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New Real-Time PCR Assay for Rapid Detection of Methicillin-Resistant *Staphylococcus aureus* Directly from Specimens Containing a Mixture of Staphylococci

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Molecular methods for the rapid identification of methicillin-resistant *Staphylococcus aureus* (MRSA) are generally based on the detection of an *S. aureus*-specific gene target and the *mecA* gene. However, such methods cannot be applied for the direct detection of MRSA from nonsterile specimens such as nasal samples without the previous isolation, capture, or enrichment of MRSA because these samples often contain both coagulase-negative staphylococci (CoNS) and *S. aureus*, either of which can carry *mecA*. In this study, we describe a real-time multiplex PCR assay which allows the detection of MRSA directly from clinical specimens containing a mixture of staphylococci in <1 h. Five primers specific to the different staphylococcal cassette chromosome *mec* (SCC*mec*) right extremity sequences, including three new sequences, were used in combination with a primer and three molecular beacon probes specific to the *S. aureus* chromosomal *orfX* gene sequences located to the right of the SCC*mec* integration site. Of the 1,657 MRSA isolates tested, 1,636 (98.7%) were detected with the PCR assay, whereas 26 of 569 (4.6%) methicillin-susceptible *S. aureus* (MSSA) strains were misidentified as MRSA. None of the 62 nonstaphylococcal bacterial species or the 212 methicillin-resistant or 74 methicillin-susceptible CoNS strains (MRCoNS and MSCoNS, respectively) were detected by the assay. The amplification of MRSA was not inhibited in the presence of high copy numbers of MSSA, MRCoNS, or MSCoNS. The analytical sensitivity of the PCR assay, as evaluated with MRSA-negative nasal specimens containing a mixture of MSSA, MRCoNS, and MSCoNS spiked with MRSA, was ~25 CFU per nasal sample. This real-time PCR assay represents a rapid and powerful method which can be used for the detection of MRSA directly from specimens containing a mixture of staphylococci.

Staphylococcus aureus is a major pathogen that causes a wide spectrum of clinical manifestations, such as wound infections, pneumonia, septicemia, and endocarditis. Beta-lactam antimicrobial agents are the preferred drugs for serious *S. aureus* infections. However, since the introduction of methicillin into clinical use, methicillin-resistant *S. aureus* (MRSA) strains have emerged worldwide as important nosocomial pathogens, and the prevalence of these strains in the community is now increasing substantially (6, 10, 18).

Methicillin resistance in *S. aureus* is caused by the acquisition of an exogenous gene, *mecA*, that encodes an additional β-lactam-resistant penicillin-binding protein (PBP), termed PBP 2a (or PBP2′) (15). The *mecA* gene is carried by a mobile genetic element, designated staphylococcal cassette chromosome *mec* (SCC*mec*), inserted near the chromosomal origin of replication (32). SCC*mec* is characterized by the presence of terminal inverted and direct repeats, a set of site-specific recombinase genes (*ccrA* and *ccrB*), and the *mec* gene complex (16). The SCC*mec* DNAs are integrated at a specific site (*attBscc*) in the methicillin-susceptible *S. aureus* (MSSA) chromosome which is located at the 3′ end of an open reading frame (ORF), *orfX*, of unknown function (16).

The rapid and accurate identification of MRSA in clinical specimens has important implications for the therapy and management of both colonized and infected patients. Numerous molecular approaches that reduce the time for identification of MRSA have been described (3, 12, 13, 26, 30, 35, 42, 46, 48, 49). However, the molecular tests developed to date for the detection of MRSA are based on the detection of an *S. aureus*-specific gene and/or *mecA*. Thus, they cannot be applied for the direct detection of MRSA from nonsterile specimens such as nasal samples without the previous isolation, capture, or enrichment of MRSA because these samples often contain both coagulase-negative staphylococci (CoNS) and *S. aureus*, either of which can carry *mecA* (1).

In this study, we describe a real-time multiplex PCR assay that is useful for the detection of MRSA directly from specimens containing a mixture of staphylococci. This assay comprises five primers specific to the various SCC*mec* right extremity (SRE) sequences, including three new sequences, in combination with a primer and three molecular beacon probes (MBPs) specific to the *S. aureus* chromosomal *orfX* gene located to the right of the SCC*mec* integration site. We have

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validated this real-time PCR assay by using a variety of gram-negative and gram-positive bacterial species as well as strains of MSSA, MRSA, methicillin-susceptible CoNS (MSCoNS), and methicillin-resistant CoNS (MRCoNS) from various countries. The assay was also used to detect MRSA directly from nasal specimens.

MATERIALS AND METHODS

Bacterial strains. The reference strains used for this study are listed in Tables 1 and 2. The staphylococcal clinical isolates used for this study are part of the SENTRY program collection and several supplier's collections. The *S. aureus* clinical isolates originated from Africa ($n = 15$) as well as the following 29 countries: Albania ($n = 2$), Argentina ($n = 50$), Australia ($n = 71$), Austria ($n = 2$), Belgium ($n = 3$), Brazil ($n = 78$), Canada ($n = 601$), Chile ($n = 42$), China ($n = 70$), Denmark ($n = 33$), Egypt ($n = 1$), France ($n = 36$), Germany ($n = 27$), Greece ($n = 4$), Ireland ($n = 5$), Israel ($n = 19$), Italy ($n = 61$), Japan ($n = 62$), Mexico ($n = 1$), The Netherlands ($n = 179$), Poland ($n = 33$), Portugal ($n = 76$), Singapore ($n = 20$), Slovenia ($n = 1$), Spain ($n = 30$), Switzerland ($n = 13$), Turkey ($n = 28$), United Kingdom ($n = 10$), and United States ($n = 535$). The CoNS clinical isolates originated from the following eight countries: Argentina ($n = 13$), Canada ($n = 95$), China ($n = 7$), Denmark ($n = 21$), France ($n = 4$), Japan ($n = 28$), United Kingdom ($n = 44$), and United States ($n = 37$). Confirmation of the identification of the staphylococcal strains was done by use of the MicroScan WalkAway Panel Type Positive Breakpoint Combo 13 system when required (Dade Behring Canada Inc., Mississauga, Ontario, Canada).

MIC determination. The MICs for oxacillin were either provided by the strain suppliers or determined in our laboratory by the Etest method (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. The results were interpreted according to the standards of the NCCLS (37). *S. aureus* strains ATCC 29213 and ATCC 43300 were used as quality controls for antimicrobial susceptibility testing.

DNA sequencing. The primers used to amplify and sequence the SRE junctions (MREJs) of various MRSA strains are listed in Table 3. The MREJ comprises the right extremity of SCC*mec*, the SCC*mec* integration site, and the *orfX* gene.

