Feasibility of Loop-Mediated Isothermal Amplification for Rapid Detection of Methicillin-Susceptible and Methicillin-Resistant *Staphylococcus aureus* in Tissue Samples

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Background: To date, few studies have investigated the feasibility of the loop-mediated isothermal amplification (LAMP) assay for identifying pathogens in tissue samples. This study aimed to investigate the feasibility of LAMP for the rapid detection of methicillin-susceptible or methicillin-resistant *Staphylococcus aureus* (MSSA or MRSA) in tissue samples, using a bead-beating DNA extraction method.

Methods: Twenty tissue samples infected with either MSSA (n = 10) or MRSA (n = 10) were obtained from patients who underwent orthopedic surgery for suspected musculoskeletal infection between December 2019 and September 2020. DNA was extracted from the infected tissue samples using the bead-beating method. A multiplex LAMP assay was conducted to identify MSSA and MRSA infections. To recognize the *Staphylococcus* genus, *S. aureus*, and methicillin resistance, 3 sets of 6 primers for the 16S ribosomal ribonucleic acid (rRNA) and the *femA* and *mecA* genes were used, respectively. The limit of detection and sensitivity (detection rate) of the LAMP assay for diagnosing MSSA and MRSA infection were analyzed.

Results: The LAMP result was positive for samples containing 10^3 colony-forming unit (CFU)/mL for 16S rRNA, 10^4 CFU/mL for *femA*, and 10^5 CFU/mL for *mecA*. The limits of detection for 16S rRNA and *femA* were not different between MSSA and MRSA. For the 10 MSSA-positive samples, the LAMP assay showed 100% positive reactions for 16S rRNA and *femA* and a 100% negative reaction for *mecA*. For the 10 MRSA-positive samples, the LAMP assay showed 100% positive reactions for 16S rRNA and *mecA* but only 90% positive reactions for *femA*. The sensitivity (detection rate) of the LAMP assay for identifying MSSA and MRSA in infected tissue samples was 100% and 90%, respectively.

Conclusions: The results of this study suggest that the LAMP assay performed with tissue DNA samples can be a useful diagnostic method for the rapid detection of musculoskeletal infections caused by MSSA and MRSA.

Keywords: *Methicillin-susceptible Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus, Loop-mediated isothermal amplification*

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Methicillin-susceptible or methicillin-resistant *Staphylococcus aureus* (MSSA or MRSA) is the most common pathogen in musculoskeletal infections.[1-4] Although early identification of the causative bacteria is essential for the treatment of *S. aureus* infections, conventional culture-based methods have limitations such as low sensitivity and a delay in diagnosis.[5] These limitations often cause difficulties in the selection of appropriate antibiotics and evaluation of the infection status intraoperatively.

Recently, genetic diagnostic tools have been developed to overcome the limitations of conventional diagnostic methods for the early detection and identification of disease-causing bacteria.[6,7] Polymerase chain reaction (PCR), which amplifies minute amounts of bacterial DNA, is a genetic diagnostic method.[7] Previous studies on the use of PCR targeting the 16S ribosomal ribonucleic acid (rRNA) gene, which is present in all bacteria, have shown excellent results for identifying infections.[8] However, PCR requires specific expensive equipment and is a technique that many orthopedic surgeons are unfamiliar with. In addition, PCR has a relatively high detection limit and requires temperature control and 4–8 hours of analysis time.[9]

The loop-mediated isothermal amplification (LAMP) assay is a rapid isothermal technique that does not require temperature control.[10] This assay has a relatively low detection limit and requires only 1–2 hours for analysis.[10,11] Previous studies have shown that the LAMP assay is useful for the rapid detection of pathogens in various fluid samples (e.g., blood, pleural, or synovial fluid).[12,13] However, in many orthopedic operations for musculoskeletal infection, it is difficult to obtain fluid samples to identify pathogens. To date, few studies have investigated the feasibility of the LAMP assay for identifying pathogens in tissue samples.

In this study, we investigated the feasibility of LAMP for the rapid detection of MSSA and MRSA in tissue samples using the bead-beating DNA extraction method. We hypothesized that the LAMP assay using infected tissue samples would show high sensitivity (detection rate) for diagnosing MSSA or MRSA infection.

