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Comparison of Adhesion and Virulence of Two Predominant Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus* Clones and Clonal Methicillin-Susceptible *S. aureus* Isolates▽

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The virulence of SCC*mec* type IV hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates belonging to the major sequence type 8 (ST8 [Lyon clone]) and to a minor upcoming clone, ST5, was compared with that of methicillin-susceptible *S. aureus* (MSSA) isolates of matching sequence types. In vitro adhesion to human airway epithelial cells (HAECs) as an indicator of dissemination and mortality in a murine sepsis model as an indicator of virulence were evaluated. Ten MRSA isolates and 8 MSSA isolates of ST8 and 8 MRSA isolates and 8 MSSA isolates of ST5 were characterized with respect to multilocus sequence type; *agr*, *spa*, and capsule typing; in vitro doubling time; toxin and adhesin gene profiles; and adherence to HAECs. Adherence was significantly lower in the MRSA ST5 group than in the ST8 groups. Infections with MRSA and MSSA isolates ST8 and ST5 were compared. No change in virulence related to the presence of SCC*mec* was observed, since ST8 but not ST5 caused a significantly lower mortality in its presence. Despite their similar genetic backgrounds, individual clonal MRSA and MSSA isolates were heterogeneous in adherence and virulence. No one of these specific virulence factors determined in vitro was related to mouse mortality. In conclusion, in a bacteremic model, mortality was dependent on the ST and was differentially modulated by SCC*mec*; within an ST, clonality was not associated with a homogenous outcome.

Staphylococcus aureus is a pathogen expressing multiple virulence factors such as adhesins, toxins, and immune evasion molecules that enable the bacteria to induce a wide variety of infections. Hospital-acquired (HA) methicillin-resistant *S. aureus* (MRSA) arose in 1961 through the acquisition of the SCC*mec* element and has become one of the most frequent pathogens responsible for HA infections worldwide, particularly in intensive care units (ICUs). Molecular characterization by multilocus sequence typing (MLST) of clinical MRSA isolates in a given area revealed that most are genetically related by sharing the same sequence type (ST), belong to one of the five major clonal complexes (CC), and disseminate within and between hospitals. We recently described and genetically characterized the HA-MRSA isolates detected in France (6). The major clone (Lyon clone) shares ST8, which belongs to CC8; a second clone shares ST5, which belongs to CC5 and is related to the New York-Japan clone and has been spreading for a few years.

Differences in pathogenicity and virulence between MRSA and methicillin-susceptible *S. aureus* (MSSA) isolates may exist but remain an unresolved question. Studies of MRSA virulence in humans suggest a greater burden for MRSA infections in terms of length of hospitalization and mortality rate, but these studies are impaired by multiple confounding factors (5, 21). First, differences in patient populations with respect to comorbidities and therapeutic options that are more or less bactericidal limit conclusions about a putative association between increased virulence and methicillin resistance. Second, pandemic MRSA clones may have specific virulence properties, as has been demonstrated for the pandemic MRSA clone in Brazil (CC8, ST239), which has an enhanced ability to adhere to and invade epithelial cells in vitro compared with sporadic MRSA isolates (3).

This study used the two major HA-MRSA clones, MRSA ST8 and ST5, detected in France and isolates of MSSA with either ST8 or ST5 to evaluate (i) adhesion to human airway epithelial cells (HAECs) as an indicator of dissemination and (ii) mortality rates induced in a murine sepsis model as an indicator of virulence. We compared the mortality rates after infection with MRSA and MSSA, both belonging to ST8 and ST5, to determine the effect of SCC*mec* and ST on virulence. To define the role of SCC*mec* in an isogenic background, we included an MRSA ST30 isolate from which SCC*mec* was cured. Finally we related epithelial cell adhesion and in vitro virulence properties to the mortality induced by different isolates of a given lineage.

