JCM
Journals.ASM.org

Multiplex Real-Time PCR Assay for Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Strains Suitable in Regions of High MRSA Endemicity

Jeong-Uk Kim,[a] Choong-Hwan Cha,[a] Hae-Kyong An,[a] Ho-Jun Lee,[a] Mi-Na Kim[b]

Department of Laboratory Medicine, Gangneung Asan Hospital, University of Ulsan College of Medicine, Gangneung, South Korea[a]; Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea[b]

A multiplex real-time PCR assay that simultaneously detects the *mecA*, staphylococcal cassette chromosome (SCC*mec*)-open reading frame X (*orfX*) junction, and staphylococcal 16S rRNA genes was developed and evaluated using 444 staphylococcal strains. We demonstrated that this assay resulted in fewer false-positive results than a single-locus real-time PCR assay that amplified the SCC*mec-orfX* junction. This assay would be useful in a clinical laboratory in a region of high endemicity for methicillin-resistant *Staphylococcus aureus* (MRSA) infections.

The spread of methicillin-resistant *Staphylococcus aureus* (MRSA) among hospital and community settings poses a threat to public health worldwide. Rapid, accurate detection and appropriate intervention reduce the prevalence of MRSA (1–3). Recently, rapid methods for molecular detection of MRSA have been developed. A single-locus real-time PCR assay that amplifies the staphylococcal cassette chromosome (SCC*mec*)-open reading frame X (*orfX*) junction was first proposed by Huletsky et al. (4), and now, there are commercially available assays that identify MRSA based on the detection of the SCC*mec-orfX* junction (5–7). These assays have an advantage over double-locus assays, based on the simultaneous detection of the *mecA* gene and a *S. aureus*-specific gene, for the direct detection of MRSA from screening specimens. Double-locus assays have been associated with false-positive MRSA detections in clinical samples, including nasal swabs that contain both methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant coagulase-negative staphylococci (MRCoNS) (8). However, false-positive MRSA results have been also reported in single-locus assays (6, 7, 9–12), for example, due to MSSA isolates containing SCC*mec* remnants that were misidentified as MRSA (11–16).

South Korea has been a region of MRSA infection endemicity for many years. The rates of methicillin resistance among *S. aureus* isolates recovered from clinical specimens ranged from 67.8% to 74.1% during the 2000s (17). SCC*mec* is a mobile element that can be inserted into and excised from the chromosome. It was reported that partial excision of SCC*mec* from epidemic MRSA strains results in MSSA isolates (13, 15). Thus, in regions of high endemicity, the single-locus assay for direct detection of MRSA may have high false-positive results because of the presence of MRSA-derived MSSA strains that carry remnants of SCC*mec* elements. To address this, we developed a multiplex real-time PCR assay that simultaneously detects the *mecA*, SCC*mec-orfX* junction, and staphylococcal 16S rRNA genes. The assay was based on the hypothesis that a pure MRSA strain has constant *mecA*, SCC*mec-orfX* junction, and 16S rRNA copy numbers, represented by the threshold cycle (C_T) value, and that the exact relationship between C_T values of each target may be established. *mecA* and SCC*mec-orfX* were targeted to reduce the false-positive MRSA results caused by the presence of SCC*mec* remnants among MSSA isolates that do not carry *mecA*. The staphylococcal 16S rRNA gene was targeted to indicate coexisting staphylococcal strains in clinical samples.

This assay was evaluated using 444 strains, which included both reference strains from various international collections and clinical isolates from laboratory collections. The reference strains were 8 strains of MRSA (CCARM 3792, CCARM 3795, CCARM 3798, CCARM 3803, CCARM 3805, CCARM 3877, CCARM 3897, and CCARM 3911), 4 strains of MSSA (KCTC 1621, KCTC 1916, KCTC 1928, and ATCC 29213), and 11 strains of methicillin-susceptible coagulase-negative staphylococci (MSCoNS) (*Staphylococcus epidermidis*, KCCM 35494; *Staphylococcus simulans*, KCCM 41686; *Staphylococcus capitis*, KCCM 41466; *Staphylococcus warneri*, KCTC 3340; *Staphylococcus haemolyticus*, KCTC 3341; *Staphylococcus xylosus*, KCTC 3342; *Staphylococcus intermedius*, KCTC 3344; *Staphylococcus saprophyticus*, KCTC 3345; *Staphylococcus cohnii*, KCTC 3574; *Staphylococcus caprae*, KCTC 3583; and *Staphylococcus auricularis*, KCTC 3584). Twenty-nine MSSA isolates carrying SCC*mec* remnants, which had been confirmed by SCC*mec* typing (18, 19), were tested as control strains. The clinical isolates consisted of 209 MRSA, 109 MSSA, and 74 MRCoNS strains and were recovered mostly from wound, sputum, blood, and urine samples. Identification and susceptibility testing of these staphylococcal isolates were performed using the MicroScan WalkAway 96 (Siemens Healthcare Diagnostics Inc., West Sacramento, CA) and the Vitek 2 (bioMérieux Inc., Durham, NC) automated identification and susceptibility testing systems.

