Journal of Microbiological Methods 167 (2019) 105746

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Rapid detection of carbapenemase-producing *Enterobacteriaceae* (CPE) using a simple DNA extraction method and Loop-mediated isothermal amplification (LAMP)

Ji Eun Lee[a], Lae Hyung Kang[b], Jung Ok Kim[a], Soon Young Paik[b], Hae Kyung Lee[c], Soo Young Kim[d], Yeon-Joon Park[a,*]

[a] Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
[b] Department of Microbiology, The Catholic University of Korea
[c] Department of Laboratory Medicine, Uijeongbu St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
[d] Department of Laboratory Medicine, St. Vincent's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

ARTICLE INFO

Keywords:
FTA card
Extraction
Carbapenemase-producing *Enterobacteriaceae* (CPE)
Loop - mediated isothermal amplification (LAMP)
Xpert Carba-R

1. Introduction

The emergence and dissemination of carbapenemase-producing *Enterobacteriaceae* (CPE) have been described worldwide (Nordmann et al., 2011) and it is important to distinguish between carbapenemase-producing carbapenem-resistant *Enterobacteriaceae* (CP CRE) and carbapenemase-non-producing carbapenem-resistant *Enterobacteriaceae* (non-CP CRE) because the carbapenemase genes CPE can be easily spread by mobile elements (Queenan and Bush, 2007). In addition, carbapenemase production is normally linked to multiple drug resistance (MDR) clones commonly associated with healthcare related infections and increased morbidity and mortality (Hernandez-Garcia et al., 2018). This resistance is mainly achieved by the production of carbapenemases, particularly *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), oxacillinase-48 (OXA48), imipenemase (IMP) and Verona imipenemase (VIM) (Nordmann et al., 2011; Wilson and Torok, 2018; Grundmann et al., 2010). Among the 4 classes of β-lactamases defined by the Ambler classification system, the carbapenemases that confer carbapenem resistance in Enterobacteriaceae belong to 3 of them: Class A (e.g., KPC, GES), Class B (e.g., NDM, IMP, VIM), and Class D (e.g., OXA) (Nordmann and Poirel, 2002; van Duin and Doi, 2017).

To prevent the spread of these CPE, early identification of carriers is essential. Although there are commercial kits such as Xpert Carba-R (Cepheid, Sunnyvale, CA) and BD MAXTM CRE assay (Becton-Dickinson, New jersey, USA) which are easy to use, they are expensive (Antonelli et al., 2016; Tato et al., 2016).

Loop-mediated isothermal amplification (LAMP) is a technique in which DNA is amplified with high specificity, sensitivity and rapidity under isothermal conditions ranging between 60 °C and 65 °C for < 1 h. LAMP employs a *Bst* DNA polymerase with strand-displacement activity, along with two inner primers (FIP, BIP) and outer primers (F3, B3), which recognize six separate regions within a target DNA. Additionally, loop-primers designed to anneal at the loop structure in LAMP amplicons, can accelerate and enhance the sensitivity of the LAMP reaction (Mori and Notomi, 2009). As this method requires only a water bath or heat block, it can be an inexpensive technique for rapid CPE detection in clinical laboratories that lack specialized equipment.

Whatman FTA Cards (GE Healthcare Life Science, Pittsburgh, USA) are a chemically treated filter paper, which lyses the cells and preserves the DNA in the filter matrix. After air-drying, the card can be folded and stored at room temperature for years. Although there are several reports about the preservation efficiency of the fecal DNA in the FTA cards (Hale et al., 2016: Song et al., 2016), to our knowledge, this is the

* Corresponding author.
E-mail address: yjpk@catholic.ac.kr (Y.-J. Park).

https://doi.org/10.1016/j.mimet.2019.105746
Received 1 May 2019; Received in revised form 15 October 2019; Accepted 15 October 2019
Available online 05 November 2019
0167-7012/

Table 1
The MICS for imipenem, ertapenem and meropenem of bacterial strains used in this study.

