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Carba PBP: a novel penicillin-binding protein-based lateral flow assay for rapid phenotypic detection of carbapenemase-producing *Enterobacterales*

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ABSTRACT Rapid phenotypic detection assays, including Carba NP and its variants, are widely applied for clinical diagnosis of carbapenemase-producing *Enterobacterales* (CPE). However, these tests are based on the acidification of the pH indicator during carbapenem hydrolysis, which limits test sensitivity and speed, especially for the detection of CPE producing low-activity carbapenem (e.g., OXA-48 variants). Herein, we developed a novel rapid and sensitive CPE detection method (Carba PBP) that could measure substrate (meropenem) consumption based on penicillin-binding protein (PBP). Meropenem-specific PBP was used to develop a competitive lateral flow assay (LFA) for meropenem identification. For the detection of carbapenemase activity, meropenem concentration was optimized using a checkerboard assay. The performance of Carba PBP was evaluated and compared with that of Carba NP using a panel of 94 clinical strains characterized by whole-genome sequencing and carbapenem susceptibility test. The limit of detection of PBP-based LFA for meropenem identification was 7 ng mL^{-1}. Using 10 ng mL^{-1} meropenem as the substrate, Carba PBP and Carba NP could detect 10 ng mL^{-1} carbapenemase within 25 min and 1,280 ng mL^{-1} CPE in 2 h, respectively. The sensitivity and specificity were 100% (75/75) and 100% (19/19) for Carba PBP and 85.3% (64/75) and 100% (19/19) for Carba NP, respectively. When compared with Carba NP, Carba PBP showed superior performance in detecting all the tested CPE strains (including OXA-48-like variants) within 25 min and presented two orders of magnitude higher analytical sensitivity, demonstrating potential for clinical diagnosis of CPE.

IMPORTANCE This study successfully achieved the goal of carbapenemase activity detection with both high sensitivity and convenience, offering a convenient lateral flow assay for clinical diagnosis of carbapenemase-producing *Enterobacterales*.

KEYWORDS carbapenemase, activity, PBP, LFA, meropenem

The emergence and spread of carbapenem-resistant *Enterobacterales* (CRE) pose a threat to public health worldwide (1). In China, the resistance rate of CRE increased by more than 60% in 2020 compared to 2015. Carbapenem-resistant *Escherichia coli* and carbapenem-resistant *Klebsiella pneumoniae* account for more than 90% of CRE and became the main target of CRE research (2). Carbapenemase-encoding genes are often located on mobile genetic elements, which promote their horizontal transfer even across different bacterial species (3). Based on the Ambler classification method, carbapenemases can be categorized into Class A (KPC, GES, etc.) serine β-lactamases, Class D (OXA-48 like) serine β-lactamases, and Class B (NDM, VIM, IMP, etc.) metallo-β-lactamases (4). To date, nearly 300 unique carbapenemases have been identified, and the number continues to increase (5). Patients infected with carbapenemase-producing

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Enterobacterales (CPE) may not show any distinct symptoms, which can delay effective treatment, even leading to sepsis (6, 7). Therefore, successful infection control is crucial to restrict the spread of CPE. Rapid and sensitive identification of carbapenemases is one of the most imperative approaches to accomplish clinical prevention and treatment of CPE.

The currently available CPE detection methods can be mainly grouped into phenotypic and genotypic methods (8). Genotypic methods, including polymerase chain reaction (PCR) methods, show high sensitivity and accuracy but are limited by high cost and specific operation (9). CARBA 5, a representative immunological method, can detect five major carbapenemases (KPC, NDM, VIM, IMP, and OXA-48-like carbapenemases) via antigen–antibody reactions on chromatographic paper. However, genotypic methods target only preselected genes or proteins and cannot detect novel or mutated carbapenemases. In contrast, phenotypic methods based on carbapenemase activity can theoretically detect almost all classes and variants of carbapenemases without genotypic identification (10). However, some phenotypic methods, including the carbapenemase inactivation method (CIM), a modified version of CIM (mCIM), and enzyme inhibitor enhancement assay, require overnight culture. Carba NP, endorsed by the Clinical and Laboratory Standards Institute (CLSI) for the rapid detection of carbapenemases (11), represents a momentous shift, as it allows for the completion of the detection process within just 2 h, resulting in substantial time and cost savings compared to the traditional mCIM method. (We have consulted with bioMérieux representatives for commercial product availability in China, and the Rapidec Carba NP is currently not available in China.) Based on the principle of acidification of the reaction medium resulting from the hydrolytic activity of carbapenemases, Carba NP can detect carbapenemases by measuring the hydrolysis of imipenem by carbapenemases; a color shift of the pH indicator (phenol red) from red to yellow following acidification can reveal the change in the pH of the medium. However, although Carba NP can produce results in 2 h, it cannot accurately detect OXA-48 (sensitivity as low as 11%) or other carbapenemases (GES and SME) (11–13), owing to the inability of phenol red to detect weak acidification caused by carbapenemases with low activity or expression levels. Hence, there is an urgent need to develop rapid and sensitive CPE detection methods based on carbapenemase activities. In this study, we proposed a novel penicillin-binding protein (PBP)-based carbapenemase detection method, namely, Carba PBP, which could rapidly detect carbapenemase activity with high sensitivity, demonstrating significant potential for clinical diagnosis of CPE.

