Original Article

Comparative Study on DNA Amplification Methods for Detection of Carbapenem-Resistant Enterobacteriaceae (CRE)

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Background: Carbapenem-resistant Enterobacteriaceae (CRE) is a resistant group of gram-negative bacteria that produces carbapenemase destroying carbapenem molecules causing drug resistances. Among them, the incidence of New Delhi metallo-beta-lactamases (bla_{NDM-1}), a metallo-carbapenemases or class B carbapenemases or metallo-β-lactamases (MBL), are increasing worldwide.

Objective: To focused on comparative study on DNA amplification methods for detection of CRE in terms of analytical sensitivity and specificity.

Materials and Methods: CRE strains (*Escherichia coli*, *Escherichia cloacae*, *Citrobacter freudii*, and *Klebsiella pneumonia*) were collected from HRH Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University, Nakhon Nayok, Thailand. All specimens were initially screened for CRE isolates by using carbapenem disks inhibition test. The polymerase chain reaction (PCR), loop mediated isothermal amplification (LAMP) and recombinant polymerase amplification (RPA) assays were further employed for identification of drug resistance bla_{NDM-1} gene among CRE isolates. The analytical sensitivity and specificity of the three amplification assays were compared and analyzed.

Results: The analytical sensitivity test of PCR, LAMP and RPA assays revealed that the limit of detection were 0.74 ng/μL, 7.4 pg/μL, and 0.74 ng/μL, respectively. Specificity test demonstrated that all three assays showed no cross hybridization to the other 15 related bacterial isolates.

Conclusion: The data pointed out that LAMP assay was 100 times more sensitive than PCR and RPA, which could be suitable for application as early detection test for carbapenem resistant bacteria. Hence, the physician can select the proper antibiotics to treat the CRE infectious bacteria, which can reduce morbidity and mortality rates in patients. In the future, LAMP will be combined with lateral flow dipstick (LFD) to improve the efficacy of the assay as not only the early detection test with high sensitivity and specificity for carbapenem resistant bacteria, but also as the convenient and rapid screening test.

Keywords: Enterobacteriaceae; Carbapenem; Carbapenem-resistant Enterobacteriaceae (CRE); New Delhi metallo-beta-lactamase (bla_{NDM-1})

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Enterobacteriaceae are a family of facultative anaerobic gram-negative, rod-shaped bacteria. They are usually found in human intestinal flora major and cause infection as both community-acquired and hospital-acquired(1). This family is comprised of various genus and species such as *Klebsiella* spp., *Escherichia* coli, *Citrobacter* spp., *Enterobacter* spp., *Salmonella* spp., *Proteus* spp., *Morganella* spp., *Serratia* spp., *Shigella* spp., *Yersinia* spp., and *Providencia* spp. The Enterobacteriaceae are the most important bacteria causing bloodstream infections, urinary tract infections (UTIs), and healthcare-associated pneumonias(2). They can normally spread to humans via water and food. However, plasmids and transposons of them are the main transferred way of multiple genetic resistant elements(3).

Carbapenem is a group of antimicrobial drugs of which chemical structure called β-lactam ring (beta-lactam) is against many types of bacteria(4). Enterobacteriaceae has three major resistant mechanisms to carbapenem such as enzyme production, efflux pumps, and porin mutations.

