Infection and Drug Resistance

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ORIGINAL RESEARCH

Design of Rapid Detection System for Five Major Carbapenemase Families (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} and $bla_{OXA-48-Like}$) by Colorimetric Loop-Mediated Isothermal Amplification

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Purpose: Carbapenemase-producing *Enterobacteriaceae* (CPE) infection constitutes a public health threat. Timely and efficient diagnosis is of paramount importance for prompt and effective therapy. In order to quickly and comprehensively detect the five major families of carbapenemases (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and $bla_{OXA-48-like}$), colorimetric loop-mediated isothermal amplification (LAMP) was employed.

Materials and Methods: Five sets of LAMP primers were designed, each of which can, respectively, amplify all the carbapenemase subtypes described in this work. Twenty whole genome sequencing-verified-"standard strains", including 1 bla_{NDM-1}, 1 bla_{NDM-5}, 1 bla_{NDM-6}, 1 bla_{NDM-7}, 2 bla_{IMP-4}, 1 bla_{IMP-8}, 2 bla_{KPC-2}, 1 bla_{KPC-3}, 1 bla_{KPC-4}, 1 bla_{KPC-5}, 1 bla_{KPC-6}, 1 bla_{KPC-7}, 1 bla_{OXA-48} and 1 $bla_{OXA-181}$ carrier, and 1 bla_{VIM} and $bla_{OXA-244}$, 1 bla_{KPC-2} and bla_{IMP-4}, 1 bla_{KPC-2} and bla_{VIM-1} and 1 bla_{KPC-2} and bla_{NDM-1}-co-carriers, were used to establish a 25-microliter visual LAMP reaction system (kept at 65°C for 30 minutes in water bath). Color change from bright pink to yellow indicated positive amplification. In addition, 126 pre-verified clinical carbapenem-resistant *Enterobacteriaceae* (CRE) isolates, including 65 CPE (23 bla_{NDM}, 2 $bla_{OXA-48-like}$, 1 bla_{KPC} and bla_{VIM}, 2 bla_{IMP}, and 37 bla_{KPC} carriers) and 61 non-CPE, were also detected.

Results: With the lowest detection limit of 10 colony forming units (CFU) per reaction for LAMP and 10^3 CFU per reaction for PCR, the LAMP system demonstrated dramatically higher sensitivity while retaining the same specificity. Furthermore, we demonstrated concordant results between the two methods for the 126 clinical isolates.

Conclusion: Therefore, LAMP could be used for rapid identification of the five major carbapenemase gene families in routine clinical laboratories.

Keywords: carbapenemase-producing *Enterobacteriaceae*, loop-mediated isothermal amplification, bla_{NDM}, bla_{KPC}, bla_{VIM}, bla_{IMP}, $bla_{OXA-48-like}$

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Introduction

Antibiotic resistance is one of the most threatening global health challenges.[1–3] Carbapenems are considered as the last line of defense against the infection of *Enterobacteriaceae* producing extended-spectrum β-lactamases (ESBLs) and AmpC cephalosporinases (AmpCs).[4,5] However, due to the wide spread of ESBLs-producing *Enterobacteriaceae* in recent years, the extensive use of carbapenems has led to the quick emergence of carbapenem-resistant

Enterobacteriaceae (CRE). Carbapenem resistance in *Enterobacteriaceae* is mainly caused by the production of carbapenemases, especially Class A *Klebsiella pneumoniae* carbapenemase (KPC), Class B New Delhi metallo-β-lactamase (NDM), Imipenemase (IMP), Verona Integron-encoded Metallo-β-lactamase (VIM), and Class D oxacillinase-48-like (OXA-48-like).[4,6] Several methods have been employed to detect carbapenemase activities, such as the Modified-Hodge test (MHT),[7,8] the Ethylenediaminetetraacetic Acid (EDTA) inhibition test,[8] MALDI-TOF MS,[9] Carba NP test (CNPt),[10,11] and the carbapenem inhibition test (CIM).[12,13] Yet, some defects, such as low specificity, low sensitivity, lack of rapidity and convenience, still remain. As the standard method for identifying carbapenemase genotypes, polymerase chain reaction (PCR) depends on both expensive instruments and specialized technicians. Therefore, an easier, more convenient, rapid and accurate carbapenemase genotyping method, which can easily be carried out in primary-level laboratories, is essential.

Loop-mediated isothermal amplification (LAMP), invented by Notomi et al in 2000,[14] is a type of in vitro amplification technique under constant temperature condition without the need of skilled operators and expensive instruments, which has the characteristics of isothermal, rapid, high specificity and sensitivity. The reaction can be completed by setting up 3 pairs of specific primers (FIP, BIP, F3, B3, LF, and LB) for target gene and amplified at 65°C for 30 minutes; moreover, the amplified products can be observed by colorimetric change or visually measured by the presence of turbidity of the byproduct (magnesium pyrophosphate). The technique has been widely used for point-of-care diagnosis and detection of viruses, bacteria and parasites involved in recurrent and emerging infectious diseases.[15–18] Recently, LAMP has been used to detect carbapenemase genes in hydroxynaphthol blue dye (HNB), gel electrophoresis, turbidity, SYBR green or calcein for the measurement of the by-products.[19–22] However, those previously reported LAMP systems could not detect all the variants that have been reported from each carbapenemase gene family. To more rapidly and comprehensively detect the most common carbapenemase gene families in *Enterobacteriaceae* (bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, and $bla_{OXA\text{-}48\text{-}like}$), we tried to develop a new colorimetric LAMP system.

