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The Usefulness of Multiplex PCR for the Identification of Bacteria in Joint Infection

Hwanjung Kim,[1] Jimyung Kim,[2]* and Chunhwa Ihm[2]

[1]*Department of Orthopedic Surgery, Eulji University Hospital, Dunsandong, Daejeon, Korea*
[2]*Department of Laboratory Medicine, Eulji University Hospital, Dunsandong, Daejeon, Korea*

Background: The diagnosis of septic arthritis (SA) relies on synovial analysis and conventional culture. But, these methods lack of sensitivity and culture is time consuming to establish a definite diagnosis. This study evaluated a new multiplex PCR assay which entailed screening PCR for Gram typing and identification PCR for species identification using two primer mixes. *Methods*: A total of 80 synovial fluid samples from patients with suspected SA were collected. Culture, multiplex PCR, and 16S rRNA gene PCR were performed. *Results*: The analytical sensitivity of multiplex PCR assay was 10^1 CFU/ml for each type of bacteria. There was no cross-reactivity with common bacterial pathogens. Bacteria were detected in 20, 25, and 26 of 80 samples for culture, multiplex PCR, and 16S rRNA gene PCR, respectively. Nineteen (95%) of 20 culture-positive samples and 6 (10%) of 60 culture-negative samples were positive for the multiplex PCR. Five of six samples which were positive only from multiplex PCR were also positive in 16S rRNA gene PCR. The multiplex PCR showed 2 false-negative in 27 true-positive samples but no false-positive. The sensitivity and specificity of the multiplex PCR were 92.6 and 100%, and the agreement with culture and 16S rRNA gene PCR were 91.3 and 96.3%, respectively. The time to detection for multiplex PCR was a maximum of 6 hr. *Conclusion*: This multiplex PCR assay offers high sensitivity and improved detection speed relative to culture. The appropriate combination of this new multiplex PCR assay with culture may contribute to the accurate and rapid diagnosis of SA. J. Clin. Lab. Anal. 24:175–181, 2010.

Key words: culture; multiplex polymerase chain reaction; septic arthritis

INTRODUCTION

The diagnosis of infectious arthritis currently relies on the isolation of organisms from aspirated joint fluid by microbiological cultures, but complete identification of the infecting microorganisms on gram staining and routine culture is difficult. Although gram stain and culture are the most commonly used methods to detect organisms, both depend on the amount of bacteria present. So, the sensitivity of gram stain has been reported in the range of 29–50% and the sensitivity of culture at 82% (1,2), and cultures may be negative for patients for whom treatment has already been initiated.

Because prompt diagnosis and early initiation of therapy are critical for improving the outcome, a sensitive diagnostic assay for the rapid diagnosis of septic arthritis (SA) is required and the use of PCR amplification of 16S rRNA gene has been proposed for the detection of eubacteria in synovial fluid (3). PCR amplification of the 16S rRNA is now an established technique for the detection of bacteria in meningitis (4), endocarditis (5), and endophthalmitis (6). Broad-based PCR demonstrated high sensitivity and specificity in bone and joint infections but relied on sequencing for definite pathogen identification (7). Recently, real-time multiprobe PCR was introduced and this assay diagnosed SA with speed and accuracy (8).

We report a novel multiplex PCR assay for early diagnosis of SA. This novel assay, based on a multiplex PCR method using dual priming oligonucleotide system,

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*Correspondence to: Jimyung Kim, Department of Laboratory Medicine, Eulji University Hospital, Dunsandong, Daejeon, Korea. E-mail: jmkim@eulji.ac.kr

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consists of an initial screening assay, permitting differentiation of gram-positive, gram-negative, and *Candida* spp. and an identification assay for definite pathogen characterization of the species. Diagnostic accuracy of this assay was evaluated in comparison to culture and 16S rRNA gene PCR methods using synovial fluid samples.