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However, the enzyme production is the main resistance mechanism that cleave the β-lactam ring, an essential component of carbapenem antibiotic[5]. The β-lactam ring is recognized to penicillin binding proteins (PBPs), by inhibiting formation of peptidoglycan of Enterobacteriaceae[6]. Carbapenem-resistant Enterobacteriaceae (CRE) bacteria are commonly colonized at intestinal of patient and can spread to blood stream as well as excreted into feces. Therefore, the early detection of CRE can support the effective treatment of the infection and reduce the spreading of CRE bacteria from feces. Additionally, the carbapenemases can be divided into three ambler categories, class A types bla_{KPC} (*Klebsiella pneumonia* carbapenemase), class B types bla_{IMP} (Imipenemase), bla_{VIM} (Verona integrin metallo-beta-lactamase), and bla_{NDM-1} (New Delhi metallo-beta-lactamase-1), and class D types bla_{OXA-23} (Oxacillinase-23), bla_{OXA-48} (Oxacillinase-48), and bla_{OXA-58} (Oxacillinase-58)[3]. Classes A and D possess a serine residue at the active site to facilitate ring opening, they are thus called serine. Class B metallo-beta-lactamases (MBLs), bla_{IMP}, bla_{VIM}, and bla_{NDM-1}, the active site of which uses zinc ions to mediate bond hydrolysis. Among of them, bla_{NDM-1}, a member of class B is the most common types of CRE found in Asia[7]. For these reasons, the present study was interested in developing the rapid method instead of conventional method (disc sensitivity) for detecting bla_{NDM-1} in clinical field.

The bla_{NDM-1} gene is a type of MBL able to hydrolyze most β-lactams, including carbapenems, but not monobactams[8,9]. The bla_{NDM-1} was first identified in a *K. pneumonia* strain isolated from a Swedish patient who had been hospitalized in New Delhi, India, in 2008[9]. The clinical samples, bla_{NDM-1} gene have also been detected in hospital sewage in several countries, including China[10,11], India[12], and Lebanon[13]. Some bla_{NDM-1}-positive strains recovered from hospital sewage belong to the Enterobacteriaceae[12,13]. Detection of bla_{NDM-1} is essential for informing therapeutic decisions as well as provides critical information in investigating outbreaks and local epidemiology of bla_{NDM-1}-positive strains[7].

At present, reference methods for detection of bla_{NDM-1} gene was based on molecular techniques due to their excellent sensitivity and specificity and robust performance[14,15]. Basically, the conventional polymerase chain reaction (PCR) is employed as the reference molecular technique. Alternatively, loop mediated isothermal amplification (LAMP) has been introduced for the rapid and sensitive detection of bla_{NDM-1} since it relied on isothermal amplification, which does not require expensive thermocyclers. In addition, LAMP has been shown to exhibit higher sensitivity for detecting bla_{NDM-1} than conventional PCR[16-18]. Currently, recombinant polymerase amplification (RPA) technique has been developed to be carried out within 20 minutes. However, the reaction could be interfered with high protein contamination causing false positive. Therefore, the RPA product should be cleaned up prior to agarose gel electrophoresis (AGE) analysis.

Hence, the present study was concentrated on comparative study on DNA amplification methods for detection of CRE in terms of analytical sensitivity and specificity, which will be further applied for early detection test of carbapenem resistant bacteria.

Materials and Methods

The present research was approved by the Ethics Committee of Srinakharinwirot University (SWUEC/E-344/2560).

Sample collection

CRE strains (*E. coli*, *E. cloacae*, *C. freudii*, and *K. pneumonia*) were collected from HRH Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University, Nakhon Nayok, Thailand. All specimens were initially screened for CRE isolates by using carbapenem disks inhibition test[19]. The three types of carbapenem sensitivity discs consisted of meropenem, imipenem, and ertapenem. The inhibition was analyzed according to Clinical and Laboratory Standards Institute (CLSI) 2018 that the carbapenem resistance strain must show resistance to at least one-third of the selected disc.

DNA extraction

The 3-5 CRE resistance colonies were selected and suspended in 200 µL of lysis buffer containing 1% Triton X-100, 10 mM Tris-HCl pH 8.0 and 1 mM Disodium ethylene diamine tetra acetate (EDTA). The suspension was mixed by shaking for 30 seconds and heated at 100°C for 20 minutes. After that, the mixture was centrifuged at 15,000 × g for five minutes. The supernatant was then kept at 4°C until use. Each DNA concentration was determined by using NanoDrop™ 2000 Spectrophotometer (Thermo Scientific; Wilmington, DE, USA).