In this study, we described the design of three sets of primers and optimized the LAMP assay for detection of each of the five carbapenemase gene families. Of note, extremely strict primer screening criteria were followed, such as TM value, primer terminal stability, GC content and primer spacing. Most importantly, we designed five sets of LAMP primers for each carbapenemase gene family, ensuring no spanning of as many as possible the mutation sites among all the different variants of each carbapenemase family. Theoretically, each set of the LAMP primers can, respectively, detect the carbapenemase subtypes as described in this work. Also, the specificity and sensitivity of the LAMP reactions were determined. Finally, this LAMP system was used for rapid identification of carbapenemase genes in 126 pre-verified clinical CRE isolates.

Materials and Methods

Bacterial Isolates

Twenty whole-genome sequencing-verified "standard strains" from the Microbiology Laboratory of the first affiliated Hospital of Chongqing Medical University, including 1 $bla_{NDM\text{-}1}$, 1 $bla_{NDM\text{-}5}$, 1 $bla_{NDM\text{-}6}$, 1 $bla_{NDM\text{-}7}$, 2 $bla_{IMP\text{-}4}$, 1 $bla_{IMP\text{-}8}$, 2 $bla_{KPC\text{-}2}$, 1 $bla_{KPC\text{-}3}$, 1 $bla_{KPC\text{-}4}$, 1 $bla_{KPC\text{-}5}$, 1 $bla_{KPC\text{-}6}$, 1 $bla_{KPC\text{-}7}$, 1 $bla_{OXA\text{-}48}$ and 1 $bla_{OXA\text{-}181}$ carriers, and 1 bla_{VIM} and $bla_{OXA\text{-}244}$, 1 $bla_{KPC\text{-}2}$ and $bla_{IMP\text{-}4}$, 1 $bla_{KPC\text{-}2}$ and $bla_{NDM\text{-}1}$ and 1 $bla_{KPC\text{-}2}$ and $bla_{VIM\text{-}1}$ co-carriers (Table 1) were collected. One hundred twenty-six pre-verified CRE clinical strains, mainly consisting of *Klebsiella pneumoniae, Escherichia coli* and *Enterobacter cloacae* isolated during 2015–2020, were further collected from the first affiliated Hospital of Chongqing Medical University (Table 2). Carba NP test[23] was performed on all the isolates to determine if there were any bacteria that appeared to produce carbapenemases by phenotypic methods but were negative by genotypic methods, or vice versa.

Preparation of Bacterial DNA

Bacterial DNA was extracted by TIANamp Bacteria DNA kit (TIANGEN BIOTECH (BEIJING) CO., LTD; Cat#DP302-02; Lot#S8230) according to the manufacturer's instructions.

Primer Design

The reference sequences of the five major carbapenemase gene families (bla_{NDM}, bla_{KPC}, bla_{VIM}, bla_{IMP}, and $bla_{OXA\text{-}48\text{-}like}$) were downloaded from GeneBank website

Table 1 "Standard Strains" Verified by Whole-Genome Sequencing*

Strain No.	Strain Species	Carbapenemase Genes
1	*Enterobacter cloacae*	bla_{NDM-1}
2	*Klebsiella pneumoniae*	bla_{KPC-2}
3	*Klebsiella pneumoniae*	bla_{KPC-2}
4	*Klebsiella pneumoniae*	bla_{IMP-4}
5	*Escherichia coli*	bla_{IMP-4}
6	*Klebsiella pneumoniae*	bla_{NDM-5}
7	*Klebsiella pneumoniae*	bla_{KPC-2} and bla_{IMP-4}
8	*Klebsiella pneumoniae*	bla_{IMP-8}
9	*Klebsiella areogenes*	bla_{KPC-2} and bla_{NDM-1}
10	*Klebsiella pneumoniae*	bla_{NDM-7}
11	*Klebsiella pneumoniae*	bla_{KPC-6}
12	*Klebsiella pneumoniae*	bla_{KPC-4}
13	*Klebsiella pneumoniae*	bla_{KPC-3}
14	*Klebsiella pneumoniae*	bla_{KPC-7}
15	*Klebsiella pneumoniae*	bla_{VIM} and $bla_{OXA-244}$
16	*Klebsiella pneumoniae*	bla_{OXA-48}
17	*Klebsiella pneumoniae*	$bla_{OXA-181}$
18	*Klebsiella pneumoniae*	bla_{KPC-5}
19	*Klebsiella pneumoniae*	bla_{NDM-6}
20	*Klebsiella pneumoniae*	bla_{KPC-2} and bla_{VIM-1}

Notes: *The accession number of the sequences of those "standard strains" was not submitted to the database.