MATERIALS AND METHODS

Bacterial isolates and materials

Recombinant KPC, NDM, VIM, IMP, and OXA-48 were produced in our laboratory using a prokaryotic expression system. Meropenem, imipenem, and bovine serum albumin (BSA) were purchased from Aladdin Co., Ltd. (Shanghai, China). Anti-His tag mouse monoclonal antibody was obtained from Thermo Fisher Scientific (China) Co., Ltd. (Shanghai, China). Mueller–Hinton agar (MHA) and Mueller–Hinton broth (MH broth) were supplied by Land Bridge Technology Co., Ltd. (Beijing, China). The Bacterial Genomic DNA Extraction Kit was purchased from Magen Biotechnology Co., Ltd. (Beijing, China). The E-test MIC test strip was obtained from Liofilchem Inc. (Roseto degli Abruzzi, Italy). The glass fiber, conjugate pad, and absorbent pads were purchased from Millipore (China) Co., Ltd. (Beijing, China). The nitrocellulose (NC) membrane was acquired from Pall (China) Co., Ltd. (Beijing, China). The microwell plate was obtained from Yunpeng Technology Development Co., Ltd. (Fujian, China). Ultrapure water (18.2 MΩ cm) was procured from a Millipore Milli-Q system and used for the preparation of all solutions. The CTS300 automatic programmable cutter was purchased from Jinbiao Biotechnology Co., Ltd. (Shanghai, China).

The bacterial isolates were isolated and then preserved for experimental use by the Second Affiliated Hospital of Zhejiang University and China Agricultural University Veterinary Teaching Hospital, China. The quality control strain *Escherichia coli* ATCC 25922 was purchased from Shanghai Prajna Biology Technique Co. Ltd (Shanghai, China). All the strains were inoculated with MHA and incubated overnight at 37°C for activation. Then, the bacterial cells were transferred into MH broth and incubated at 37°C until the culture reached an optical density ($OD_{600\ nm}$) of 0.6–0.8. Subsequently, the bacterial genomic DNA was extracted using the Bacterial Genomic DNA Extraction Kit and sequenced (Beijing Sinobiocore Biotechnology Co. Ltd., Beijing, China).

Lateral flow assay for meropenem detection

The lateral flow assay (LFA) strip consisted of three basic components, namely, sample pad, NC membrane, and absorption pad, all of which were attached onto a PVC support with approximately 1–2 mm overlap between two adjacent parts. The glass fiber pretreated with phosphate buffer (containing 2% Tween-20) was used as the sample pad. The NC membrane (PALL vivid 120) was sprayed with penicillin G-BSA conjugate (Pen G-BSA, 1.6 mg mL^{-1}) to form the test line (T) and with anti-His tag mouse mAb (1 mg mL^{-1}) to form the control line (C), both with a volume of 0.8 $\mu L \cdot cm^{-1}$. The assembled card was cut to a strip of 3 mm width and stored at 4°C until use.

Meropenem standard was diluted with 200 µL of bacteria lysis buffer (20 mM PBS containing 1% casein, 0.1% Triton X-100, and 0.1 mM $ZnSO_4$) to different concentrations (0–14 ng mL^{-1}) in a microwell plate. Then, 10 µL of gold nanoparticle-labeled PBP (GNP-PBP 4) was added to the prepared meropenem solutions and incubated at 25°C for 5 min. Subsequently, the LFA strips were inserted into each microwell and allowed to stand for 5 min. Each concentration of meropenem was tested in triplicate. A standard curve for meropenem detection was obtained with the concentration of meropenem (ng mL^{-1}) as the abscissa and the intensity of the test line as the ordinate using Origin 8.0 (Origin Lab Corp, Northampton, MA, USA).

Carba PBP

To determine the optimal substrate concentration, gradient-diluted meropenem (0, 5, 10, 20, 40, and 80 ng mL^{-1}) was respectively mixed with gradient-diluted recombinant KPC-2 carbapenemase protein (0, 10, 20, and 40 ng mL^{-1}) and 200 µL of bacteria lysis buffer in a microwell plate and incubated at 37°C for 20 min. Then, 10 µL of GNP-PBP 4 was added to the reaction mixtures and incubated at room temperature for 5 min. After that, the LFA strips were inserted into the microwells and allowed to stand for 5 min.

Evaluation of the sensitivity and specificity of Carba PBP

Ten-fold dilutions of CPE expressing a variety of carbapenemases were individually inoculated into 200 µL of running buffer (bacteria lysis buffer containing 10 ng mL^{-1} meropenem) and subjected to Carba PBP as well as CLSI-recommended Carba NP.

Detection of clinical isolates using Carba PBP

A loopful (1.0 µL) of bacterial cells cultured overnight on MHA plates was scraped off, suspended in a microwell tube containing 200 µL of running buffer, mixed for 5 s by vortexing, and further incubated at room temperature for 15 min. Then, the reaction mixtures were examined by Carba PBP and Carba NP, and the performances of both methods were compared. The Carba NP testing process starts after the sample preparation stage, wherein bacterial colonies that have been previously developed on the culture medium are employed for detection. This process provides results in approximately 2 h. Likewise, the detection workflow is applicable to the Carba PBP test, which, in turn, delivers results in as little as 25 min.

RESULTS

Principles of CPE detection by Carba PBP

As shown in Fig. 1, Carba PBP was designed to detect carbapenemase activity by measuring meropenem consumption. Meropenem LFA was based on the competitive inhibition of the binding of antigen PenG-BSA to GNP-PBP 4 by meropenem. Before carbapenemase hydrolysis, the presence of a sufficient amount of meropenem [above the limit of detection (LOD) of meropenem LFA] as a substrate for the hydrolysis reaction produced a colorless test line. However, after carbapenemase hydrolysis, the residual meropenem in the LFA detection range produced a visible test line. If the sample was carbapenemase-negative, a sufficient amount of meropenem remained to form the GNP-PBP 4–meropenem complex, which resulted in no binding reaction between GNP-PBP 4 and PenG-BSA, thus producing a colorless test line. Nevertheless, a visible control line emerged because both the GNP-PBP 4 and GNP-PBP 4–meropenem complex were trapped by anti-His tag mAb. In contrast, if the sample was carbapenemase-positive, the residual meropenem rarely formed the GNP-PBP 4–meropenem complex, and thus a sufficient amount of GNP-PBP 4 was combined with PenG-BSA and anti-His tag mAb to produce visible test and control lines.