The MREJ fragments to be sequenced were amplified on a PTC-200 thermocycler (MJ Research Inc., Watertown, Mass.), using purified genomic DNA. Genomic DNAs were purified by use of a Gnome kit (Qbiogene Inc., Carlsbad, Calif.) according to the manufacturer's instructions. Depending on the MRSA strain, amplification products of 1.2 or >8 kb were obtained. Amplification products of 1.2 kb were amplified as previously described (29), whereas those of >8 kb were amplified with Herculase Enhanced DNA polymerase (Stratagene, La Jolla, Calif.). Purification of the amplification products and sequencing reactions were performed as previously described (29).

Primers and probes. A multiple nucleotide sequence alignment of the MREJ sequences of several staphylococcal species obtained for this study or available from public databases was performed with the Pileup program from the GCG package (version 10; Accelrys, San Diego, Calif.). This alignment allowed the design of a set of PCR primers specific to the various SRE sequences (mecii574, meciii519, meciv511, mecv492, and mecvii512) as well as a primer specific to *S. aureus orfX* (Xsau325). This set of primers was used in combination with three MBPs (XsauB5-FAM, XsauB8-FAM, and XsauB9-FAM) targeting *orfX* sequences within the MREJ amplification products, allowing the detection of all *S. aureus orfX* variants. A fourth MBP (PSARM-TET) was used to detect pSARM internal control amplification products. MBPs were designed as previously described (4). Primers were designed with the help of Oligo Primer Analysis software, version 6.22 (Molecular Biology Insights, Cascade, Colo.). Primers were synthesized with a DNA synthesizer (model 391; PE Applied Biosystems, Foster City, Calif.) and MBPs were obtained from Biosearch Technologies (Novato, Calif.). The PCR primers and probes used for this study are listed in Table 3 and are shown in Fig. 1.

Construction of internal control. An internal control was constructed as described previously (28). A 324-bp DNA fragment consisting of a 276-bp sequence not found in MRSA flanked by the sequences of the meciv511 and mecv492 primers (Table 3) was used as a template for the internal control. This fragment was cloned into the pCR2.1 vector (Invitrogen, Burlington, Ontario, Canada) and isolated as previously described (28). The purified recombinant plasmid, named pSARM, was linearized with BamHI (New England Biolabs, Mississauga, Ontario, Canada) and serially diluted. The concentration of the linearized plasmid was optimized to permit the amplification of the 324-bp internal control product without a significant detrimental effect on MRSA-specific amplification.

PCR assay. For all bacterial strains, amplification was performed with either purified genomic DNAs or crude DNA extracts prepared from bacterial suspensions whose turbidity was adjusted to that of a 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 bacteria per ml. Crude DNA extracts were prepared for PCRs by a rapid DNA extraction method (Infectio Diagnostic Inc., Ste-Foy, Canada) (28). One microliter of purified genomic DNA or of a crude DNA extract was transferred directly to a 24-μl PCR mixture containing oligonucleotide primers and probes (Table 3), four deoxyribonucleoside triphosphates (Pharmacia Biotech, Baie d'Urfé, Québec, Canada), a 10 mM Tris-HCl buffer (pH 9.0), 4.75 mM $MgCl_2$, 2.5 mg of bovine serum albumin per ml, and 1.25 U of *Taq* DNA polymerase (Roche, Mississauga, Ontario, Canada) combined with a TaqStart antibody (Clontech Laboratories, Palo Alto, Calif.). The internal control (pSARM) was included in the PCR assay to ensure that there was no significant PCR inhibition by the test sample. The thermal cycling protocol was as follows: 3 min at 95°C for initial denaturation followed by 48 cycles of three steps consisting of 5 s at 95°C for denaturation, 15 s at 60°C for annealing, and 20 s at 72°C for extension. Real-time detection of the PCR products was performed on a Smart Cycler (Cepheid, Sunnyvale, Calif.) by measuring the fluorescence signal emitted by the MBPs hybridized to their targets at the end of each annealing step. The specificity and ubiquity (i.e., the ability to detect all or most MRSA strains) (5) of the PCR assay were verified by use of a panel of 22 gram-negative bacterial species, 40 gram-positive non-staphylococcal bacterial species, 14 species (212 strains) of MRCoNS, 27 species (74 strains) of MSCoNS, 569 MSSA isolates, and 1,657 MRSA strains from various geographic areas. For determination of the analytical sensitivity of the PCR assay, 1-μl samples of serial twofold dilutions of purified genomic DNA (ranging from 64 to 1 genome copy per μl) from MRSA strains of different MREJ types were used to determine the minimal number of genomes which were detected. To verify that MRSA amplification was not inhibited in the presence of DNAs from MSSA, MRCoNS, and MSCoNS, we performed the following experiment. The equivalent of 10 genome copies of DNA from MRSA strain ATCC 43300 (carrying MREJ type ii) per PCR was amplified by the real-time PCR assay in the presence of 0, 10, 10^2, 10^3, and 10^4 genome copies of DNA (per PCR) from either (i) MSSA strain ATCC 29213 (MREJ negative), (ii) methicillin-resistant *Staphylococcus epidermidis* strain ATCC 35983 (carrying MREJ type ii), or (iii) methicillin-susceptible *S. epidermidis* strain ATCC 14990 (MREJ negative).

MREJ and SCC*mec* typing. The MREJ types of the MRSA strains described in this study were determined by sequence analysis or by examining the PCR amplification products generated by the multiplex PCR assay described above by standard agarose gel electrophoresis (44). The SCC*mec* types and subtypes of the MRSA strains described in this study were identified by using a previously described typing method (38). Primers mecIVc70 (5′-TGGGGTATTTTTATC TTCAACTC-3′) and mecIVc1079 (5′-TGGGATTTTAAAGCAGAATATCA-3′) were designed to identify SCC*mec* type IVc based on the SCC*mec* sequence of MRSA strain MR108 (25). Primers mecIVd26 (5′-ACGGGAGATTAGGA GATGTTAT-3′) and mecIVd307 (5′-CAGCCATCAATTTTGTTTCACC-3′) were designed to identify SCC*mec* type IVd based on the SCC*mec* sequence of MRSA strain JCSC 4469 (GenBank accession number AB097677).