Table 2 Pre-Verified Clinical CRE Strains Tested in This Study

Strains	β-Lactamase Genes
Carbapenemase producers (65)	
Enterobacter cloacae (10)	bla_{NDM}
Escherichia coli (7)	bla_{NDM}
Citrobacter freundii (2)	bla_{NDM}
Enterobacter hormaechei (3)	bla_{NDM}
Enterobacter kobei (1)	bla_{NDM}
Klebsiella pneumoniae (36)	bla_{KPC-2}
Citrobacter freundii (1)	bla_{KPC-2}
Klebsiella pneumoniae (2)	bla_{IMP-4}
Klebsiella pneumoniae (1)	bla_{KPC-2} and bla_{VIM-1}
Klebsiella pneumoniae (1)	bla_{OXA-23}
Klebsiella oxytoca (1)	bla_{OXA-23}
***Non-carbapenemase producers* (61)**	
***ESBLs producers* (17)**	
Escherichia coli (6)	bla_{CTX-M}
Klebsiella pneumoniae (6)	bla_{CTX-M}
Klebsiella areogenes (1)	bla_{CTX-M}
Enterobacter cloacae (1)	bla_{CTX-M}
Klebsiella pneumoniae (1)	bla_{SHV}
Klebsiella pneumoniae (2)	$bla_{CTX-M-like}$, bla_{SHV}
***AmpC producers* (3)**	
Enterobacter cloacae (1)	bla_{DHA-1}
Klebsiella areogenes (1)	bla_{DHA-1}
Klebsiella pneumoniae (1)	bla_{DHA-1}
ESBLs & AmpC producers	
Enterobacter cloacae (1)[a]	bla_{CTX-M}, bla_{DHA-1}
***Non-ESBLs& non-pAmpC producers* (41)**	
Enterobacter cloacae (8) *Klebsiella pneumoniae* (18) *Escherichia coli* (6) *Klebsiella areogenes* (5) *Citrobacter freundii* (3) *Enterobacter hormaechei* (1)	

Notes: [a]The strains that produce carbapenemase genes and ESBLs at the same time are only counted in the number of carbapenemase-producing strains.

(https://www.ncbi.nlm.nih.gov/genbank/), and LAMP primers were designed by PrimerV5 (http://primerexplorer.jp/lampv5e/index.html). With the pre-defined primer designing principles: no mutation sites to be involved at the 3′-end of the forward primer, optimal TM values, terminal stability of the primers, and no secondary structures between the primers, the selected primers could theoretically be used to detect much more reported variants of each carbapenemase family gene group as compared to those of the previous studies. So far, according to the Beta-Lactamase DataBase (BLDB) (http://www.bldb.eu/Enzymes.php), bla_{NDM} has 31 variants, bla_{KPC} has 79 variants, bla_{IMP} has 89 variants and bla_{VIM} has 73 variants. The sequences of the mutants of $bla_{OXA-48-like}$ were compared with the sequence of bla_{OXA-48}. The primer sets of this study could detect all the reported variants of bla_{NDM} and bla_{KPC}, the bla_{IMP} group including bla_{IMP-1}-bla_{IMP-14}, and bla_{IMP-22}, bla_{IMP-32}, bla_{IMP-33}, bla_{IMP-48}, and bla_{IMP-68}, the bla_{VIM} group including all the reported variants except for bla_{VIM-7}, bla_{VIM-10}, bla_{VIM-51} and bla_{VIM-65}, and the $bla_{OXA-48-like}$ group including bla_{OXA-48}, $bla_{OXA-181}$, $bla_{OXA-232}$, $bla_{OXA-204}$, $bla_{OXA-162}$, and $bla_{OXA-244}$. The LAMP primers were listed in Table 3 and

Table 3 Nucleotide Sequences of the LAMP Primers for Detection of bla_{NDM}, bla_{KPC}, bla_{VIM}, bla_{IMP} and $bla_{OXA-48-Like}$ Gene Families