MATERIALS AND METHODS

***S. aureus* isolates.** During a survey in ICUs of Edouard Herriot Hospital (a 1,100-bed University Hospital located in Lyon, France), we collected 17 MRSA

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isolates responsible for bloodstream infections representing two different MRSA clones (11). The major one called, the Lyon clone, comprised 13 of the 17 isolates, which shared the *agr* type 1 allele, ST8, CC8, *spa* type t008 or relatives, and SCC*mec* type IV and were positive for the staphylococcal enterotoxin A (*sea*) gene. The minor clone, related to the New York-Japan clone, was found in 3 of the 17 isolates, which shared the *agr* type 2 allele, ST5, CC5, *spa* type t002 or relatives, and SCC*mec* type IV and were positive for the *tst* gene and the *egc* locus. Epidemiological studies revealed that the Lyon clone was the predominant MRSA clone in France, whereas the ST5 MRSA clone was emergent and has been spreading throughout France for some years (6, 9). For this study, we randomly selected 10 of the 13 MRSA ST8 Lyon clone isolates and the 3 MRSA ST5 isolates positive for the *tst* gene and the *egc* locus. To analyze the effect of the SCC*mec* cassette on adhesion and virulence, we completed the first strain selection by adding eight MSSA ST8 and eight MSSA ST5 isolates, also originating from bloodstream infections. The isolates either were collected by the National Center for Staphylococci located in Lyon, France, during a national survey (in 2006 to 2007) or were spontaneously referred to the Center (in the period 2003 to 2007). Five MRSA ST5 isolates were taken from a national survey to complete the first selection of three MRSA ST5 isolates. Thus, the study included 10 MRSA and 8 MSSA isolates of ST8 and 8 MRSA and 8 MSSA isolates of ST5. Finally, we used as an isogenic control pair an MRSA ST30 isolate (WSPPA) and its isogenic mutant from which SCC*mec* was cured (ME230) (2, 20).

Characterization of the genetic background and virulence factors. All strains were characterized by MLST, *spa* typing, and accessory gene regulator (*agr*) allele typing as previously described (1–4). Capsular typing was based on serotyping and PCR as previously described (22). Each isolate was genetically characterized by adhesin gene profiling (*fnbA* and *fnbB* [encoding fibronectin binding proteins A and B, respectively], *clfA* and *clfB* [encoding clumping factors A and B, respectively], *eno* [encoding laminin binding protein], *ebpS* [encoding elastin binding protein], *cna* [encoding collagen binding protein], and *bbp* [encoding bone sialoprotein binding protein]), and toxin gene content (*tst*; staphylococcal enterotoxins and enterotoxin-like *sea*, *seb*, *sec*, *sed*, *seh*, *selK*, *selL*, *selM*, *selO*, *selP*, *selQ*, and *selR*) with multiplex PCR (13, 14). Positive isolates for *selM* and *selO* were considered to be also positive for the *egc* locus, since these genes belong to the *egc* operon. Delta-toxin production was determined in an agar plate assay testing synergy with a beta-toxin reference strain as previously described; isolates without delta-toxin production were considered to have dysfunction of the *agr* locus (19). Production of alpha-toxin was determined by incubating the strains overnight on sheep blood agar plates at 37°C. Superantigen expression was measured by using Ridascreen set A, B, C, D, and E (R-Biopharm AG, Darmstadt, Germany).

Doubling time. Bacterial doubling time was determined as previously described, with minor modifications (10). Overnight cultures were diluted 1:100 in 5 ml of brain heart infusion (BHI) medium and grown on for 3 h at 37°C and 180 rpm. These cultures were seeded at a 1:100 dilution and a 1:4 culture/flask ratio and then incubated at 37°C and 180 rpm for further 4 h. Optical density was measured every 30 min for 4 h. Values were converted into $\log_2$ values, and the doubling time was calculated as the reciprocal of the slope.

Determination of adhesion to HAECs. Adhesion to HAECs was analyzed as previously described (7). Experiments were performed in triplicates, and the number of adherent bacteria per cell was averaged for at least 30 cells per strain.