PCR amplification of bla_{NDM-1} gene

PCR amplification was achieved by using

primers designed based on *bla*$_{NDM-1}$ gene (Gen-bank accession on. Genbank; KY074638). The forward and reverse primers were analyzed by using software Primer explorer V5 programed (http://primerexplorer.jp/lampv5e/index.html). The condition of PCR amplification for *bla*$_{NDM-1}$ gene was prepare in 25 µL reaction containing 4 ng of genomic DNA, 10X PCR buffer, 50 mM $MgCl_2$, 10 mM dNTPs, Taq polymerase (Invitrogen, USA) and 10 µM of each primer. The PCR reaction was performed by using C1000 Touch™ Thermal Cycler (Bio-Rad Laboratories Ltd.; California, USA). The steps of PCR amplification contained pre-denaturation at 94°C for five minutes followed by 30 cycles of denaturation at 94°C for one minute, annealing at 58°C for one minute and extension at 72°C for one minute, and finally post-extension at 72°C for another five minutes. The PCR products were analyzed by using 2% AGE in 0.5X Tris/Borate/EDTA (TBE) buffer at 100 volts. The DNA pattern was observed under UV light by using gel-doc (UVITEC Cambridge).

LAMP amplification of *bla*$_{NDM-1}$ gene

LAMP amplification was accomplished by using primers designed based on *bla*$_{NDM-1}$ gene (Gen-bank accession on. Genbank; KY074638). The forward and reverse primers as well as forward inner and reverse inner primers were analyzed by using software Primer explorer V5 programed (http://primerexplorer.jp/lampv5e/index.html) (Under submission). The condition of LAMP amplification for *bla*$_{NDM-1}$ gene was prepared in 25 µL reaction containing 4 ng of genomic DNA, 10X thermal, 5M Betaine, 25 mM dNTP, 100 µM MgSO4, Bst DNA polymerase (New England Biolabs, Ipswich, MA, USA). 10 µM of outer primer (NDM-1-F3 and NDM-1-B3) and 100 µM of inner primer (NDM-1-FIP and NDM-1-BIP). The reaction mixture was incubated at 63°C for 60 minutes by using C1000 Touch™ Thermal Cycler (Bio-Rad Laboratories Ltd.; California, USA). It was analyzed by 2% AGE in 0.5X TBE buffer at 100 volts. The DNA pattern was observed under UV light by using gel-doc (UVITEC Cambridge).

RPA amplification of *bla*$_{NDM-1}$ gene

RPA was achieved by using primers designed based on *bla*$_{NDM-1}$ gene (Gen-bank accession on. Genbank; KY074638). The forward and reverse primers were analyzed by using software Primer explorer V5 programed (http://primerexplorer.jp/lampv5e/index.html). The condition of RPA for *bla*$_{NDM-1}$ gene was prepared in 50 µL reaction containing 4 ng of genomic DNA, RPA kit [(TwistAmp® Basic kit Quick Guide). The reaction mixture was incubated at 33°C for 20 minutes. The RPA product was isolated by using GenepHIow™ Gel/PCR kit (Geneaid) and analyzed by using 2% AGE in 0.5X TBE buffer at 100 volts. The DNA pattern was observed under UV light by using gel-doc (UVITEC Cambridge).

Analytical sensitivity test

The analytical sensitivity test was performed by 1:10 serial dilution of the standard DNA prior to testing with PCR, LAMP, and RPA assays. Standard DNA measured the concentration start 0.74 ng/µL by using nanodrop and performed by 1:10 by Sterile deionized water into 10 times. Every dilution was used as a template for evaluating of the sensitivity of PCR, LAMP, and RPA methods.

