Comparison of Conventional PCR, Multiplex PCR, and Loop-Mediated Isothermal Amplification Assays for Rapid Detection of *Arcobacter* Species

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This study aimed to develop a loop-mediated isothermal amplification (LAMP) method for the rapid detection of *Arcobacter* species. Specific primers targeting the 23S ribosomal RNA gene were used to detect *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii*. The specificity of the LAMP primer set was assessed using DNA samples from a panel of *Arcobacter* and *Campylobacter* species, and the sensitivity was determined using serial dilutions of *Arcobacter* species cultures. LAMP showed a 10- to 1,000-fold-higher sensitivity than multiplex PCR, with a detection limit of 2 to 20 CFU per reaction *in vitro*. Whereas multiplex PCR showed cross-reactivity with *Campylobacter* species, the LAMP method developed in this study was more sensitive and reliable than conventional PCR or multiplex PCR for the detection of *Arcobacter* species.

*A*rcobacter species are Gram-negative, non-spore-forming, motile, spiral-shaped bacteria that require a low-oxygen environment and usually blood-based or complex media for their initial culture [1]. *Arcobacter* organisms were first isolated as aerotolerant *Campylobacter*-like microorganisms from aborted bovine and pig fetuses in 1977 and were assigned to the family *Campylobacteraceae* [2, 3]. In 1991, the aerotolerant campylobacters were reclassified, giving rise to the new genus *Arcobacter*, belonging to the class *Epsilonproteobacteria* [4, 5]. *Arcobacter* species have high oxygen tolerance and can grow between 15 and 30°C, whereas *Campylobacter* species can grow only at 37°C and require a strict microaerophilic environment [5]. *Arcobacter nitrofigilis* and *Arcobacter cryaerophilus* were previously included in the genus *Campylobacter* and later reassigned to the genus *Arcobacter*. In the past decade, the genus *Arcobacter* has been expanded to include 14 species with the following chronological order of discovery: *Arcobacter butzleri*, *Arcobacter skirrowii*, *Arcobacter cibarius*, *Arcobacter halophilus*, *Arcobacter mytili*, *Arcobacter thereius*, *Arcobacter marinus*, *Arcobacter trophiarum*, *Arcobacter defluvii*, *Arcobacter molluscorum*, *Arcobacter bivalviorum*, and *Arcobacter venerupis* [5–15].

Among the *Arcobacter* species described to date, *A. butzleri*, *A. skirrowii*, and *A. cryaerophilus* are considered human pathogens causing gastroenteritis or bacteremia [16]. The clinical features of *A. butzleri* infection include watery and persistent diarrhea with abdominal pain [17]. Presently, *Arcobacter* species are a serious potential concern in food safety because they can contaminate animal-origin foods and cause human diseases [17, 18]. *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* were detected first in chicken and later in pork, beef, untreated water, and contaminated foods [19].

Selective culture methods for isolating *Arcobacter* species have been developed and compared in previous studies [20–23]. PCR, multiplex PCR, real-time PCR, and multiplex real-time PCR for detecting *Arcobacter* species were developed, targeting the 16S or 23S rRNA, *rpoB-rpoC*, and *gyrA* genes [24–26]. Loop-mediated isothermal amplification (LAMP) was recently developed for on-the-spot inspections [27]. Importantly, this approach requires only simple equipment, such as a heating block and water bath. The method uses four or six different primers specifically designed

to recognize six distinct regions in the target sequence (Eiken Chemical Co., Ltd.) and uses *Bst* DNA polymerase to interact with the template DNA during DNA replication. *Bst* DNA polymerase has a very high activity; thus, vast amounts of high-molecular-weight DNA can be produced within a short time [28], with the reaction proceeding at a constant temperature. LAMP was first introduced in 2000, and it has been successfully used for the detection of many pathogens [28–31].

Therefore, this study aimed to develop a rapid and sensitive LAMP technique for the detection of *Arcobacter* species and to compare the sensitivities of LAMP and multiplex PCR in the detection of *Arcobacter* in broth and chicken.