Genes	Primers	Sequence (5′-3′)	Length	Spanning Position on the Gene
bla_{NDM}	F3	CATTAGCCGCTGCATTGATG	20	47–66
	B3	CCGCCATCCCTGACGATC	18	234–251
	FIP	ATCGCCAAACCGTTGGTCGCC-CGGTGAAATCCGCCCG	37	124–144, 84–99
	BIP	TGGTTTTCCGCCAGCTCGC-AGCGACTGCCCCGAAAC	36	146–164, 206–222
	LF	TCCATTTGCTGGCCAATCG	19	101–119
	LB	ACCGAATGTCTGGCAGCACA	20	165–184
bla_{KPC}	F3	TGGACACACCCATCCGT	17	269–285
	B3	GGAACGTGGTATCGCCG	17	462–478
	FIP	ACCGTCATGCCTGTTGTCAGAT-CGGCAAAAATGCGCTGGT	40	332–353, 288–305
	BIP	AATACAGTGATAACGCCGCCGC-CGCATGAAGGCCGTCAG	39	380–401, 439–455
	LF	GAGATGGGTGACCACGGA	18	306–323
	LB	CAATTTGTTGCTGAAGGAGTTGGGC	25	402–426
bla_{VIM}	F3	GGTTGTATACGTCCCGTCAG	20	552–571
	B3	GTGCTTTGACAACGTTCGC	19	757–775
	FIP	GATCGGCATCGGCCACGTTC-GGTGGTTGTGCCGTTCAT	38	627–646, 586–603
	BIP	AACACTACCCGGAAGCAGAGGT-TGTGTGCTGGAGCAAGTCT	41	683–704, 738–756
	LF	GCAGACGTGCTTGACAACTC	20	604–623
	LB	TCATTCCCGGGCACGGT	17	707–723
bla_{IMP}	F3	GCGTTGTTCCTAAACATGG	19	140–158
	B3	ATACGTGGGGATGGATTG	18	328–345
	FIP	CCACAAACCAAGTGACTAACTTTTC-TTGTTCTTGTAGATGCTGAAG	46	223–247, 164–184
	BIP	AACGTGGCTATAAAATAAAAGGCAG-AGAATTAAGCCACTCTATTCCG	47	248–272, 306–327
	LF	GCCGTAAATGGAGTGTCAATTAGAT	25	188–212
	LB	CTCATTTTCATAGTGACAGCACGG	24	281–304
$bla_{OXA-48-like}$	F3	AATAGCTTGATCGCCCTC	18	226–243
	B3	CCATAATCGAAAGCATGTAGC	21	414–434
	FIP	GATTCCAAGTGGCGATATCGC-GGCGTGGTTAAGGATGAAC	40	299–319, 250–268
	BIP	TAATCACCGCGATGAAATATTCAGT-CTTGCTCATACGTGCCTC	43	332–356, 394–411
	LF	TGTCCATCCCACTTAAAGACTTG	23	271–293
	LB	CCTGTTTATCAAGAATTTGCCCGC	24	361–384

Notes: The reference sequences used in LAMP primer design were bla_{NDM-1} (KJ018857), bla_{KPC-2} (LDDY01000008), bla_{VIM-1} (AJ278514), bla_{IMP-4} (AF244145), and bla_{OXA-48} (LN864820).

Abbreviations: BIP, backward inner primer; B3, backward outer primer; FIP, forward inner primer; F3, forward outer primer; LB, loop backward primer; LF, loop forward primer.

synthesized by Sangon Biotech (Shanghai, China) Co., Ltd.

Colorimetric LAMP Assay

LAMP reaction was performed in a total volume of 25 μL, comprising of 2.5 μL 10×LAMP Primer Mix [2 μM each of outer primer (F3, B3), 16 μM each of inner primer (FIP, BIP), 8 μM each of loop primer (LF, LB)], 12.5 μL WarmStart Colorimetric LAMP 2X Master Mix (NEW ENGLAND BioLabs® Inc), 9 μL dH_2O, 1 μL target DNA template. The amplification was carried out at 65°C for 30 minutes. Aseptic distilled water was used as the negative control. Color change from bright pink to yellow indicates a positive reaction.

Comparison of the Specificity and Sensitivity Between the Colorimetric LAMP and PCR

To estimate the specificity of the colorimetric LAMP assay for the detection of the five major carbapenemase gene families, we used the standard strain containing only one