MATERIALS AND METHODS

Patients

A total of 80 synovial fluid samples (one sample per patient) were collected from patients who presented with suspected SA between July 2007 and October 2008. Patients' clinical and other laboratory variables suggested a higher likelihood of SA and all of the samples were taken for diagnostic purposes. These processes followed the protocol approved by the Eulji University Hospital Institutional Review Board. Samples were from 63 knees, 10 hips, 6 ankles, and 1 shoulder. Specimens obtained by needle aspiration were placed in sterile tubes and delivered to the laboratory within 2 hr of collection. The samples were divided into three parts: for culture, for PCR, and for storage at −20°C.

Bacterial Isolation and Identification

Direct microscopic examination after gram staining was performed to note the presence of bacteria. The samples were inoculated on 5% sheep blood and chocolate agar, incubated at 37°C in a 5% CO_2 atmosphere and anaerobic atmosphere for 48 hr. Pure bacterial cultures were identified using a commercially available Vitek biochemical assay (bioMerieux Vitek Inc., Hazelwood, MO).

Extraction of DNA

Each 500 μl of synovial fluid samples was centrifuged at 3,200 × *g* for 10 min and the pellet was resuspended in 200 μl of phosphate buffered saline (Bioneer, Daejeon, Korea). DNA was extracted using a QIAamp DNA mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. An additional incubation at 95°C for 15 min was performed following proteinase K digestion to ensure complete lysis of bacterial cells. The extracted DNA was stored at −20°C before undergoing PCR testing. We tested for the presence of bacteria using both screening multiplex PCR and 16S rRNA gene PCR.

Multiplex PCR

A total of 64 clinically relevant bacterial organisms including the six most common SA-related organisms (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Escherichia coli*, and *Pseudomonas aeruginosa*) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). DNA was extracted from the organisms to test the analytical sensitivity of gram-positive and gram-negative/*Candida* spp. primers, as well as pathogen-specific primers. The new multiplex PCR was performed using the primers mentioned in Table 1.

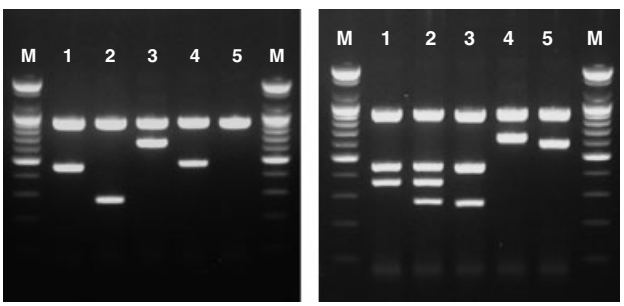
First, two types (Group Screening 1 and 2) of Seeplex Sepsis screening assay (Seegene Inc., Seoul, Korea) were undertaken on 3 μl of DNA to detect pathogens, classifying into the gram-positive group and the gram-negative/*Candida* spp. group. The Seeplex Sepsis screening assay covered only 64 species including 22 species *Staphyloccus* spp., 24 species of *Streptococcus* spp., 2 species of *Enterococcus* spp., 10 species of gram-negative bacteria, and 6 species of fungi (Table 2). The PCR mixture contained 4 μl of multiplex primer sets (5 primer sets including 1 internal control primer set in Group Screening 1 and 17 primer set including 1 internal control primer set in Group Screening 2, respectively), 10 μl of master mix (hot start *Taq* DNA polymerase and dNTP are included in the reaction buffer), and 3 μl of 8-methoxypsoralen (8-Mop). Positive samples by the screening assay were further analyzed with a second multiplex PCR using adequate Seeplex Sepsis identification assay (Seegene Inc.) for definite identification of a few common bacterial species. We performed Identification 2 if 458 bp band was observed in Group Screening 1 and Identification 3 if 271 bp band was seen. And Identification 1, 4, and 5 were performed, respectively, according to the positive band's size (580, 467, and 335 bp band) observed in Group Screening 2. For Identification 1 and 2, the number of primer sets including 1 internal control primer set was 7, and 6 for Identification 3, 4, and 5. An identification assay for *Staphylococcus* could detect three species (*S. aureus*, *S. epidermidis*, *Staphylococcus haemolyticus*) and an identification assay for *Streptococcus* could detect 4 species (*Streptococcus pneumoniae*, *Streptococcus agalactiae*, *S. pyogenes*, *Streptococcus mitis*). Samples in which a positive band was detected for screening PCR but not for identification PCR were considered as other *Staphylococcus* and *Streptococcus* spp. according to the results for screening PCR. Identification assays for gram-negative bacteria and fungi could accurately detect species covered in screening PCR. After preheating at 95°C for 15 min, 40 amplification cycles of 30 sec at 94°C, 1.5 min at 63°C, 1.5 min at 72°C were carried out in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, Canada). The final cycle ended with a 10 min extension at 72°C.