Evaluation of the sensitivity of the PCR assay by using nasal samples. The minimal number of CFU that can be detected in nasal specimens was evaluated by using 18 nasal swabs obtained from nine volunteers (2 nasal swabs/volunteer) who were not colonized by MRSA. Nasal specimens were collected with a collection and transport system for aerobes (Venturi Transystem; Copan Canada, Richmond Hill, Ontario, Canada). A swab was carefully inserted a short distance into each nostril and gently rotated for 5 s. Swabs were inserted into the transport medium immediately after samples were obtained. Each swab was streaked directly on mannitol salt agar to determine the presence of MSSA, MRSA, MRCoNS, or MSCoNS. The agar medium was examined after 1 and 2 days of incubation for typical staphylococcal colonies. The identification of suspicious staphylococcal colonies was based on catalase production, slide agglutination (Staphaurex; Murex Biotech Limited), and the tube coagulase test. Identification of and oxacillin MIC determinations for the staphylococcal isolates were performed as described above. None of the specimens contained MRSA isolates, whereas 10 specimens contained MSCoNS isolates, 4 specimens contained MRCoNS isolates, and 4 specimens contained both MSSA and MSCoNS isolates. Nasal specimens from the 18 swabs were resuspended in buffer and pooled. The pooled MRSA-negative specimens, which contained a mixture of MSSA, MRCoNS, and MSCoNS, were divided into 18 aliquots. The samples were spiked with serial 10-fold dilutions of MRSA strain ATCC 43300 during the logarithmic phase of growth (optical density at 600 nm of ~0.6) in phosphate-buffered saline. Each 10-fold dilution was added directly to the clinical specimens and processed prior to PCR amplification by a rapid DNA extraction method (28). The number of CFU was estimated by standard plating procedures.

TABLE 1. Reference *S. aureus* strains used for this study[a]

Source or strain no.	Source or strain no.
HARMONY collection of European epidemic MRSA strains (type designation)[b]	1163/98 (S. German EMRSA II d1)
96158 (B)	1869/98 (N. German EMRSA I d)
97117 (A)	134/93 (N. German EMRSA I)
97118 (A)	1000/93 (Hannover area EMRSA III)
97120 (B)	1450/94 (N. German EMRSA I a)
97151 (B)	825/96 (Berlin epidemic EMRSA IV)
97392 (B)	842/96 (Berlin epidemic EMRSA IV a)
97393 (A)	2594-1/97 (S. German EMRSA II a)
BM10827 (C)	1155-2/98 (S. German EMRSA II)
162 (A)	1442/98 (Hannover area EMRSA III a)
920 (B)	234/95 (N. German EMRSA I b)
95035 (A)	37481 (Seinajoki E 14)
97121 (B)	54511 (Turku I E6)
BM10828 (C)	54518 (Turku II E7)
BM10882 (C)	96/32010 (EMRSA-16)
97S97 (Belgian epidemic clone 1a)	99/579 (EMRSA-16/a3)
98S46 (Belgian epidemic clone 3b)	5 (E1)
97S96 (Belgian epidemic clone 1a)	6 (D)
97S98 (Belgian epidemic clone 1b)	13 (A′)
97S99 (Belgian epidemic clone 2a)	14 (A′)
97S100 (Belgian epidemic clone 2b)	18 (A)
97S101 (Belgian epidemic clone 3a)	20 (E)
359/96 (Berlin epidemic EMRSA IVc)	25 (F^I)
792/96 (Berlin epidemic EMRSA IVd)	30 (G)
844/96 (Berlin epidemic EMRSA IVb)	33 (F)
1966/97 (Hannover area EMRSA IIIc)	54 (B)
2594-2/97 (S. German EMRSA IIb)	60 (A″)
131/98 (S. German EMRSA II d2)	80 (E)
406/98 (N. German EMRSA I c1)	98 (C)
408/98 (N. German EMRSA I c2)	Public collections (type designation)
61974 (Helsinki I E1)	ATCC 6538[c]
62176 (Kotka E10)	ATCC 13301[c]
62305 (mecA- Tampere I E12)	ATCC 23235[c]
62396 (Helsinki II E2)	ATCC 25923[c]
75541 (Tampere II E13)	ATCC 27660[c]
75916 (Helsinki V E5)	ATCC 29737[c]
76167 (Kemi E17)	ATCC 29213[c]
98442 (Helsinki VI E19)	ATCC 29247[c]
98514 (Helsinki VII E20)	ATCC 33591
98541 (Lohja E24)	ATCC 33592
3717 (EMRSA-GR1b)	ATCC 33593
3680 (EMRSA-GR1)	ATCC 43300
3713 (EMRSA-GR1a)	ATCC BAA-38 (Archaic clone of MRSA)[d]
HS 2 (I)	ATCC BAA-39 (Hungarian clone of MRSA)[d]
AO 17934/97 (II)	ATCC BAA-40 (Portuguese clone of MRSA)[d]
N8-890/99 (Sa 543 VI)	ATCC BAA-41 (New York clone of MRSA)[d]
N8-3756/90 (Sa544 I)	ATCC BAA-42 (Pediatric clone of MRSA)[d]
9805-01937 (V)	ATCC BAA-43 (Brazilian clone of MRSA)[d]
AK 541 (IV)	ATCC BAA-44 (Iberian clone of MRSA)[d]
ON 408/99 (VII)	CCUG 41787 (Sa 501 V)[b]
AO 9973/97 (III)	CCUG 38266 (II)[b]
98/10618 (EMRSA-15/b2)	NCTC 8325[c]
98/26821 (EMRSA-15/b3)	NCTC 11939 (EMRSA-1)[b]
98/24344 (EMRSA-15/b7)	
99/1139 (EMRSA-16/a2)	Canadian epidemic MRSA strains[e]
99/159 (EMRSA-16/a14)	CMRSA-1
M307 (EMRSA-3)	CMRSA-2
90/10685 (EMRSA-15)	CMRSA-3
98/14719 (EMRSA-15/b4)	CMRSA-4
872/98 (Hannover area EMRSA IIIb)	CMRSA-5
1155-1/98 (S. German EMRSA II c)	CMRSA-6

[a] All *S. aureus* strains are resistant to oxacillin except where otherwise indicated.
[b] Information on these strains and type designations based on pulsed-field gel electrophoresis are from http://www.phls.co.uk/inter/harmony/menu.htm.
[c] Susceptible to oxacillin (MSSA).
[d] Information on these strains and type designations based on pulsed-field gel electrophoresis are from reference 7.
[e] Information on these strains and type designations based on pulsed-field gel electrophoresis are from reference 47.