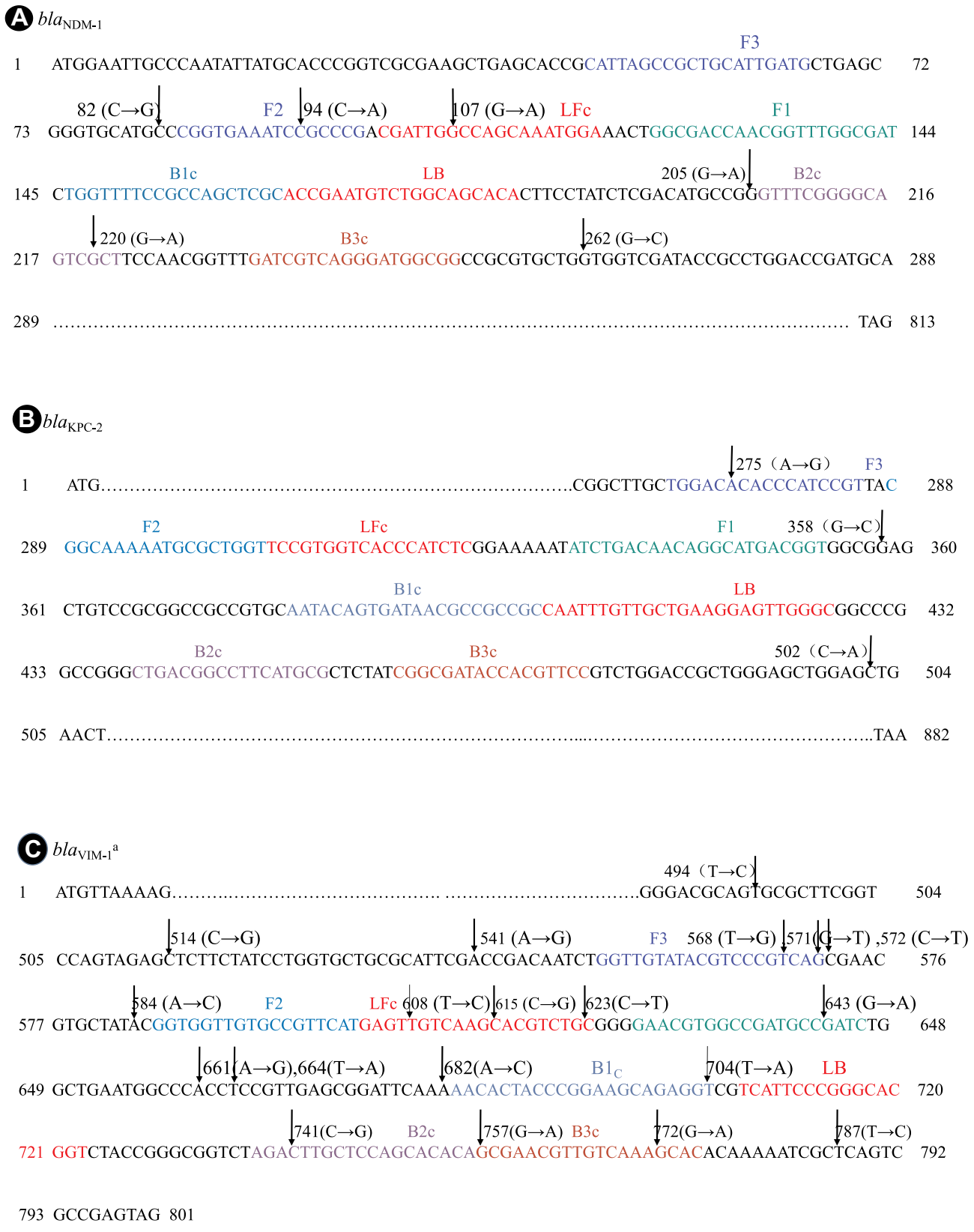

Figure 1 Continued.

Figure 1 Schematic diagram of the spanning range of the primers and the associated mutation sites for each carbapenemase gene class.

Notes: (**A**–**E**): bla_{NDM-1}, bla_{KPC-2}, bla_{VIM-1}, bla_{IMP-4}, bla_{OXA-48}. No mutation occurred at the end of 3′ end of the selected primers of bla_{NDM-1} and bla_{KPC-2}. [a]G→T at the 3′ end of F3 primer, bla_{VIM-1} changed to bla_{VIM-7} at this position, theoretically, bla_{VIM-7} could not be detected by our primer. Similarly, bla_{VIM-1} changed to bla_{VIM-10} at the 3′ end of B1c primer (T →A) in BIP primer and bla_{VIM-1} changed to bla_{VIM-51} and bla_{VIM-65} at the 3′ end of LF primer (C→T). [b]After comparing the gene sequences of bla_{IMP} families, our LAMP primers can only detect theoretically bla_{IMP-1}- bla_{IMP-14}, and bla_{IMP-22}, bla_{IMP-32}, bla_{IMP-33}, bla_{IMP-48} and bla_{IMP-68}. [c]A→T at the 3′ end of LF primer, bla_{OXA-48} changed to $bla_{OXA-324}$ at this position, theoretically, $bla_{OXA-324}$ could not be detected. Mutation sites out of the spanning range of the selected primers were not listed.

specific carbapenemase gene. Strains without the specific genes being detected were also selected to verify the specificity of the LAMP primers. Experimental sensitivity of the LAMP assay was ascertained by a series of DNA template extracted from 10^1 to 10^8 CFU/reaction from an initial concentration of 10^9 CFU/mL bacteria. The lowest value of positive result is the limit of detection. As a standard for assessing the colorimetric LAMP system, PCR was used to detect the potential presence of the five carbapenemase gene families. The evaluation experiments of sensitivity and specificity were repeated thrice to ensure reproducibility.

Ethical Considerations

For this study, samples were collected at the microbiology laboratory of the hospital, with no contact with the patient. This study was retrospective with no patient identification performed during data collection. Therefore, the

Chongqing Medical University Institutional Review Board and the Biomedical Ethics Committee determined that this research was exempt from approval.

Results

Specificity of the Colorimetric LAMP

The spanning sites where the LAMP primers are located in each carbapenemase gene family are listed as in Figure 1. As shown in Figure 2, we only observed color change from bright pink to yellow when the specific LAMP primers for the target gene were added, thus demonstrating 100% specificity of the colorimetric LAMP system in detecting the five carbapenemase gene families.