The reference strains and the clinical isolates were grown on blood agar plates (Asan Pharmaceutical, Seoul, South Korea) at 37°C for 24 h. Two or three bacterial colonies of the reference

Received 21 September 2012 Returned for modification 11 November 2012 Accepted 17 December 2012

Published ahead of print 26 December 2012

Address correspondence to Jeong-Uk Kim, jukim@gnah.co.kr.

doi:10.1128/JCM.02495-12

TABLE 1 The real-time PCR primers and probes for the detection of MRSA

Oligonucleotide	Sequence (5′→3′)[a]	Concn (μM)	Target(s)[b]
FSCC_A	GCGGAGGCTAACTATGTCAA	0.5	I, II, IVa, IVb, IVc, IVg, VI, VIII
FSCC_B	ATATGTAATTCCTCCACATCTCATT	0.5	III, V, VII
FSCC_C	GGCTGAAGTAACCGCATCA	0.5	IVe
FSCC_D	TTCATAATATGTGCTACGCAATC	0.5	X
FSCC_E	CGGCAATTCTCATAAACCTC	0.5	IX, XI
B*orfX*	GCAAAATGACATTCCCACA	0.5	*orfX*
P*orfX*	HEX-TCAATTAACACAACCCGCATCAT-BHQ1	0.2	*orfX*
F*mecA*	GAATGCAGAAAGACCAAAGC	0.5	*mecA*
B*mecA*	TTCTTTGGAACGATGCCTAT	0.5	*mecA*
P*mecA*	FAM-TTGGCCAATACAGGAACAGCA-BHQ1	0.2	*mecA*
F16SrRNA	CTTACCAAATCTTGACATCCTTT	0.5	16S rRNA
B16SrRNA	CTCGTTGCGGGACTTAAC	0.5	16S rRNA
P16SrRNA	Cy5.5-CGTCAGCTCGTGTCGTGAGAT-BHQ2	0.2	16S rRNA

[a] HEX, hexachloro-6-carboxyfluorescein; FAM, 6-carboxyfluorescein; BHQ1, black hole quencher 1; BHQ2, black hole quencher 2.

[b] The Roman numerals indicate the SCC*mec* types amplified by the primer.

strains and isolates were harvested with a 1-μl loop and suspended in 0.5 ml of distilled water. The suspension was heated in a boiling water bath for 10 min and centrifuged at 13,000 × *g* for 5 min. The supernatant was used for the real-time PCR.

Base sequences of the SCC*mec*-*orfX* junction, *mecA*, and staphylococcal 16S rRNA genes were obtained from NCBI GenBank and aligned with Sequencher 5.0 software (Gene Codes Co., Ann Arbor, MI). Based on sequence alignment, we identified regions of interest and designed primers and probes manually or with the Primer 3 program (http://frodo.wi.mit.edu/primer3/). The real-time PCR primers and probes designed and used in this study are shown in Table 1.

The real-time PCRs were conducted with a Rotor-Gene Q real-time PCR instrument (Qiagen Inc., Germantown, MD). The PCR mixture contained 0.5 μl of primer-probe mix, 5 μl of 2× Rotor-Gene Multiplex PCR master mix (Qiagen Inc., Germantown, MD), and 1.0 μl of template DNA in a total volume of 10 μl. The PCR parameters were 95°C for 5 min followed by 40 cycles of 95°C for 15 s and 60°C for 15 s, and green, yellow, and crimson fluorescence were measured. After completion of PCR, C_T values of the *mecA*, SCC*mec*-*orfX*, and 16S rRNA genes were recorded from the Rotor-Gene Q software. Statistical tests, including determinations of the *r* correlation coefficient and descriptive statistics, were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL). *P* values below the 5% level were considered statistically significant.

The analytical sensitivity of the real-time PCR was determined by 10-fold serial dilutions of a subculture of MRSA strain CCARM 3792. The strain was grown overnight on a blood agar plate, suspended in saline to a density equivalent to a 0.5 McFarland turbidity number, and serially diluted 10-fold from 10^2 to 10^7. DNA was extracted from 200 μl of the bacterial dilutions using the QIAcube with a QIAamp DNA minikit (Qiagen Inc., Germantown, MD) and eluted in 50 μl. In parallel, 100 μl of the dilutions was plated on blood agar and incubated at 37°C for 24 h. Thereafter, CFU were counted.

