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Rapid PCR Detection of *Staphylococcus aureus* Clonal Complex 398 by Targeting the Restriction-Modification System Carrying *sau1-hsdS1*▽

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A PCR targeting *sau1-hsdS1* was developed for rapid detection of *Staphylococcus aureus* clonal complex 398 (CC398). High sensitivity (100%) and specificity (100%) were shown by evaluating the test on a large strain collection (n = 1,307). We recommend this test for accurate, rapid, and inexpensive diagnosis of methicillin-resistant *S. aureus* (MRSA) CC398 in hospitals and on farms.

Methicillin-resistant *Staphylococcus aureus* (MRSA) belonging to clonal complex 398 (CC398) has emerged in livestock worldwide and is presently regarded as an important zoonotic agent (3, 8, 10, 11). CC398 strains are not typeable by standard SmaI pulsed-field gel electrophoresis (PFGE) analysis (1) and are currently identified at the clonal level by *spa* typing or multilocus sequence typing (MLST). In this study, a lineage-specific PCR was developed for rapid detection of *S. aureus* CC398 based on the principle that clonal differences within *S. aureus* are reflected in the sequence of *sau1-hsdS1*, a gene responsible for the restriction modification specificity of this bacterial species (12). As the sequence of *sau1-hsdS1* in CC398 was unknown, as the first step we studied the sequence variability of this gene in CC398. A universal reverse primer (5′-CAATTTGTCGGTCGAGTTTGCTG-3′) was designed for amplification of an approximately 530-bp region in *sau1-hsdS1* by aligning publically available *sau1-hsdS1* sequences (GenBank accession numbers DQ309449 to DQ309455) and used with the forward universal *sau1-hsdS1* AF primer described by Cockfield et al. (2) (5′-AGGGTTTGAAGGCGAA TGGG-3′). The amplicon sequences obtained from eight CC398 isolates displaying distinct *spa* types (t011, t034, t108, t567, t571, t1255, t1793, and t2876) were sequenced (TAG Copenhagen, Copenhagen, Denmark) and showed 100% identity and no homology to any publically available bacterial sequences (www.ncbi.nlm.nih.gov/blast). A CC398-specific reverse primer (CC398r1 [5′-CAGTATAAAGAGGTGACATG ACCCCT-3′]) was designed to amplify a 296-bp fragment of the *sau1-hsdS1* gene in combination with the AF primer (final primer concentration of 0.2 μM) using AmpliTaq Gold (Applied Biosystems). The following conditions were used: 12 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 61°C, and 1 min at 72°C, and a final extension at 72°C for 10 min. The PCR test was evaluated using a large collection of strains (n = 1,307) comprising over 10 clonal complexes (Table 1). The collection included 40 human and 1,072 animal CC398 isolates representative of 13 *spa* types (t011, t034, t108, t567, t571, t899, t1255, t1344, t1456, t1793, t2330, t2876, and t4652) and 75 human and 160 animal non-CC398 isolates provided by the four laboratories involved in the study. All isolates had been previously characterized by *spa* typing (5), and clonal complex associations were determined using http://spaserver.ridom.de and by comparing MLST mapping of previous isolates with similar or related *spa* types with subsequent clustering by using eBURST v3 (4, 7). The results of the PCR validation showed 100% specificity (235/235) and 100% sensitivity (1,072/1,072). A multiplex PCR version allowing differentiation between MRSA and non-MRSA isolates belonging to CC398 was obtained by coupling the primers targeting *sau1-hsdS1* with *mecA*-specific primers (6) (mecup1 [5′-GGGATCATAGCGT CATTATTC-3′] and mecup2 [5′-AACGATTGTGACACGA TAGCC-3′]) (see Fig. 1). The size of the CC398-specific amplicon (527 bp) is easily distinguishable from the predicted product sizes of hospital-acquired *S. aureus* lineages by the use of the existing restriction-modification typing scheme of Cockfield et al. (2). This allows a potential expansion of the current typing scheme for rapid identification of livestock-associated MRSA CC398 in hospital settings.

For a quicker turnaround time, the conventional PCR was converted to a real-time platform. Performance of the real-time PCR was validated using a separate strain collection consisting of 77 CC398 and 18 non-CC398 isolates (CC5, -8, -9, -22, -30, -45, -80, and -121) of human and animal origin, confirming the excellent results obtained by the conventional PCR (100% sensitivity and specificity). Real-time PCR (RT-PCR) was performed using 96-well plates on an Mx3000P platform (Stratagene) and Maxima SYBR green-ROX quantitative PCR (qPCR) master mix (Fermentas) with a 0.5 μM concen-

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TABLE 1. *S. aureus* strain collection used for validation of the CC398-specific PCR

Origin (no. of isolates)	Clonal complex[b]	*spa* type(s) (no. of isolates where ≥10)	No. of isolates of indicated clonal complex type	No. of *sau1-hsdS1* PCR-positive results
Cattle (8)	CC80	t527	1	0
	CC9	t2839	1	0
	ND[a]	t524, 2873, t3046	6	0
Dog (9)	CC15	t084, t774	2	0
	CC20	t091	1	0
	CC25	t227	1	0
	CC30	t3055	1	0
	CC5	t548	1	0
	CC8	t030	1	0
	ND	t1335, t1651	2	0
Goat (1)	ND	t1166	1	0
Horse (13)	CC15	t084	1	0
	ND	t1166, t1294, t2112, t2484, t3043, t3044	12	0
Human (115)	CC22	t005, t022, t032, t223, t541	10	0
	CC30	t012, t016, t019, t318	10	0
	CC398	t011, t034 (35), t108, t567, t571	40	40
	CC45	t015, t065, t230	7	0
	CC5	t001, t002, t003, t041, t045	20	0
	CC8	t008, t024, t037, t064	21	0
	CC80	t044, t376	5	0
	ST152/377	t355	2	0
Poultry (114)	CC5	t002 (64), t306, t2049	66	0
	CC8	t304	1	0
	CC9	t1430	19	0
	CC80	t203	1	0
	CC398	t011 (11), t034, t108, t567, t1456, t4652	25	25
	ND	t324, t2038	2	0
Sheep (5)	ND	t2678, t3042, t3045, t3047	5	0
Swine (1,042)	CC1	t127	2	0
	CC5	t151, t2164	2	0
	CC30	t1333, t2840	8	0
	CC398	t011 (404), t034 (13), t108 (575), t571, t899, t1255, t1344, t1793, t2330, t2876	1,007	1,007
	CC9	t337 (21), t899[c], t2839	23	0