Strain names	Species (n)	β-lactamase genes	MIC (μg/ml)		
			IPM	ERT	MEM
Carbapenemase producers (42)					
E_CRE11	*Pseudomonas aeruginosa*	IMP-1	> 16	> 8	32
Inf_23	*Escherichia coli*	IMP-1	1	4	1
ST_386	*Escherichia coli*	IMP-1	0.25	0.5	0.5
ST_82	*Pseudomonas aeruginosa*	IMP-1	> 16	> 8	> 32
16,088	*Pseudomonas aeruginosa*	IMP-6	16	> 8	> 32
16,085	*Pseudomonas aeruginosa*	IMP-6	> 16	> 8	> 32
16,104	*Pseudomonas aeruginosa*	IMP-26	> 16	> 8	> 32
18CRE209	*Klebsiella oxytoca*	IMP	4	4	4
Inf_8	*Klebsiella pneumoniae*	VIM-1	> 16	> 8	> 32
Inf_1	*Klebsiella oxytoca*	VIM-1	2	0.5	1
Inf_20	*Enterobacter cloacae*	VIM-1	4	1	2
E_CRE13	*Citrobacter freundii*	VIM-2	> 16	> 8	32
E_CRE17	*Pseudomonas aeruginosa*	VIM-2	> 16	> 8	32
ST_81	*Pseudomonas aeruginosa*	VIM-1	> 16	> 8	> 32
16,086	*Pseudomonas aeruginosa*	VIM-2	16	> 8	4
16,105	*Pseudomonas aeruginosa*	VIM-3	16	> 8	4
16CRE59	*Klebsiella pneumoniae*	KPC-2	> 16	> 8	> 32
16CRE70	*Klebsiella pneumoniae*	KPC-2	16	> 8	> 32
16CRE78	*Klebsiella pneumoniae*	KPC-2	16	> 8	> 32
16CRE80	*Klebsiella pneumoniae*	KPC-2	> 16	> 8	> 32
16,052	*Klebsiella pneumoniae*	KPC-2	0.5	0.125	0.5
16,044	*Escherichia coli*	KPC-2	4	8	2
17CRE21	*Klebsiella pneumoniae*	KPC-2	> 16	> 8	> 32
ST_400	*Klebsiella pneumoniae*	KPC-3	0.25	> 8	4
Inf_2	*Acinetobacter baumannii*	NDM-1	> 16	> 8	> 32
17CRE110	*Morganella morganii*	NDM-1	16	2	4
ST_340	*Klebsiella pneumoniae*	NDM-1	4	> 8	2
ST_398	*Klebsiella pneumoniae*	NDM-1	2	8	2
16,046	*Escherichia coli*	NDM-1	16	> 8	4
17CRE160	*Escherichia coli*	NDM-5	16	> 8	32
17CRE158	*Escherichia coli*	NDM-5	> 16	> 8	> 32
17CRE168	*Escherichia coli*	NDM-5	16	> 8	> 32
ST_263	*Klebsiella pneumoniae*	OXA-48	> 16	> 8	32
17CRE216	*Escherichia coli*	OXA-48	2.0	> 8	4.00
17CRE207	*Escherichia coli*	OXA-48	0.5	0.125	0.06
16CRE58	*Escherichia coli*	OXA-48	8	32	4
Inf_7	*Klebsiella pneumoniae*	OXA-48	2	2	0.5
Inf_10	*Citrobacter freundii*	OXA-48	2	1	0.5
16R61	*Klebsiella pneumoniae*	OXA-232	2	> 8	16
ST_459	*Klebsiella pneumoniae*	OXA-232	1	> 8	4
16,045	*Escherichia coli*	OXA-232	1	4	0.5
16,053	*Klebsiella pneumoniae*	OXA-232	4	4	1
Non-carbapenemase producers (22)					
Carbapenemase resistance (7)					
17CRE38	*Escherichia coli*		8	16	4
17CRE75	*Enterobacter aerogenes*		1	8	0.5
17CRE80	*Enterobacter aerogenes*		2	4	0.5
17CRE82	*Klebsiella pneumoniae*		8	> 8	4
17CRE86	*Klebsiella pneumoniae*		2	16	2
17CRE118	*Enterobacter amnigenus 2*		8	> 8	8
17CRE204	*Enterobacter amnigenus 2*		16	> 8	8
ESBL producers (7)					
15JMHS2	*Escherichia coli*	CTX-M-9	0.25	0.125	0.25
15JMHS13	*Escherichia coli*	CTX-M-1, TEM	0.25	0.125	0.25
15BDC2	*Klebsiella pneumoniae*	CTX-M-1, SHV, TEM	0.25	2	0.25
SCServ5	*Klebsiella pneumoniae*	CTX-M-1, SHV, TEM	0.5	0.25	0.25
15BDC12	*Klebsiella pneumoniae*	CTX-M-1, SHV	0.25	0.25	0.25
SCServ3	*Klebsiella pneumoniae*	TEM	0.50	0.125	0.5
ATCC700603	*Klebsiella pneumoniae*	SHV	0.25	0.125	0.5
pAmpC producers (3)					
JMHS14	*Escherichia coli*	CIT, TEM	0.25	0.125	0.5
USH5	*Escherichia coli*	CIT	0.50	0.125	0.5
EMary14	*Klebsiella pneumoniae*	DHA	1	1	0.5
ESBL & pAmpC producers (2)					
15GM1	*Escherichia coli*	CTX-M-9, CIT	1	0.5	0.25
BSB7	*Klebsiella pneumoniae*	CTX-M-1, DHA	1	0.5	0.25
Non-ESBL & non-AmpC producers (3)					
EMary 13	*Escherichia coli*		0.25	0.125	0.5
HWDB14	*Klebsiella pneumoniae*		0.25	0.125	0.5
ATCC25922	*Escherichia coli*		0.25	0.015	0.03