Analytical specificity test

The analytical specificity test was accomplished by testing the primers against 16 strains separated into two groups including ten strains of non-Enterobacteriaceae (*E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, *S. agalactiae* ATCC 12386, *S. pneumonia* ATCC 49619, *E. casseliflavus* ATCC 700327, *A. baumannii* ATCC 196606, *S. aureus* ATCC 29213, *S. pyogenes* ATCC 19615, and *S. maltophilia* ATCC 17666) and six strains of Enterobacteriaceae (positive *bla*$_{NDM-1}$ gene, *K. pneumonia* ATCC 700603, *S. sonnei* ATCC 25931, *E. coli* ATCC 35218, *P. mirabilis* ATCC 43071, and *E. hormaechei* ATCC 700323), which were used as templates for evaluation of the specificities of PCR, LAMP, and RPA methods.

Clinical sample testing

One hundred ten species CRE extracted DNA Bacteria samples were derived from the hospital (Medical centers, HRH Princess Maha Chakri Sirindhorn, Nakhon Nayok). All samples were tested with PCR, LAMP, and RPA with specific primer for *bla*$_{NDM-1}$ gene and compared with universal primer for standard method (PCR) from EUCAST. The results were calculated in the sensitivity, specificity, and relative trueness followed by ISO16140-2 (ISO, 2016).

Sensitivity of the alternative method=SEalt
(PA+PD) / (PA+ND+PD) ×100
Specificity of the alternative method=SPalt
1–P0 / N ×100%
Relative trueness (%R)=RT
(PA+ND) / (N) ×100

False positive ratio of the alternative method=FPR

FP / NA ×100

PA=positive agreement

PD=positive deviation

NA(FP)=negative agreement due to false-positive alternative method result

NA=negative agreement due to false-negative alternative method result

ND=negative deviation

N=number of samples

P0=total number of false positive results before confirmation result

Results

PCR of *bla*$_{NDM-1}$ gene

PCR amplification of CRE strains (*E. coli*, *E. cloacae*, *C. freudii*, and *K. pneumonia*) containing *bla*$_{NDM-1}$ gene generated the PCR product of 204 bp in size (Figure 1-3).

LAMP of *bla*$_{NDM-1}$ gene

The CRE strains (*E. coli*, *E. cloacae*, *C. freudii*, and *K. pneumonia*) CRE strains containing *bla*$_{NDM-1}$ gene generated the LAMP ladder product (Figure 4-6).

RPA of *bla*$_{NDM-1}$ gene

The RPA of CRE strains (*E. coli*, *E. cloacae*, *C. freudii*, and *K. pneumonia*) containing *bla*$_{NDM-1}$ gene generated the RPA product of 204 bp in size (Figure 7-9).

Analytical sensitivity and specificity assays of PCR, LAMP, and RPA based on *bla*$_{NDM-1}$ gene

Sensitivity test of PCR, LAMP, and RPA for amplification of CRE isolates containing *bla*$_{NDM-1}$ gene revealed that the limit of detection was 0.74 ng/µL (Figure 2), 7.4 pg/µL (Figure 5), and 0.74 ng/µL (Figure 8), respectively. For specificity test, 16 strains of microorganisms were initially screened to CRE property by using carbapenem disks inhibition assay, while meropenem, imipenem, and ertapenem were used as control (the results were not shown). Only one strain, *K. pneumonia* was shown positive *bla*$_{NDM-1}$ gene strain. In parallel, the analytical specificity

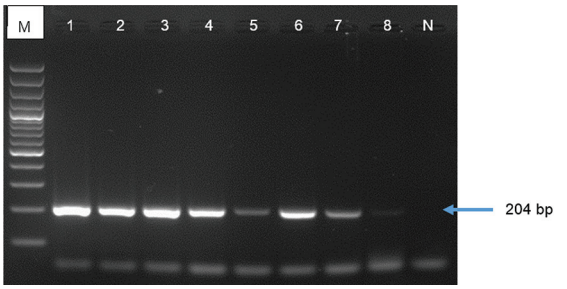

Figure 1. Gel electrophoresis pattern of PCR amplification of *bla*$_{NDM-1}$ gene with 204 bp PCR product in size. Lane M=100 bp λ ladder marker. Lanes 1-2 represent *E. coli*, Lanes 3-4 represent *E. cloacae*, Lane 5 represent *K. pneumonia*, Lane 6 represent *C. freudii*, Lanes 7-8 represent *K. pneumonia*. Lanes 1-8 were CRE positive control. Lane N represents negative control.