The real-time PCR assay was initially evaluated with 8 MRSA, 4

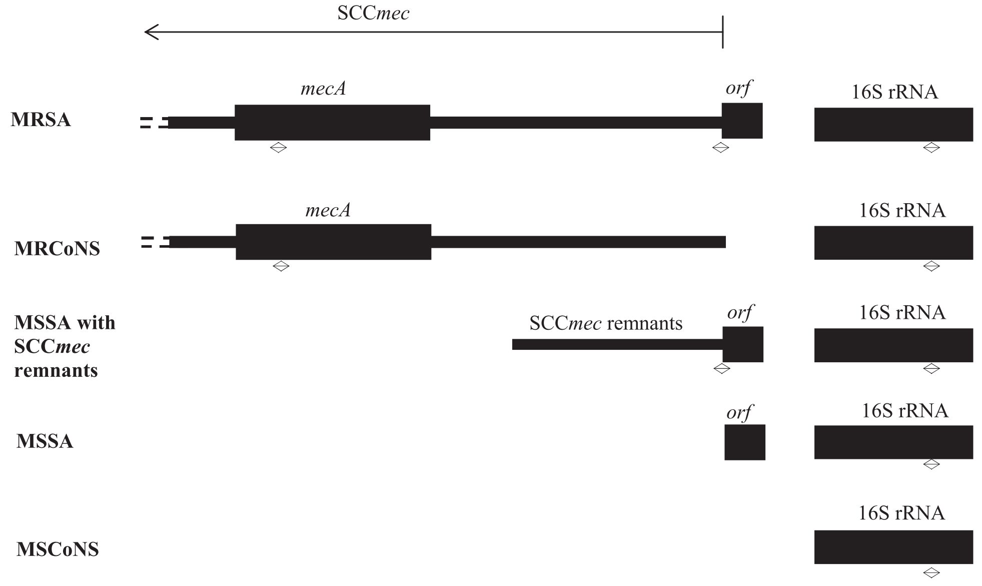

FIG 1 Schematic diagram showing the relevant genetic elements detected by the multiplex real-time PCR assay in MRSA, MRCoNS, MSSA with SCC*mec* remnants, MSSA, and MSCoNS. The two-way arrows indicate the amplified regions.

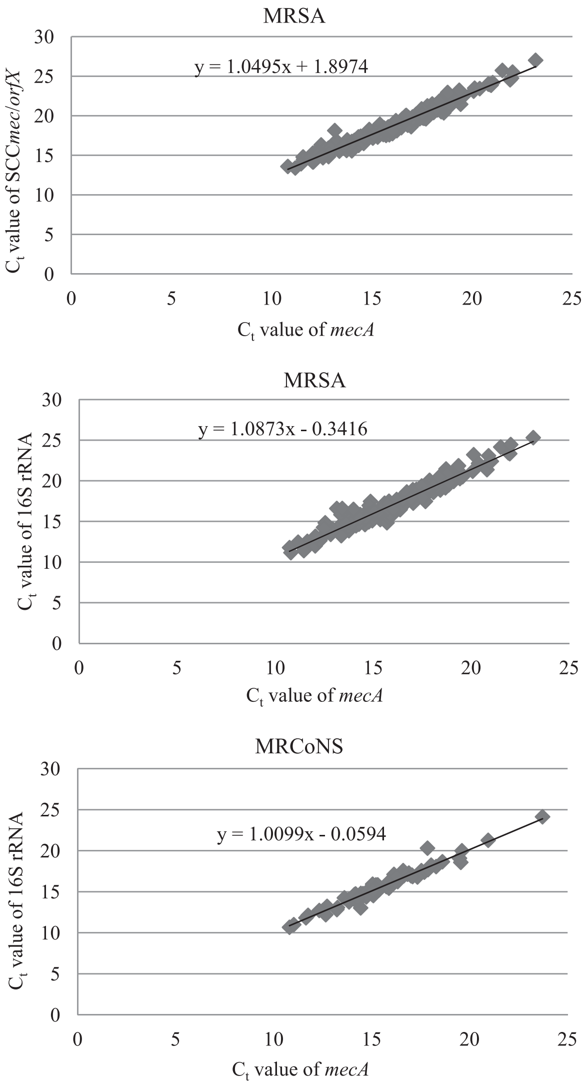

FIG 2 Correlation between C_T values of *mecA*, SCC*mec-orfX*, and 16S rRNA genes in 209 MRSA and 74 MRCoNS isolates.