METHODS

This study was conducted after obtaining approval from the Institutional Review Boards (IRB No. 2020AS0143 and IRB No. 2020GR0317). Infected tissue samples were obtained during orthopedic operations for suspected musculoskeletal infection after obtaining informed consent from patients between December 2019 and September 2020 at

two hospitals (Korea University Guro Hospital and Korea University Ansan Hospital). Patients who did not agree to donate tissue samples, presented difficulty in collecting tissue samples, and/or used antibiotics within 2 weeks before surgery were excluded from this study.

Two tissue samples from the same location were collected from each patient. One tissue was submitted for standard microbiological tissue culture, and the other was stored at −80°C for the LAMP assay in the tissue bank of each hospital. Thus, 20 tissue samples with MSSA (n = 10) or MRSA (n = 10) were collected. The preoperative demographic data of the enrolled patients are summarized in Table 1.

Table 1. Sex, Age, Diagnosis, and Infection Site in Patients with Infected Tissues

Sample	Sex	Age (yr)	Diagnosis	Infection site
MSSA1	Female	81	Periprosthetic joint infection	Rt knee
MSSA2	Female	44	Chronic osteomyelitis	Rt knee
MSSA3	Male	48	Diabetes mellitus foot	Rt foot
MSSA4	Male	48	Diabetes mellitus foot	Lt foot
MSSA5	Male	26	Infected epidermoid cyst	Lt 1st toe
MSSA6	Male	50	Diabetes mellitus foot	Lt foot
MSSA7	Male	45	Chronic osteomyelitis	Lt cuboid
MSSA8	Male	53	Chronic osteomyelitis	Lt tibia
MSSA9	Male	31	Chronic osteomyelitis	Rt tibia
MSSA10	Male	59	Chronic osteomyelitis	Lt tibia
MRSA1	Female	80	Diabetes mellitus foot	Lt 2nd toe
MRSA2	Female	50	Diabetes mellitus foot	Rt foot
MRSA3	Female	66	Infective myofasciitis	Lt thigh
MRSA4	Male	40	Acute postoperative infection	Rt tibia
MRSA5	Female	68	Infective bursitis	Lt ankle
MRSA6	Female	79	Periprosthetic joint infection	Lt hip
MRSA7	Male	52	Diabetes mellitus foot	Lt 4th toe
MRSA8	Male	53	Diabetes mellitus foot	Lt 4th toe
MRSA9	Male	27	Chronic osteomyelitis	Rt femur
MRSA10	Female	20	Chronic osteomyelitis	Lt ankle

MSSA: methicillin-susceptible *Staphylococcus aureus*, MRSA: methicillin-resistant *Staphylococcus aureus*, Rt: right, Lt: left.

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Tissue Sample Preparation and DNA Extraction

Thawed tissue samples were homogenized in 800 μL of lysis buffer (FACS lysing solution; BD Bioscience, San Jose, CA, USA) using a tissue grinding tube (Bioprep-A20, Allsheng, Hangzhou, China) and homogenizer (Bioprep-6 homogenizer, Allsheng). The tissue grinding tube contained 3- and 6-mm metal beads and 1.4-mm ceramic beads. Homogenization was performed for 10 cycles of 30 seconds at a speed of 6 m/sec (Fig. 1). Next, the homogenate was centrifuged for 30 seconds at 5,000 rpm, and 800 μL of the supernatant was decanted for DNA extraction. DNA extraction was conducted using an NX-48 bacterial DNA kit (Genolution Inc., Seoul, Korea) and Nextractor NX-48 automated extractor (Genolution Inc.). The process, from tissue sample preparation to DNA extraction, was completed within 1 hour.

LAMP Assay

To recognize the *S. genus, S. aureus,* and methicillin resistance, we used 3 LAMP primer sets targeting the 16S rRNA and *mecA* and *femA* genes each; these were developed by Baek et al.[14] and Lin et al.[15] (Table 2). All LAMP primers were synthesized by Macrogen, Inc. (Seoul, Korea). LAMP assays were performed using an RNA amplification kit (Eiken Chemical Company, Tokyo, Japan). The LAMP reaction mixture was prepared with 12.5 μL of reaction buffer, 2 μL of enzyme mix, 1 μL of fluorescence detection reagent, 4 μL of distilled water, 2.5 μL of primer mix, and 3 μL of genomic DNA (final reaction volume: 25 μL). The composition of the LAMP primer mix included 2 μM of

2 outer primers (F3 and B3), 16 μM of 2 inner primers (FIP and BIP), and 5 μM of loop primers (loop B and loop F). The reaction tubes were loaded into a thermocycler (CFX-96, Bio-Rad, Korea) and incubated at 64°C for 60 minutes.