Meropenem detection by LFA

The overall performance of LFA for meropenem detection was analyzed with different GNP-PBP 4 concentrations (2.3, 4.6, 9.2, and 18.4 μg mL^{-1}). As meropenem could inhibit the binding of GNP-PBP 4 to the coated antigen PenG-BSA immobilized on the test line, the competition for free meropenem in the mixture as well as the sensitivity of LFA both increased with decreasing GNP-PBP 4 concentrations. As shown in Fig. 2, the LFA sensitivity was the highest when 2.3 μg mL^{-1} GNP-PBP 4 was used, and the cutoff value observed by the naked eye was 7 ng mL^{-1}. Therefore, 2.3 μg mL^{-1} GNP-PBP 4 was utilized in the subsequent experiments.

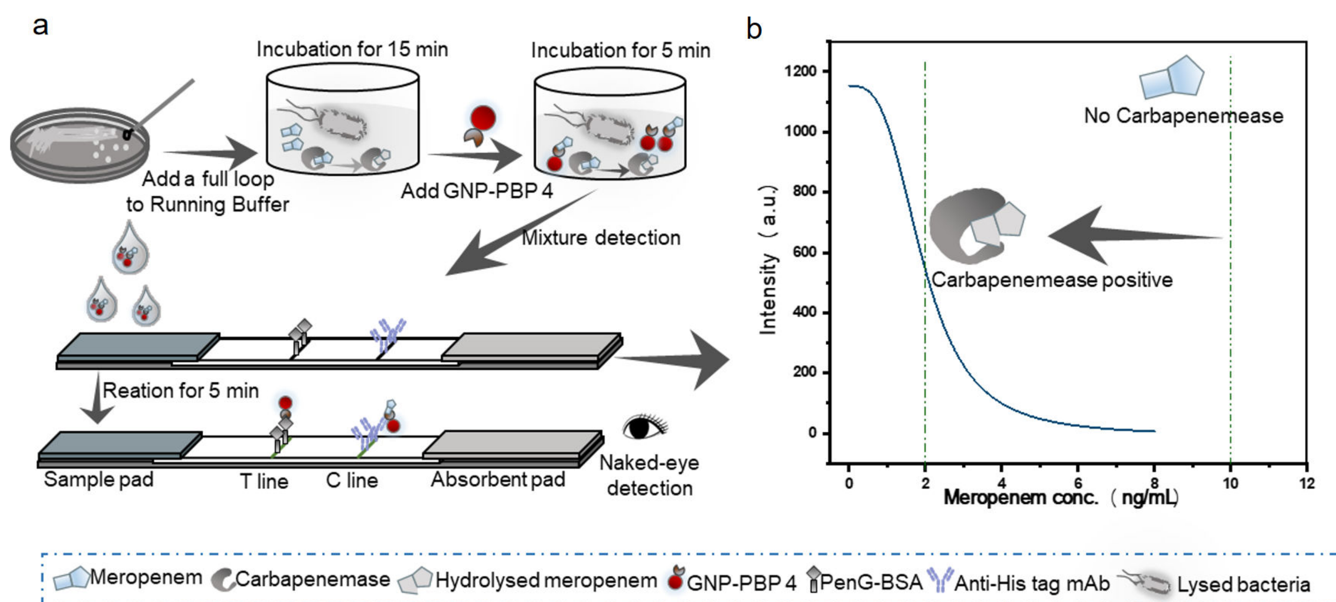

FIG 1 Schematic illustration of the detection procedure of the Carba PBP (a) and (b) comparison schematic of T line intensity under the "no carbapenemase" and "carbapenemase-positive" situation: in the absence of carbapenemase, there is no signal on the T line. In the presence of carbapenemase, the meropenem was hydrolyzed by carbapenemase, which resulted in a lower amount of meropenem (marked arrow in Fig. 1b) and further led to an increased color intensity on the T-line.