TABLE 1. Targets for Bacterial Detection by the Seeplex Sepsis Screening and Identification Test

Organism	Target DNA	Accession No.	Length (bp)
Group screening 1			
Enterococcus faecium/faecalis			650
E.faecium	ddl	AY489046	
E.faecalis	ace	AF260879	
Staphylococcus spp.	gap	AF270050	458
Streptococcus spp.	tuf	AF124225	271
Group screening 2			
Fungi			580
Candida albicans	phr1	M90812	
Candida tropicalis	erg11	AY942643	
Candida parapsilosis	top2	AB049144	
Candida glabrata	ssk2	EF193045	
Candida krusei	ABC1	FJ445767	
Aspergillus fumigatus	tyr1	AJ293806	
Gram-negative bacteria 1			467
Enterobacter aerogenes	tolC	AJ306390	
Serratia marcescens	chiC	AF454464	
Klebsiella pneumoniae	lamB	X66952	
Enterobacter cloacae	UDP	Z11835	
Klebsiella oxytoca	mdh	AY367380	
Gram-negative bacteria 2			335
Pseudomonas aeruginosa	algD	Y00337	
Escherichia coli	lamB	M26131	
Proteus mirabilis	ureR	Z18752	
Stenotrophomonas maltophilia	smeD	AJ252200	
Acinetobacter baumannii	trpE	NC_009085	
Identification 1			
Candida albicans	phr1	M90812	649
Candida tropicalis	erg11	AY942643	513
Candida parapsilosis	top2	AB049144	410
Candida glabrata	ssk2	EF193045	313
Candida krusei	ABC1	FJ445767	225
Aspergillus fumigatus	tyr1	AJ293806	152
Identification 2			
Enterococcus faecium/faecalis			650
E.faecium	ddl	AY489046	
E.faecalis	ace	AF260879	
Staphylococcus spp.	gap	AF270050	458
Staphylococcus aureus	nuc	EF529597	361
Staphylococcus epidermidis	fmhB	AF269600	280
Staphylococcus haemolyticus	fmhA	AP006716	202
Identification 3			
Streptococcus agalactiae	cfb	X72754	730
Streptococcus pneumoniae	ply	EF413947	552
Streptococcus pyogenes	rpoB	NC_004070	445
Streptococcus mitis	gyrB	AB238626	351
Streptococcus spp.	tuf	AF124225	271
Identification 4			
Enterobacter aerogenes	tolC	AJ306390	603
Serratia marcescens	chiC	AF454464	466
Klebsiella pneumoniae	lamB	X66952	350
Enterobacter cloacae	ompX	M33878	284
Klebsiella oxytoca	mdh	AY367380	200
Identification 5			
Pseudomonas aeruginosa	algD	Y00337	655
Escherichia coli	lamB	M26131	496
Proteus mirabilis	ureR	Z18752	335
Stenotrophomonas maltophilia	ITS	AY116914	200
Acinetobacter baumannii	trpE	NC_009085	156