MSSA, and 11 MSCoNS reference strains and 29 MSSA control strains carrying SCC*mec* remnants. Only the expected PCR products were amplified from each reference strain. However, SCC*mec-orfX* was not detected in 6 of 29 control strains. The results of the evaluation of 392 clinical isolates were as follows. Three targets were simultaneously detected in all 209 (100%) MRSA isolates and in 4 (5.4%) MRCoNS isolates. Of the 109 MSSA isolates, the *mecA* and 16S rRNA genes were detected at the same time in 2 (1.8%) isolates, and both the SCC*mec-orfX* and 16S rRNA genes were detected in 11 (10.1%) isolates. The C_T values of *mecA* were compared to the C_T values of the SCC*mec-orfX* and 16S rRNA genes. The correlation coefficient determined between *mecA* C_T values and SCC*mec-orfX* C_T values was high for MRSA isolates ($r = 0.959$; $P < 0.0001$), and correlation coefficients determined between *mecA* C_T values and 16S rRNA C_T values were high for MRSA isolates ($r = 0.970$; $P < 0.0001$) and MRCoNS isolates ($r = 0.963$; $P < 0.0001$). The results are shown in Fig. 1 and 2 and Table 2. Thus, the C_T differences between the SCC*mec-orfX* and *mecA* genes (C_{TSCC}) and between the 16S rRNA and *mecA* genes (C_{T16S})

TABLE 2 MRSA detection results for clinical isolates by multiplex real-time PCR[a]

		mecA		SCC*mec-orfX*		16S rRNA			
Species	No. of isolates	No. of isolates showing positive result	Threshold cycle (C_T) value	No. of isolates showing positive result	Threshold cycle (C_T) value	No. of isolates showing positive result	Threshold cycle (C_T) value	C_T difference between SCC*mec-orfX* and *mecA* (C_{TSCC})	C_T difference between 16S rRNA and *mecA* (C_{T16S})
MRSA	209	209	16.71 ± 2.44	209	19.02 ± 2.59	209	17.35 ± 2.73	2.70 ± 0.50 (1.58 ~ 4.99)	1.08 ± 0.70 (−0.86 ~ 3.45)
MSSA	109	2	35.40 ± 1.36	11	29.32 ± 2.45	109	17.95 ± 2.87		−18.58 ± 1.28 (−19.48 ~ −17.67)
MRCoNS	74	74	15.48 ± 2.36	4	34.98 ± 2.35	74	15.61 ± 2.41	17.78 ± 1.84 (15.37 ~ 19.69)	0.10 ± 0.51 (−1.41 ~ 2.46)

[a] The *mecA*, SCC*mec-orfX*, and 16S rRNA threshold cycle values represent the means ± standard deviations. The C_{TSCC} and C_{T16S} threshold cycle values represent ranges of means ± standard deviations.

were used to assess the presence of MRSA. A $C_{TSCC} \geq 4.7$ (mean + 4 standard deviations [SD]) indicated that MRSA and staphylococci other than MRSA were present simultaneously, whereas a $C_{T16S} \leq -1.72$ (mean − 4 SD) indicated that MRSA and staphylococci lacking the *mecA* gene coexisted.

The detection limit of the assay was determined using genomic DNA purified from a 1:10^4 dilution of a stock solution of MRSA strain CCARM 3792 and was found to be 20 CFU per PCR.