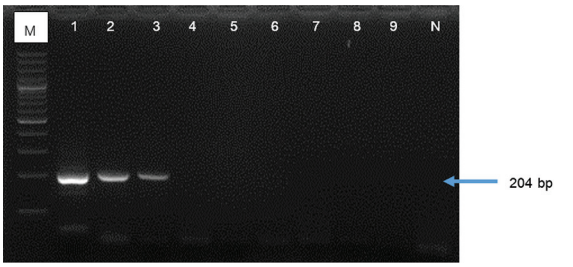

Figure 2. The analytical sensitivity test of *bla*$_{NDM-1}$ gene using PCR showing the limit of detection at 0.74 ng/µL. Lane M=100 bp λ ladder marker. Lanes 1-9 represent 74 ng/µL, 7.4 ng/µL, 0.74 ng/µL, 74 pg/µL, 7.4 pg/µL, 0.74 pg/µL, 74 fg/µL, 7.4 fg/µL, and 0.74 fg/µL. Lane N represents negative control.

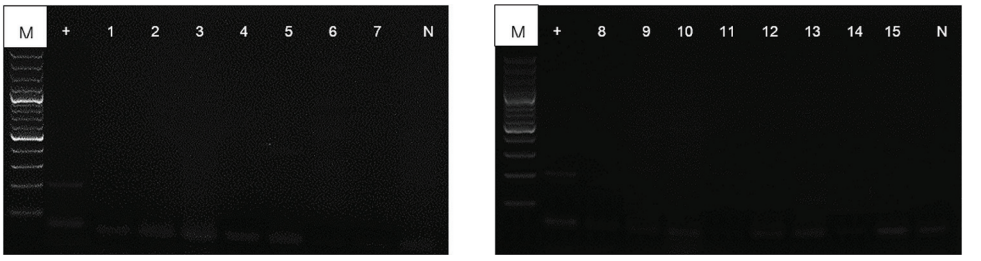

Figure 3. The specificity test of PCR for detection of *bla*$_{NDM-1}$ gene. Lane M=100 bp λ ladder marker, Lane + represents positive *bla*$_{NDM-1}$ gene of 204 bp. Lanes 1-15 represent *E. faecalis* (ATCC 29212), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923), *S. agalactiae* (ATCC 12386), *K. pneumonia* (ATCC 700603), *S. pneumonia* (ATCC 49619), *S. sonnei* (ATCC 25931), *E. coli* (ATCC 35218), *E. casseliflavus* (ATCC 700327), *P. mirabilis* (ATCC 43071), *A. baumannii* (ATCC 196606), *S. aureus* (ATCC 29213), *E. hormaechei* (ATCC 700323), *S. pyogenes* (ATCC 19615), *S. maltophilia* (ATCC 17666), respectively. Lane N represents negative control.

test based on PCR, LAMP, and RPA demonstrated no cross-amplification to host DNA and other microorganisms such as *E. faecalis* (ACTT 29212), *P. aeruginosa* (ACTT 27853), *S. aureus* (ATCC 25923), *S. agalactiae* (ACTT 12386), *K. pneumonia* (ATCC 700603), *S. pneumonia* (ATCC 49619), *S. sonnei* (ATCC 25931), *E. coli* (ATCC 35218), *E. casseliflavus* (ATCC 700327), *P. mirabilis* (ATCC