The results were determined by visual observation of the color change by naked eye in a single-blind manner. The green color change of the pH indicator was interpreted as positive for amplification of DNA. The LAMP assay was defined as positive for MSSA infection if the result was positive for 16S rRNA and *femA* and negative for *mecA*. However, it was defined as positive for MRSA infection if the result was positive for 16S rRNA, *femA*, and *mecA* (Fig. 2).

Table 2. Sequences of Primers for the LAMP Assay Used to Detect MSSA and MRSA

Target gene	Primer	Sequence (5′→3′)
16S rRNA*	F3	TGGAATTCCATGTGTAGCGG
	B3	AGGCGGAGTGCTTAATTGC
	FIP	TCGCACATCAGCGTCAGTTACA-ATGCGCAGAGATATGGAGGA
	BIP	AGATACCCTGGTAGTCCACGCC-CACTAAGGGGCGGAAACC
	LF	CCAGAAAGTCGCCTTCGCCACT
	LB	AAACGATGAGTGCTAAGTGTTAGG
femA†	F3	ATGCTGGTGGTACATCAA
	B3	TGGTTTAATAAAGTCACCAACAT
	FIP	GGTCAATGCCATGATTTAATGCATA-GCATTCCGTCATTTTGCC
	BIP	CAGAAGATGCTGAAGATGCTGG-TCAATAATTTCAGCATTGTAACC
	LF	AATCATTTCCCATTGCACT
	LB	TGTAGTTAAATTCAA
*mecA**	F3	TGATGCTAAAGTTCAAAAGAGT
	B3	GTAATCTGGAACTTGTTGACC
	FIP	AGGTGTGCTTACAAGTGCTAATAAT-CAACATGAAAAATGATTATGGCT
	BIP	TGACGTCTATCCATTTATGTATGGC-AGGTTCTTTTTTATCTTCGGTTA
	LF	TGAGGGTGGATAGCAGTACC
	LB	TGAGTAACGAAGAATAT

LAMP: loop-mediated isothermal amplification, MSSA: methicillin-susceptible *Staphylococcus aureus*, MRSA: methicillin-resistant *Staphylococcus aureus*, rRNA: ribosomal RNA.
*LAMP primers described by Baek et al. (2019).[14] †LAMP primers described by Lin et al. (2017).[15]
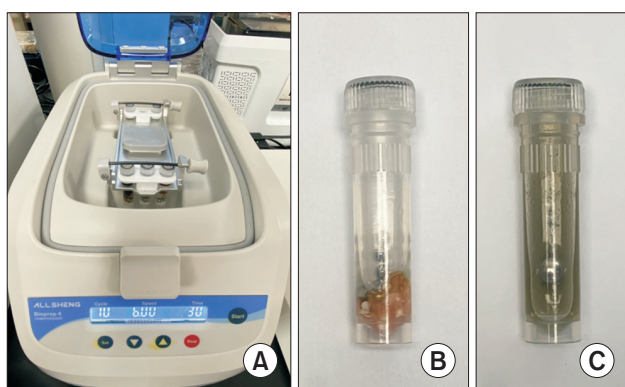
Fig. 1. Tissue sample preparation for loop-mediated isothermal amplification assay. Each tissue sample was homogenized in 800 μL of lysis buffer using a tissue grinding tube and homogenizer. Homogenization was performed for 10 cycles of 30 seconds at a speed of 6 m/sec. (A) Homogenizer (Bioprep-6 homogenizer, Allsheng). (B) Tissue grinding tube containing tissue sample, lysis buffer, and ceramic and metal beads. (C) Homogenate.

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Limit of Detection of LAMP Assay

To determine the limit of detection (LOD), MSSA or MRSA suspension (1.0×10^7 colony-forming units [CFU]/mL) was diluted 10 times in steps to 1.0×10^1 CFU/mL, and the LAMP assay was performed for 16S rRNA, *femA*, and *mecA* at each concentration.

Statistical Methods

Tissue samples with MSSA or MRSA were defined as positive for infection in this study. To investigate the feasibility of the LAMP assay for identifying MSSA and MRSA in tissue samples, the sensitivity (detection rate) of the assay was calculated for each bacterium. The sensitivity and specificity of the LAMP primers for the target genes were also calculated to evaluate the validity of the LAMP primers.