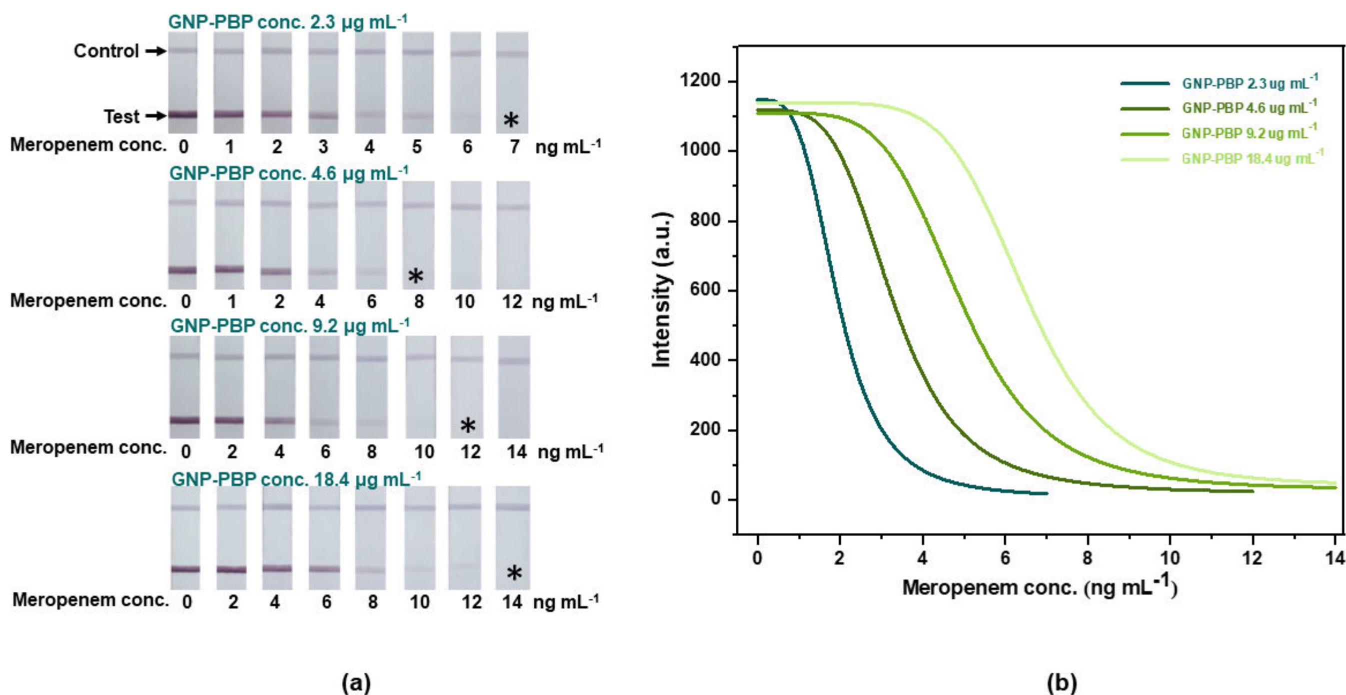

FIG 2 Performance of LFA in detecting meropenem under different GNP-PBP 4 concentrations. (a) LOD of LFA under different GNP-PBP 4 concentrations (2.3, 4.6, 9.2, and 18.4 μg mL^{-1}); (b) calibration curve of meropenem detection by PBP-based LFA under different GNP-PBP 4 concentrations.

Carba PBP

In the present study, KPC-2, one of the most common carbapenemases, was employed to optimize the concentration of meropenem used in Carba PBP. As the expression level of KPC-2 in clinical strains was unclear, a checkerboard assay was utilized. It can be observed from Fig. 3a that the Carba PBP could detect KPC-2 at a concentration as low as 20 ng mL^{-1} with the addition of 10 ng mL^{-1} meropenem. However, with increasing meropenem concentrations, the test line disappeared in the presence of 20 ng mL^{-1} KPC-2, and the LOD increased. Therefore, 10 ng mL^{-1} was selected as the optimal substrate concentration. Subsequently, the other experimental conditions (phosphate buffer, NaCl, and Triton X-100 concentrations) were optimized (see web-only Fig. S1 to S3). Besides, the effects of key factors influencing carbapenemase activity, including incubation temperature and time, on CPE detection by Carba PBP were also examined. To facilitate point-of-care examination, room temperature was employed as the incubation temperature, and the bacterial samples were incubated for 5, 10, 15, and 20 min. As shown in Fig. 3b, with the increase in incubation time, the color intensity gradually increased, and the LFA could detect all the CPE samples within 15 min of incubation. Thus, room temperature and incubation time of 15 min were concluded to be the optimal conditions for the detection of carbapenemase hydrolysis by Carba PBP.

Analytical sensitivity of Carba PBP and Carba NP

The analytical sensitivity of Carba PBP and Carba NP was determined by a simple test using serially diluted representative carbapenemases (KPC, NDM, VIM, IMP, and OXA-48) in the concentration range of 10–20,480 ng mL^{-1}. As shown in Fig. 4, Carba PBP could detect 10 ng mL^{-1} carbapenemase within 25 min, whereas the LOD of Carba NP was 1,280 ng mL^{-1} after 2 h of the reaction. The analytical sensitivity of Carba PBP was two orders of magnitude higher than that of Carba NP.