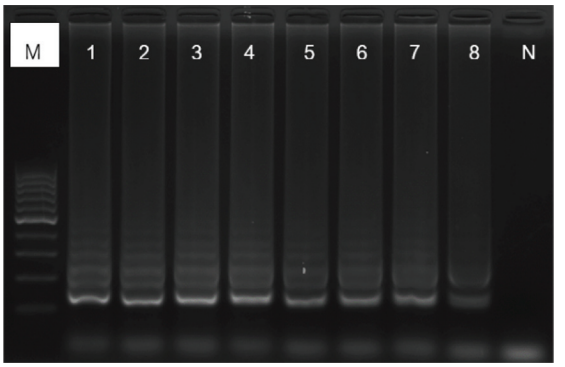

Figure 4. The gel electrophoresis pattern of LAMP for detection of bla_{NDM-1} gene. Lane M represents 100 bp λ ladder marker. Lanes 1-2 represent *E. coli*, Lanes 3-4 represent *E. cloacae*, Lane 5 represent *K. pneumonia*, Lane 6 represent C. freudii, Lanes 7-8 represent K. pneumonia. Lanes 1-8 were CRE positive control. Lane N represents negative control.

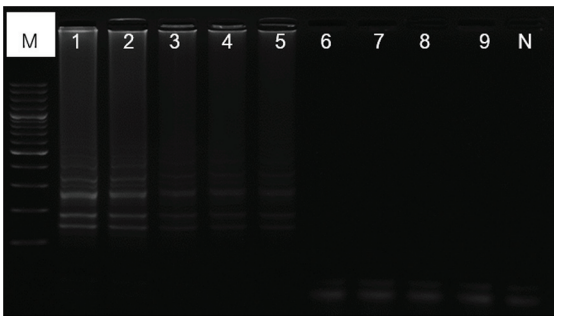

Figure 5. The analytical sensitivity test of LAMP for amplification of bla_{NDM-1} gene showed the limit of detection at 7.4 pg/μL. Lane M represents 100 bp λ ladder marker. Lanes 1-9 represent 74 ng/μL, 7.4 ng/μL, 0.74 ng/μL, 74 pg/μL, 7.4 pg/μL, 0.74 pg/μL, 74 fg/μL, 7.4 fg/μL, and 0.74 fg/μL. Lane N represents negative control.

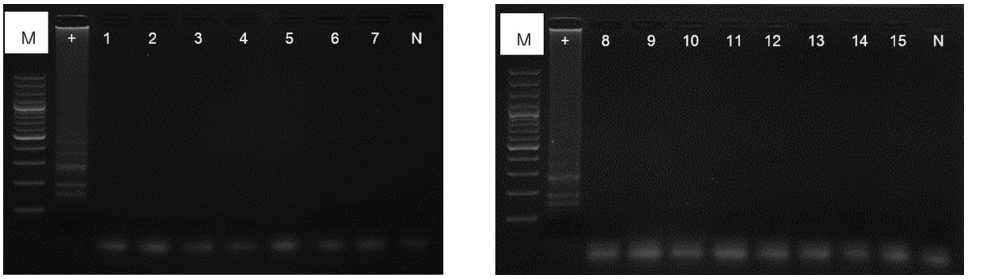

Figure 6. The analytical specificity test of LAMP on amplification of bla_{NDM-1} gene. Lane M=100 bp λ ladder marker. Lane + represents positive bla_{NDM-1} gene, Lanes 1-15 represent *E. faecalis* (ATCC 29212), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923), *S. agalactiae* (ATCC 12386), *K. pneumonia* (ATCC 700603), *S. pneumonia* (ATCC 49619), *S. sonnei* (ATCC 25931), *E. coli* (ATCC 35218), *E. casseliflavus* (ATCC 700327), *P. mirabilis* (ATCC 43071), *A. baumannii* (ATCC 196606), *S. aureus* (ATCC 29213), *E. hormaechei* (ATCC 700323), *S. pyogenes* (ATCC 19615), *S. maltophilia* (ATCC 17666), respectively. Lane N represents negative control.