TABLE 2. Sixty-Four Species of Organism Covered by Seeplex Sepsis Screening PCR

Staphylococcus spp. (22)	*S.aureus*, *S.epidermidis*, *S.haemolyticus*, other 19 *Staphylococcus* spp.
Streptococcus spp. (24)	*S.pneumoniae*, *S.agalactiae*, *S.pyogenes*, *S.mitis*, other 20 *Streptococcus* spp.
Enterococcus spp. (2)	*E.faecium*, *E.faecalis*
Gram-negative bacteria (10)	*E.coli*, *P.mirabilis*, *P.aeruginosa*, *A.baumannii*, *S.maltophilia* *K.pneumoniae*, *K.oxytoca*, *S.marcescens*, *E.cloacae*, *E.aerogenes*
Fungi (6)	*C.albicans*, *C.tropicalis*, *C.parapsilosis*, *C.glabrata*, *C.krusei*, A.fumigatus



(A) (B)

Fig. 1. (**A**) Screening multiplex PCR assay for five samples. M, size markers (each 100 bp); 1. *Staphylococcus* spp.; 2. *Streptococcus* spp.; 3. *Enterococcus* spp.; 4. Gram-negative bacteria; and 5. Negative. (**B**) Identification multiplex PCR assay for five samples. M, size markers (each 100 bp); 1. *S. aureus*; 2. *S. aureus*/*S. epidermidis*; 3. *S. pyogenes*; 4. *E. faecium*; and 5. *E. aerogenes*.

The multiplex PCR products were visualized using a MCE-202 microchip electrophoresis system (Shimazu corporation, Kyoto, Japan) (Fig. 1).

16S rRNA Gene PCR

To clarify the result of multiplex PCR, 16S rRNA gene PCR was run in parallel with screening multiplex PCR. Extracted DNA was PCR amplified with the 536F and rp2 (Bioneer) primer pair targeting the 16S rRNA gene as previously described (7,9). The sequences of the primers were as follows: forward primer (536F), 5′-CAGCAGCCGCGGTAATAC-3′; the reverse primer (rp2), 5′-ACGGCTACCTTGTTACGACTT-3′. A sample of culture proven *S. aureus* infection collected from a patient and a sample of osteoarthritis collected from a patient were used as a positive and negative control, respectively. To avoid amplification of possible bacterial contaminants, UV light irradiation for 5 min together with 8-Mop was used in the reaction mixture before addition of the extracted DNA and the procedure was done again if amplication of negative control occurred.

Comparison of Tests

Positive results isolated in at least two assays were considered to be a true-positive. If a microorganism was isolated in only one assay or was not detected in all assays, the interpretation of results was determined according to the clinical diagnosis based on synovial fluid analysis (10). The accuracies of culture, multiplex PCR, and 16S rRNA gene PCR were determined by sensitivity and specificity. All samples with discordant findings between culture and PCR results were inoculated in an enriched broth for 24 hr and were plated on a 5% sheep blood agar to assess bacterial growth. Amplified PCR products from discordant clinical samples were sequenced by accessing the GeneBank (www.ncbi.nlm.nih.gov) database via the BLAST program.

RESULTS

Analytical Sensitivity and Specificity of Multiplex PCR

A single isolated colony of each organism (*S. aureus, S. agalactiae*, *Enterococcus faecium*, *E. coli*, *P. aeruginosa*, and *Candida albicans*) was inoculated in tryptic soy broth and incubated at 37°C overnight. The sensitivity of the multiplex PCR assay was assessed with serial dilution of each of these strains in culture-negative and DNA-free synovial fluid. The detection limits of multiplex PCR were 10^1 CFU/ml for SA-related organisms and there was no difference between single targeted PCR and multiplex PCR. DNA was also extracted from other 26 species (*Neisseria gonorrhoeae*, *E. avium*, *Salmonella enteritica*, *Hemophilus infleuenza*, *Burkholderia cepacia*, etc.) beyond the listed 64 species to test the analytical specificity. All Gram typing primers and pathogen-specific primers correctly identified their respective target organisms.