TABLE 2. Reference strains other than *S. aureus* used for this study

Bacterial group or strain	Bacterial group or strain
CoNS	*Enterococcus faecium* ATCC 19434
S. arlettae ATCC 43957 (MSCoNS)	*Enterococcus flavescens* ATCC 49996
S. auricularis ATCC 33753 (MSCoNS)	*Enterococcus gallinarum* ATCC 49573
S. capitis subsp. *capitis* ATCC 27840 (MSCoNS)	*Enterococcus hirae* ATCC 8043
S. capitis subsp. *ureolyticus* ATCC 49326 (MSCoNS)	*Enterococcus mundtii* ATCC 43186
S. caprae ATCC 35538 (MSCoNS)	*Enterococcus pseudoavium* ATCC 49372
S. carnosus subsp. *carnosus* ATCC 51365 (MSCoNS)	*Enterococcus raffinosus* ATCC 49427
S. chromogenes ATCC 43764 (MSCoNS)	*Enterococcus saccharolyticus* ATCC 43076
S. cohnii subsp. *cohnii* ATCC 29974 (MSCoNS)	*Enterococcus solitarius* ATCC 49428
S. delphini ATCC 49171 (MSCoNS)	*Gemella haemolysans* ATCC 10379
S. epidermidis ATCC 12228 (MSCoNS)	*Kocuria rhizophila* ATCC 9341
S. epidermidis ATCC 14990 (MSCoNS)	*Lactobacillus crispatus* ATCC 33820
S. epidermidis ATCC 35983 (MRCoNS)	*Leifsonia aquatica* ATCC 14665
S. epidermidis ATCC 35984 (MRCoNS)	*Listeria monocytogenes* ATCC 15313
S. equorum ATCC 43958 (MSCoNS)	*Streptococcus agalactiae* ATCC 13813
S. felis ATCC 49168 (MSCoNS)	*Streptococcus anginosus* ATCC 33397
S. gallinarum ATCC 35539 (MSCoNS)	*Streptococcus bovis* ATCC 33317
S. haemolyticus ATCC 29970 (MSCoNS)	*Streptococcus constellatus* subsp. *constellatus* ATCC 27823
S. hominis subsp. *hominis* ATCC 27844 (MSCoNS)	*Streptococcus cristatus* ATCC 51100
S. hominis subsp. *hominis* ATCC 35982 (MSCoNS)	*Streptococcus intermedius* ATCC 27335
S. hyicus ATCC 11249 (MSCoNS)	*Streptococcus mitis* ATCC 49456
S. intermedius ATCC 29663 (MSCoNS)	*Streptococcus mutans* ATCC 25175
S. kloosii ATCC 43959 (MSCoNS)	*Streptococcus parasanguinis* ATCC 15912
S. lentus ATCC 29070 (MSCoNS)	*Streptococcus pneumoniae* ATCC 6303
S. lugdunensis ATCC 43809 (MSCoNS)	*Streptococcus pyogenes* ATCC 19615
S. pasteuri ATCC 51129 (MSCoNS)	*Streptococcus salivarius* ATCC 7073
S. pulvereri ATCC 51698 (MSCoNS)	*Streptococcus sanguinis* ATCC 10556
S. saccharolyticus ATCC 14953 (MSCoNS)	*Streptococcus suis* ATCC 43765
S. saprophyticus ATCC 43867 (MSCoNS)	
S. saprophyticus ATCC 35552 (MSCoNS)	Gram-negative bacteria
S. saprophyticus ATCC 15305 (MSCoNS)	*Acinetobacter baumannii* ATCC 19606
S. schleiferi subsp. *coagulans* ATCC 49545 (MSCoNS)	*Bordetella pertussis* ATCC 9797
S. schleiferi subsp. *schleiferi* ATCC 43808 (MSCoNS)	*Burkholderia cepacia* ATCC 25416
S. sciuri subsp. *sciuri* ATCC 29060 (MRCoNS)	*Citrobacter freundii* ATCC 8090
S. sciuri subsp. *sciuri* ATCC 29062 (MSCoNS)	*Enterobacter cloacae* ATCC 13047
S. simulans ATCC 27848 (MSCoNS)	*Escherichia coli* ATCC 25922
S. warneri ATCC 27836 (MSCoNS)	*Haemophilus influenzae* ATCC 9007
S. xylosus ATCC 29971 (MRCoNS)	*Hafnia alvei* ATCC 13337
	Klebsiella pneumoniae ATCC 13883
Nonstaphylococcal gram-positive bacteria	*Moraxella catarrhalis* ATCC 43628
Abiotrophia defectiva ATCC 49176	*Neisseria gonorrhoeae* ATCC 35201
Bacillus cereus ATCC 13472	*Neisseria meningitidis* ATCC 13077
Corynebacterium bovis ATCC 7715	*Pasteurella aerogenes* ATCC 27883
Corynebacterium cervicis NCTC 10604	*Proteus mirabilis* ATCC 25933
Corynebacterium flavescens ATCC 10340	*Providencia stuartii* ATCC 33672
Corynebacterium genitalium ATCC 33031	*Pseudomonas aeruginosa* ATCC 35554
Enterococcus avium ATCC 14025	*Pseudomonas fluorescens* ATCC 13525
Enterococcus casseliflavus ATCC 25788	*Salmonella choleraesuis* subsp. *choleraesuis* ATCC 14028
Enterococcus cecorum ATCC 43198	*Serratia marcescens* ATCC 13880
Enterococcus dispar ATCC 51266	*Shigella sonnei* ATCC 29930
Enterococcus durans ATCC 19432	*Stenotrophomonas maltophilia* ATCC 13637
Enterococcus faecalis ATCC 19433	*Yersinia enterocolitica* ATCC 9610

Nucleotide sequence accession numbers. GenBank accession numbers for the *S. aureus* MREJ sequences are as follows: for strain ATCC 33592, AY267373; for strain ATCC BAA-40, AY267374; for strain CMRSA-1, AY267375; for strain CCRI-1263, AY267376; for strain CCRI-1311, AY267377; for strain CCRI-1331, AY267379; for strain CCRI-1377, AY267380; for strain CCRI-2025, AY267381; for strain CCRI-8895, AY267382; for strain CCRI-8903, AY267383; and for strain CCRI-9583, AY267384.

RESULTS

New SRE sequences of MRSA. A molecular method named *mec* right extremity polymorphism (MREP) typing, which takes advantage of the polymorphism among SRE sequences, was previously developed (17, 23). This method combines primers specific to each distinct SRE sequence with a primer specific to the *S. aureus* chromosome located to the right of the SCC*mec* integration site to detect the different SCC*mec* types encountered. MREP types i, ii, and iii were defined according to the respective SCC*mec* types I, II, and III (17, 23). By using this strategy, we have developed a new set of primers for the specific identification of MRSA by using the known SRE sequences of SCC*mec* types I, II, III, IVa, IVb, and IVc (2, 23–25, 32, 34). The first primer, mecii574, recognizes the right extremity sequences of SCC*mec* types I, II, IVa, IVb, and IVc. The right extremities of SCC*mec* types II, IVa, IVb, and IVc are identical but differ from the right extremity of SCC*mec*