RESULTS

LOD of LAMP Assay

The LODs of the LAMP assay for 16S rRNA, *femA*, and *mecA* of MSSA and MRSA are shown in Table 3. The LAMP results were positive for simple samples containing 10^3 CFU/mL for 16S rRNA, 10^4 CFU/mL for *femA*, and 10^5 CFU/mL for *mecA* (Table 3, Fig. 3). The LODs of 16S rRNA and *femA* were not different between MSSA and MRSA.

Sensitivity (Detection Rate) of LAMP Assay for Identifying MSSA and MRSA

For the 10 MSSA-positive samples, the LAMP assay showed 100% positive reactions for 16S rRNA and *femA*

and a 100% negative reaction for *mecA* (Table 4). For the 10 MRSA-positive samples, the LAMP assay showed 100% positive reactions for 16S rRNA and *mecA* but only 90% for *femA*. For the *femA*-negative MRSA sample, the LAMP assay showed a positive reaction for 16S rRNA and *mecA* (Table 4). The sensitivity (detection rate) of the LAMP assay for diagnosing MSSA and MRSA in infected tissue samples was 100% and 90%, respectively.

Sensitivity and Specificity of LAMP Primers for 16S rRNA, *femA*, and *mecA*

The sensitivity and specificity of LAMP primers for 16S rRNA, *femA*, and *mecA* in 10 MSSA and 10 MRSA samples are summarized in Table 5. The sensitivities of the LAMP primers for 16S rRNA and *femA* were 100% and 95%, respectively. The sensitivity and specificity of the
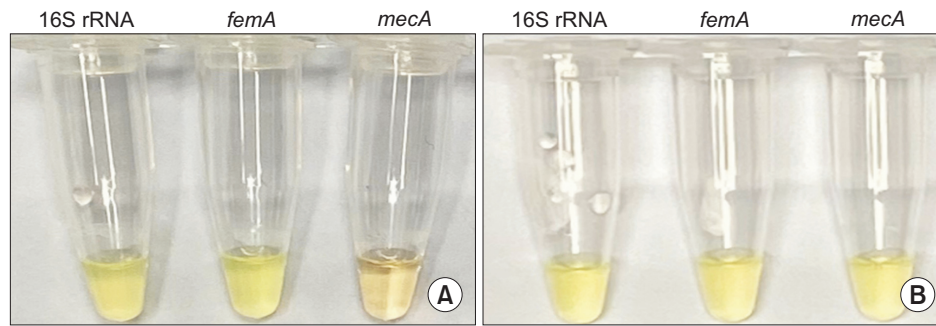
Table 3. Detection Limits of the LAMP Assay for 16S rRNA and *femA* and *mecA* Genes of MSSA and MRSA

Gene	Detection limit (CFU/mL)	
	MSSA	MRSA
16S rRNA	10^3	10^3
femA	10^4	10^4
mecA	NA	10^5

LAMP: loop-mediated isothermal amplification, rRNA: ribosomal RNA, MSSA: methicillin-susceptible *Staphylococcus aureus*, MRSA: methicillin-resistant *Staphylococcus aureus*, CFU: colony-forming unit, NA: not applicable.
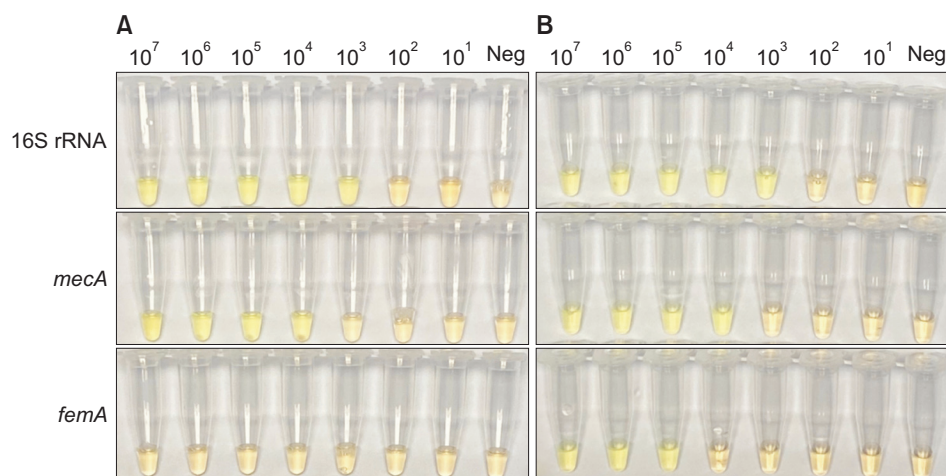
Fig. 2. Typical findings of the loop-mediated isothermal amplification (LAMP) assay for detecting methicillin-susceptible *Staphylococcus aureus* (A) and methicillin-resistant *Staphylococcus aureus* (B). (A) LAMP assay shows positive results for 16S ribosomal RNA (rRNA) and *femA* and a negative (Neg) result for *mecA*. (B) LAMP assay shows positive results for 16S rRNA, *femA*, and *mecA*. LAMP results were determined by naked-eye detection under normal light. The green color change of the pH indicator was interpreted as positive for the amplification of DNA.

