPC	KPC	KPC
8-8247-kp	KPC-2	category A	KPC	KPC	KPC	KPC
9-8046-kp	KPC-2	category A	KPC	KPC	KPC	KPC
10-9261-kp	NDM-1	category B	NDM	NDM	NDM	NDM
11-4963-kp	NDM-1	category B	NDM	NDM	NDM	NDM
12-6310-kp	NDM-1	category B	NDM	NDM	NDM	NDM
13-7353-kp	NDM-1	category B	NDM	NDM	NDM	NDM
14-4530-kp	NDM-1	category B	NDM	NDM	NDM	NDM
15-4658-kp	NDM-1	category B	NDM	NDM	NDM	NDM
16-6441-kp	NDM-1	category B	NDM	NDM	NDM	NDM
17-7984- <i>E. coli</i>	NDM-1	category B	NDM	NDM	NDM	NDM
18-8961-kp	KPC-2	category A	KPC	KPC	KPC	KPC
19-7862- <i>E. coli</i>	NDM-1	category B	NDM	NDM	NDM	NDM
20-8024-kp	KPC-2	category A	KPC	KPC	KPC	KPC
21-7926-kp	KPC-2	category A	KPC	KPC	KPC	KPC
22-4420-kp	NDM-1	category B	NDM	NDM	NDM	NDM
23-8986-kp	KPC-2	category A	KPC	KPC	KPC	KPC
24-9990-kp	KPC-2	category A	KPC	KPC	KPC	KPC
25-7005-kp	NDM-1	category B	NDM	NDM	NDM	NDM
26-7615-kp	KPC-2	category A	KPC	KPC	KPC	KPC
27-4480-kp	KPC-2	category A	KPC	KPC	KPC	KPC
28-9899- <i>E. coli</i>	NDM-1	category B	NDM	NDM	NDM	NDM
29-4650-kp	KPC-2	category A	KPC	KPC	KPC	KPC
30-7972-kp	KPC-2	category A	KPC	KPC	KPC	KPC
31-6993- <i>E. coli</i>	NDM-1	category B	NDM	NDM	NDM	NDM
32-4610-kp	KPC-2	category A	KPC	KPC	KPC	KPC
33-7985-kp	KPC-2	category A	KPC	KPC	KPC	KPC
34-7407-kp	NDM-1	category B	NDM	NDM	NDM	NDM
35-4812- <i>E. coli</i>	NDM-1	category B	NDM	NDM	NDM	NDM
36-7467-kp	NDM-1	category B	Not detected	Not detected	NDM	NDM
37-7973-kp	KPC-2	category A	KPC	KPC	KPC	KPC
38-6887-kp	NDM-1	category B	NDM	NDM	NDM	NDM
39-6186-kp	KPC-2	category A	KPC	KPC	KPC	KPC
40-9059-kp	KPC-2	category A	KPC	KPC	KPC	KPC
41-7519-kp	KPC-2	category A	KPC	KPC	KPC	KPC
42-8918-kp	NDM-1	category B	NDM	NDM	NDM	NDM
43-9777-kp	KPC-2	category A	KPC	KPC	KPC	KPC
44-6434-kp	KPC-2	category A	KPC	KPC	KPC	KPC
45-6440-kp	NDM-1	category B	NDM	NDM	NDM	NDM
46-7820-kp	KPC-2	category A	KPC	KPC	KPC	KPC
47-6071-kp	KPC-2	category A	KPC	KPC	KPC	KPC

Table V
Continued.

No.	WGS	APB/EDTA	NG-test Carba 5	PCR	DETECTR	gene- Xpert
48-6322-kp	KPC-2	category A	KPC	KPC	KPC	KPC
49-4865- <i>E. coli</i>	NDM-1	category B	NDM	NDM	NDM	NDM
50-8052-kp	NDM-1	category B	NDM	NDM	NDM	NDM
51-4906-kp	KPC-2	category A	KPC	KPC	KPC	KPC
52-7979-kp	KPC-2	category A	KPC	KPC	KPC	KPC
53-4669- <i>E. coli</i>	NDM-1	category B	NDM	NDM	NDM	NDM
54-4947-kp	NDM-1	category B	NDM	NDM	NDM	NDM
55-7052-kp	NDM-1	category B	NDM	NDM	NDM	NDM
56-10015-kp	NDM-1	category B	NDM	NDM	NDM	NDM
57-4919-kp	KPC-2	category A	KPC	KPC	KPC	KPC
58-7380- <i>E. coli</i>	NDM-1	category B	NDM	NDM	NDM	NDM

issue is linked to its principle, which relies on antibody detection of expressed proteins.