TABLE 3. Oligonucleotides used for this study

Oligonucleotide	Oligonucleotide sequence (5′ → 3′)	Nucleotide position	Amplification product size (bp)
S. aureus orfX-specific primer			
Xsau325	GGATCAAACGGCCTGCACA	325[a]	
SCC*mec*-specific primers			
mecii574	GTCAAAAATCATGAACCTCATTACTTATG	472,[b] 574[c]	176[d] or 278[e]
meciii519	ATTTCATATATGTAATTCCTCCACATCTC	519[f]	223[g]
meciv511	CAAATATTATCTCGTAATTTACCTTGTTC	511[h]	215[i]
mecv492	CTCTGCTTTATATTATAAAATTACGGCTG	492[j]	196[k]
mecvii512	CACTTTTTATTCTTCAAAGATTTGAGC	512[l]	214[m]
S. aureus orfX-specific probes			
XsauB5-FAM[n]	CCCGCGCGTAGTTACTGCGTTGTAAGACGTCCGCGGG[o]	346[a]	
XsauB8-FAM[n]	CCCGCGCATAGTTACTGCGTTGTAAGACGTCCGCGGG[o]	346[p]	
XsauB9-FAM[n]	CCCGCGCGTAGTTACTACGTTGTAAGACGTCCGCGGG[o]	346[q]	
Internal control probe			
PSARM-TET[r]	CCGGCGATGCCTCTTCACATTGCTCCACCTTTCCTCGCCGG[o]	2,038[s]	
Sequencing primers			
IS431626	TCTACGGATTTTCGCCATGC	626[t]	1,200[u]
Xsau401	ATCAAATGATGCGGGTTGTGT	401[a]	
mecA1059	AACAGGTGAATTATTAGCACTTGTAAG	1,059[v]	>8,000[w]
SA0022Sau2673	GAGGACCAAACGACATGAAAATC	2,673[x]	
Internal sequencing primers			
meciv1013	CAATCGGTATCTGTAAATATCAAAT	1,013[h]	
meciv1411	TCGCATACCTGTTTATCTTCTACT	1,411[h]	
meciv916	TTGGTTCCATCTGAACTTTGAG	916[h]	
mecvii1126	ACTAGAATCTCCAAATGAATCCAGT	1,126[l]	
mecv1029	TTTAAATTCAGCTATATGGGGAGA	1,029[j]	
mecv968	TTCCGTTTTGCTATTCCATAAT	968[j]	
tetK1169	CCTCTGATAAAAAACTTGTGAAAT	1,169[y]	
tetK136	ACTACTCCTGGAATTACAAACTGG	136[y]	
Xsau193	GCCAAAATTAAACCACAATCCAC	193[a]	
Xsau367	CATTTTGCTGAATGATAGTGCGTA	367[a]	

[a] Nucleotide position in *orfX* (start codon = nt 1 to 3) from GenBank accession no. AP003129.
[b] Nucleotide position in SCC*mec* from the *orfX* gene (start codon = nt 1 to 3) from GenBank accession no. AB033763.
[c] Nucleotide position in SCC*mec* from the *orfX* gene (start codon = nt 1 to 3) from GenBank accession no. AP003129.
[d] Amplification product size generated with primer Xsau325 using DNA from MRSA with MREJ type i.
[e] Amplification product size generated with primer Xsau325 using DNA from MRSA with MREJ type ii.
[f] Nucleotide position in SCC*mec* from the *orfX* gene (start codon = nt 1 to 3) from GenBank accession no. AB037671.
[g] Amplification product size generated with primer Xsau325 using DNA from MRSA with MREJ type iii.
[h] Nucleotide position in the SCC*mec* from the *orfX* gene (start codon = nt 1 to 3) from GenBank accession no. AY267374.
[i] Amplification product size generated with primer Xsau325 using DNA from MRSA with MREJ type iv.
[j] Nucleotide position in the SCC*mec* from the *orfX* gene (start codon = nt 1 to 3) from GenBank accession no. AY267381.
[k] Amplification product size generated with primer Xsau325 using DNA from MRSA with MREJ type v.
[l] Nucleotide position in the SCC*mec* from the *orfX* gene (start codon = nt 1 to 3) from GenBank accession no. AY267375.
[m] Amplification product size generated with primer Xsau325 using DNA from MRSA with MREJ type vii.
[n] FAM, 6-carboxylfluorescein.
[o] The underlined sequences constitute the stem of each molecular beacon.
[p] Nucleotide position in *orfX* (start codon = nt 1 to 3) from GenBank accession no. AB037671.
[q] Nucleotide position in *orfX* (start codon = nt 1 to 3) from GenBank accession no. AP004822.
[r] TET, tetrachloro-6-carboxylfluorescein.
[s] Nucleotide position from GenBank accession no. D83207.
[t] Nucleotide position in the IS*431* transposase gene (start codon = nt 1 to 3) from GenBank accession no. AB037671.
[u] Amplification product size generated with primer Xsau401.
[v] Nucleotide position in *mecA* (start codon = nt 1 to 3) from GenBank accession no. X52593 (35).
[w] Amplification product size generated with primer Xsau401 or SA0022Sau2673.
[x] Nucleotide position from the *SA0022 orf* (start codon = nt 1 to 3) from GenBank accession no. AP003129.
[y] Nucleotide position in *tetK* (start codon = nt 1 to 3) from GenBank accession no. S67449.

type I by a 102-bp insertion (2, 23–25, 32, 34). The second primer, meciii519, was specific to the right extremity sequence of SCC*mec* type III. These two primers were used in combination with a primer specific to the *S. aureus orfX* gene, located to the right of the SCC*mec* integration site (Xsau325). We used this set of primers to amplify the MREJs of a variety of MRSA strains. We found that 15 of 206 MRSA strains tested could not be amplified with this assay (data not shown). The MREJs of 11 of these 15 strains were sequenced in order to establish the new sequences at the right extremity of SCC*mec* to allow the development of a PCR assay to detect more MRSA strains. The MREJ sequences obtained for these 11 MRSA strains were compared to sequences available from public databases.