Comparison of Culture and Molecular Methods

Among the 80 samples, 15 (18.7%) were positive for gram staining and 20 (25.0%) were culture-positive. Twenty culture-positive results showed the following organisms: ten *S. aureus*, four coagulase negative staphylococcus (CNS), two *S. pyogenes*, one *S. agalactiae*, one *Streptococcus intermedius*, one *E. faecium*, one *Enterobacter cloacae*, and one *Salmonella* spp. One patient had a polymicrobial infection (*S. aureus* and *E. cloacae*).

All culture-positive samples showed positive results by 16S rRNA gene PCR without any discrepancy, but Gram typing primers were in concordance with the culture results in 19 samples (95.0%) and were negative in 1 sample (culture-positive for *Salmonella* spp.) as shown in Table 3. Of 19 screening PCR positive-samples, pathogen-specific primers were in concordance with culture results in 18 samples. One was reported positive for *S. aureus* in culture but showed coinfection for *S. aureus*/*S. epidermidis* in identification multiplex PCR. Compared to 16S rRNA PCR, the screening multiplex PCR showed the same positive results in 19 samples.

Fifty four of the 60 culture-negative samples tested negative and 6 culture-negative samples (10.0%) had positive results in the screening multiplex PCR. Six screening PCR positive samples were identified with three other *Streptococcus* spp., two *S. aureus* and one *E. aerogenes* in the identification multiplex PCR, but 1 sample identified with *S. aureus* showed negative in 16S rRNA gene PCR. Fifty three of the 54 culture-negative samples showed the same negative results in both 16S rRNA gene PCR and screening multiplex PCR, but 1 out of 54 samples was only positive for 16S rRNA gene PCR, being screening multiplex PCR negative.

Discordant Multiplex PCR Results

Seven samples showed discordant culture and screening multiplex PCR results. One was reported culture-positive for *Salmonella* spp. group D, but was negative with multiplex PCR. Six samples were reported negative by culture, but were positive by multiplex PCR. Additional culturing for six culture negative samples did not show any growth. Among them, three patients have been receiving antibiotic therapy at the time of sampling.

Two samples showed different results between culture and identification multiplex PCR although congruent results were yielded between culture and screening multiplex PCR. One sample which was reported as *S. aureus* by culture was actually other *Staphylococcus* spp. in identification multiplex PCR, but repeat culture and sequencing of this sample confirmed that it was *S. aureus*. Another sample reported positive for *S. aureus* in culture but coinfection for *S. aureus* and *S. epidermidis* in identification multiplex PCR was revealed as mixed growth of *S. aureus* and *S. epidermidis* in a reculture.

Of culture-negative samples, two samples showed discordant results between 16S rRNA gene PCR and multiplex PCR. One sample that showed a positive result only for 16S rRNA gene PCR was identified with *Mycobacteria tuberculosis* and one sample that showed a positive result only for multiplex PCR were identified as *S. aureus* by sequencing (Table 4). A mycobacterial culture for one sample, detected as *M. tuberculosis* in 16S rRNA gene PCR, showed a positive result after 5 weeks.

TABLE 3. Multiplex PCR (Screening) Results Vs. Culture and 16S rRNA Gene PCR in Synovial Fluid Samples

	No. of samples with the following culture result		No. of samples with the following 16S rRNA gene PCR result		
Multiplex PCR result	Positive	Negative	Positive	Negative	Total no. of samples
Positive	19	6	24	1	25
Negative	1	54	2	53	55
Total no. of samples	20	60	26	54	80