MATERIALS AND METHODS

Bacterial strains and DNA extraction. Forty-two *Arcobacter* species and 27 food-borne pathogens were used in this study (Table 1). *Arcobacter* species were cultured using *Arcobacter* selection broth (Oxoid, Basingstoke, United Kingdom) or with added agar technical (Oxoid, United Kingdom) at 37°C for 48 h. Other pathogens were cultured in brain heart infusion broth at 37°C for 24 h. Bacterial DNA was extracted with an AccuPrep genomic DNA extraction kit (Bioneer, Daejeon, South Korea).

Multiplex PCR for *Arcobacter* species. As multiplex PCR for *Arcobacter* species was widely used in previous studies [23, 26, 32, 33], the primers ARCO (5′-CCT GGA CTT GAC ATA GTA AGA ATG A-3′), BUTZ (5′-CGT ATT CAC CGT AGC ATA GC-3′), SKIR (5′-GGC GAT TTA CTG GAA CAC A-3′), CRY1 (5′-TGC TGG AGC GGA TAG AAG TA-3′), and CRY2 (5′-AAC AAC CTA CGT CCT TCG AC-3′) were used to detect *Arcobacter* species in this study. The multiplex PCR mixture contained 1 mM concentrations of deoxynucleoside triphosphate s (dNTPs) (Bioneer), 2 μl of 10× reaction buffer (Tris [pH 9.0], 15 mM MgCl₂) (Bioneer), a 1.25 μM concentration of each primer, 0.05 U/μl Top polymerase

TABLE 1 Reference strains for the sensitivity and specificity tests of LAMP used in this study

Species	Strain(s)[a]
Arcobacter butzleri	ATCC 49616; CAU 076046, 076048, 076050, 080083, 080084, 080086, 080089, 080090, 080092, 080097, 080098, 080099, 080100, 080101, 080105, 080137, 080138, 080142, 080145, 080150, 080159, 080161, 080163, 080165, 080166, 080169, 080170, 080171, 080173, 080174, 080175, 080176, 080177, 080178, 080179, 080180
Arcobacter cryaerophilus	ATCC 43158
Arcobacter skirrowii	ATCC 51132; CAU 090017, 090019, 090023
Escherichia coli O157:H7	ATCC 43889, 43890
Listeria monocytogenes	ATCC 15313, 19117, 19114
Salmonella enterica serovar Typhimurium	ATCC 43971, 19585
Staphylococcus aureus	KACC 10778, 10768, 10196; ATCC 49444,12600
Vibrio parahaemolyticus	KCCM 4664, ATCC 43996, KCTC 2471
Bacillus cereus	ATCC 13061, 10879
Campylobacter jejuni	ATCC 33291; NCCP 10402, 10276, 11211, 10672
Campylobacter coli	NCCP 11191
Helicobacter pylori	ATCC 43504, 49503; KTCC B0233, B0322

[a] ATCC, American Type Culture Collection; CAU, Chung-Ang University; KACC, Korean Agricultural Culture Collection; KCCM, Korean Culture Center of Microorganisms; KCTC, Korean Collection of Type Culture; NCCP, National Culture Collection for Pathogens.

(Bioneer), and 2 μl of DNA template, with deionized water added to bring the total reaction volume to 20 μl. Multiplex PCR was performed on an MJ mini personal thermal cycler (Bio-Rad, Mexico) with the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 61°C for 45 s, extension at 72°C for 45 s, and final extension at 72°C for 10 min. PCR products were visualized on a 1.2% agarose gel using polyacrylamide gel electrophoresis (Bioneer).