Consequently, when β -lactamase is inadequately expressed or has amino acid substitutions at the major epitopes recognized by the antibody, there is a possibility of a false negative result (Zhang et al. 2022). The GeneXpert Carba-R assay is a fluorescence quantitative PCR that directly uses raw samples without needing overnight incubation. This technique surpasses NG-test Carba 5 in specimen detection and accuracy, making it a preferred option for high-risk patients such as immunosuppressed or bone marrow transplant patients. However, its high cost limits its widespread use in clinical practice. PCR only requires the extraction of nucleic acids from the test bacteria, with results typically available within 1–2 h. The speed, efficiency, and simplicity of operation are the primary factors driving PCR's widespread adoption as a zymotype detection method in laboratory settings.

Nevertheless, PCR demands a substantial investment in equipment, reagents, and skilled personnel, and it is typically utilized in well-equipped and established laboratories. Additionally, the interpretation of results often involves running agarose gel electrophoresis to assess the amplified products. Moreover, the precision of each detection method hinges on the specific type of carbapenemase being targeted. Hence, there is a pressing demand for the development of novel nucleic acid detection techniques that are rapid, specific, sensitive, and cost-effective, particularly those suitable for versatile on-site diagnostic purposes.

The rapid detection of nucleic acids plays a crucial role in various applications within human health and biotechnology. CRISPR-Cas-based methods, in particular, are undergoing testing for the management of genetic disorders, infectious diseases, and a wide range of other medical conditions (Salsman and Dellaire

2017; Li et al. 2018; Lambert et al. 2020;). In 2017, Doudna and coworkers (Chen et al. 2018) introduced a CRISPR-Cas-based tool known as DNA endonuclease-targeted CRISPR trans reporter (DETECTR). The technique relies on the collateral cleavage activity of Cas12a protein, which is triggered by Cas12a's recognition of target RNA. DETECTR, a widely adopted detection method in recent times, enables high-throughput processing from sample collection to obtaining results. Its performance is comparable to PCR while minimizing operation handling time. The principle of DETECTR involves amplifying the target gene at a constant temperature and subsequently activating the paracrine effect through protein-specific cleavage of the target gene. This process enables detection within just 1 h, without the need for precision instruments. Expanding the application of CRISPR-Cas technology into the realm of molecular diagnostics, it has been reported (Tsou et al. 2019) that DETECTR has been employed for the detection of human papillomavirus (HPV) and the differentiation between HPV16 and HPV18. DETECTR is a rapid detection platform based on CRISPR-Cas12a gene editing and isothermal amplification. It shows minimal susceptibility to gene mutations and demonstrates robust performance in detecting KPC-2 and NDM-1. Indeed, its results are highly consistent with the WGS results, with a sensitivity and specificity of 100%. The disadvantage of this method is that after RPA amplification, the cap of the reaction tube must be opened for product transfer, leading to a higher risk of aerosol contamination and potentially increasing the likelihood of false-positive results (Jiang et al. 2023).

Nevertheless, this study has several limitations. First, due to regional variations (Ma et al. 2023), the sample size collected was restricted to encompass only KPC-2- and NDM-1-producing strains. Hence, it was

not feasible to assess the efficacy of these methods in detecting VIM, OXA-48, and IMP carbapenemases. In this scenario, the strains were limited to *bla*_{KPC-2} and NDM-1, thereby preventing the evaluation of differences between the five methods for detecting carbapenemase variants.

The effectiveness of different methods such as APB/EDTA, PCR, NG-test Carba 5, GeneXpert Carba-R, and DETECTR in detecting carbapenemases was evaluated, using WGS as the benchmark. All five methods showed strong accuracy and adherence to standards. Nevertheless, the sensitivity of each method to different carbapenemases varied. The APB/EDTA method could only determine the classification of the resistant gene but not the zymotype. Regarding the detection of KPC carbapenemases, PCR, NG-test Carba 5, GeneXpert Carba-R, and DETECTR all achieved a 100% sensitivity rate. However, when it came to detecting NDM carbapenemases, DETECTR, and GeneXpert Carba-R outperformed PCR and NG-test Carba 5.

Ethical statement

All procedures performed in this study involving human participants were in compliance with the ethical standards of the Medical Ethics Committee of Anhui Medical University (Reference number: LISC20210802) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Author contributions

The collection of specimens, conduct of experiments, article writing and translation, and chart compilation for this study were collaboratively completed by JZ, NNG, RDL, GL, GPL, and JLS. During the initial stages of the research, NNG collected specimens, extracted nucleic acids and wrote the paper. JZ contributed to the overall experiment development, while GL, RDL and GPL supported in icon arrangement and translation. The rest of the team participated in revising the article. JLS provided experimental ideas, guided the experimental direction, and contributed to manuscript revisions. All team members were involved in project development and design, as well as reviewed the initial draft.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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