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Fig. 3. Images of 16S ribosomal RNA (rRNA), *mecA*, and *femA* loop-mediated isothermal amplification products of methicillin-susceptible *Staphylococcus aureus* (A) and methicillin-resistant *Staphylococcus aureus* (B) isolates at serially diluted concentrations (~10^1 to 10^7 CFU/mL). The green color change of the pH indicator was interpreted as positive for the amplification of DNA. CFU: colony-forming unit.

Table 4. Results of the LAMP Assay to Detect rRNA and *femA* and *mecA* Genes

Sample	16S rRNA	*femA*	*mecA*
MSSA1	+	+	−
MSSA2	+	+	−
MSSA3	+	+	−
MSSA4	+	+	−
MSSA5	+	+	−
MSSA6	+	+	−
MSSA7	+	+	−
MSSA8	+	+	−
MSSA9	+	+	−
MSSA10	+	+	−
MRSA1	+	+	+
MRSA2	+	+	+
MRSA3	+	+	+
MRSA4	+	+	+
MRSA5	+	+	+
MRSA6	+	+	+
MRSA7	+	+	+
MRSA8	+	−	+
MRSA9	+	+	+
MRSA10	+	+	+

LAMP: loop-mediated isothermal amplification, rRNA: ribosomal RNA, MSSA: methicillin-susceptible *Staphylococcus aureus*, MRSA: methicillin-resistant *Staphylococcus aureus*.

Table 5. Sensitivity and Specificity of LAMP Primers for 16S rRNA and *femA* and *mecA* Genes

Target gene	Sensitivity	Specificity
16S rRNA	100 (20/20)	NA
femA	95 (19/20)	NA
mecA	100 (10/10)	100 (10/10)

Values are presented as percent (number).
LAMP: loop-mediated isothermal amplification, rRNA: ribosomal RNA, NA: not applicable.

LAMP primer for *mecA* was 100% for all the samples.

DISCUSSION

The most important finding of this study is that the LAMP assay showed high sensitivity (detection rate) for determining the presence of MSSA or MRSA in infected tissue samples. To the best of our knowledge, this is the first experimental study to investigate the feasibility of the LAMP assay for diagnosing MSSA and MRSA infections in tissue samples. To extract bacterial DNA from the infected tissue, tissue samples were homogenized using a tissue grinding tube and homogenizer, and the homogenate was centrifuged before DNA extraction. The LAMP assay detected MSSA or MRSA infection in almost all (19/20) of the tissue samples in this study. Therefore, the results imply that a sufficient amount of DNA can be extracted and amplified via the tissue preparation and bead-beating DNA extraction methods used.

Increased incidence of human diseases caused by food-borne pathogens is considered a major threat for public health worldwide. Accordingly, molecular diag-

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nostic technologies for the rapid detection of food-borne pathogens have been developed. During the past few decades, numerous PCR-based assays have been proposed for the rapid detection of food-borne pathogens. However, the disadvantages of PCR-based assays (time consumption for determination, risk of cross contamination, low detection limit, and need for expensive equipment and reagents) posed obstacles for their broader application.[16] The LAMP assay, which was developed by Notomi et al.[17] in 2000, has emerged as an alternative to PCR-based methods and been widely used for microbial identification and diagnosis, as well as for the surveillance of infection diseases.[16] Currently, LAMP is being used for the detection and identification of causative pathogens in patients with microbial infectious diseases, as it offers advantages of high sensitivity, specificity, rapid delivery of results, and cost-effectiveness.[16]

Previous studies have also indicated the diagnostic value of the LAMP assay for identifying MSSA or MRSA infection in human samples. Henares et al.[13] reported a LAMP assay using pleural and synovial fluid samples with sensitivity and specificity values of 83.3% and 97.8%, respectively, for *S. aureus* detection. Misawa et al.[12] also reported a LAMP assay for identifying MRSA in blood samples that showed 92.3% sensitivity and 100% specificity. In our study, the sensitivity (detection rate) of the LAMP assay for diagnosing MSSA and MRSA in infected tissue samples was 100% and 90%, respectively. Therefore, the LAMP assay can be considered a feasible diagnostic method for the rapid detection of *S. aureus* in tissue samples.