first report of using the FTA card for DNA extraction directly from rectal swab specimens.

Here, we introduce a simple DNA extraction method using the FTA card combined with the LAMP reaction which can detect carbapenemase genes within 1 h directly from rectal swab specimens.

2. Material and methods

2.1. Bacterial isolates

Bacterial collection of 42 *Enterobacteriaceae* and *Pseudomonas* spp. isolates harboring various carbapenemase genes (5 NDM-1, 3 NDM-5, 5 IMP-1, 2 IMP-6, 1 IMP-26, 3 VIM-2, 4 VIM-1, 1 VIM-3, 7 KPC-2, 1 KPC-3, 6 OXA-48 and 4 OXA-232) and 22 *Enterobacteriaceae* isolates without carbapenemase genes (2 ATCC, 7 CRE, 5 ESBL, 2 ESBL & pAmpC, 3 non-ESBL and 3 non-ESBL & AmpC) were included in this study (Table 1). The minimum inhibitory concentrations (MICs) of carbapenems were determined by broth microdilution method (CLSI, 2015).

2.2. Development LAMP reaction

The LAMP primer sets comprised two inner primers (FIP and BIP), two outer primers (F3 and B3) and two loop primers (LF and LB) for detecting the bla_{NDM}, bla_{OXA-48}, bla_{VIM}, bla_{IMP} and bla_{KPC} groups. The primers for each target gene were designed by LAMP Designer 1.10 (PREMIER Biosoft international). For the other variants of each gene those that are not included in our out tested strains, we performed in silico analysis using the cut off value of ≥80% identity (Srisrattakarn et al., 2017) (Table 2): NDM group including $bla_{NDM-1}$to bla_{NDM-16} except bla_{NDM-10}; OXA-48 group including bla_{OXA-48}, $bla_{OXA-162}$, $bla_{OXA-163}$, $bla_{OXA-181}$, $bla_{OXA-199}$, $bla_{OXA-204}$, $bla_{OXA-232}$, $bla_{OXA-244}$, $bla_{OXA-245}$, $bla_{OXA-247}$, $bla_{OXA-370}$, $bla_{OXA-405}$ and $bla_{OXA-438}$; VIM group including bla_{VIM-1} to bla_{VIM-46} except bla_{VIM-7} (81% difference in sequence), $bla_{VIM-21,22}$; IMP group including bla_{IMP-1} to bla_{IMP-6} and bla_{IMP-26} (IMP-5 has 19 sequences different, 92%); KPC group including bla_{KPC-1} to bla_{KPC-24} except bla_{KPC-14} (loss G of six). To check the cross-reactivity between primers, each primer was tested with other carbapenemase producing strains.

Each LAMP assay was performed in 25 μl reactions which included 12.5 μl of NEB's WarmStart LAMP 2× Master Mix (New England Biolabs, Frankfurt am Main, Germany), 2.5 μl of LAMP primer mix (10×) (0.2 μM of the forward (F3) and backward (B3) primers, and 1.6 μM forward internal primer (FIP) and backward internal primer (BIP) primers and 0.4 μM of the backward loop (BL) and forward loop

Table 2
Nucleotide sequences of the LAMP primers for detection of IMP, VIM, KPC, NDM and OXA.