LAMP for *Arcobacter* species. LAMP primers specific for the *A. butzleri* 23S gene (GenBank no. FN600698.1) were designed by the online LAMP primer design software PrimerExplorer V4 (http://primerexplorer .jp) and were as follows: F3, 5′-ACT GTG ACA ACC AGG AGG TT-3′; B3, 5′-TCC AAC GCT CCT TAC CGG-3′; FIP, 5′-CGC GCA GAA TCA CTA GAC CAG TGG CTT AGA AGC AGC CAT CC-3′; and BIP, 5′-AAC GGG GCT AAG ATG TAC ACC GAC GCT GAA TAG AAC GCT CTC-3′. LAMP was carried out in a total reaction volume of 25 μl with a final concentration of 2 μM for both FIP and BIP and 0.2 μM for both F3 and B3, 0.8 mM concentrations of dNTPs (Bioneer), 2.5 μl of 1× ThermoPol reaction buffer [20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100] (New England BioLabs Inc., Ipswich, United Kingdom), 8 U of *Bst* DNA polymerase large fragment (New England BioLabs Inc.), 2 μl of DNA template, and diethylpyrocarbonate (DEPC)-treated deionized water to bring the reaction volume to 25 μl. The reaction mixture was incubated at 61°C for 50 min and heated at 80°C for 5 min to terminate the reaction. The LAMP reactions were conducted using an MJ mini personal thermal cycler (Bio-Rad) for temperature control.

Sensitivity and specificity of LAMP and multiplex PCR. The specificity and sensitivity of the LAMP and multiplex PCR methods used in this study were determined using reference strains, including *A. butzleri* ATCC 49616, *A. skirrowii* ATCC 51132, and *A. cryaerophilus* ATCC 43158 (Table 1). To determine the detection limit of LAMP for *Arcobacter* species in chicken, 10 g of chicken skin and meat not containing *Arcobacter* species was spiked with 1 ml of *A. butzleri*, *A. skirrowii*, or *A. cryaerophilus* cultures at 10^4 to 10^8 CFU/ml. Each sample was homogenized for 2 min in a stomacher bag with 90 ml of 0.1% peptone water. One milliliter of homogenate was transferred to a 1.5-ml Eppendorf tube. The samples were centrifuged at 13,000 rpm for 15 min. The supernatant was discarded, and the bacterial pellet was resuspended in 100 μl of sterile water and boiled for 10 min to lyse the cells. The suspension was centrifuged at 8,000 rpm for 2 min, and the supernatant was used as a DNA template. From each sample, 2 μl of the supernatant was used for LAMP and PCR (26).

To confirm the LAMP amplification product specificity, restriction fragment length polymorphism (RFLP) analysis was performed with the restriction enzyme HindIII. The specific restriction sites in the LAMP products were analyzed and selected with NEB cutter V2.0 (http://tools .neb.com/NEBcutter2). Briefly, the DNA products amplified by LAMP were digested with 1 U/μl HindIII (Promega, Madison, WI) following the manufacturer's standard protocol. HindIII can specifically digest the DNA sequence 5′-AAGCTT-3′. The final digestion products were expected to be 147, 198, and 249 bp.

Experimental design. Thirty fresh whole chickens were purchased from several local supermarkets in Gyeonggi province. The chickens were kept at 4°C and analyzed within 12 h of purchase. Five grams of skin from each chicken was collected in a stomacher bag with 45 ml of 0.1% peptone water and homogenized for 2 min. From each sample, bacterial DNA was extracted by boiling for 10 min. Another 1-ml homogenate of each sample was transferred to 9 ml of *Arcobacter* broth containing C.A.T. supplement (Oxoid) and incubated at 30°C in an incubator. After 3, 6, and 24 h of incubation, the DNA was extracted from 1 ml of culture broth. After 24 h of incubation, the cultured broth was filtered with a 0.45-μm sterile syringe filter and inoculated on an *Arcobacter* selective agar. After an additional 48 h of incubation at 30°C, single colonies were isolated for further analysis.

For accurate comparison, the sensitivities of multiplex PCR and LAMP were compared to that of a conventional PCR method developed in a previous study (34). The primers Arc1 (5′-AGA ACG GGT TAT AGC TTG CTA T-3′) and Arc2 (5′-GAT ACA ATA CAG GCT AAT CTC T-3′) were used for conventional PCR, which generated a 181-bp DNA product for *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* (34). Bacterial culture, conventional PCR, multiplex PCR, and LAMP were compared for the detection of *Arcobacter* spp. in the cultured samples at 0, 3, 6, and 24 h of incubation.