Determination of virulence in a murine sepsis model. Female C57BL/6 and BALB/c mice, 6 to 8 weeks old, were maintained under specific-pathogen-free conditions in the Animal House of the Department of Biomedicine, University Hospital Basel, Switzerland. All experiments were performed in accordance with the Swiss Veterinary Law. Before intravenous challenge, *S. aureus* isolates were grown freshly from frozen stock cultures (−70°C) prepared using the Microbank (Basel, Switzerland) system (Pro Lab Microbank bacterial preservation system green). One cryoculture bead per strain was incubated in 1 ml of BHI broth (BBL Becton Dickinson, MD) for 7 h at 37°C. Cultures were then diluted 1:100 in 5 ml of BHI broth and incubated overnight at 37°C. Bacteria were then centrifuged at 4,000 × *g* for 10 min, and the pellet was washed twice and resuspended in pyrogen-free 0.9% NaCl before use. Mice were injected with MRSA and MSSA inocula ranging from 5.1×10^7 to 1.4×10^8 CFU (median) in a volume of 200 μl via the caudal vein. The median inocula were as follows: MRSA ST8, 1.4×10^8 CFU; MSSA ST8, 6.8×10^7 CFU; MRSA ST5, 6.8×10^7 CFU; and MSSA ST5, 5.1×10^7 CFU. The median inocula for MRSA ST30 WSPPA and ME230 were 3×10^7 CFU in C57BL/6 mice and 3×10^6 CFU in BALB/c mice. A further subgroup of 15 BALB/c mice were infected with MRSA ST8 at a median inoculum of 2.8×10^6 CFU. Control mice were injected with 200 μl of 0.9% saline. Weight change and clinical severity were determined 1, 2, 5, and 8 days after infection. Disease severity was assessed by the following scoring system: 0, normal activity; 1, trembling and weakness; 2, piloerection and decreased activity; 3, constrained movements and strongly decreased activity; and 4, no movement. The mice were sacrificed by intraperitoneal injection of 100 mg/kg pentobarbital (Abbott Laboratories) when they reached a score of 4, and surviving mice were sacrificed on day 8 after infection. Four to five mice per group were used.

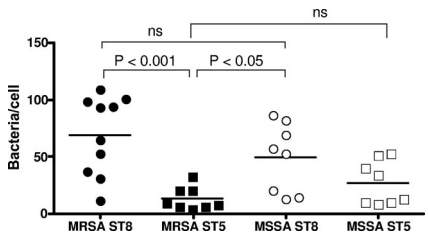


FIG. 1. Adherence of 10 MRSA ST8 (closed circles), 8 MRSA ST8 (closed squares), 8 MSSA ST8 (open circles), and 8 MSSA ST5 (open squares) isolates to human airway epithelial cells. Statistical significance by analysis of variance: MRSA ST8 versus MRSA ST5, $P < 0.001$; MRSA ST8 versus MSSA ST 5, $P < 0.05$; and MRSA ST5 versus MSSA ST8, $P < 0.05$. ns, not significant.

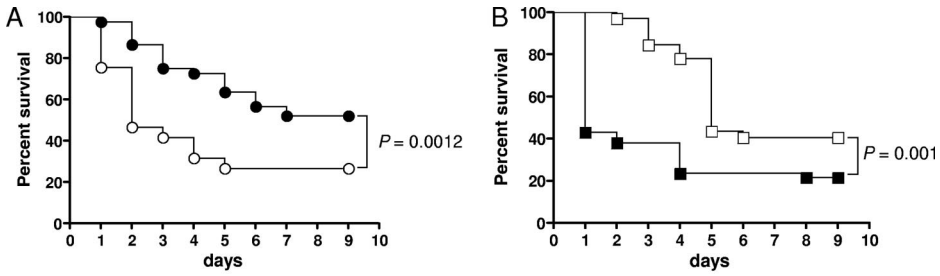
Data analysis. Data were analyzed with SPSS and Graph Pad Prism 4 software. Categorical data were analyzed with Fisher's exact test. Continuous variables were compared using the Mann-Whitney U test for two groups and with the Kruskall Wallis test for more than two groups. *P* values of <0.05 were considered to denote statistical significance. Survival differences between mice challenged with the different groups were analyzed during the infection process with the log rank test and a Kaplan-Meier survival curve. Overall mortality after infection was determined by chi-square contingency test.

RESULTS

Adhesion of ST8 and ST5 isolates to HAECs. MRSA and MSSA, each of ST8 and ST5, respectively, representing the prevalent clones in French ICUs, were first compared in their adherence to HAECs. Adherence was widely variable in MRSA ST8 and in MSSA ST8 (Fig. 1). The median value was significantly lower for MRSA ST5 than for both MRSA ST8 ($P < 0.001$) and MSSA ST8 ($P < 0.05$) but was similar to that of MSSA ST5. The median adhesion of all ST5 isolates (MRSA and MSSA) was significantly lower than that of all ST8 isolates ($P < 0.0002$ [data not shown]).