Target gene		Primer Sequences (5′ → 3′)	Position	Length
IMP	F3	GGGCGTTGTTCCTAAACA	138	18
	B3	TTGTGGCTTGAACCTTACC	400	19
	FIP	CGCTCCACAAACCAAGTGACTAATGCTGAGGCTTACCTAATTG		43
	BIP	CATTTTCATAGCGACAGCACGGTGCATACGTGGGGATAGAT		41
	Loop F	TCTTTAGCCGTAAATGGAGTGT	218	22
	Loop B	GCGGAATAGAGTGGCTTAATTC	305	22
VIM	F3	GGTGAGTATCCGACAGTCA	91	19
	B3	GTCATGAAAGTGCGTGGA	351	18
	FIP	GGTAGACCGCGCCATCAACAGATTGCCGATGGTGTT		36
	BIP	TGTCCGTGATGGTGATGAGTTGCAATCTCCGCGAGAAGTG		40
	Loop F	ACGACTGCGTTGCGATAT	184	18
	Loop B	CTTTTGATTGATACAGCGTGGG	241	22
KPC	F3	GGTTCCGTGGTCACCCATC	303	19
	B3	GCAGAGCCCAGTGTCAGTTT	590	20
	FIP	CGCCCAACTCCTTCAGCAGCCGCCGTGCAATACAGT		36
	BIP	CGGCCTTCATGCGCTCTATGGATGGCGGAGTTCAGCTC		38
	Loop F	AATTGGCGGCGGCGTTAT	406	18
	Loop B	CGATACCACGTTCCGTCTGG	465	20
NDM	F3	AAATGGAAACTGGCGACC	113	18
	B3	CAGGTTGATCTCCTGCTTG	333	19
	FIP	CGGCATGTCGAGATAGGAAGTGGGTTTGGCGATCTGGTTT		40
	BIP	CGTGCTGGTGGTCGATACCTCCAGTTGAGGATCTGGG		37
	Loop F	CCAGACATTCGGTGCGAG	177	18
	Loop B	CTGGACCGATGACCAGAC	276	18
OXA	F3	ATTATCGGAATGCCTGCG	43	18
	B3	CGATTCCAAGTGGCGATAT	320	19
	FIP	TGCTTGGTTCGCCCGTTTAATCACAGGGCGTAGTTGT		37
	BIP	CCCAATAGCTTGATCGCCCTCATCCCACTTAAAGACTTGGTG		42
	Loop F	TGCTGCTTATTCTCATTCCAGA	158	22
	Loop B	ATTTGGGCGTGGTTAAGGAT	245	20

(FL) primers), 0.5 μl of WarmStart LAMP Fluorescent Dye and finally 2 μl of template DNA was added. The reaction was performed in a Bio-Rad CFX Real-Time PCR detection system at 65 °C for 1 h. The fluorescence channel was selected for SYBR detection and a reading was taken every minute until reaching 200,000 RFU which was considered as a positive signal for amplification. For each run, a no template control and a positive template control were included.

2.3. Conventional PCR

For conventional PCR, an AccuPower PCR PreMix (Bioneer, Deajeon, Korea) was prepared and DNA was amplified using 10 pmoles of F3 and B3. PCR condition was pre-denaturation at 94 °C for 5 min, denaturation at 95 °C for 20 s, and 30 cycles of annealing at 60 °C for 20 s. A no template control (NTC) and positive control for each gene (DNA extracted from isolate harboring the target carbapenemase gene) were included.

2.4. Analytical sensitivity and specificity of LAMP assay

For analytical sensitivity testing, DNAs extracted from bacterial collection of 42 CPEs which were investigated or collected from the previous studies were used. DNA was extracted by a simple boiling method. Briefly, a single colony was suspended in 0.45% NaCl solution and was adjusted to a 0.5 McFarland (McF) standard and boiled for 10 min. After 5 min centrifugation, the supernatant was 10-fold diluted and used as the DNA template (10^5 to 10^0 for reaction).

The analytical sensitivity of the LAMP assay was compared with conventional PCR. Both the LAMP and conventional PCR were performed in triplicate and the lowest CFU/reaction was defined as where the gene was detected at least two times.

Analytical specificity was determined using 22 carbapenemase-negative strains under the same condition. In addition, to check the cross-reactivity between each set of LAMP primers and carbapenemase genes, each carbapenemase-harboring isolate was tested with the four kinds of LAMP primers targeting other genes. For example, a KPC-producing isolate was tested with LAMP primers targeting IMP, VIM, NDM, and OXA type carbapenemase gene and also for other carbapenemase-producing isolates.