Statistical analysis. Fisher's exact test was used to analyze the comparative detection of conventional PCR, multiplex PCR, and LAMP in experimentally *A. butzleri*-challenged samples at each time point. Analyses were carried out using SAS 9.2 software (SAS Institute Inc., Cary, NC). *P* values less than 0.05 were considered statistically significant.

RESULTS

Specificity of LAMP and multiplex PCR. To evaluate the specificity of the primer set, we used multiplex PCR and LAMP to detect DNAs extracted from 42 bacterial strains of *Arcobacter* and 27 strains of other food-borne bacteria (Table 2). Multiplex PCR detected and differentiated *A. butzleri*, *A. skirrowii*, and *A. cryaerophilus* in the 42 *Arcobacter* strains used in this study. In addition, multiplex PCR detected 5 strains of *Campylobacter jejuni* and 1

TABLE 2 LAMP and multiplex PCR detection with bacterial DNA isolated from reference strains[a]

Species	No. positive for *Arcobacter* DNA/no. tested by:	
	LAMP	Multiplex PCR
A. butzleri	37/37	37/37
A. skirrowii	4/4	4/4
A. cryaerophilus	1/1	1/1
C. jejuni	1/5	5/5
C. coli	0/1	1/1
H. pylori	0/4	0/4
E. coli O157:H7	0/2	0/2
L. monocytogenes	0/3	0/3
S. Typhimurium	0/2	0/2
S. aureus	0/5	0/5
V. parahaemolyticus	0/3	0/3
B. cereus	0/2	0/2

[a] The multiplex PCR developed by Houf et al. (26) to detect and differentiate *Arcobacter* species.

strain of *Campylobacter coli* as false positives for *Arcobacter* species. Other food-borne pathogens were not detected by multiplex PCR. The size of the DNA product amplified from *Campylobacter* spp. was matched with the expected 641-bp amplicon from *A. skirrowii* (Fig. 1A).

The LAMP method developed in this study detected 37 *A. butzleri*, 4 *A. skirrowii*, and 1 *A. cryaerophilus* strains. The other food-borne pathogens, except *C. jejuni* NCCP 10672, were not amplified by LAMP (Table 2) (Fig. 1B). RFLP analysis of the LAMP products amplified from 42 *Arcobacter* strains, including 3 reference strains, yielded three fragment sizes: 147, 198, and 249 bp (Fig. 1C). The LAMP products of *C. jejuni* NCCP 10672 were not digested with HindIII (Fig. 1C). The specificities of multiplex PCR and LAMP are summarized in Table 2.

Sensitivity of LAMP and multiplex PCR. The sensitivities of LAMP and multiplex PCR were determined by serial 10-fold dilutions of *Arcobacter* reference strains (Fig. 2). The detection limit of LAMP in cultured broth was 2 CFU per reaction for *A. butzleri* ATCC 49616 and *A. skirrowii* ATCC 51132, and 20 CFU per reaction for *A. cryaerophilus* ATCC 43158. However, the detection limit of multiplex PCR in cultured broth was 20 CFU per reaction for *A. butzleri*, 20 CFU per reaction for *A. skirrowii*, and 2×10^4 CFU per reaction for *A. cryaerophilus*. The detection sensitivity of LAMP in cultured broth was 10-fold higher for *A. butzleri* and *A. skirrowii* and 1,000-fold higher for *A. cryaerophilus* than that of multiplex PCR.

The detection limits of LAMP and multiplex PCR were also determined in chicken spiked with an *Arcobacter* reference strains (Fig. 3). The detection limit of LAMP in chicken was 2×10^2 CFU per reaction for *A. butzleri* ATCC 49616, *A. skirrowii* ATCC 51132, and *A. cryaerophilus* ATCC 43158. However, the detection limit of multiplex PCR in chicken was 2×10^3 CFU per reaction for *A. butzleri* and *A. skirrowii* and 2×10^4 CFU for *A. cryaerophilus*. The detection sensitivity of LAMP for *Arcobacter* species was 10- to 100-fold higher than that of multiplex PCR.