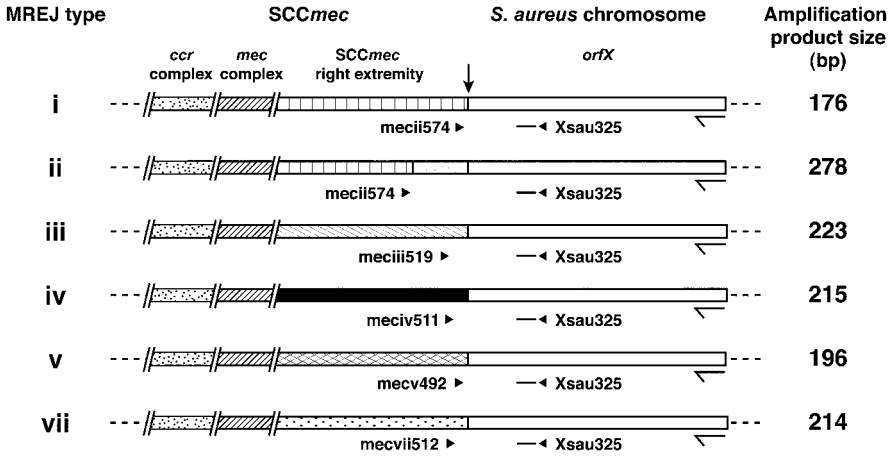

FIG. 1. Schematic representation of the MREJs of MRSA strains with MREJ types i to vii. Each MREJ type exhibits a distinct SRE sequence, with the exception of MREJ types i and ii, which differ by a 102-bp insertion. The positions of forward (▶) and reverse (◀) primers and of internal probes (bars) included in the MRSA assay are shown. Three MBPs, XsauB5, XsauB8, and XsauB9, were used to detect the MRSA-specific amplification products. The locations within SCC*mec* of the *crr* complex and the *mec* complex containing *mecA* are shown, but these genetic elements are not drawn to scale. The vertical arrow at the top indicates the SCC*mec* integration site within the chromosomal *S. aureus orfX* gene. The half-arrows show the positions of the *orfX* start codon and the direction of transcription. More details regarding primers and probes are found in Table 3.

Portions of the MREJ sequence corresponding to the *orfX* gene for these 11 MRSA strains had identities ranging from 97 to 100% with the publicly available *orfX* sequences of *S. aureus* and from 79.1 to 82.5% with those of other staphylococcal species (*S. epidermidis*, *S. hominis*, and *S. haemolyticus*). The characteristic right chromosome-SCC*mec* junction *attL* sequence, which comprises the inverted repeat IRscc-R and the direct repeat DRscc (23), was present within the 3′ end of the *orfX* gene for all 11 strains. However, the DNA sequence within the right extremity of SCC*mec* was shown to be very different from those of types I, II, III, IVa, IVb, and IVc which were previously described (2, 23–25, 32, 34). Three novel SRE sequences were characterized. These sequences were designated MREJ types iv, v, and vii. The MREJs comprising distinct MREP types were named according to the previously described MREP numbering scheme (17, 23). Hence, MREP type i comprises MREJ type i, MREP type ii comprises MREJ type ii, and so on, up to MREP type vii.

The SRE sequences obtained for strains ATCC 33592, ATCC BAA-40, CCRI-1331, CCRI-8895, and CCRI-8903 were nearly identical to each other and exhibited 100% identity with a DNA fragment found within SCC*mec* type III of MRSA strain 85/2082, which includes the sequence located between the second and third IS*431* copies, the inverted repeat sequence of IS*431*, and part of the IS*431* transposase gene sequence (23). However, our sequence data revealed the location of this fragment at the right extremity of SCC*mec* to be adjacent to the DRscc. These new sequences were designated MREJ type iv because the SRE sequences for these five MRSA strains were different from those of SCC*mec* type I from MRSA strain NCTC 10442, SCC*mec* type II from MRSA strain N315, SCC*mec* type III from MRSA strain 85/2082, SCC*mec* type IVa from MRSA strain CA05, SCC*mec* type IVb from MRSA strain 8/6P, and SCC*mec* type IVc from MRSA strain MR108 (23, 25, 34).

The SRE sequences obtained for strains CCRI-1263, CCRI-1311, CCRI-1377, and CCRI-2025 were nearly identical to each other, were different from those of all four SCC*mec* types and MREJ type iv, and consequently, were designated MREJ type v. The SRE sequence of MREJ type v did not show any significant homology with any published sequences when compared with publicly available sequences by use of BLAST.

The SRE sequences obtained for strains CCRI-9583 and CMRSA-1 were also different from those of all four SCC*mec* types and MREJ types iv and v, and consequently, were designated MREJ type vii. Upon a BLAST search, the SRE sequence of MREJ type vii was also shown to be unique, exhibiting no significant homology to any published sequences.

Four of the 15 MRSA strains that were not detected by the PCR assay were not sequenced, as they were shown to carry either MREJ type v or vii based on agarose gel electrophoresis analysis of the amplification products.

Comparison of MREJ and SCC*mec* types of MRSA strains. The MREJ types defined for several MRSA strains in this study were compared with the SCC*mec* types established for these strains by using a previously described SCC*mec* typing method (38). As shown in Table 4, there was no correlation between the MREJ and SCC*mec* types. The MRSA strain with MREJ type i had SCC*mec* type I and those with MREJ type iii all had SCC*mec* type III. It was not possible to identify the SCC*mec* type of the epidemic strain ATCC BAA-42, which carries MREJ type ii. This strain was shown to carry a class B *mec* gene complex. However, primers targeting the *ccrA* and *ccrB* genes used for this typing method (38) did not generate any amplification products. All other MRSA strains with MREJ type ii carried SCC*mec* type I, II, or IVd. The two MRSA strains with the new MREJ type vii defined in this study carried SCC*mec* type II, whereas MRSA strains with the new MREJ type v had either SCC*mec* type IVa or IVc. MRSA strains with the new MREJ type iv carried SCC*mec* type III or IVa.

Analytical sensitivity and specificity of real-time multiplex PCR assay. By using the three new SRE sequences for MRSA, we developed a real-time multiplex PCR assay containing six

TABLE 4. Comparison of MREJ and SCC*mec* typing for the MRSA strains described in this study, including well-known epidemic clones

MRSA strain number	MREJ type	SCC*mec* type
ATCC BAA-38	i	I
CMRSA-2	ii	II
CMRSA-4	ii	II
CMRSA-5	ii	IVd[a]
ATCC BAA-41	ii	II
ATCC BAA-42	ii	ND[b]
ATCC BAA-44	ii	I
ATCC BAA-39	iii	III
ATCC BAA-43	iii	III
CMRSA-3	iii	III
ATCC 33592	iv	III
ATCC BAA-40	iv	III
CCRI-1331	iv	IVa
CCRI-8895	iv	III
CCRI-8903	iv	III
CCRI-1263	v	IVc[c]
CCRI-1311	v	IVa
CCRI-1377	v	IVa
CCRI-2025	v	IVa
CMRSA-1	vii	II
CCRI-9583	vii	II

[a] SCC*mec* subtype IVd was defined by using primers mecIVd26 and mecIVd307 as described in Materials and Methods.
[b] It was not possible to define the SCC*mec* type of this strain by using the previously described *ccrA*- and *ccrB*-specific primers (39). This strain carries novel *ccrA* and *ccrB* gene sequences (39).
[c] SCC*mec* subtype IVc was defined by using primers mecIVc70 and mecIVc1079 as described in Materials and Methods.