2.5. DNA extraction from the rectal swab specimens using the FTA card

We optimized the DNA preparation process. 100 μl of rectal swab specimens in transport medium (Copan, Murrieta, USA) was mixed with 500 μl of buffer ATL (Qiagen, Hilden, Germany, Cat No./ID: 19076) and heated in a heating block for 5 min at 95 °C. After centrifugation at 12,000 *g* for 5 min, the supernatant was transferred into a tube containing proteinase K (15 μl) and 200 μl of buffer AL (Qiagen, Cat No./ID: 19075) and incubated at 70 °C for 10 min. For extraction with the FTA card, 4-mm disks were punched out by using a hole punch and placed in a 1.5-ml micro tube. Fifty μl of pretreated sample was loaded onto the FTA card and the FTA card was washed with 100 μl of Whatman FTA purification reagent (GE Healthcare Life Science, Pittsburgh, USA). Then, the FTA card was transferred into 1.5-ml micro tube and soaked with 50 μl of 1 mM TE buffer (10 mM Tris-HCL pH 8.0; 1 mM EDTA pH 8.0). After 5 min, the FTA card was removed and the eluate was used for the amplification reaction.

2.6. Comparison of DNA extraction efficiency

To estimate the DNA extraction efficiency of the novel method using the FTA card, we spiked ten-fold serial dilutions of the KPC-positive strain corresponding to the 10^4 to 10^0 CFU/reaction into the rectal swab. From the same amount (100 μl) of the spiked specimens, DNA was extracted with the novel, developed method and using a QiAmp DNeasy Stool Kit (Qiagen, Hilden, Germany). In addition, the Xpert Carba-R assay was also performed according to the manufacturer's instruction.

2.7. Comparison with Xpert Carba-R in clinical specimens

Currently, in our hospital, carriers of CPE are screened by Xpert Carba-R assay (Cepheid) from the rectal swab of the patients. Therefore, to compare the clinical sensitivity and specificity of the newly developed method with the Xpert Carba-R assay, we used the left-over specimens which were frozen in −70 °C deep freezer after the Xpert Carba-R assay was performed. We selected 15 specimens (three specimens for each of 5 carbapenemase genes (IMP, VIM, KPC, NDM, and OXA type), which had showed Ct values between 25 and 35 and tested them with our newly developed method.

In addition, to evaluate the clinical specificity, 45 rectal swab specimens which had showed negative results by the Xpert Carba-R assay were tested with the LAMP using DNA extracted by newly developed the FTA card method.

2.8. Statistical analysis

For each 1:10 serial dilution (ranging from 10^4 CFU/rxn to 10^0 CFU/rxn) of spiked KPC-producing isolate, the correlation analysis was carried out between the time to amplification detection (t_{amp}) of LAMP assay and threshold cycle (Ct) of Xpert Carba-R assay.

3. Results

3.1. Analytical sensitivity and specificity of the LAMP assay

LAMP and conventional PCR were compared for 42 carbapenemase-producing isolates. LAMP reaction showed higher or similar sensitivity than PCR according to the isolates (Table 3). All the carbapenemase-non-producing isolates gave negative results. In addition, each LAMP primer did not show cross-reactivity with other carbapenemase gene-producing isolates.

3.2. Comparison of DNA extraction efficiency

To estimate the DNA extraction efficiency of the novel method using the FTA card, we compared it with QIAmp DNeasy kit and Xpert Carba-R assay. While the lowest concentration of detection was 10^1 CFU/reaction for QIAmp DNeasy kit, that of our novel method was 10^0CFU/reaction (Table 4). We also observed a very strong correlation between t_{amp} of the LAMP assay and Ct value of the Xpert Carba- R assay in the same set of spiked rectal swab specimens ($R^2 = 0.963$).

3.3. Comparison with Xpert Carba-R in clinical specimens

With the cut-off value for positivity as ≤40 min, Among the 45 carbapenemase-negative specimens, some of them showed the positive reaction but the reaction occurred after 40 min. Therefore, we set the cut-off value for positivity as ≤40 min. And the 15 clinical specimens (3 specimens for each carbapenemase gene) which showed Ct value of between 25 and 35 by Xpert Carba-R, the highest Ct value of the specimens our novel method detected carbapenemase was 33.2 and 33.4 for KPC and OXA type, but it was < 31 for NDM, IMP and VIM.