Real-time PCR assays, including the BD GeneOhm MRSA assay and Cepheid's Xpert MRSA assay that target the *orfX* of *S. aureus* and the right-extremity junction of SCC*mec*, are currently used for infection control (20–23). Previous studies reported false-negative and -positive results in these single-locus assays ranging from 0.0% to 7.3% and from 0.0% to 5.4%, respectively (4, 6, 24–26). False-negative and -positive results can affect the whole infection control program, bringing about the spread of MRSA in the hospital and unnecessary isolation and decolonization procedures. If a MRSA with an unknown SCC*mec* type is present in the samples, there could be false-negative results. Currently, 11 different types of SCC*mec* have been recognized in *S. aureus* (http://www.sccmec.org/); we designed 5 forward primers, 1 reverse primer, and 1 probe to detect all known types of SCC*mec*. SCC*mec* is a 21- to 67-kb genetic fragment that integrates into the chromosome of MRSA at the integration site sequence for SCC (ISS), which is located at the 3′ end of *orfX*, and carries the central determinant for broad-spectrum β-lactam resistance encoded by the *mecA* gene (27–29). It is unstable and able to be excised. Excision of the SCC*mec* can be complete or partial, with some elements left behind at the ISS. Since MRSA strains are resistant to multiple drugs, the excision of SCC*mec* from such isolates results in MSSA isolates retaining resistance to antibiotics other than ß-lactams (12–16, 30). In the study on determining the proportion and diversity of multidrug-resistant MSSA (MR-MSSA) strains derived from MRSA strains, Donnio et al. investigated 247 MR-MSSA isolates from 60 French hospitals using the IDI-MRSA real-time PCR assay, the forerunner of the BD GeneOhm MRSA assay, and found that 68% of isolates were positive (15). According to Shore's study, 7 MR-MSSA isolates harboring SCC*mec* remnants identified by SCC*mec* typing PCR were tested with the BD GeneOhm MRSA and Xpert MRSA assays, and 3 isolates yielded positive results in both assays (14). In the present study, 29 MSSA isolates tested as control strains were MR-MSSA and carried SCC*mec* remnants that had been confirmed by the multiplex PCR-based SCC*mec* typing. In 23 of 29 MR-MSSA control strains, the SCC*mec-orfX* junction was detected. Six control strains with negative results might contain SCC*mec* remnants that lacked the target-specific region of 5 forward primers. The possibility of the presence of SCC*mec* remnants in MR-MSSA should always be considered when a real-time PCR assay targeting the SCC*mec-orfX* junction is used for the rapid detection of MRSA from clinical specimens, since this might give a high number of false-positive results.

To our knowledge, the incidence of false positives has not been reported in South Korea for single-locus real-time PCR assays. We expected the false-positive results to be higher than in countries of low MRSA infection endemicity; as predicted, they were as high as 10.1% in the clinical isolates of staphylococci that had been consecutively collected in our laboratory over 3 months. If such a high rate of false-positive results occurs, the diagnostic value of single-locus real-time PCR assays seems unsatisfactory for a laboratory in a region of high MRSA infection endemicity. Therefore, we considered simultaneous amplification of the *mecA* gene and SCC*mec-orfX* junction to rule out MSSA isolates that carry SCC*mec* remnants and lack the *mecA* gene. However, this could also lead to a false-positive result when MRCoNS and MSSA carrying SCC*mec* remnants coexist in the clinical samples. Thus, the staphylococcal 16S rRNA gene was added to the targets to lessen false-positive results. In the case of a pure MRSA strain, relative quantifications of the three target genes would be constant, whereas in cases of mixed populations of MRCoNS and MSSA carrying SCC*mec* remnants, they would be mostly variable.

Three primer-probe pairs targeting the 16S rRNA gene were designed. Of those, the pair having a C_T value very close to the C_T of *mecA* was chosen. In most of the MRSA isolates, the C_T of SCC*mec* was the largest, followed by those of the 16S rRNA and *mecA* genes. The *mecA* gene was chosen as a reference gene for relative quantifications. Consequently, the C_T differences between the SCC*mec-orfX* and *mecA* genes (C_{Tscc}) and between the 16S rRNA and *mecA* genes (C_{T16S}) were constant. We tested whether mixed populations of MRCoNS and MSSA carrying SCC*mec* remnants can be distinguished by relative quantifications. Mixed cocktails of staphylococcal genomic DNA samples were made to amplify the three targets, including mixtures of genomic DNA from MRSA and staphylococci other than MRSA and mixtures of MRCoNS and MSSA carrying SCC*mec* remnants. Then, the simulated samples were analyzed. The results of analysis for mixtures of genomic DNA from MRCoNS and MSSA carrying SCC*mec* remnants are shown in Table 3. The data showed that MRCoNS and MSSA carrying SCC*mec* remnants were simultaneously present only with $C_{TSCC} \geq 4.7$ and $C_{T16S} \leq -1.72$, and mixed populations of MRSA and MRCoNS could not be differentiated from those of MRCoNS and MSSA carrying SCC*mec* remnants with $C_{TSCC} \geq 4.7$ and $C_{T16S} \geq -1.71$. The data from other mixed DNA samples were in accord with proposed C_T calculations; a $C_{TSCC} \geq 4.7$ indicated that MRSA and staphylococci other than MRSA were present simultaneously, whereas a $C_{T16S} \leq -1.72$ indicated that MRSA and staphylococci lacking the *mecA* gene coexisted.