The LODs of LAMP primers for the 16S rRNA and *femA*, and *mecA* genes have been previously reported. Xu et al.[18] reported that LAMP was positive for samples containing 10^4 CFU/mL for 16S rRNA, 10^4 CFU/mL for *femA*, and 10^5 CFU/mL for *mecA*. Lin et al.[15] also reported LODs of LAMP assays of 10^4 CFU/mL for 16S rRNA and *femA* and 10^5 CFU/mL for *mecA*. The detection limit of the LAMP assay was reported to be 10^4 CFU/mL for 16S rRNA, *femA*, and *mecA* in the study by Baek et al.[14] In the current study, the results of the LOD test were comparable with those from previous studies (10^3 CFU/mL for 16S rRNA, 10^4 CFU/mL for *femA*, and 10^5 CFU/mL for *mecA*) (Table 3). Moreover, the LAMP primers showed an excellent detection rate for the target gene (Table 5). Therefore, the LAMP primers used in this study can be considered effective for diagnosing MSSA and MRSA infections.

In this study, all experimental processes, including tissue sample preparation, DNA extraction, and LAMP assay, were completed within 2 hours. Rapid detection of disease-causing bacteria in tissue samples can be helpful for the diagnosis and treatment of musculoskeletal infections. Rapid detection also makes it possible to use appropriate antibiotics in the early stages of infection. In addition, the results of LAMP assays can be used to determine the treatment plan when a clear distinction between aseptic and septic loosening is not possible after joint replacement surgery. Diagnosis of periprosthetic joint infection is still challenging, and routine diagnostic tests have demonstrated high false-negative rates.[19] Although the choice of treatment for septic loosening is re-implantation after removing the prosthesis and eradicating the causative bacteria,[20-22] strikingly high mortality rates have been reported before re-implantation.[23] Therefore, rapid and direct detection of pathogens using LAMP assays can be helpful in preventing the unnecessary removal of prostheses. Moreover, it can also help confirm the eradication of the causative bacteria before re-implantation.

In this study, the LAMP primers targeted only the 16S rRNA and *femA* genes of MSSA and MRSA. *S. aureus* is the most common pathogen in musculoskeletal infections; however, various other pathogens can also cause such infections.[1-3] Although LAMP primers targeting the 16S rRNA gene can be used to evaluate the presence of infection, they are limited in terms of identifying the causative bacteria. This is one of the major limitations of genetic diagnostic methods, such as PCR and LAMP, compared with that of culture-based methods. To overcome this limitation, multiplex LAMP assays are required for identifying various pathogens that commonly cause musculoskeletal infections simultaneously. Therefore, we are planning to evaluate the diagnostic value of the LAMP assay for identifying other bacteria such as *Streptococcus*, *Enterococcus*, and *Klebsiella* species in tissue samples in the future.

This study has several limitations. First, the sample size was relatively small. However, it was difficult to obtain a large number of samples because infected tissue samples were collected prospectively in this study. Second, other diagnostic values such as the specificity and the positive and negative predictive values of the LAMP assay could not be evaluated because this study was conducted with tissue samples in which MSSA or MRSA was identified in tissue culture. However, this study focused on evaluating the sensitivity (detection rate) of the LAMP assay because the primary aim was to evaluate whether a sufficient amount of bacterial DNA could be extracted from infected tissue samples for this assay. Further studies will be required to confirm the false-negative rate of the LAMP assay using noninfected tissues obtained from clean sur-

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geries. Finally, in this study, it was not possible to compare the diagnostic value of the LAMP assay with that of other diagnostic methods, such as culture and PCR analysis.

In conclusion, the LAMP assay performed with tissue DNA samples can be a useful diagnostic method for the rapid detection of musculoskeletal infections caused by MSSA and MRSA. Further research is needed to evaluate the diagnostic value of the LAMP assay for identifying other pathogenic bacteria in tissue samples.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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