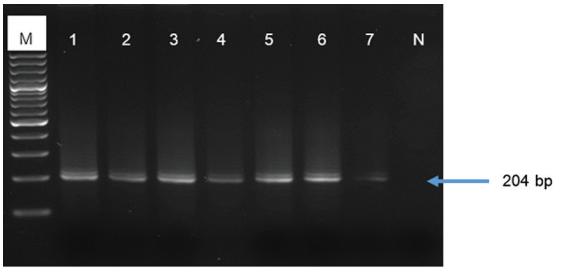

Figure 7. Gel electrophoresis pattern of 204 bp in size RPA products amplified from CRE strains containing bla_{NDM-1} gene. Lane M represents 100 bp λ ladder marker. Lanes 1-2 represent *E. coli*, Lanes 3-4 represent *E. cloacae*, Lane 5 represent *K. pneumonia*, Lane 6 represent *C. freudii*, Lane 7 represent *K. pneumonia*. Lanes 1-7 were CRE positive control. Lane N represents negative control.

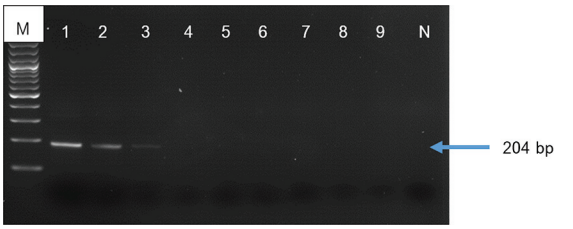

Figure 8. The analytical sensitivity test of RPA for amplification of bla_{NDM-1} gene with the limit of detection at 0.74 ng/μL. Lane M represents 100 bp λ ladder marker. Lane 1-9 represent 74 ng/ μL, 7.4 ng/μL, 0.74 ng/μL, 74 pg/μL, 7.4 pg/μL, 0.74 pg/μL, 74 fg/μL, 7.4 fg/μL, and 0.74 fg/μL. Lane N represents negative control.

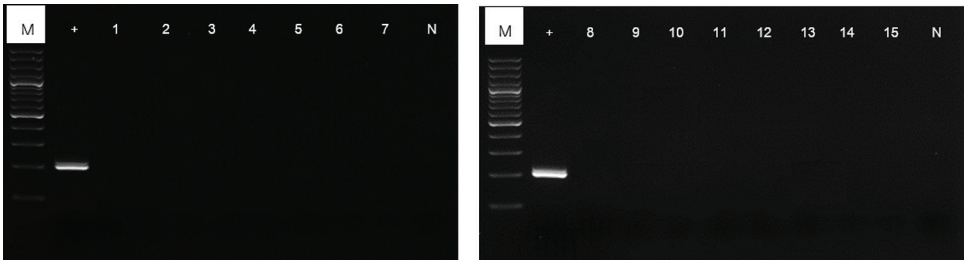

Figure 9. The analytical specificity test of RPA on amplification of bla_{NDM-1} gene. Lane M represents 100 bp λ ladder marker. Lane + represents positive bla_{NDM-1} gene of 204 bp product in size. Lanes 1-15 represent *E. faecalis* (ATCC 29212), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923), *S. agalactiae* (ATCC 12386), *K. pneumonia* (ATCC 700603), *S. pneumonia* (ATCC 49619), *S. sonnei* (ATCC 25931), *E. coli* (ATCC 35218), *E. casseliflavus* (ATCC 700327), *P. mirabilis* (ATCC 43071), *A. baumannii* (ATCC 196606), *S. aureus* (ATCC 29213), *E. hormaechei* (ATCC 700323), *S. pyogenes* (ATCC 19615), and *S. maltophilia* (ATCC 17666), respectively. Lane N represents negative control.