Detection of *Arcobacter* species in chickens from retail markets. Ten (33.3%) *Arcobacter butzleri* and three (10.0%) *A. cryaerophilus* strains were isolated by bacterial culture using *Arcobacter* selective medium from 30 chickens purchased from retail

markets. Culture homogenates from the 30 chicken samples were analyzed by conventional PCR, multiplex PCR, and LAMP at 0, 3, 6, and 24 h of incubation (Table 3). Although *Arcobacter* species were not detected in chicken homogenates without incubation by
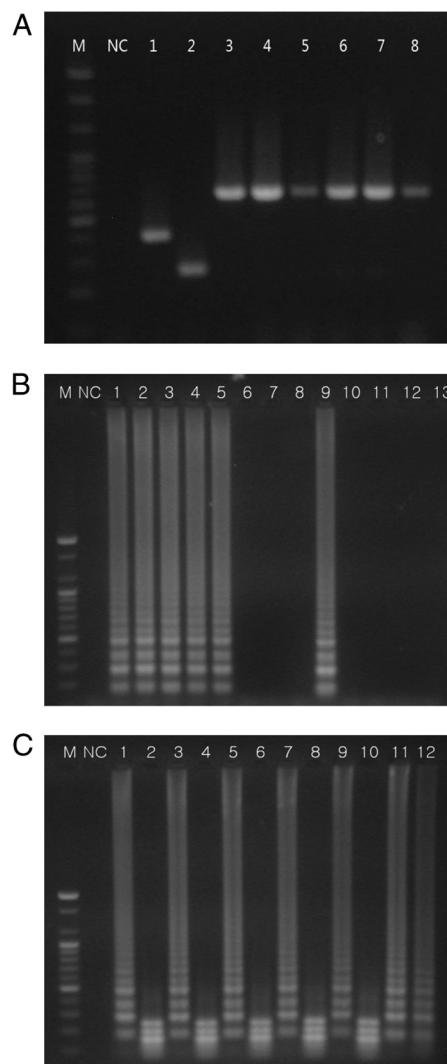