Effect of the SCC*mec* cassette on virulence in ST8 and ST5 lineages. MRSA and MSSA isolates of the same ST were then used to infect C57BL/6 mice, and the mortality rates were determined. When comparing genetically similar strains differing with respect to the presence or absence of SCC*mec*, it was evident that SCC*mec* had a variable influence upon outcome depending on the ST lineage. As a group, MRSA ST8 induced a lower mortality than MSSA ST8 (Fig. 2A). Conversely, MRSA ST5 induced a higher mortality than MSSA ST5 (Fig. 2B). Thus, we may conclude that the observed high mortality of MRSA ST5 was not related to SCC*mec*, since both MRSA ST5 and MRSA ST8 isolates have this determinant but only MRSA ST5 induced a higher mortality than its SCC*mec*− counterparts. This conclusion was corroborated by survival studies with an MRSA ST30 isolate and its isogenic mutant, from which SCC*mec* was cured. C57BL/6 and BALB/c mice infected with either strain showed similar rates of mortality (data not shown).

Virulence of individual isolates of ST8 and ST5 lineages. Mortality was analyzed for each group of mice infected with



Overall mortality

MRSA ST8	MSSA ST8	*P*
21/44 (48%)	30/41 (73%)	0.026

Overall mortality

MRSA ST5	MSSA ST5	*P*
33/42 (79%)	19/32 (59%)	0.123

FIG. 2. (A) Survival of 44 and 41 mice infected with 10 MRSA ST8 isolates (closed circles) and 8 MSSA ST8 isolates (open circles), respectively. $P = 0.0012$ by log rank test in Kaplan-Meier analysis. Four to five mice were infected with each strain. (B) Survival of 42 and 32 mice infected with eight MRSA ST5 isolates (closed squares) and eight MSSA ST5 isolates (open squares), respectively. $P = 0.001$ by log rank test in Kaplan-Meier analysis. Four to five mice were infected with each strain.

the different isolates of each lineage. Results obtained with both ST8 (Fig. 3) and ST5 (Fig. 4) strains with and without SCC*mec* showed a similar, striking variability of mortality rates between the isolates of any given ST. Three of the 10 isolates belonging to MRSA ST8 were never lethal, whereas 7 were lethal for 50 to 100% of the mice. Similarly, for MSSA ST8 the mortality rate varied from 20% with one strain to 100% with four strains (Fig. 3). Among MRSA ST5, which as a group was most virulent, only one isolate was lethal for 20% of the mice and seven isolates were lethal for 50 to 100% of the mice. Finally, MSSA ST5 contained two nonlethal and three 100% lethal isolates among eight strains.