Much Higher Sensitivity of the Colorimetric LAMP Compared with Traditional PCR

As depicted in Figure 3, while more than 10^3 CFU/reaction was required for PCR amplification of all the five carbapenemase gene families, positive LAMP results could be achieved with only 10^1 CFU/reaction for bla_{KPC} (Figure 3B), bla_{IMP} (Figure 3D), and $bla_{OXA\text{-}48\text{-}like}$ (Figure 3E), and 10^2 CFU/reaction for bla_{NDM} (Figure 3A) and bla_{VIM} (Figure 3C).

Higher Proficiency of the Colorimetric LAMP in Detecting Carbapenemase Gene Families in Clinical Isolates

The five carbapenemase gene families in a total of 126 pre-verified clinical CRE isolates were detected by both the colorimetric LAMP assay and the traditional PCR assay. Both of them identified 65 carbapenemase-carriers, including 23 strains with bla_{NDM}, 37 with bla_{KPC}, 2 with $bla_{OXA\text{-}48\text{-}like}$, 2 with bla_{IMP} and 1 with bla_{KPC} and bla_{VIM}, indicating 100% concordance in the detection proficiency between the two methods. Of note, with its simplicity and rapidity, the colorimetric LAMP assay demonstrated much higher proficiency in detecting carbapenemase genotypes in clinical isolates when compared with traditional PCR and subsequent sequencing.

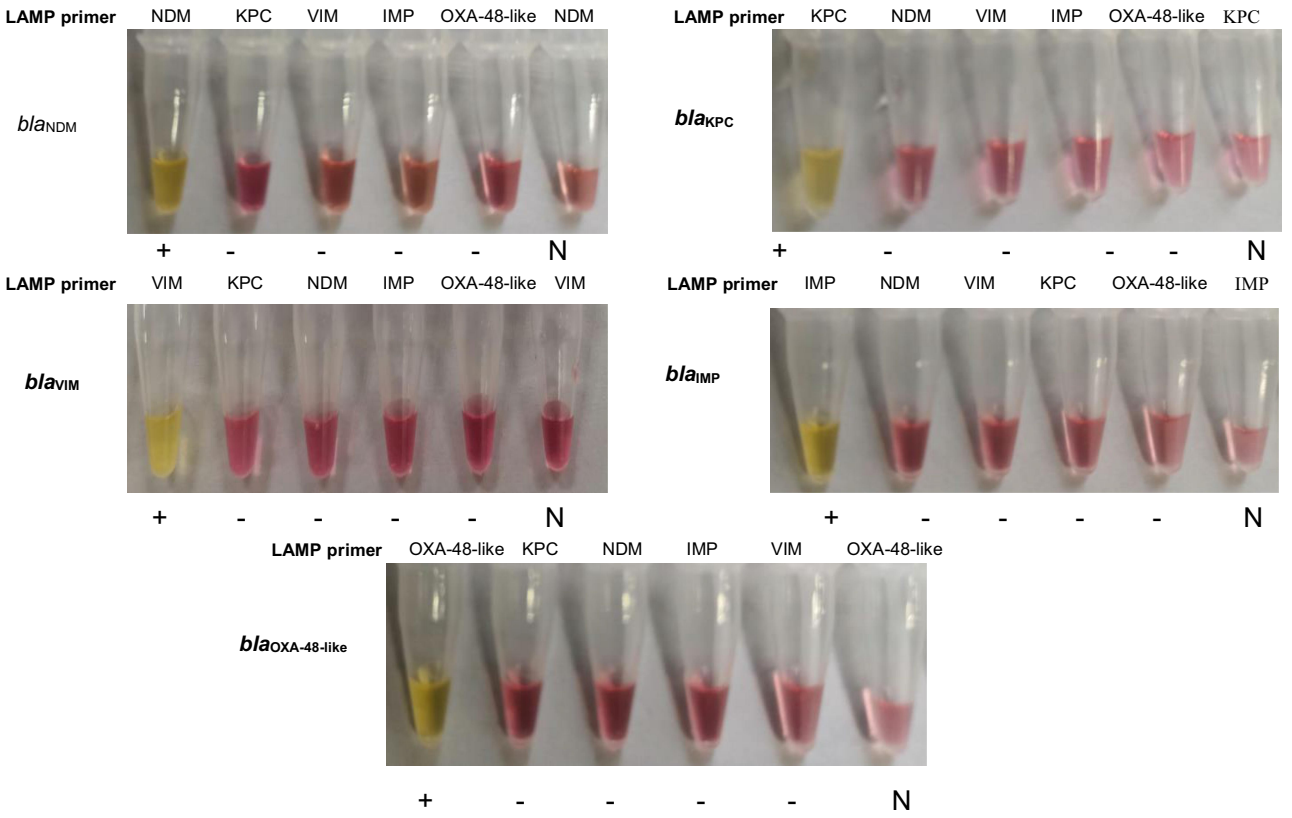

Figure 2 The specificity of the LAMP primers.