FIG 1 Specificity of multiplex PCR and LAMP. (A) PCR. Lane M, 100-bp DNA marker; lane NC, negative control; lane 1, *A. butzleri* ATCC 49616; lane 2, *A. cryaerophilus* ATCC 43152; lane 3, *A. skirrowii* ATCC 51132; lane 4, *C. jejuni* ATCC 33291; lane 5, *C. jejuni* NCCP 10402; lane 6, *C. jejuni* NCCP 10276; lane 7, *C. jejuni* NCCP 10672; lane 8, *C. coli* NCCP 11191. (B) LAMP. Lane M, 100-bp DNA marker; lane NC, negative control; lane 1, *A. butzleri* ATCC 49616; lane 2, *A. butzleri* CAU 076046; lane 3, *A. skirrowii* ATCC 51132; lane 4, *A. skirrowii* CAU 090029; lane 5, *A. cryaerophilus* ATCC 43152; lane 6, *C. jejuni* ATCC 33291; lane 7, *C. jejuni* NCCP 10402; lane 8, *C. jejuni* NCCP 10276; lane 9, *C. jejuni* NCCP 10672; lane 10, *C. jejuni* NCCP 11211; lane 11, *C. coli* NCCP 11191; lane 12, *H. pylori* ATCC 43504; lane 13, *E. coli* O157:H7 ATCC 43889. (C) Restriction fragment length polymorphism of LAMP products. Lane M, 100-bp DNA marker; lane NC, negative control; lane 1, *A. butzleri* ATCC 49616 LAMP; lane 2, *A. butzleri* ATCC 49616 LAMP product digested with HindIII; lane 3, *A. butzleri* CAU 076046 LAMP; lane 4, *A. butzleri* CAU 076046 LAMP product digested with HindIII; lane 5, *A. skirrowii* ATCC 51132 LAMP; lane 6, *A. skirrowii* ATCC 51132 LAMP product digested with HindIII; lane 7, *A. skirrowii* CAU 090017 LAMP; lane 8, *A. skirrowii* CAU 090017 LAMP product digested with HindIII; lane 9, *A. cryaerophilus* ATCC 43152 LAMP; lane 10, *A. cryaerophilus* ATCC 43152 LAMP product digested with HindIII; lane 11, *C. jejuni* NCCP 10672 LAMP; lane 12, *C. jejuni* NCCP 10672 LAMP product digested with HindIII.
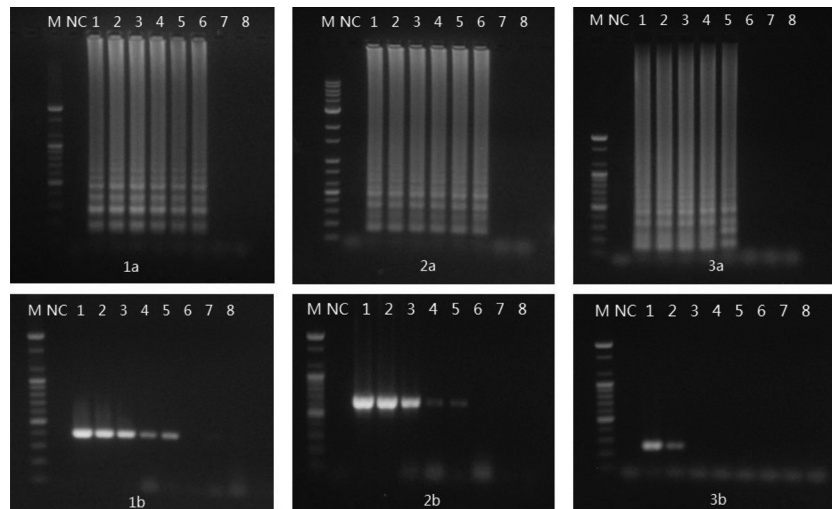
FIG 2 Comparison of LAMP and multiplex PCR for the detection of *Arcobacter* species in broth. (Panels 1a and 1b) *A. butzleri* ATCC 49616; (panels 2a and 2b) *A. skirrowii* ATCC 51132; (panels 3a and 3b) *A. cryaerophilus* ATCC 43152. Lane M, 100-bp DNA marker; lane NC, negative control; lane 1, 2×10^5 CFU per reaction; lane 2, 2×10^4 CFU per reaction; lane 3, 2×10^3 CFU per reaction; lane 4, 2×10^2 CFU per reaction; lane 5, 2×10^1 CFU per reaction; lane 6, 2 CFU per reaction; lane 7, 2×10^{-1} CFU per reaction; lane 8, 2×10^{-2} CFU per reaction.

conventional PCR, homogenates incubated for 3, 6, and 24 h showed positivity for 6, 18, and 27 of 30 chickens, respectively. Whereas 9 of 30 chicken homogenates were positive for *Arcobacter* species by multiplex PCR at 0 h of incubation, homogenates incubated for 3, 6, and 24 h showed positivity for 19, 22, and 28 of 30 chickens, respectively. In LAMP detection, homogenates incubated for 0, 3, 6, and 24 h showed positivity for 20, 24, 27, and 28 of 30 chickens, respectively. The detection rate of LAMP was significantly higher than rates of conventional PCR and multiplex PCR at 0, 3, and 6 h incubation ($P < 0.05$). However, the detection rates of conventional PCR, multiplex PCR, and LAMP at 24 h incubations were not significantly different.

In homogenates cultured for 24 h, multiplex PCR detected both *A. butzleri* and *A. cryaerophilus* in 10 samples, and simulta-neously detected *A. butzleri*, *A. cryaerophilus*, and *A. cryaerophilus* in five chickens. Five chickens were positive only for *A. butzleri*, and four chickens were positive for both *A. butzleri* and *A. skirrowii*. Two chickens were positive for either *A. skirrowii* or *A. cryaerophilus*.