In this study, unexpected amplimers were obtained in 4 MRCoNS isolates and 2 MSSA isolates. A total of 4 MRCoNS isolates yielded simultaneous amplification of the three targets, and the C_{TSCC} values were 19.69, 17.50, 18.55, and 15.37; C_{T16S} values were 0.61, −0.97, −0.31, and −0.09, respectively. In 2 MSSA isolates, the *mecA* was amplified and the C_{T16S} values were −17.67 and −19.48, respectively. In order to know whether or not these 6 isolates were unusual genotypic strains, stored isolates were regrown on blood agar plates for 24 h. Template DNA was extracted from a single colony. Only expected products were amplified from the reprepared samples; both the *mecA* and 16S rRNA genes were detected in 4 MRCoNS isolates and the 16S rRNA gene in 2 MSSA isolates. The results showed that the unexpected amplimers were due to the mixed staphylococci. Consequently, 4 MRCoNS isolates showing amplification of all of the three targets would be mixtures of MRSA and MRCoNS or of MRCoNS and MSSA carrying SCC*mec* remnants. In South Korea, it is assumed that MSSA strains carrying SCC*mec* remnants comprise approximate 3% of the *S. aureus* isolates recovered from clinical specimens because about 30% of *S. aureus* isolates are MSSA and 10% of MSSA carry SCC*mec* remnants. Becker's study reported that nasal cocolonization by MSSA and MRCoNS was observed in 3.4% of patients (8). Furthermore, it was known from analyzing

TABLE 3 Results of the multiplex real-time PCR assay for mixtures of genomic DNA from MRCoNS and MSSA carrying SCC*mec* remnants

DNA sample[a]	Proportion		C_T difference between SCC*mec-orfX* and *mecA* (C_{TSCC})[b]	C_T difference between 16S rRNA and *mecA* (C_{T16S})[b]
	MRCoNS (%)	MSSA (%)		
A	95	5	15.99 ± 1.48 (13.03 ~ 16.94)	0.87 ± 0.50 (0.20 ~ 1.34)
B	90	10	14.60 ± 1.52 (11.75 ~ 16.15)	0.74 ± 0.17 (0.51 ~ 0.92)
C	80	20	13.15 ± 1.74 (10.33 ~ 15.34)	0.90 ± 0.44 (0.36 ~ 1.38)
D	70	30	12.32 ± 1.96 (9.36 ~ 15.00)	0.59 ± 0.23 (0.19 ~ 0.85)
E	60	40	11.54 ± 1.79 (8.79 ~ 14.29)	0.45 ± 0.51 (−0.13 ~ 1.10)
F	50	50	12.25 ± 4.42 (8.34 ~ 20.75)	0.46 ± 0.62 (−0.65 ~ 1.01)
G	40	60	10.41 ± 2.41 (7.38 ~ 14.59)	0.27 ± 0.22 (−0.01 ~ 0.57)
H	30	70	9.25 ± 2.23 (6.46 ~ 12.43)	−0.19 ± 0.40 (−0.72 ~ 0.22)
I	20	80	7.81 ± 2.00 (4.94 ~ 9.71)	−0.56 ± 0.91 (−1.85 ~ 0.56)
J	10	90	6.91 ± 1.64 (4.85 ~ 8.59)	−1.55 ± 1.00 (−2.81 ~ −0.47)
K	5	95	5.66 ± 1.34 (3.84 ~ 7.01)	−2.06 ± 0.69 (−2.99 ~ −0.95)

[a] Six samples each of 11 types of DNA were used.

[b] The threshold cycle values represent ranges of means ± standard deviations.

simulated samples that MRCoNS must comprise more than 90% of a mixed population to have a C_{TSCC} value over 15. Accordingly, it is reasonable to assume that there is very little chance of amplifying all three of the targets from mixed populations of MRCoNS and MSSA carrying SCC*mec* remnants. In the case of 2 MSSA isolates showing amplification of the *mecA*, it would seem that they contained mixed populations of MRCoNS and MSSA.

In summary, as a preliminary study for the introduction of a direct MRSA molecular detection system to our laboratory in a region with high MRSA infection endemicity, a multiplex real-time PCR assay that simultaneously detects the *mecA*, SCC*mec-orfX* junction, and staphylococcal 16S rRNA genes was developed and evaluated using 444 staphylococcal strains. The key issue was whether this assay can reduce false positives caused by MSSA carrying SCC*mec* remnants. The evaluation data showed that this assay resulted in fewer false-positive results than a single-locus real-time PCR assay that amplified the SCC*mec-orfX* junction. This assay would be useful in a clinical laboratory in a region with high MRSA infection endemicity, although further evaluation with clinical specimens is necessary before it can be applied in the laboratory.