Table 3
Comparison of analytical sensitivity of the LAMP assay and PCR.

CPE genes (No)	Limit of detection (CFU/reaction) (number of isolates)	
	LAMP	Conventional PCR
IMP-1 (5)	10^1 (2), 10^2 (3)	10^1 (2), 10^2 (3)
IMP-6 (2)	10^1 (2)	10^1 (2)
IMP-26 (1)	10^3 (1)	10^3 (1)
VIM-1 (4)	10^1 (3), 10^2 (1)	10^1 (4)
VIM-2 (3)	10^0 (2), 10^2 (1)	10^2 (3)
VIM-3 (1)	10^1 (1)	10^1 (1)
KPC-2 (7)	10^0 (3), 10^1 (2), 10^2 (2)	10^2 (3), 10^3 (2), 10^4 (1)
KPC-3 (1)	10^0 (1)	10^3 (1)
NDM-1 (5)	10^0 (1), 10^1 (1), 10^2 (3)	10^1 (1), 10^2 (3), 10^3 (1)
NDM-5 (3)	10^0 (1), 10^1 (2)	10^2 (3)
OXA-48 (6)	10^0 (1), 10^1 (1), 10^2 (4)	10^1 (1), 10^2 (5)
OXA-232 (4)	10^0 (2), 10^1 (1), 10^2 (1)	10^2 (1), 10^3 (2), 10^4 (1)

Table 4
Comparison of DNA extraction efficiency in a rectal swab specimen spiked with KPC-producing isolate.

CFU/rxn	FTA card(LAMP, time)	Commercial kit(LAMP, time)	Xpert Carba-R(real-time PCR, Ct)
10^4	23.37	23.78	21.6
10^3	26.47	27.1	26.3
10^2	30.28	31.36	28.3
10^1	32.76	33.91	31.6
10^0	34.84	Not detected	35.6

4. Discussion

In this study, we developed a LAMP method and combined it with the simple DNA extraction method using the FTA card.

To compare the analytical sensitivity of the LAMP assay with conventional PCR, LAMP and conventional PCR were performed for 42 carbapenemase-producing isolates. LAMP reaction showed higher or similar sensitivity than PCR according to the isolates (Table 3). All the carbapenemase-non-producing isolates gave negative results. In addition, each LAMP primer did not show cross-reactivity with other carbapenemase gene-producing isolates. Compared to Srisrattakarn's study which detected CPE genes with LAMP where it detected 10^5 CFU/ml (10^2 CFU/reaction) for IMP, VIM and KPC and only NDM was detected to 10^3 CFU/ml (10^0 CFU/reaction) (Srisrattakarn et al., 2017), our method showed higher sensitivity ($10^{0–3}$ CFU/reaction) according to the isolates. This might be due to superiority in the primers picked up in this study or fluorescence can detect signals at much lower concentrations (10–500 nM) than hydroxynaphthol blue dye observed with naked eye.

In addition, by in silico analysis, we confirmed that the primers developed in this study can detect most of the variants of carbapenemase genes, indicating that the primers developed in this study are superior to those developed by Cheng et al. (2014) which can detect only two (IMP-4 and IMP-38) out of the many variants of IMP type carbapenemase genes. Moreover, the primers developed by Cheng et al. could not detect OXA type carbapenemase gene.

In comparison with conventional PCR, the limit of detection (LOD) showed a variation between isolates harboring identical genotype of carbapenemase with both LAMP and conventional PCR (Table3). We presume that this variation might be due to the difference in the copy number of the gene in each isolate which can affect any molecular method.

The detection of CPE in rectal swab specimens by molecular methods usually requires a highly efficient DNA extraction method because of the presence of inhibitors in samples. In this study, we compared the DNA extraction efficiency of the novel method using the FTA card with QIAmp DNeasy stool kit while the detection limit of the FTA card method was 10^0CFU/reaction that of QIAamp DNeasy stool kit was 10^1CFU/reaction. This is in line with the previous study where the efficacy of DNA extraction using the FTA card was compatible with that of QIAamp stool mini kit (Subrungruang et al., 2004) but our novel method showed higher efficiency than commercial kit. Moreover, while the method used by Subrungruang et al. requires two times of drying and thus it takes about 3 h, our novel method eliminated the drying step and thus it takes only 40 min. Interestingly, although LAMP assay is known as qualitative method, we found the time to amplification detection (t_{amp}) was shorter when there was higher concentration of

Table 5
Evaluation of clinical sensitivity in three specimens which showed positive reaction by Xpert Carba-R with Ct value between 25 and 35 for each target gene.