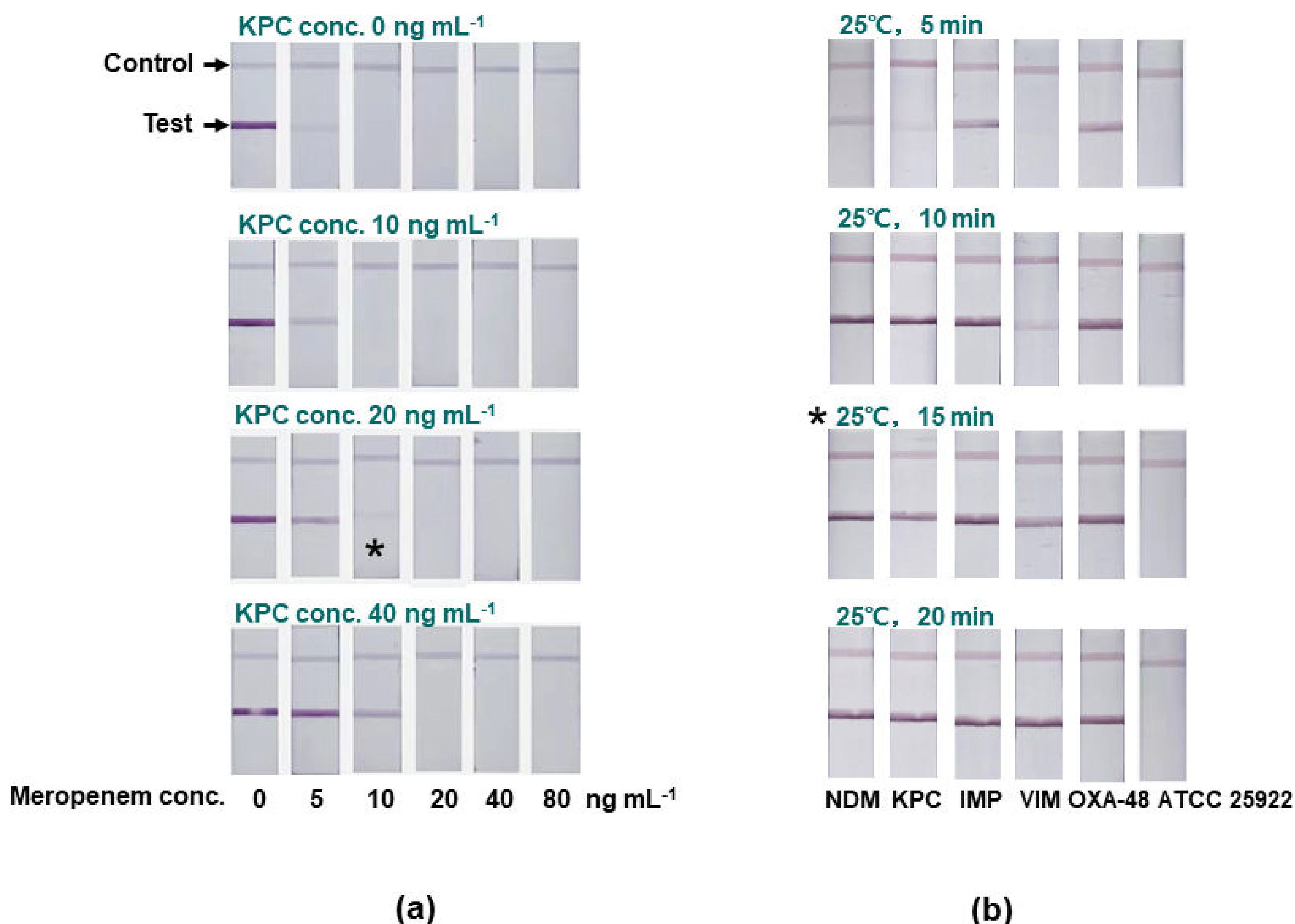

FIG 3 Development of Carba PBP assay and optimization of experimental conditions. (a) Checkerboard assay under different concentrations of KPC (0, 10, 20, and 40 ng mL^{-1}) and meropenem (0, 5, 10, 20, 40, and 80 ng mL^{-1}), and the optimal concentration for KPC and meropenem was marked with * in Fig. 3a. (b) Optimization of incubation time under room temperature (25°C), and the optimal incubation time was marked with * in Fig. 3b. (The used NDM, KPC, IMP, VIM, and OXA-48 CPE strains in Fig. 3b were marked with * in Table 1.)

Detection of clinical isolates by Carba PBP

As shown in Table 1, a total of 94 (93 from clinical and a single β-lactamase-negative *E. coli* reference strain ATCC 25922) strain isolates were examined by both Carba PBP and Carba NP. The β-lactamase gene content of those tested isolates was determined by whole-genome sequencing (Illumina NovaSeq 6000) (more details can be found in Supplementary Materials). Whole-genome sequencing combined with carbapenem susceptibility test were utilized as reference methods to confirm CPE. Carba PBP could detect all 75 CPEs, including 14 NDM-1-, 18 NDM-5-, 3 NDM-9-, 10 IMP-4-, 1 IMP-26-, 5 VIM-1-, 20 KPC-2-positive isolates, 4 multi-carbapenemase producers, and 9 OXA-48-type positive *Klebsiella pneumoniae* strains. All tests were conducted in triplicate with the Carba PBP and Carba NP tests, giving replicable results. In contrast, Carba NP failed to detect nine OXA-48-positive *K. pneumoniae* strains and two NDM-1-positive strains (*Enterobacter cloacae* and *Proteus mirabilis*). With regard to the detection of the 19 non-CPEs, both Carba PBP and Carba NP presented negative results. The sensitivity and specificity of Carba PBP were both 100%, whereas those of Carba NP were 85.3% and 100%, respectively.