DISCUSSION

Arcobacter species are considered a major public health concern ([18]). In severe chronic human diseases caused by *Arcobacter* spp., the rapid detection and treatment of *Arcobacter* infection are required ([35]). Nine *A. butzleri* isolates were cultured from diarrheal stool samples in Turkish hospitals, although *A. butzleri* was first isolated from the blood of a Korean patient with liver cirrhosis ([36–38]). Bacterial culture is not practical for the rapid detection of
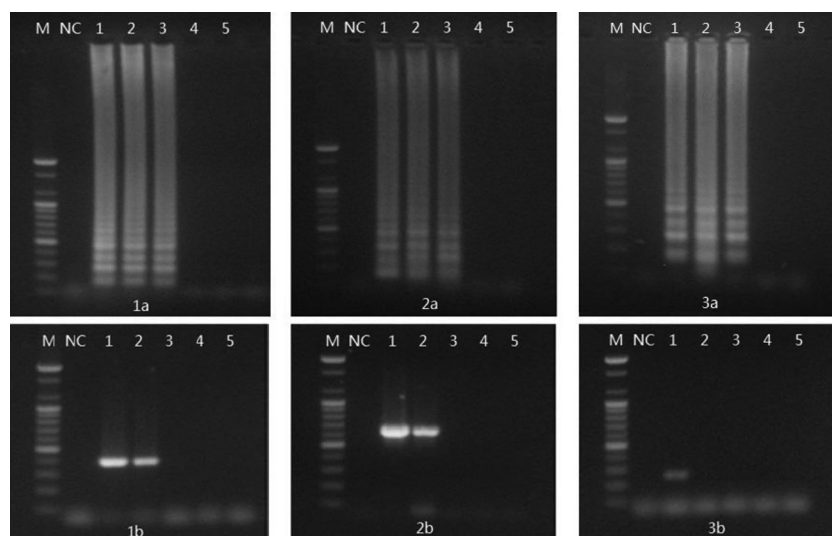

FIG 3 Comparison of LAMP and multiplex PCR for the detection of *Arcobacter* species in chicken. (Panels 1a and 1b) *A. butzleri* ATCC 49616; (panels 2a and 2b) *A. skirrowii* ATCC 51132; (panels 3a and 3b) *A. cryaerophilus* ATCC 43152. Lane M, 100-bp DNA marker; lane NC, negative control; lane 1, 2×10^4 CFU per reaction; lane 2, 2×10^3 CFU per reaction; lane 3, 2×10^2 CFU per reaction; lane 4, 2×10^1 CFU per reaction; lane 5, 2 CFU per reaction.

TABLE 3 Comparative detection of *Arcobacter* species in 30 chickens purchased from retail markets

Incubation time (h)	No. of positive chickens			
	Bacterial culture	Conventional PCR[a]	Multiplex PCR[b]	LAMP
0		0	9	20
3		6	19	24
6		18	22	27
24	13	27	28	28

[a] The conventional PCR developed by Gonzalez et al. (34).
[b] The multiplex PCR developed by Houf et al. (26).

Arcobacter species because of their slow growth in *Campylobacter* blood-free selective medium; therefore, conventional PCR, multiplex PCR, and enterobacterial repetitive intergenic consensus (ERIC)-PCR were used in previous studies for the rapid detection of *Arcobacter* spp. (23, 35, 36). In this study, five *C. jejuni* strains and one *C. coli* strain were detected as false positives by multiplex PCR described previously (26). The *Campylobacter* species amplicons were matched with the expected size of the *A. cryaerophilus* amplicon. Because of a lack of a simple identification system for *Arcobacter* spp., multiplex PCR was widely used for the detection of *Arcobacter* spp. in previous studies (19, 23, 25, 26). However, the cross-reactivity of multiplex PCR with *Campylobacter* spp. has not been addressed thus far. A high rate of contamination with *Campylobacter* in chicken was also reported in a previous study (39). Thus, it is possible that the prevalence data obtained from multiplex PCR reflected *Campylobacter* contamination. Whereas *Campylobacter* spp. could be cultured only under anaerobic conditions, *Arcobacter* species were differentiated by bacterial culture under aerobic conditions. Further, given that bacterial isolation takes 2 to 4 days and anaerobic chamber culture requires skilled microbiologists, bacterial culture is not practical for on-the-spot inspection of *Arcobacter* contamination.