CPE gene	Xpert Carba -R	FTA Card
	(Real-time PCR, Ct)	(LAMP, minute showing positivity)
IMP	25.7	14.7
	30.4	20.6
	33.5	Negative
VIM	27.2	11.4
	31.4	35.4
	34.1	Negative
KPC	26.8	11.8
	30	13.2
	33.2	28.2
NDM	27.2	18.8
	30.6	35.8
	33	Negative
OXA	28.8	12.6
	30.1	16
	33.4	20.4

target DNA and the t_{amp} and the Ct value of the Xpert Carba-R assay showed strong correlation (R2 = 0.964). Aglietti et al. (2019) also reported that they observed very strong correlation (R2 > 0.95) between the t_{amp} in LAMP assay and Ct value of the qPCR in the same set of serially diluted DNA samples.

Finally, to investigate the clinical usefulness of this method, we tested the clinical rectal swab specimens for which the Xpert Carba-R assay was requested to investigate the CPE carriers. For KPC and OXA genes, the LAMP reaction combined with the novel DNA extraction method detected the target genes < 40 min even in specimens with Ct value by the Xpert Carba-R assay was 33–34. However, for NDM, IMP and VIM, the novel method detected target genes for specimens of which Ct value by the Xpert Carba-R assay was < 31 (Table 5). We could not elucidate the reason for the lower sensitivity in detecting NDM, IMP, and VIM but we presume that it might due to a copy number variation because in evaluating the analytical sensitivity with bacterial isolates with same genotype, the LOD was variable; for instance, of the five NDM-1 isolates, the LOD for three isolates were 10^2 CFU/reaction, but for the remaining two isolates, it was 10^1 CFU/reaction and 10^0 CFU/reaction (Table 3).

Xpert Carba-R assay is a rapid, accurate method which demonstrated 100% positive percentage of agreement (PPA, sensitivity) for targets KPC, NDM and OXA-48 except for VIM (PPA, 60%) and a very high negative percentage of agreement (NPA, specificity) ranging between 98.9 and 99.9% relative to the culture and sequencing (Moore et al., 2017). In another study by Tato et al. (2016), the sensitivity, specificity, and positive and negative predictive values of the Xpert Carba-R assay compared to those of the reference culture and sequencing results were 96.6% (95% confidence interval [CI], 92.2% to 98.9%), 98.6% (95% CI, 97.1% to 99.4%), 95.3%, and 99.0%, respectively. Seven specimens were considered to be false positives and the Ct value ranged from 26.2 to 28.2 for KPCs and were > 31 for the remaining carbapenemases). In our clinical microbiology laboratory, of the 9537 rectal swab specimens requested for Xpert Carba-R assay between July 2018 and April 2019, the number of positive specimens for KPC, OXA, NDM, IMP, and VIM was 255, 112, 99, 19 and 6, respectively. In addition, among the 491 specimens determined positive by Xpert Carba-R assay, 58 (18 IMP, 4 VIM, 11 KPC, 13 NDM and 12 OXA) were false-positive results compared with culture combined with PCR method. Of note, the Ct value of most (53 out of 58) of the false-positive cases were high (> 31). Taking into account that 122 out of the 491 positive specimens showed Ct value of > 31, the false positive rate is very high (about 43%, 53/122) in specimens showing high Ct value (> 31). This finding indicates that the inability to detect carbapenemase gene in specimens with Xpert Carba-R Ct value of > 31 might be clinically non-significant.

In conclusion, the results presented in this study show that this novel DNA extraction method combined with LAMP assay will be rapid, cost-effective assay which can replace the Xpert Carba -R assay especially in clinical settings with limited resources.

Declaration of Competing Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

Acknowledgements

This work was supported by a research grant from the Korea Health Industry Development Institute (HI16C0443).