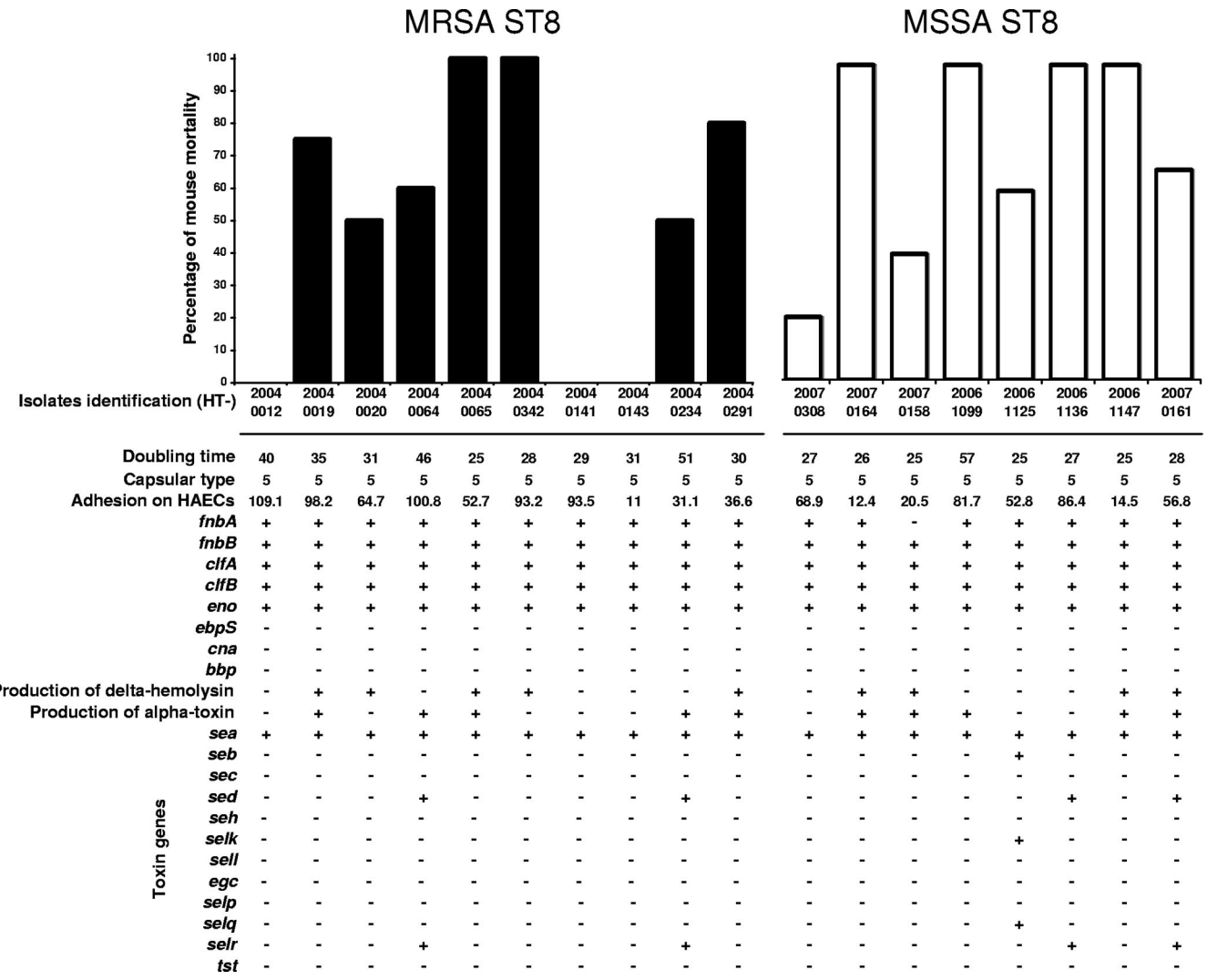
To find an explanation for the variable mortality of genetically related strains, the in vitro growth, adhesion to HAECs, adhesin and toxin gene profiles, as well as toxin production of MRSA ST8 and MRSA ST5 were related to the outcome as presented in Fig. 3 and 4. The doubling times of all groups of strains were similar: 31.00 ± 2.67 min and 26.50 ± 3.87 min for MRSA ST8 and MRSA ST5, respectively; and 26.50 ± 3.87 min and 30.5 ± 1.35 for MSSA ST8 and MSSA ST5, respectively. Isolates belonging to the ST8 and ST5 groups harbored capsular type 5. Comparison of the adhesion gene profiles revealed that MRSA and MSSA of both ST8 and ST5 harbored the *fnbA*, *fnbB*, *clfA*, *clfB*, and *eno* genes, with one exception in the MSSA ST8 group, which lacked *fnbA*. Furthermore, all ST8 isolates harbored the *sea* gene and all ST5 isolates harbored the *tst* and *ebpS genes* and the *egc* locus. As shown in Fig. 3 and 4, no specific virulence factor was able to discriminate between lethal and nonlethal ST8 or ST5 isolates. In particular, the three nonlethal isolates of MRSA ST8 (Fig. 3) and the two nonlethal isolates of MSSA ST5 (Fig. 4) did not differ in adhesin and toxin expression from their lethal counterparts. To evaluate the role of *tst* for mortality in the ST5 isolates, a group of four mice was infected with the isogenic *tst* deletion mutant of MRSA ST5 isolate HT 2003-0749. The mortality induced by the *tst* deletion mutant was even higher (50% [data not shown]) than that of isolates expressing *tst* (20% for isolate HT 2003-0749 [Fig. 4]). This is in agreement with the observation that *tst* suppresses exoprotein expression (23).

Effect of host genotype on outcome after infection with MRSA ST8. To exclude that differences in lethality between MRSA ST8 isolates were solely visible in the C57BL/6 strain, we infected the more susceptible inbred mouse strain BALB/c with 8 of the same 10 MRSA ST8 isolates. Five of seven strains which were highly virulent in C57BL/6 mice were also the most virulent strains in BALB/c mice, and the three isolates which were nonlethal in C57BL/6 mice (Fig. 3) were also the least virulent strains in BALB/c mice. Mortality in BALB/c mice was significantly higher for lethal than for nonlethal MRSA, as determined in C57BL/6 mice (80% versus 33%; $P = 0.0445$ [data not shown]).