REFERENCES

1. **Bootsma MC, Diekmann O, Bonten MJ.** 2006. Controlling methicillin-resistant *Staphylococcus aureus*: quantifying the effects of interventions and rapid diagnostic testing. Proc. Natl. Acad. Sci. U. S. A. **103**:5620–5625.
2. **Harbarth S, Masuet-Aumatell C, Schrenzel J, Francois P, Akakpo C, Renzi G, Pugin J, Ricou B, Pittet D.** 2006. Evaluation of rapid screening and pre-emptive contact isolation for detecting and controlling methicillin-resistant *Staphylococcus aureus* in critical care: an interventional cohort study. Crit. Care **10**:R25. doi:10.1186/cc3982.
3. **Chowers MY, Paitan Y, Gottesman BS, Gerber B, Ben-Nissan Y, Shitrit P.** 2009. Hospital-wide methicillin-resistant Staphylococcus aureus control program: a 5-year follow-up. Infect. Control Hosp. Epidemiol. **30**: 778–781.
4. **Huletsky A, Giroux R, Rossbach V, Gagnon M, Vaillancourt M, Bernier M, Gagnon F, Truchon K, Bastien M, Picard FJ, van Belkum A, Ouellette M, Roy PH, Bergeron MG.** 2004. New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. J. Clin. Microbiol. **42**:1875–1884.
5. **Peterson LR, Liesenfeld O, Woods CW, Allen SD, Pombo D, Patel PA, Mehta MS, Nicholson B, Fuller D, Onderdonk A.** 2010. Multicenter evaluation of the LightCycler methicillin-resistant *Staphylococcus aureus* (MRSA) advanced test as a rapid method for detection of MRSA in nasal surveillance swabs. J. Clin. Microbiol. **48**:1661–1666.
6. **Rossney AS, Herra CM, Brennan GI, Morgan PM, O'Connell B.** 2008. Evaluation of the Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) assay using the GeneXpert real-time PCR platform for rapid detection of MRSA from screening specimens. J. Clin. Microbiol. **46**:3285–3290.
7. **Rossney AS, Herra CM, Fitzgibbon MM, Morgan PM, Lawrence MJ, O'Connell B.** 2007. Evaluation of the IDI-MRSA assay on the SmartCycler real-time PCR platform for rapid detection of MRSA from screening specimens. Eur. J. Clin. Microbiol. Infect. Dis. **26**:459–466.
8. **Becker K, Pagnier I, Schuhen B, Wenzelburger F, Friedrich AW, Kipp F, Peters G, von Eiff C.** 2006. Does nasal cocolonization by methicillin-resistant coagulase-negative staphylococci and methicillin-susceptible *Staphylococcus aureus* strains occur frequently enough to represent a risk of false-positive methicillin-resistant *S. aureus* determinations by molecular methods? J. Clin. Microbiol. **44**:229–231.
9. **Laurent C, Bogaerts P, Schoevaerdts D, Denis O, Deplano A, Swine C, Struelens MJ, Glupczynski Y.** 2010. Evaluation of the Xpert MRSA assay for rapid detection of methicillin-resistant *Staphylococcus aureus* from nares swabs of geriatric hospitalized patients and failure to detect a specific SCC*mec* type IV variant. Eur. J. Clin. Microbiol. Infect. Dis. **29**:995–1002.
10. **Oberdorfer K, Pohl S, Frey M, Heeg K, Wendt C.** 2006. Evaluation of a single-locus real-time polymerase chain reaction as a screening test for specific detection of methicillin-resistant *Staphylococcus aureus* in ICU patients. Eur. J. Clin. Microbiol. Infect. Dis. **25**:657–663.
11. **Stamper PD, Louie L, Wong H, Simor AE, Farley JE, Carroll KC.** 2011. Genotypic and phenotypic characterization of methicillin-susceptible *Staphylococcus aureus* isolates misidentified as methicillin-resistant *Staphylococcus aureus* by the BD GeneOhm MRSA assay. J. Clin. Microbiol. **49**:1240–1244.
12. **Wong H, Louie L, Lo RY, Simor AE.** 2010. Characterization of *Staphylococcus aureus* isolates with a partial or complete absence of staphylococcal cassette chromosome elements. J. Clin. Microbiol. **48**:3525–3531.
13. **Donnio PY, Oliveira DC, Faria NA, Wilhelm N, Le Coustumier A, de Lencastre H.** 2005. Partial excision of the chromosomal cassette containing the methicillin resistance determinant results in methicillin-susceptible *Staphylococcus aureus*. J. Clin. Microbiol. **43**:4191–4193.
14. **Shore AC, Rossney AS, O'Connell B, Herra CM, Sullivan DJ, Humphreys H, Coleman DC.** 2008. Detection of staphylococcal cassette chromosome mec-associated DNA segments in multiresistant methicillin-susceptible *Staphylococcus aureus* (MSSA) and identification of *Staphylococcus epidermidis* ccrAB4 in both methicillin-resistant *S. aureus* and MSSA. Antimicrob. Agents Chemother. **52**:4407–4419.
15. **Donnio PY, Fevrier F, Bifani P, Dehem M, Kervegant C, Wilhelm N, Gautier-Lerestif AL, Lafforgue N, Cormier M, Le Coustumier A.** 2007. Molecular and epidemiological evidence for spread of multiresistant methicillin-susceptible *Staphylococcus aureus* strains in hospitals. Antimicrob. Agents Chemother. **51**:4342–4350.
16. **Lindqvist M, Isaksson B, Grub C, Jonassen TO, Hallgren A.** 2012. Detection and characterisation of SCC*mec* remnants in multiresistant methicillin-susceptible *Staphylococcus aureus* causing a clonal outbreak in a Swedish county. Eur. J. Clin. Microbiol. Infect. Dis. **31**:141–147.