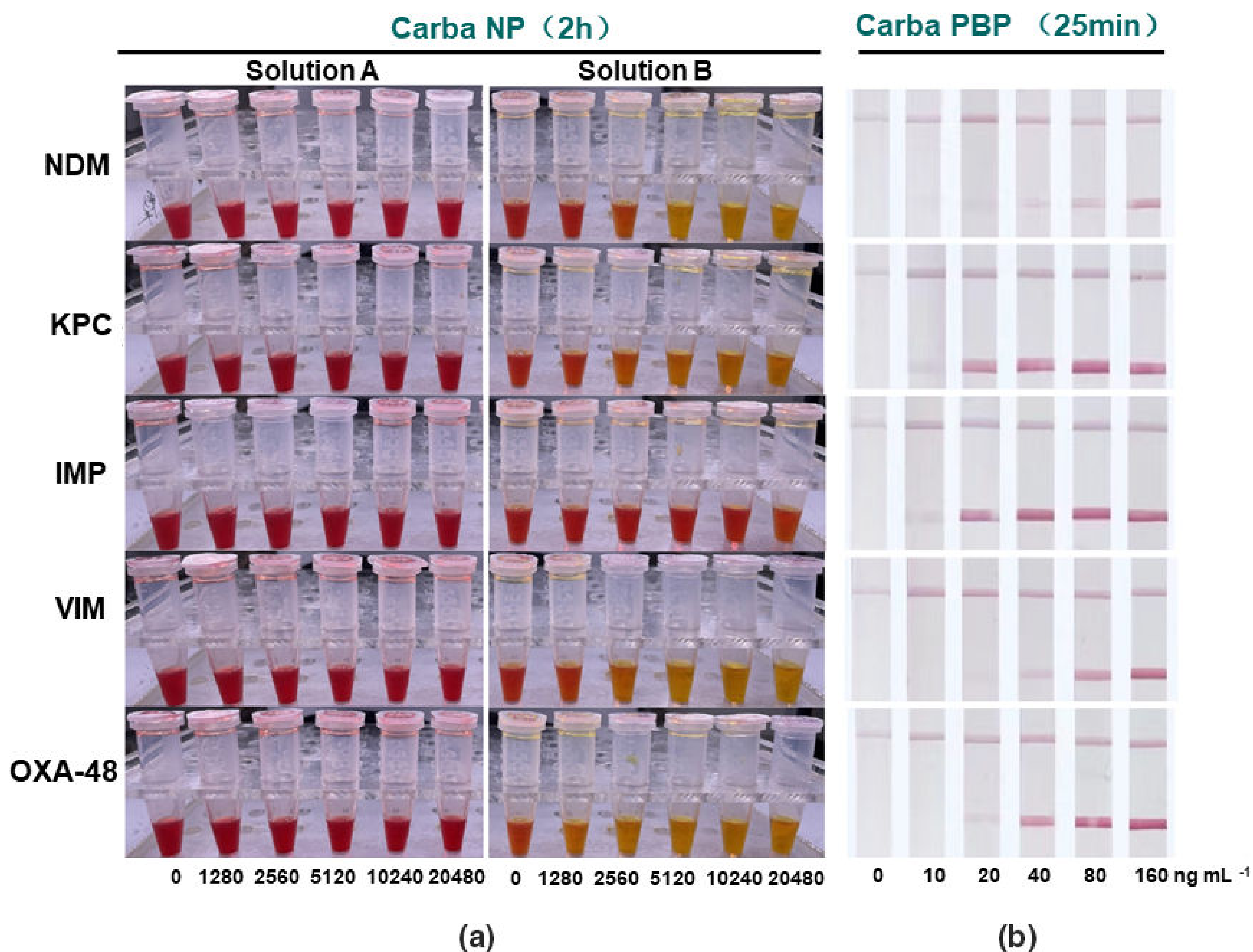

FIG 4 Analytical sensitivity of (a) Carba NP and (b) Carba PBP in detecting serially diluted recombinant KPC, NDM, VIM, IMP, and OXA-48 in the concentration range of 0-20,480 ng mL^{-1}.

DISCUSSION

Carba NP is recommended by the CLSI as a confirmatory test for carbapenemase production (11). As a colorimetric assay for carbapenemase activity, Carba NP can detect most of the prevalent carbapenemases (14). However, the sensitivity of Carba NP in detecting carbapenemases with low activity or expression level (e.g., OXA-48-like carbapenemases) is as low as 11% (11, 13). Moreover, owing to the lower β-lactamase activity in mucoid isolates than in the non-mucoid isolates, Carba NP has produced false-negative results for some strains of *Providencia rettgeri* and *P. mirabilis*, especially for mucoid isolates, even when expressing NDM carbapenemase (15, 16). In the present study, the homemade Carba NP test failed to detect nine OXA-48-type positive strains and two NDM-1-positive strains. The CLSI Carba NP method used in our study had a sensitivity of 85.3% (64/75) and a specificity of 100% (19/19), which is consistent with other data reported in the literature (17).

In order to improve performance, including sensitivity and efficiency, various modified Carba NP methods have been developed. For instance, the CarbAcineto NP test, with modified lysis conditions and higher bacterial inoculum, exhibited 87.7% sensitivity for CPE isolates (89.2% of Carba NP) (18, 19). Another modified Carba NP assay, using bath sonication instead of centrifugation and utilizing imipenem/cilastatin as the substrate, detected OXA-48 at mg mL^{-1} level (20), presenting its low analytical sensitivity.

TABLE 1 Detection of clinical isolates by Carba PBP and Carba NP[a]

Species	β-Lactamase content	Sequence type	MIC, μg mL^{-1}			Carba PBP	Carba NP
			IMI	MER	ERT		
E. coli	**NDM-1** + SHV-12 + TEM-1B	ST2003	> 32	> 32	> 32	+	+
	NDM-1 + CTX M-27 + SHV-12	ST131	3	< 1	3	+	+
	NDM-1 + CTX M-27*	ST131	> 32	4	2	+	+
	NDM-1 + CTX M-15	ST167	3	< 1	2	+	+
	NDM-5 + CTX M-64 + + OXA-1TEM-1B	ST10	> 32	2	6	+	+
	NDM-5 + CMY-2 + CTX M-15 + OXA-1 + TEM-1B	ST410	5	3	12	+	+
	NDM-5 + CTX M-55 + TEM-1B	ST156	4	2	14	+	+
	NDM-5 + CTX M-199 + OXA-1 + TEM-1B	ST405	2	2	16	+	+
	NDM-5+CMY-2 + CTX M-15 + OXA-1 + TEM-1B	ST410	1.5	< 1	8	+	+
	NDM-5 + CTX M-55 + TEM-141;	ST224	12	4	6	+	+
	NDM-5 + CTX M-65 + TEM-1B	ST167	1	1	4	+	+
	NDM-5 + CTX M-3+SHV-106 + TEM-1B	–	6	2	6	+	+
	NDM-9 + CTX M-55 + OXA-1	ST1266	12	3	4	+	+
	NDM-9 + OXA-10	ST10	16	4	12	+	+
	IMP-4 + OXA-1*	ST131	8	12	3	+	+
	IMP-4 + CMY-42 + CTX M-14 + CTX M-15 + OXA-1	ST167	4	4	6	+	+
	IMP-4 + CMY-42 + CTX M-14 + CTX M-15 + OXA-1	ST167	8	16	1.5	+	+
	VIM-1*	–	6	1.5	< 1	+	+
	KPC-2 + CTX M-14	ST405	> 32	> 32	> 32	+	+
	KPC-2 + CTX M-24 + CTX M-3	ST131	4	< 1	2	+	+
	KPC-2 + **IMP-4** + CTX M-15 + OXA-1 + SHV-106 + TEM-1B	ST131	4	< 1	< 1	+	+
	KPC-2	ST48	8	< 1	1.5	+	+
	OXA-181 + TEM-1A	ST361	> 32	> 32	> 32	+	–
	OXA-181 + CMY-2	ST410	2	< 1	2	+	–
K. pneumoniae	**NDM-1** + CTX M-15 + SHV-12 + TEM-1B	ST846	12	3	3	+	+
	NDM-1	ST76	3	< 1	3	+	+
	NDM-1 + CTX M-3 + SHV-187 + TEM-1B	ST152	4	3	2	+	+
	NDM-1 + CTX M-15 + SHV-1 + TEM-1B	ST846	8	6	3	+	+
	NDM-1 + SHV-187	ST111	8	3	1.5	+	+
	NDM-5 + SHV-182 + TEM-1A	ST340	12	8	> 32	+	+
	NDM-5 + DHA-1 + SHV-187 + TEM-1B	ST831	12	4	4	+	+
	NDM-5 + DHA-1 + SHV-187 + TEM-1B	ST831	16	4	8	+	+
	NDM-5 + CTX M-15 + OXA-1 + SHV-106 + TEM-1B	ST307	8	2	4	+	+
	NDM-5 + CTX M-3 + SHV-187 + TEM-1B	ST534	> 32	2	4	+	+
	NDM-5 + DHA-1 + OXA-1 + SHV-11	ST147	> 32	> 32	> 32	+	+
	NDM-5 + CTX M-1 + OXA-1 + SHV-106 + TEM-1B	ST307	> 32	4	6	+	+
	NDM-5 + CTX M-55 + TEM-1B	ST2083	6	6	> 32	+	+
	NDM-5 + CTX M-3 + SHV-106 + TEM-1B	–	12	2	6	+	+
	NDM-5 + SHV-110	ST190	3	3	3	+	+
	IMP-4 + CTX M-15 + OXA-1 + SHV-106 + TEM-1B	ST307	6	6	3	+	+
	IMP-4 + SHV-187	ST29	3	< 1	3	+	+
	VIM-1 + CTX M-15 + SHV-190 + TEM-1C	ST23	4	< 1	< 1	+	+
	VIM-1 + SHV-12 + SHV-187	ST68	> 32	3	1	+	+
	VIM-1 + SHV-187	ST63	> 32	2	1.5	+	+
	KPC-2*	ST131	4	1	1.5	+	+
	KPC-2 + SHV-106	ST15	> 32	> 32	> 32	+	+
	KPC-2 + CTX M-15 + OXA-1 + SHV-106 + TEM-1B	ST15	> 32	12	> 32	+	+
	KPC-2 + CTX M-65 + TEM-1B	ST11	> 32	> 32	> 32	+	+
	KPC-2 + CTX M-65 + SHV-182 + TEM-1B	ST11	> 32	> 32	> 32	+	+
	KPC-2 + CTX M-15 + OXA-1 + SHV-106 + TEM-1B	ST15	6	> 32	> 32	+	+