DISCUSSION

In this study, we have demonstrated that while MRSA and MSSA ST8 isolates displayed similar adhesion to HAECs in vitro, the MRSA ST8 isolates collectively exhibited a lower virulence than the MSSA isolates in a murine sepsis model. Conversely, the minor MRSA ST5 clone in France exhibited low adhesion to HAECs in vitro and displayed significantly greater lethality in the sepsis model than MSSA ST5 isolates.

The mechanism involved in the selection of pandemic MRSA clones is not clear. The worldwide dissemination of MRSA clones may be dependent on particular adhesive properties that enable bacterial colonization of mucosal and skin surfaces. Indeed, Amaral et al. showed that adhesion to a bronchial epithelial cell line of the Brazilian MRSA pandemic clone was higher than that of sporadic MRSA and clinical MSSA isolates (3). In contrast, Aathithan et al. found similar adhesion to the liver epithelial cell line HepG2 when comparing two pandemic MRSA clones in the United Kingdom with the MSSA strains Wood 46 and Cowan I (1). Our study is the first to compare levels of adhesion of genetically similar groups of clinical MRSA and MSSA isolates to primary human airway epithelia. We found generally lower adhesion in lineage 5 than 8 and the lowest values for the MRSA ST5 group. The latter was not explained by a different adhesin gene profile. Future investigations on adhesin protein expression of MRSA ST5



Isolates identification (HT-)	2004 0012	2004 0019	2004 0020	2004 0064	2004 0065	2004 0342	2004 0141	2004 0143	2004 0234	2004 0291	2007 0308	2007 0164	2007 0158	2006 1099	2006 1125	2006 1136	2006 1147	2007 0161
Doubling time	40	35	31	46	25	28	29	31	51	30	27	26	25	57	25	27	25	28
Capsular type	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Adhesion on HAECs	109.1	98.2	64.7	100.8	52.7	93.2	93.5	11	31.1	36.6	68.9	12.4	20.5	81.7	52.8	86.4	14.5	56.8
fnbA	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
fnbB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
clfA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
clfB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
eno	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ebpS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cna	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bbp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Production of delta-hemolysin	-	+	+	-	+	+	-	-	-	+	-	+	+	-	-	-	+	+
Production of alpha-toxin	-	+	-	+	+	-	-	-	+	+	-	+	+	+	-	-	+	+
sea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
seb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
sec	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
sed	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	+
seh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
selk	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
sell	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
egc	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
selp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
selq	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
selr	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	+
tst	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Toxin genes

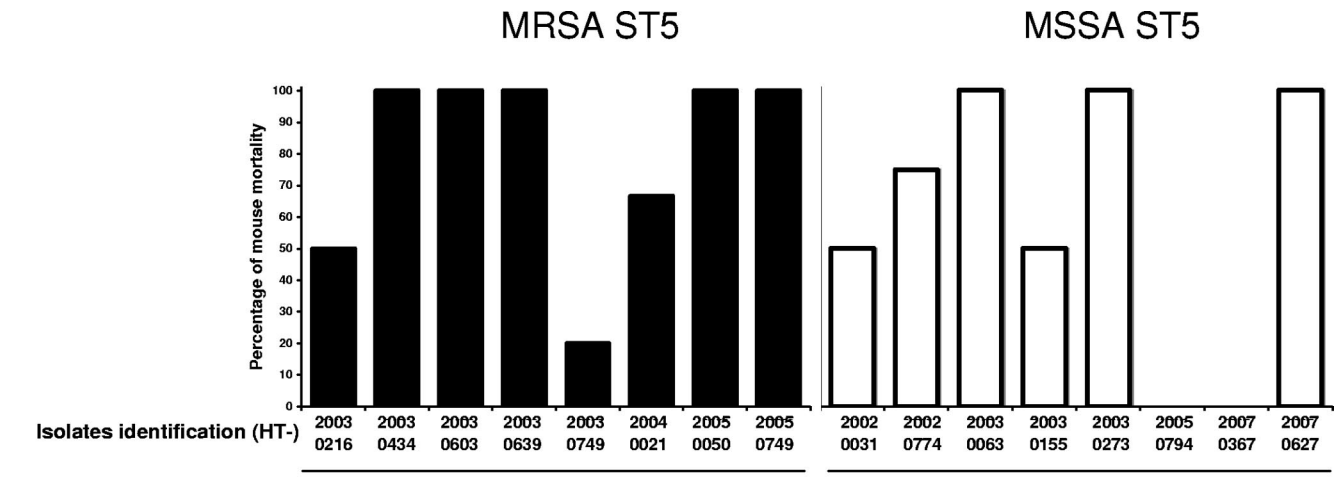
FIG. 3. Individual genotypic and phenotypic characteristics of the MRSA and MSSA ST8 isolates.

and blockade of adhesin receptors on airway epithelial cells will help link ST and dissemination.