17. **Korea Centers for Disease Control and Prevention.** 2010. 2010 laboratory surveillance for vancomycin resistant staphylococcus aureus. VRSA Newsl. **10**:3.
18. **Chen L, Mediavilla JR, Oliveira DC, Willey BM, de Lencastre H, Kreiswirth BN.** 2009. Multiplex real-time PCR for rapid Staphylococcal cassette chromosome mec typing. J. Clin. Microbiol. **47**:3692–3706.
19. **Milheiriço C, Oliveira DC, de Lencastre H.** 2007. Update to the multiplex PCR strategy for assignment of mec element types in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **51**:3374–3377.
20. **Ho TH, Huang YC, Lin TY.** 2011. Evaluation of the BD GeneOhm StaphSR assay for detection of *Staphylococcus aureus* in patients in intensive care units. J. Microbiol. Immunol. Infect. **44**:310–315.
21. **Keshtgar MR, Khalili A, Coen PG, Carder C, Macrae B, Jeanes A, Folan P, Baker D, Wren M, Wilson AP.** 2008. Impact of rapid molecular screening for meticillin-resistant *Staphylococcus aureus* in surgical wards. Br. J. Surg. **95**:381–386.
22. **Kluytmans J.** 2007. Control of meticillin-resistant *Staphylococcus aureus* (MRSA) and the value of rapid tests. J. Hosp. Infect. **65**(Suppl 2):100–104.
23. **Schulz M, Nonnenmacher C, Mutters R.** 2009. Cost-effectiveness of rapid MRSA screening in surgical patients. Eur. J. Clin. Microbiol. Infect. Dis. **28**:1291–1296.
24. **Kolman S, Arielly H, Paitan Y.** 2010. Evaluation of single and double-locus real-time PCR assays for methicillin-resistant *Staphylococcus aureus* (MRSA) surveillance. BMC Res. Notes **3**:110. doi:10.1186/1756-0500-3-110.
25. **Ornskov D, Kolmos B, Bendix Horn P, Nederby Nielsen J, Brandslund I, Schouenborg P.** 2008. Screening for methicillin-resistant *Staphylococcus aureus* in clinical swabs using a high-throughput real-time PCR-based method. Clin. Microbiol. Infect. **14**:22–28.
26. **Söderquist B, Neander M, Dienus O, Zimmermann J, Berglund C, Matussek A, Molling P.** 2012. Real-time multiplex PCR for direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical samples enriched by broth culture. APMIS **120**:427–432.
27. **Hiramatsu K, Cui L, Kuroda M, Ito T.** 2001. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol. **9**:486–493.
28. **Hiramatsu K, Katayama Y, Yuzawa H, Ito T.** 2002. Molecular genetics of methicillin-resistant *Staphylococcus aureus*. Int. J. Med. Microbiol. **292**: 67–74.
29. **International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC).** 2009. Classification of staphylococcal cassette chromosome mec (SCC*mec*): guidelines for reporting novel SCCmec elements. Antimicrob. Agents Chemother. **53**: 4961–4967.
30. **Donnio PY, Louvet L, Preney L, Nicolas D, Avril JL, Desbordes L.** 2002. Nine-year surveillance of methicillin-resistant *Staphylococcus aureus* in a hospital suggests instability of *mecA* DNA region in an epidemic strain. J. Clin. Microbiol. **40**:1048–1052.