Notes: DNA templates with $bla_{NDM\text{-}1}$, $bla_{KPC\text{-}2}$, $bla_{IMP\text{-}4}$, $bla_{OXA\text{-}48}$, and $bla_{VIM\text{-}1}$ were used, respectively. When different LAMP primers were added to each tube, only the reaction tubes containing the specific LAMP primers for the gene were amplified. In the last tube, sterile distilled water was added instead of template to rule out non-specific amplification.

Abbreviation: N, negative control.

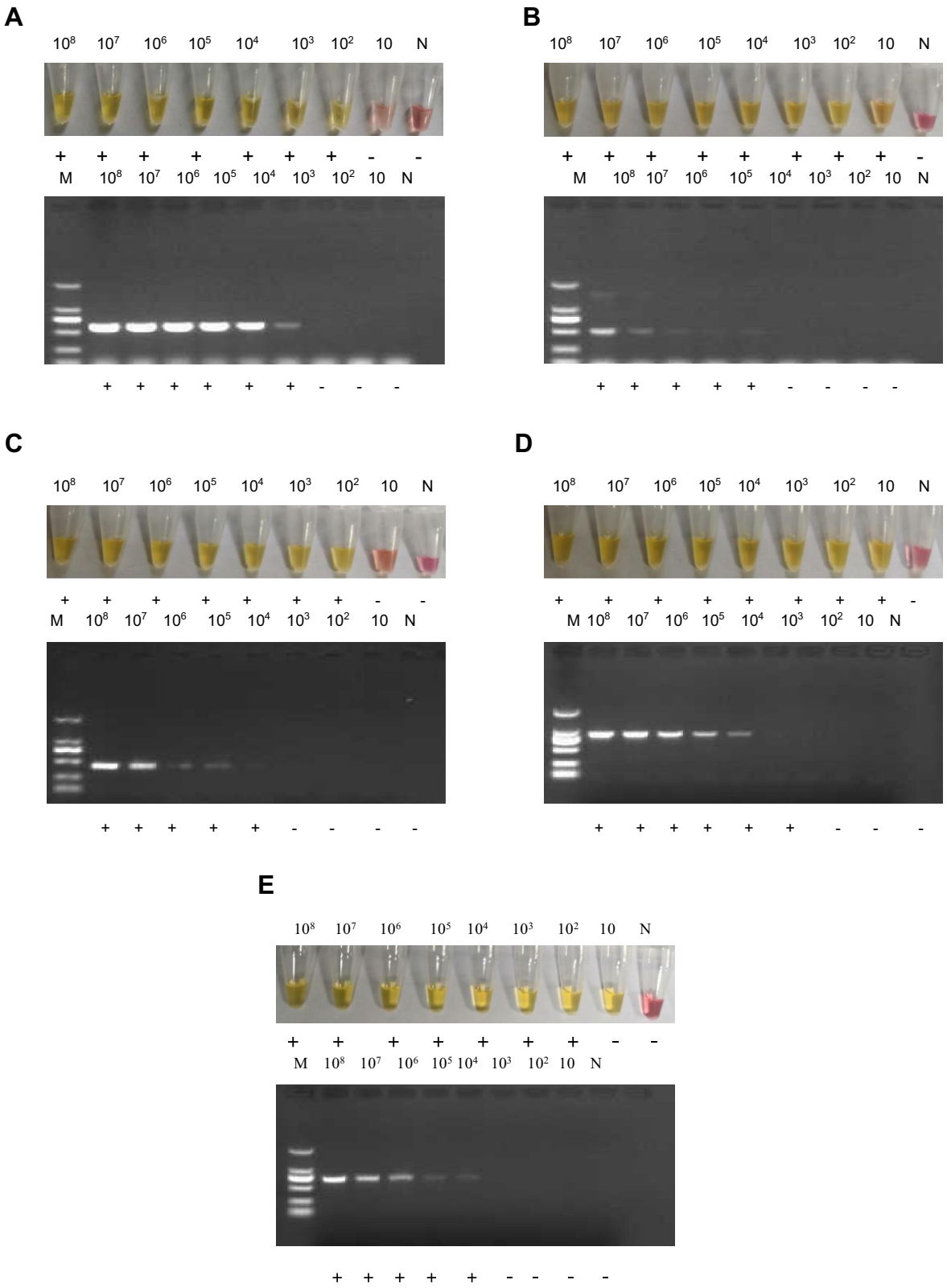

Figure 3 The comparison of the sensitivity between the LAMP reaction and PCR for detection of the five carbapenemase gene families.
Notes: The detections of the bla_{NDM} gene (**A**), bla_{KPC} (**B**), bla_{IMP} (**C**), $bla_{OXA\text{-}48\text{-}like}$ (**D**), and bla_{VIM} (**E**) were carried out in duplicate for each dilution of DNA template.
Abbreviation: N, negative control.