(*Continued on next page*)

TABLE 1 Detection of clinical isolates by Carba PBP and Carba NP[a] (*Continued*)

Species	β-Lactamase content	Sequence type	MIC, µg mL^{-1}			Carba PBP	Carba NP
			IMI	MER	ERT		
	KPC-2 + SHV-106	ST2237	24	2	3	+	+
	KPC-2 + CTX M-3 + SHV-61	ST494	> 32	< 1	1.5	+	+
	KPC-2 + TEM-1B	ST37	> 32	16	6	+	+
	KPC-2 + CTX M-15 + OXA-1 + SHV-106 + TEM-1B	ST307	8	1.5	2	+	+
	KPC-2 + CTX M-15 + OXA-1 + SHV-106 + TEM-1B	ST307	6	< 1	1.5	+	+
	KPC-2 + SHV-106	ST15	> 32	> 32	> 32	+	+
	KPC-2 + CTX M-65 + TEM-1B	ST11	> 32	> 32	> 32	+	+
	KPC-2 + CTX M-55 + SHV-187	ST1	3	1	6	+	+
	OXA-48 + CTX-M-14b + CTX M-55+SHV-11*	ST85	16	1.5	6	+	–
	OXA-48 + CTX-M-14b + CTX M-55+SHV-11	ST94	6	0.75	4	+	–
	OXA-48 + CTX-M-14b + SHV-145 + TEM-1B	ST115	6	1	6	+	–
	OXA-48 + CTX-M-14b + CTX M-55+SHV-11	ST90	12	0.75	4	+	–
	OXA-48 + CTX-M-14b + SHV-145 + TEM-1B	ST118	4	1	6	+	–
	OXA-181 + OXA-10 + SHV-85	ST37	> 32	> 32	> 32	+	–
	OXA-232 + CTX M-15 + SHV-106	ST15	4	4	> 32	+	–
E. cloacae	**NDM-1** + ACT-7 + DHA-1 + SHV-12 + SFO-1 + TEM-1B	ST51	6	6	> 32	+	–
	NDM-1 + **VIM-1** + ACT-16 + DHA-1 + SHV-12 + TEM-1B	ST171	8	< 1	1.5	+	+
	NDM-9 + ACT-16 + CTX M-65	ST114	3	1.5	4	+	+
	IMP-4 + ACT-7	ST133	> 32	> 32	6	+	+
	IMP-4 + ACT-5 + SHV-12 + TEM-1B	ST97	> 32	> 32	4	+	+
	IMP-26 + CMH-3 + CTX M-14 + TEM-1B	ST513	< 1	1.5	4	+	+
	KPC-2 + ACT-7 + **OXA-1** + TEM-1B	ST93	6	1	2	+	+
	IMP-4 + **NDM-1** + ACT-16 + CTX M-15 + SHV-12	ST231	> 32	> 32	3	+	+
	NDM-1 + **IMP-4** + **KPC-2** + OXY-5–1	ST167	8	6	12	+	+
P. mirabilis	**NDM-1** + OXA-10 + OXA-1 + TEM-1B	–	> 32	1.5	< 1	+	–
K. pneumoniae	CTX-M-3 + SHV-186 + TEM-1B	ST34	< 1	< 1	< 1	–	–
	CTX-M-1 + SHV-103	ST685	< 1	< 1	< 1	–	–
	CTX-M-3 + SHV-187 + TEM-1B	ST1836	< 1	< 1	< 1	–	–
	SHV-2	ST534	< 1	< 1	< 1	–	–
	CTX-M-14	–	< 1	< 1	< 1	–	–
	CTX-M-3 + SHV-28 + TEM-1B	ST1106	< 1	< 1	< 1	–	–
	CTX-M-3 + SHV-40 + TEM-1B	ST2074	< 1	< 1	< 1	–	–
	CTX-M-15 + SHV-27	ST661	< 1	< 1	< 1	–	–
	CTX-M-27 + PLA-5A	–	< 1	< 1	< 1	–	–
	CTX-M-3 + OKP A-8 + TEM-1B	ST1040	< 1	< 1	< 1	–	–
	CTX-M-3TEM-1B	–	< 1	< 1	< 1	–	–
	CTX-M-27 + SHV-187	ST309	< 1	< 1	< 1	–	–
	CTX-M-14 + SHV-106	ST101	< 1	< 1	< 1	–	–
E. coli	CTX-M-65 + TEM-1B	ST10	< 1	< 1	< 1	–	–
	CTX-M-14 + TEM-1B	ST10	< 1	< 1	< 1	–	–
	CTX-M-55	ST3107	< 1	< 1	< 1	–	–
	CTX-M-27 + TEM-1A	ST1602	< 1	< 1	< 1	–	–
	TEM-1B	ST48	< 1	< 1	< 1	–	–
Reference E. coli strain	ATCC 25922	–	< 1	< 1	< 1	–	–