In the present study, whereas *Campylobacter* strains were detected by multiplex PCR, LAMP specifically detected only *Arcobacter* species except *C. jejuni* NCCP 10672. Because *Arcobacter* and *Helicobacter* also belong to the genus *Campylobacter* of the rRNA superfamily VI, 23S ribosomal RNA gene sequences show high homology between *Campylobacter* and *Arcobacter* (15). As LAMP primers were designed in 23S rRNA genes of *Arcobacter* species, LAMP in this study did not show the cross-reactivity with *Campylobacter* or *Helicobacter* species. Of all the reference strains tested, only *C. jejuni* NCCP 10672 showed a false-positive reaction by LAMP; however, the LAMP product of this strain was not digested with restriction enzyme HindIII. Thus, RFLP analysis of LAMP products clearly differentiated *Arcobacter* spp. and *Campylobacter* spp.

The detection limit of LAMP was determined both *in vitro* and in food. With DNA isolated from cultured bacteria, LAMP could detect as little as 2 CFU per reaction. This value is similar to those reported in previous studies, which detected 1, 20, 7.9, and 3.8 CFU per reaction for *Escherichia coli* O157:H7, *Plesiomonas shigelloides*, *C. jejuni*, and *C. coli*, respectively (30, 31, 40). The sensitivity of the LAMP method developed in this study was 10- to 1,000-fold higher than that of conventional PCR or multiplex PCR (30, 40). In concordance with the results of previous studies, LAMP showed 100-fold-greater sensitivity than conventional PCR or multiplex PCR.

Because of the high prevalence of *Arcobacter* in chicken meat (19), the sensitivity of LAMP for the detection of *Arcobacter* spp. in chicken was assessed. The detection sensitivity of LAMP was 100-fold higher than that of multiplex PCR for *A. cryaerophilus* and 10-fold higher for *A. butzleri* and *A. skirrowii*. Its detection limit was as low as 200 CFU per reaction in chicken spiked with *Arcobacter* species. According to previous studies, food components can inhibit or interfere with DNA amplification in LAMP and PCR assays for the detection of *Campylobacter* spp. (41). In the present study, the blood components or nucleic acids from the chicken samples were considered PCR inhibitors; thus, they may explain the difference in LAMP or PCR sensitivity between the bacterial culture and chicken samples.

This study showed that 93.3% of the chickens sampled were contaminated with *Arcobacter* species, with *A. butzleri* being the most prevalent species. Although the *Arcobacter* contamination rates obtained in this study were consistent with those in previous reports (23, 42), studies in the United States and Japan showed much lower rates than those reported herein (43, 44). In contrast to molecular detection, bacterial culture for *Arcobacter* spp. took at least 3 to 4 days and had low sensitivity. Although conventional PCR could not detect *Arcobacter* spp. in all samples without enrichment, the combination of bacterial culture with multiplex PCR and LAMP showed a higher detection rate. Compared with multiplex PCR, the detection rate of LAMP was significantly increased by 3 to 6 h of enrichment ($P < 0.05$). Thus, the LAMP assay developed in this study proved to be more rapid, specific, and sensitive than conventional PCR or multiplex PCR.

In contrast to PCR and real-time PCR, the detection time of LAMP is less than 1 h because the target gene is amplified under isothermal conditions. LAMP is more specific than PCR, because 4 or 6 primers used in LAMP increase specificity (45). Although short detection time and specificity of LAMP are the major advantages, continuous amplification of the target gene by LAMP may be confused with nonspecific PCR products on gel electrophoresis. In order to overcome this disadvantage of LAMP, alternative LAMP-based detection techniques were recently proposed (45–48). In one variation that does not use gel electrophoresis, a positive reaction was determined by Ca^{2+} precipitation and SYBR green fluorescent or color dye with naked-eye inspection (45, 47). In addition, real-time LAMP and probe-based LAMP have also been reported (46, 48). To enhance the specificity of LAMP, probe-based LAMP may be developed for the detection of *Arcobacter* in future research.

In conclusion, the LAMP assay developed in this research was able to detect *Arcobacter* spp. rapidly and reliably *in vitro* and in chicken samples. This method could be used for the rapid diagnosis of *Arcobacter* spp. infections in food poisoning cases or for on-the-spot inspection of slaughterhouses.

ACKNOWLEDGMENT

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