Discussion

With the wide spread of carbapenemase genes, CPE is becoming an increasingly serious world health concern. Infections caused by CPE will not only prolong the length of hospital stays but also increase the medical expenses of patients whose immune systems are compromised. Rapid and accurate identification of drug resistance genotypes can provide a basis for rational clinical use of antibiotics.

In the past decade, bacterial culture must precede the routine diagnostic methods used to detect the carbapenem-resistant organisms. MHT,[7] EDTA inhibition test,[8] MALDI-TOF MS,[9] CNPt[10,11] and CIM[12,13] were commonly employed to detect the carbapenemase activity. However, some of these phenotypic tests usually require expensive instruments and special cultural conditions, and furthermore, the time required to complete some of these tests is usually 16–20 hours.[21] Molecular diagnostic methods remain the gold standard for the identification of carbapenemase genes. However, quantitative measurements of target genes are not always straightforward in PCR analyses, and the presence of the amplified products has to be confirmed by both gel electrophoresis and subsequent sequencing. Therefore, the LAMP method that requires only water bath or heating block to obtain a constant temperature is an alternative molecular tool for identifying carbapenemase genes in the primary-level laboratories.

As a simple and rapid identification method, LAMP has been widely used in the diagnosis of carbapenemase genes. However, as the key in DNA amplification using the LAMP method, appropriate primer design is essential. Over 89 IMP, 73 VIM, 79 KPC, 31 NDM, and 26 OXA-48-like carbapenemases have been described as of the date of March 01, 2021 (http://www.bldb.eu/F-BLDB.php). Many of the variations within the carbapenemase gene families matter when it complicates the design of comprehensive PCR detection methods, necessitating repeated "tweaking" as new variants are added to the detection repertoire. Through Beta-Lactamase DataBase (http://www.bldb.eu/F-BLDB.php) search and review, we comprehensively concluded all the information on the mutation sites and the primers spanning these mutation sites were discarded during the optimal primer screening, so as to cover as many as possible the gene variants by the designed primers. Previous studies showed that the number of genotypes that could be detected by the reported LAMP system was limited, from the initial $bla_{\text{NDM-1}}$ by Liu et al,[22] to the detection of $bla_{\text{OXA-23}}$, $bla_{\text{VIM-2}}$, and $bla_{\text{IMP-1}}$ by Kim et al,[21] and the detection of $bla_{\text{NDM-1}}$ through $bla_{\text{NDM-9}}$, $bla_{\text{KPC-2}}$ through $bla_{\text{KPC-15}}$, $bla_{\text{VIM-2}}$, and $bla_{\text{IMP-4}}$ by Chen et al.[20] Later, LAMP-HNB was used to detect $bla_{\text{NDM-1}}$ through $bla_{\text{NDM-9}}$, $bla_{\text{NDM-11}}$ through $bla_{\text{NDM-16}}$, $bla_{\text{OXA-48}}$, $bla_{\text{VIM-1}}$ through $bla_{\text{VIM-46}}$ (except for $bla_{\text{VIM-7}}$, $bla_{\text{IMP-14}}$) and $bla_{\text{KPC-2}}$ through $bla_{\text{KPC-24}}$ (except for $bla_{\text{KPC-20}}$ and $bla_{\text{KPC-23}}$) groups by Srisrattakarn et al.[19] The LAMP system of the present study was demonstrated to be capable of specifically detecting all the reported subtypes of the five major carbapenemase genes as described in this work, which was consistent with the results of PCR and subsequent sequencing. After demonstration of the specificity of the respective LAMP primers for bla_{NDM}, bla_{KPC}, bla_{IMP}, bla_{VIM}, and $bla_{\text{OXA-48-like}}$, we further tested the sensitivity of the LAMP system, and the results showed higher sensitivity for LAMP when compared with that of the conventional PCR method, which was consistent with the results of previous studies.[19,20] In this study, using the WarmStart master LAMP mixed with the chromogenic reagent, primers and templates were added to the sealed reaction tank and amplified at 65°C for 30 minutes, which greatly reduced the possible pollution caused by repeated lid opening operations. And compared with other detection methods, such as turbidity, gel electrophoresis, real-time turbidimeter, SYBR Green or calcein, using this reagent to visually observe the color change to read the results is safer, more simple and rapid. Previous studies used betaine, magnesium sulfate and other substances, but other aspects such as the operational factors during reagent preparation may increase the unreliability of the results. Therefore, our results further supported the use of more convenient chromogenic mixed reagents for LAMP detection.

The colorimetric LAMP system has been demonstrated to be a simple and fast carbapenemase identification method that can be carried out in conventional laboratories without special instruments, and the results provided can lead to the rational use of antibiotics in clinic. Nevertheless, one major limitation of the present LAMP method is that amplification for each carbapenemase gene has to be run individually (ie 1 reaction/gene) as no multiplex LAMP reaction is possible at present. Then, 5 reactions are necessary to define what carbapenemase gene(s) is/are present in one isolate vs 1 reaction in a multiplex amplification, leading to a complication when a high number of isolates have to be tested.

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Disclosure

All authors reported no conflicts of interest in this work.

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