References

Aglietti, C., Luchi, N., Pepori, A.L., Bartolini, P., Pecori, F., Raio, A., Capretti, P., Santini, A., 2019. Real-time loop-mediated isothermal amplification: an early-warning tool for quarantine plant pathogen detection. AMB Express 9, 50–63.
Antonelli, A., Arena, F., Giani, T., Colavecchio, O.L., Valeva, S.V., Paule, S., Boleij, P., Rossolini, G.M., 2016. Performance of the BD MAX instrument with check-direct CPE real-time PCR for the detection of carbapenemase genes from rectal swabs, in a setting with endemic dissemination of carbapenemase-producing Enterobacteriaceae. Diagn. Microbiol. Infect. Dis. 86, 30–34.
Cheng, C., Zheng, F., Rui, Y., 2014. Rapid detection of blaNDM, blaKPC, blaIMP, and blaVIM carbapenemase genes in bacteria by loop-mediated isothermal amplification. Microb. Drug Resist. 20, 533–538.
CLSI, 2015. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow Aerobically; Approved Standard-Tenth Edition. M07-A10. Clinical and Laboratory Standards Institute.
Grundmann, H., Livermore, D.M., Giske, C.G., Canton, R., Rossolini, G.M., Campos, J., Vatopoulos, A., Gniadkowski, M., Toth, A., Pfeifer, Y., Jarlier, V., Carmeli, Y., 2010. Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 15, 46.
Hale, V.L., Tan, C.L., Niu, K., Yang, Y., Cui, D., Zhao, H., Knight, R., Amato, K.R., 2016. Effects of filed conditions on fecal microbiota. J. Microbiol. Methods 130, 180–188.
Hernandez-Garcia, M., Perez-Viso, B., Carmen Turrientes, M., Diaz-Agero, C., Lopez-Fresnena, N., Bonten, M., Malhotra-Kumar, S., Ruiz-Garbajosa, P., Canton, R., 2018. Characterization of carbapenemase-producing Enterobacteriaceae from colonized patients in a university hospital in Madrid, Spain, during the R-GNOSIS project depicts increased clonal diversity over time with maintenance of high-risk clones. J. Antimicrob. Chemother. 73, 3039–3043.
Moore, N.M., Canton, R., Carretto, E., Peterson, L.R., Sautter, R.L., Traczewski, M.M., 2017. Rapid identification of five classes of carbapenem resistance genes directly from rectal swabs by use of the Xpert Carba-R assay. J. Clin. Microbiol. 55, 2268–2275.
Mori, Y., Notomi, T., 2009. Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. J. Infect. Chemother. 15, 62–69.
Nordmann, P., Poirel, L., 2002. Emerging carbapenemases in Gram-negative aerobes. Clin. Microbiol. Infect. 8, 321–331.
Nordmann, P., Naas, T., Poirel, L., 2011. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg. Infect. Dis. 17, 1791–1798.
Queenan, A.M., Bush, K., 2007. Carbapenemases: the versatile beta-lactamases. Clin. Microbiol. Rev. 20, 440–458.
Song, S.J., Amir, A., Metcalf, J.L., Amato, K.R., Xu, Z.Z., Humphrey, G., Knight, R., 2016. Preservation Methods Differ in Fecal Microbiome Stability, Affecting Suitability for Field Studies. (Pii:e00021–16).
Srisrattakarn, A., Lulitanond, A., Wilailuckana, C., Charoensri, N., Wonglakorn, L., Saenjamla, P., Chaimanee, P., Daduang, J., Chanawong, A., 2017. Rapid and simple identification of carbapenemase genes, bla NDM, bla OXA-48, bla VIM, bla IMP-14 and bla KPC groups, in Gram-negative bacilli by in-house loop-mediated isothermal amplification with hydroxynaphthol blue dye. World J. Microbiol. Biotechnol. 33, 130.
Subrungruang, I., Mungthin, M., Chavalitshewinkoon-Petmitr, P., Rangsin, R., Naaglor, T., Leelayoova, S., 2004. Evaluation of DNA extraction and PCR methods for detection of Enterocytozoon bienuesi in stool specimens. J. Clin. Microbiol. 42, 3490–3494.
Tato, M., Ruiz-Garbajosa, P., Traczewski, M., Dodgson, A., McEwan, A., Humphries, R., Hindler, J., Veltman, J., Wang, H., Canton, R., 2016. Multisite evaluation of Cepheid Xpert Carba-R assay for detection of carbapenemase-producing organisms in rectal swabs. J. Clin. Microbiol. 54, 1814–1819.
Van Duin, D., Doi, Y., 2017. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence 8, 460–469.
Wilson, H., Torok, M.E., 2018. Extended-spectrum beta-lactamase-producing and carbapenemase-producing Enterobacteriaceae. Microb. Genom. 4, 7.