Table 1. Clinical samples are examined using to detect the bla_{NDM-1} gene on carbapenem-resistance, all assays (PCR, LAMP, and RPA) were compared using universal primers via PCR and designed primers. There are 110 clinical isolates of Enterobacteriaceae (positive= 75 isolates and negative=35 isolates)

DNA amplification methods	No. positive bla_{NDM-1}/ No. total sample	No. negative bla_{NDM-1}/ No. total sample	Sensitivity (%)	Specificity (%)	Relative trueness (%)	Process time (minutes)
PCR primer standard	75/ 75	0/ 35	100	100	100	90
PCR	75/ 75	0/ 35	100	100	100	90
LAMP	75/ 75	0/ 35	100	100	100	60
RPA	75/ 75	0/ 35	100	100	100	20

PCR=polymerase chain reaction; LAMP=loop mediated isothermal amplification; RPA=recombinant polymerase amplification

43071), *A. baumannii* (ATCC 196606), *S. aureus* (ATCC 29213), *E. hormaechei* (ATCC 700323), *S. pyogenes* (ATCC 19615), or *S. maltophilia* (ATCC 17666) (the results shown as Figure 3, 6, and 9, respectively).

Preliminary clinical samples

One hundred ten clinical isolates were screening tested with disc sensitivity on carbapenem-resistance Enterobacteriaceae prior to extract DNA and perform with the developed method including PCR, LAMP, and RPA with specific bla_{NDM-1} gene primer compared with universal EUCAST primer.

Discussion

Until now, CRE are both effects for gram-negative bacteria and gram-positive bacteria. Although new antibiotics have been continuously developed for treatment, the bacteria have adapted themself to be new antibiotic resistance.

Concerning limit of detection of PCR, LAMP, and RPA assays, the 0.74 ng/μL, 7.4 pg/μL and 0.74 ng/μL were observed, respectively. It is noted that LAMP showed 100 times more sensitive than PCR and RPA. Hence, it is suitable for use as early detection test for CRE strains containing bla_{NDM-1} gene. Furthermore, all DNA amplification assays such as PCR, LAMP, and RPA, showed no cross amplification to a target isolate and non-target other 15 related bacterial isolates, indicating that all assay primers are specific for the bla_{NDM-1} gene. Moreover, 110 isolates from clinical samples tested carbapenem-resistance Enterobacteriaceae, with 35 isolates detected as negative and 75 isolates detected as positive bla_{NDM-1} gene on PCR, LAPM, and RPA with specific primer for bla_{NDM-1} gene and compared with universal primer for standard method (PCR) from EUCAST as shown in Table 1.

To develop an effective screening test, the factors of timesaving and its convenience should be included. Among three DNA amplification assays, RPA is the most rapid when compared to PCR and LAMP. However, the assay is still costly due to the patented recombinase polymerase. In similarity to LAMP, RPA is the isothermal amplification that can be conducted at 37°C for 20 minutes, which is convenient to perform the reaction while LAMP can be completed at 60°C to 65°C for 60 minutes. Both LAMP and RPA assays do not require the complicated and expensive equipment since only a simple heating

box is necessary for LAMP and RPA process while PCR relied on expensive PCR Thermal cycler.

Conclusion

According to the present study, the PCR, LAMP, and RPA based methods could detect CRE strains containing bla_{NDM-1} gene with high specificity. Among them, LAMP is suitable for further development as the rapid and highly sensitive early screening test that can help the physician to choose the proper antibiotic to treat the infection by CRE bacteria and can reduce morbidity and mortality rates.

What is already known on this topic?

The study on DNA amplification methods for detection of CRE in terms of analytical sensitivity and specificity.

The PCR, LAMP, and RPA based methods could detect CRE strains containing bla_{NDM-1} gene with high specificity.

What this study adds?

For the limit of detection of PCR, LAMP, and RPA assays, the 0.74 ng/μL, 7.4 pg/μL and 0.74 ng/μL were observed, respectively.

It is noted that LAMP showed 100 times more sensitive than PCR and RPA.

LAMP is suitable for further development as the rapid and highly sensitive early screening test.

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Conflicts of interest

The authors declare no conflict of interest.

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