TABLE 4. Discrepant Results Between Culture and PCR

No	Culture	16S rRNA gene PCR	Screening multiplex PCR	Identification multiplex PCR	Comments
Discordant culture and screening multiplex PCR results					
1	Negative	+	STA	*S. aureus*	
2	Negative	+	STREP	other *Streptococcus*	
3	Negative	+	STREP	other *Streptococcus*	
4	Negative	+	STREP	other *Streptococcus*	
5	Negative	+	GNB	*E. aerogenes*	
6	Negative	−	STAU	*S. aureus*	Sequenced; *S. aureus*
7	*Salmonella* spp.	+	ND	Not performed	
Discordant culture and identification multiplex PCR results					
8	*S. aureus*	+	STAU	*CNS*	Sequenced; *S. aureus*
9	*S.aureus*	+	STAU	*S. aureus*/*S. epidermidis*	additional culture; *S. aureus*/*S. epidermidis*
Discordant culture and 16S rRNA gene PCR results					
10	Negative	+	ND	Not performed	additional culture; *M. tuberculosis*

STA, *Staphylococcus*; STREP, *Streptococcus*; GNB, gram-negative bacteria; ND, not detected band; +, positive; −, negative

Clinical Sensitivity and Specificity (Table 5)

Among 80 samples, 27 were considered as true-positive (20 culture-positive, 26 16S rRNA gene PCR-positive, 25 multiplex PCR-positive) and 53 were true-negative. The sensitivity and specificity of culture were 74.1 and 100%, respectively and the sensitivity of 16S rRNA gene PCR and multiplex PCR were 96.2 and 92.6%, respectively. The agreement of screening multiplex PCR with culture was 91.3% and the agreement with 16S rRNA gene PCR was 96.3%.

Assay Performance Time

The time to detection was 3.5–6 hr, which included DNA extraction (40 min) and multiplex PCR amplification (165 min).

DISCUSSION

Most SA develops as a result of the hematogenous seeding of the synovial membrane or following trauma. In addition, arthritis associated with joint surgery has been increasing (10). The most common etiologic agent in SA is *S. aureus* and *Streptococcus* spp. Gram-negative bacilli account for 10–20% of the cases and the most common gram-negative organisms are *P. aeruginosa* and *E. coli*. And 10% of SA have polymicrobial infections (11). In this study, 29 organisms were isolated when the results of the culture and molecular methods were combined and the most common organisms were *Staphylococcus* spp. and *Streptococcus* spp. with 58.6% (17/29) and 24.1% (7/29), respectively. Other isolates were gram-negative bacilli (10.3%, 3/29), enterococcal species (3.4%, 1/29) and mycobacteria (3.4%, 1/29). The majority of staphylococcal infection was due to *S. aureus* (70.5%, 12/17) but CNS accounted for a relatively high proportion (29.4%) of staphylococcal infection compared with the previous report (12). Other gram-negative bacilli besides *P. aeruginosa* and *E. coli* were commonly isolated and polymicrobial infection was proven in 2 (7.4%) of 27 patients with culture or PCR positive results.

We demonstrated the utility of a multiplex PCR assay which could be a useful adjunct for rapid, accurate

TABLE 5. Comparison of Sensitivity and Specificity of Culture, 16S rRNA gene PCR and Screening Multiplex PCR in Synovial Fluid Samples

		No. of samples with the clinical conclusion			
Multiplex PCR result		Positive	Negative	Sensitivity (%)	Specificity (%)
Culture	Positive	20	0	74.1	100
	Negative	7	53		
16S rRNA gene PCR	Positive	26	0	96.2	100
	Negative	1	53		
Multiplex PCR	Positive	25	0	92.6	100
	Negative	2	53		
Total no. of samples		27	53		

diagnosis of SA. In comparison with conventional gram staining and culture assay, multiplex PCR had the capacity for early pathogen characterization, especially in gram-positive strains and showed high sensitivity and specificity. But, there is a limitation to the detection of some gram-negative strains and other microorganisms not included in the target pathogen of the assay kit. These strains can be detected in broad-based 16S rRNA gene PCR. The sensitivity of multiplex PCR was higher than conventional culture and was similar to 16S rRNA gene PCR, and the agreement with other methods was excellent at more than 90%.