[a]"-" indicates no result.

In all these methods, the composition of bacterial lysis buffer has been improved to completely lyse the bacterial cells and increase their quantity (19, 21), ultimately enhancing the detectable signal. However, phenol red is not sufficiently sensitive to detect weak acidification, which limits the analytical sensitivity of the Carba NP methods. Due to the unavailability of RAPIDEC Carba NP in China, there is no way to compare the performance of Carba NP and RAPIDEC Carba NP in detecting the same strains under the

same experimental conditions. The sensitivity and specificity of the RAPIDEC Carba NP test were both 96%, according to Laurent Poirel and Patrice Nordmann (22). The detection performance of RAPIDEC Carba NP and Carba NP has been compared in another study. It was found that RAPIDEC Carba NP showed better performance for the rapid and efficient detection of carbapenemase-producing *Enterobacteriaceae* compared to the homemade Carba NP. Sensitivity and specificity were 99% and 100%, respectively, for the RAPIDEC Carba NP test and 96.8% and 100% for the homemade Carba NP test, respectively (23). Overall, our Carba PBP exhibited better performance than homemade Carba NP and RAPIDEC Carba NP as reported in the literature in terms of detection time and overall performance.

At present, significant efforts have been made to establish rapid and sensitive methods for CPE detection. For instance, a novel LFA for the detection of generic β-lactamase activity was developed using a cephalexin-functionalized GNP as the β-lactamase (including carbapenemases) probe(24), which could selectively bind to PBPs coated on the test line on the LFA. When exposed to β-lactamases, including carbapenemases, the hydrolyzed probe could no longer bind to the PBPs, resulting in a colorless test line. This developed LFA could detect NDM-1 up to a concentration of 110 nM in urine. However, detection of other carbapenemases (including OXA-48) and β-lactamase-producing strains using this novel LFA has not been reported.

In the present study, a novel LFA, named Carba PBP, was developed for sensitive and specific detection of carbapenemase activity by switching the detection target to measure meropenem consumption. Meropenem was more efficient (98% sensitivity and 100% specificity) against *Enterobacteriaceae* than imipenem in some carbapenemase activity detection(25), and the stability of the meropenem solution was better. Considering the specificity and the relatively stable physical and chemical properties(26), meropenem was eventually selected as the substrate of Carba PBP. Owing to the high sensitivity of competitive inhibition, the developed assay could detect the substrate at the ng mL^{-1} level, thus achieving highly sensitive detection of carbapenemase activity and 100% sensitivity for clinical isolate detection.

The major steps involved in the development of Carba PBP were the establishment of a highly sensitive meropenem detection method and optimization of meropenem concentration as the carbapenemase substrate. Considering its naturally high affinity to carbapenems (27, 28) , PBP 4 was chosen to develop a sensitive LFA for meropenem detection. In general, the substrate is the most important component, besides the enzyme itself, in assays for the detection of enzyme activity (29). Theoretically, higher substrate concentrations can result in increased enzyme hydrolysis, leading to higher detection sensitivity, whereas lower substrate concentrations can cause lower detection sensitivity. Hence, in the present study, an optimal meropenem concentration of 10 ng mL^{-1} was employed in Carba PBP for the detection of various carbapenemases. The results revealed that the analytical sensitivity of Carba PBP was two orders of magnitude higher than that of Carba NP, leading to rapid detection (25 min for Carba PBP vs 2 h for Carba NP). More importantly, Carba PBP was more sensitive in detecting clinical isolates and could detect OXA-48-like variants, when compared with Carba NP. In conclusion, a sensitive and rapid phenotypic detection method, named Carba PBP, was developed in the present study for detecting CPE by measuring meropenem consumption based on PBP 4. The developed assay presented improved sensitivity and produced visual results within 25 min, when compared with Carba NP, demonstrating significant potential for clinical diagnosis of CPE.

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DATA AVAILABILITY

Raw sequencing data of 93 strains used in this study have been submitted to Sequence Read Archive (SRA) under accession number PRJNA1024657.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Additional experimental details and supplemental figures (JCM00120-23-S0001.docx). Antimicrobial susceptibility testing, preparation of gold nanoparticle-labeled Penicillin Binding Protein and optimization of running buffer.

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