primers and four probes. The detection limit of the assay was determined by using genomic DNAs purified from 24 MRSA strains of different MREJ types and was found to be two to eight genome copies per PCR (Fig. 2). The specificity of the PCR assay was tested by using 40 nonstaphylococcal gram-positive bacterial species, 22 gram-negative bacterial species, 212 MRCoNS isolates, 74 MSCoNS isolates, and 569 MSSA strains from various geographic areas (Tables 1, 2, and 5). None of the nonstaphylococcal MRCoNS and MSCoNS strains tested cross-reacted with the PCR assay. Among the 569 MSSA strains tested, 26 (4.6%) were misidentified as MRSA based on the PCR assay. The ubiquity of the PCR assay was tested with a variety of MRSA strains originating from various geographic areas, including well-known epidemic clones (Tables 1 and 5). Of the 1,657 MRSA strains tested, 1,636 (98.7%) were specifically detected by the PCR assay. Only 1.3% of these MRSA strains, representing a broad variety of origins, were not detected by the assay. An agarose gel analysis of the PCR amplification products revealed that there was no amplification of the genomic DNAs from these 21 strains (data not shown). The amplification of MRSA (~10 genome copies per PCR) was not inhibited in the presence of increasing concentrations (up to 10^4 genome copies per PCR) of MSSA, MRCoNS, or MSCoNS (data not shown).

Detection of MRSA directly from nasal specimens. The detection limit of the PCR assay with nasal specimens was evaluated by using 18 MRSA-negative nasal specimens obtained from volunteers. The MRSA-negative specimens, which contained a mixture of MSSA, MRCoNS, and MRCoNS, were spiked with serial 10-fold dilutions of MRSA in the logarithmic phase of growth. The detection limit of the PCR assay was ~25 CFU of MRSA per nasal swab. There was no PCR inhibition for the 18 nasal samples tested based on the amplification efficiency of the internal control (data not shown).

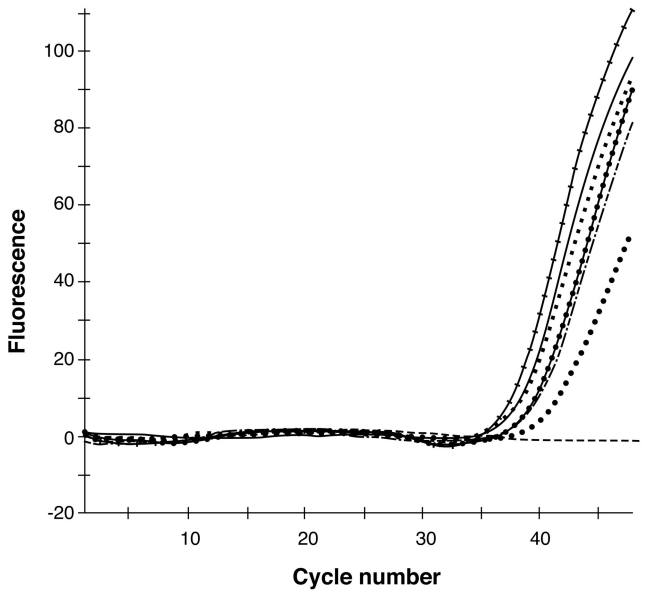

FIG. 2. Example showing the FAM fluorescence detection of MRSA, using 10 copies of genomic DNAs purified from MRSA strains with MREJ types i (solid line), ii (dashed line with dots), iii (circles), iv (solid line with circles), v (solid line with hash marks), and vii (squares). Dashed line, negative control.

DISCUSSION

The emergence of methicillin resistance in *S. aureus* is of great concern, as MRSA strains are often multidrug resistant (6). Infections with MRSA are known to be associated with considerable morbidity and mortality (8). Many studies have shown that effective control measures, including the systematic screening of persons exposed to MRSA, can confine or even eliminate the nosocomial spread of MRSA (31, 36, 41, 43, 50). However, standard culture methods for the identification of *S. aureus* and the determination of oxacillin susceptibility are time-consuming, usually requiring 2 to 4 days. For these reasons, it has become important to develop rapid diagnostic tests for the detection of MRSA.

The molecular detection of MRSA directly from clinical

TABLE 5. Evaluation of MRSA PCR assay using DNAs from a variety of methicillin-susceptible and methicillin-resistant staphylococcal strains

Staphylococcal strain type (no. of strains)[a]	No. (%) with PCR result	
	Positive	Negative
MRSA (1,657)	1,636 (98.7)	21 (1.3)
MSSA (569)	26 (4.6)	543 (95.4)
MRCoNS (212)	0	212 (100)
MSCoNS (74)	0	74 (100)

[a] Reference strains used are listed in Tables 1 and 2. The origins of the staphylococcal clinical isolates are described in Materials and Methods.

specimens containing a mixture of staphylococci, such as screening swabs from anterior nares, represents an important challenge for the rapid detection of MRSA carriers (26). To overcome this challenge, we have developed a multiplex PCR assay which provides a link between *mecA* and the *S. aureus* chromosome. This assay is based on the integration site of the SCC*mec* DNA containing *mecA* in the *S. aureus* chromosome and uses a previously described strategy (17, 23). By using this PCR strategy, we found that 15 of 206 MRSA strains were not detected, suggesting that new SCC*mec* or new SRE sequences were present in these strains. Nucleotide sequencing of the MREJs found in these MRSA strains revealed three new SRE sequences, designated MREJ types iv, v, and vii. The SRE sequences with MREJ types v and vii did not show any significant homology with sequences in databases, whereas those with MREJ type iv exhibited nearly 100% identity with a portion of the SCC*mec* type III carried by MRSA strain 85/2082 (23). This SRE sequence was also recently found at the right extremity of the SCC*mec* of MRSA strain HDG2 (39).