In seven patients, the presence of bacteria was proven only by 16S rRNA gene PCR and/or multiplex PCR, and five of seven patients showed positive results in both PCR assays. However, two patients had positive results in one PCR assay. Such a discrepancy between PCR and culture results was previously observed (13) and the sensitivity of 16S rRNA gene PCR (96.2%) and multiplex PCR (92.6%) was higher compared with culture (74.1%). Seven culture-negative patients were finally diagnosed with SA because they showed classic symptoms, laboratory findings in synovial fluid, and improved clinical course after appropriate antibiotic treatment. In particular, same positive results from two different PCR assays could be considered as true-positive and PCR results supported clinical diagnosis in five patients. So we believe that this discrepancy results from the lack of sensitivity of the culture rather than PCR contamination though the presence of a pathogen could not be proven in repeat culturing in most patients except for *M. tuberculosis*. The possible explanations for the false negativity of culture include superior sensitivity of PCR over culture, administration of antibiotics before sample collection, and fastidious organisms. We suggested that antibiotic therapy partly affected the false-negative culture result because 3 (42.9%) of 7 culture-negative patients had received antibiotics before the sampling for this study. In addition, we know that conventional culture has limitation in detecting fastidious organisms such as *M. tuberculosis*.

The multiplex PCR showed different results with 16S rRNA gene PCR in three samples and two pathogens detected in 16S rRNA gene PCR could not be isolated in multiplex PCR. We suggest that it is impossible to detect these organisms using multiplex PCR because they are not covered in the assay panel (*Salmonella* spp. and *M. tuberculosis*). Therefore, this new multiplex PCR assay is adequate for identifying most common SA-related pathogens such as *Staphylococcus* spp. and *Streptococcus* spp., but has a possibility of providing false-negative results when detecting other pathogens. However, this limitation of multiplex PCR can be overcome if cultures are performed together. The 16S rRNA gene PCR assay failed to detect *S. aureus* in one sample and this finding has been attributed to the sensitivity of PCR, discussed in a previous report (7).

Although the 16S rRNA gene PCR has been widely used to identify infecting bacteria, it cannot differentiate a monomicrobial and polymicrobial infection and identify pathogen species without additional assays. The current method is composed of Gram typing detection and additional species identification and could solve these limitations of universal PCR, although the detection of a few microorganisms might be missed.

The real-time PCR assay has been evaluated for the detection of *Staphylococcus* genus and *S. aureus* in clinical specimen and has been shown to have clinical applicability (14,15) but the rapid identification of other microorganisms was recently reported in a few studies using quantitative real-time PCR (8,16). This multiplex PCR was a novel assay adopting different multiplexing technique with real-time PCR and detection limit and assay time of the assay were comparable to previously reported multiprobe PCR. Also, our panel of pathogen-specific probes was sufficient to detect 70–80% of etiologic organisms for SA (12). The complete process could be achieved within a maximum of 6 hr and this performance time was significantly shorter than the 2 days required for conventional culture. The two-step PCR process (first, a screening PCR is performed and

then if the screening PCR is positive, an identification PCR is followed by pathogen-specific primers) could reduce unnecessary PCR testing and offer rapid results for early initiation of adequate therapy. In addition, the multiplex PCR method was easy to set up as a routine laboratory test and the clinical sensitivity of this assay was excellent compared with the reported bacterial 23S or 16S rDNA PCR for joint infection, especially in the detection of gram-positive organisms (17,18).

In conclusion, our findings illustrate that a multiplex PCR assay is a rapid, sensitive, and specific molecular method for the detection and identification of micro-organisms in SA. Although conventional culture is still important to determine antimicrobial susceptibility, the appropriate combination of this new multiplex PCR assay and conventional culture may contribute in accurate and rapid diagnosis of SA.

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