[a] ND, not determined (but non-CC398).
[b] Clonal complex associations were determined using http://spaserver.ridom.de and by comparing the results of MLST mapping of previous isolates with similar or related *spa* types with subsequent clustering by the use of eBURST v3 (http://eburst.mlst.net).
[c] MLST typed.

tration of each primer in a final volume of 12.5 μl. PCR conditions were 5 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 57°C, and 1 min at 72°C. The specificity of the amplification products was confirmed by gel electrophoresis and melting curve analysis (Fig. 1).

The very high sensitivity and specificity of the singleplex and multiplex PCR methods presented in this study are explained by the observation that the *sau1-hsdS1* sequence in CC398 is highly conserved and differs significantly from the homologous sequence in other *S. aureus* lineages. van Wamel et al. (9) recently proposed four PCRs to identify CC398 isolates. The two that showed 100% accuracy when tested with a collection of 133 isolates were A07, representing gene SAPIG2195, and C01, representing gene SAPIG2194. Both genes are carried on a transposon and are therefore unsafe for use as a stable marker of lineage. Our results confirm the general findings by Waldron et al. indicating that *sau1-hsdS1* is highly clonal specific and exhibits very high sequence homology within lineages. The sequence variability of the gene in CC398 showed 100% nucleotide identity across eight different CC398-associated *spa* types and 100% homology to SAPIG0500 from the sequenced ST398 genome (GenBank accession number AM990992). Sequence analysis of the 64 publicly available *S. aureus* whole-genome sequencing projects did not show any similarity to existing *sau1-hsdS* sequences (data not shown). Accordingly, the gene appears to be a very conserved and discriminatory epidemiological marker for clonal identification of *S. aureus* CC398.

Molecular typing of MRSA is an important tool for epidemiological surveillance and for development of infection control measures aimed at preventing dissemination within hospitals as well as from the community to hospitals. In developing such control measures, it is necessary to identify genetic markers allowing rapid and reliable MRSA identification at the CC level and easy communication of results between laboratories. Due to the increasing public health concern associated with

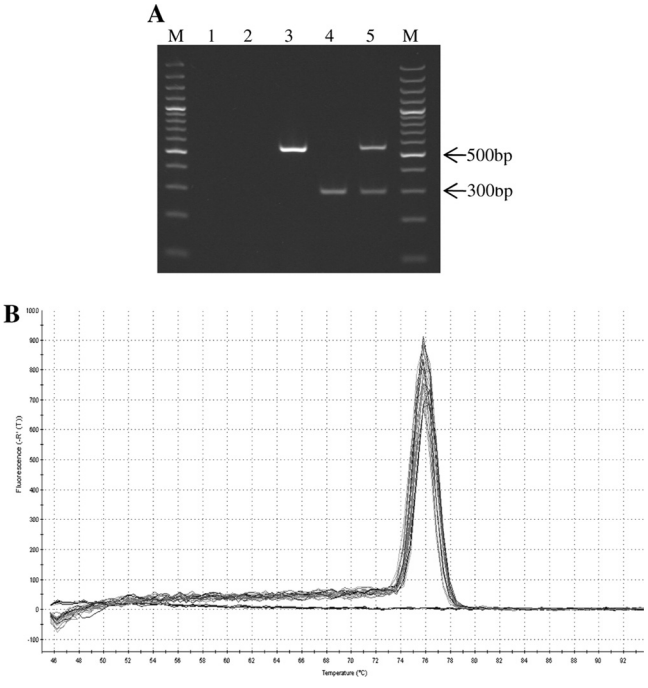

FIG. 1. (A) Duplex PCR for CC398 *sau1-hsdS1* and *mecA* detection. Lane M, 100-bp DNA ladder; lane 1, negative control (water); lane 2, non-CC398 methicillin-susceptible *S. aureus* (strain ATCC 6538); lane 3, non-CC398 methicillin-resistant *S. aureus* (strain ATCC 33591); lane 4, CC398 methicillin-sensitive *S. aureus* (SSI 52615); lane 5, CC398 methicillin-resistant *S. aureus* (KVL 288). (B) Real-time PCR melting curve analysis showing the melting temperature (T_m) of the amplicons. ($-R'(T)$), negative derivative of fluorescence with respect to temperature.

this livestock-associated MRSA strain, the need for rapid methods for MRSA identification at the CC level is no longer limited to human medicine but is now extended to MRSA surveillance of living animals, farm environments, and animal food products. Therefore, the PCR test presented here has important applications in both human and veterinary public health. We recommend the use of this test for rapid, accurate, and inexpensive identification of MRSA CC398 in human diagnostic specimens as well as in any human, animal, food, and environmental samples analyzed for surveillance purposes in hospitals or on farms.

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A Danish patent application based on the findings of this study was filed in June 2009 (PA 2009 00767).

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