It was possible to assign an SCC*mec* type for most of the MRSA strains with the new MREJ types described in this study, thereby showing that strains with new SRE sequences do not carry a new SCC*mec* but rather have structural variations at the SCC*mec* right extremity. We found that MRSA strains with the new MREJ types v and vii carried SCC*mec* types IV (IVa or IVc) and II, respectively, whereas MRSA strains with the new MREJ type iv had SCC*mec* type III or IVa. It has been shown that SCC*mec* type III of MRSA strain 85/2082 comprises two separate SCC*mec* or SCC elements that were sequentially integrated in the chromosome. This was assessed by the presence of a 15-bp direct repeat sequence between the second and third copies of IS*431* within the SCC*mec* of this strain (23). A region identical to the one found between the second and third copies of IS*431* of SCC*mec* type III of MRSA strain 85/2082 is present at the right extremity of SCC*mec* of the MRSA strains with MREJ type iv carrying SCC*mec* type III described in this study. This suggests that this genetic arrangement may be the result of an excision of the second SCC element in MRSA strains carrying MREJ type iv or that MRSA strains with MREJ type iv may be the ancestral recipients of the second SCC element. SCC*mec* types I, II, and III have been typically associated with MREJ types i, ii, and iii, respectively (17, 23). Surprisingly, the prototype of the MRSA Iberian clone of MRSA ATCC BAA-44, which was shown to have SCC*mec* type I, carries MREJ type ii. Another exception is MRSA strain 93/H44, which was shown to carry SCC*mec* type III and MREJ type i (23). It was not possible to define the SCC*mec* type for the prototype of the pediatric clone of MRSA ATCC BAA-42 carrying MREJ type ii. This strain was shown to carry an SCC*mec* containing a class B *mec* gene complex, but no amplification product was detected with the *ccr*-specific primers described by Okuma et al. (38). The following three types of *ccr* gene complexes have been described: (i) type 1, containing the *ccrA1* and *ccrB1* genes; (ii) type 2, containing the *ccrA2* and *ccrB2* genes; and (iii) type 3, containing the *ccrA3* and *ccrB3* genes (38). It was recently shown that MRSA strain ATCC BAA-42 carries new *ccrA* and *ccrB* gene sequences (39). The *ccrA* gene of this strain exhibits 58 to 61% identity with the *ccrA1*, *ccrA2*, and *ccrA3* genes, whereas the *ccrB* gene of this strain exhibits 70.2 to 73% identity with the *ccrB1*, *ccrB2*, and *ccrB3* genes (23, 34). Therefore, according to the typing nomenclature defined by Okuma et al. (38), this strain carries a new *ccr* gene complex, and consequently, a new SCC*mec* type.

The sequence of the complete SCC*mec* was not characterized for the strains described in this study, but our findings suggest that more polymorphisms are found at the right extremity of SCC*mec* than was originally suggested (17, 23).

After the discovery of new SRE sequences in MRSA strains, we developed a multiplex PCR assay which combines a primer specific to *S. aureus orfX* with a set of primers specific to each SRE sequence and with four MBPs for real-time fluorescence detection of MRSA. None of the gram-negative or gram-positive bacterial species tested other than *S. aureus*, including a variety of MRCoNS and MSCoNS strains, were detected with the PCR assay, showing that it was specific and did not cross-react with bacterial species other than *S. aureus*. We then compared conventional culture methods and PCR for the identification of *S. aureus* and the determination of susceptibility to oxacillin for 2,226 *S. aureus* isolates from various geographic areas. Whereas 1,636 (98.7%) of the 1,657 MRSA strains were correctly identified by the PCR assay, 26 (4.6%) of the 569 MSSA strains tested were misidentified as MRSA. The absence of the *mecA* gene in these 26 MSSA strains was confirmed by use of a previously described *mecA*-specific assay (35; also data not shown). This incorrect identification with the PCR assay could be explained by the presence of a residual SCC*mec* right extremity fragment following the deletion of a chromosomal region containing *mecA* or the presence of an SCC which does not contain *mecA* (23, 25). An analysis by agarose gel electrophoresis of the amplification products generated with DNAs from these MSSA strains showed that they carry SCC fragments with different MREJ types (i, ii, iii, or vii). The spontaneous loss of the *mecA* region in vitro (i) during the storage or long-term cultivation of MRSA strains in antibiotic-free medium or (ii) from cultures which were starved, grown at an elevated temperature, or given small doses of UV radiation has already been described (14, 19, 20, 22, 40). The deletion of a large chromosomal region has been identified for some strains with a deletion of *mecA*. The deletion was shown to start precisely from the left boundary of IS*431mec* and to extend leftwards for various distances beyond the *mecA* gene (51), suggesting that a residual SCC*mec* right extremity fragment would still be present in these deletants.

The deletion of the *mec* region from MRSA strains has also been observed in vivo, but the deleted *mec* fragments have not been characterized in most cases (9, 11, 21, 33). On the other hand, it was recently shown that two MSSA strains, which were originally shown to be MRSA based on *spa* sequence typing, had a deletion of the complete SCC*mec* (45). For these MRSA strains, the SCC*mec* would have been completely excised from the chromosome by the Ccr recombinases, which are specifically involved in the integration and excision of SCC*mec* in the *S. aureus* chromosome (27). In this study, the PCR detection of 26 *mecA*-negative *S. aureus* strains suggests that an SCC fragment is present in the chromosome. Some of these MSSA strains were isolated concurrently with an MRSA strain from the same patient's specimen. If a deletion event has occurred in these strains, it would be interesting to know if it was produced in vivo or in vitro during cultivation. Work is in progress

to further characterize the deletion fragment of these MSSA strains and to verify if the MSSA and MRSA strains which occur together in the same patient are of the same genetic background.

Twenty-one (1.3%) of the MRSA strains tested in this study were not detected by the PCR assay. However, for all of these MRSA strains except one, it was possible to assign an SCC*mec* type (data not shown). The lack of amplification of the MREJ fragments of these strains may be attributable to the presence of polymorphisms in the region recognized by the amplification primers or to new SRE sequences. It should be noted that these strains do not seem to represent epidemic clones in the respective geographic areas from which they originated. Sequencing of the MREJs of these strains is in progress.

To further evaluate the usefulness of the real-time PCR assay for the detection of MRSA directly from nonsterile specimens containing a mixture of staphylococci, we performed amplification of a low genome copy number of MRSA (i.e., ~10) in the presence of up to 10^4 genome copies of MSSA, MSCoNS, or MRCoNS. The data showed that the amplification of MRSA was not inhibited in the presence of these potentially competing DNAs, suggesting that the assay could be applied for the detection of MRSA directly from clinical samples containing mixed populations of staphylococci. We have used this assay to detect MRSA directly from nasal specimens. The PCR assay was performed directly from nasal material prepared with a rapid specimen preparation protocol and allowed the detection of MRSA in <1 h. The detection limit of the PCR assay was found to be ~25 CFU per nasal swab. A clinical study with >100 nasal swabs obtained during an MRSA surveillance program showed that the sensitivity of the PCR assay (>97%) compared to that of the standard culture method was sufficient to detect MRSA directly from nasal specimens (A. Huletsky, P. Lebel, M. Gagnon, M. Bernier, K. Truchon, V. Rossbach, F. Gagnon, N. Boucher, L. Guay, F. J. Picard, and M. G. Bergeron, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. D-2006, 2002).

With this study, we have developed a powerful real-time multiplex PCR assay which can be used to detect MRSA directly from nonsterile clinical specimens containing a mixture of staphylococci in <1 h. As opposed to all other published molecular methods for the detection of MRSA, this assay does not require any previous isolation, capture, or enrichment of the bacteria, thus reducing the number of sample preparation steps and the time to results. The use of this rapid PCR assay should help to reduce the workload associated with MRSA surveillance programs and the spread of MRSA in clinical settings.

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