Dissemination of pandemic MRSA clones would not be a problem per se if *S. aureus* would not be such a virulent bacterium. To assess the contribution of SCC*mec* to virulence, we performed studies with genetically closely related strains of the ST8 and ST5 lineages with and without SCC*mec* by using a well-defined sepsis model in inbred mice.

In this model, the MRSA ST8 clone isolates killed fewer mice than the MSSA ST8 isolates. This supports the previous results of Mizobuchi et al., who compared the virulence of 13 highly resistant MRSA isolates and 7 MSSA isolates from patients with various clinical syndromes (17) and found that MRSA isolates were less virulent than MSSA. However, it should be noted that the strains in the above mentioned study were not genetically characterized. Peacock et al. found that a strain belonging to an epidemic MRSA clone showed similar lethality to three clinically significant MSSA strains after intraperitoneal or intravenous inoculation of mice (18). Our comparative study of MRSA and MSSA infection in mice is strengthened by the genetic homogeneity of the MSSA isolates and their genetic similarity to the MRSA isolates. Since our MRSA ST5 and MSSA ST8 isolates were equivalently virulent, we can conclude that acquisition of SCC*mec* type IV per se is not associated with a loss of virulence. Furthermore, our in vivo results complemented our in vitro growth data showing that clonal MRSA and MSSA isolates of either ST8 or ST5 had similar doubling times. It is possible that a potential initial fitness burden (15) due to SCC*mec* acquisition was overcome by compensatory mutations. It is also possible that only those clones survived which, despite acquisition of resistance, retained rapid growth (4, 16). To draw a clear conclusion regarding the effect of SCC*mec* upon virulence, sepsis was induced with an MRSA ST30 isolate and its isogenic mutant, from which SCC*mec* was cured. Results from these experiments support our conclusion that SCC*mec* per se has no effect on virulence, since in both mouse strains C57BL/6 and BALB/c, the survival rates of mice infected with the wild-type and mutant isolates were similar.

When investigating virulence factors, which could contribute



Isolates identification (HT-)	2003 0216	2003 0434	2003 0603	2003 0639	2003 0749	2004 0021	2005 0050	2005 0749	2002 0031	2002 0774	2003 0063	2003 0155	2003 0273	2005 0794	2007 0367	2007 0627
Doubling time	26	26	49	28	26	27	27	26	32	36	28	28	32	31	30	23
Capsular type	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Adhesion on HAECs	6.3	20	32.2	20.4	7.6	6	3.8	9.3	9.5	52.7	40	8.2	12.6	33.9	9.9	50.7
Adhesin genes *fnbA*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
fnbB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
clfA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
clfB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
eno	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ebpS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
cna	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bbp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Production of delta-hemolysin	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
Production of alpha-toxin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Toxin genes *sea*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
seb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
sec	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+
sed	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
seh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
selk	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
sell	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
egc	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
selp	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-
selq	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
selr	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
tst	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

FIG. 4. Individual genotypic and phenotypic characteristics of the MRSA and MSSA ST5 isolates.

to lethal outcome, MRSA and MSSA isolates with a homogeneous genetic background as determined by methods described in this current study, had a surprisingly heterogeneous outcome. We searched for known factors contributing to this variable virulence. Most clinical *S. aureus* isolates have serotype 5 (CP5) or serotype 8 (CP8) capsules. By comparing the biological activities of isogenic mutants with CP5, CP8, or no capsule in a mouse model, Watts et al. showed that CP5-positive strains were more virulent than both CP8-positive strains and CP-negative strains (24). We did not find a correlation between CP5 positivity and mouse lethality in our study, which may well be due to other confounding factors in the clonal strains.

MRSA ST8 isolates shared the *sea* gene and produced SEA in vitro, and MRSA ST5 isolates shared the *tst* gene and produced TSST-1 in vitro. Previously, we assumed that SEA was involved in the pathogenesis of *S. aureus* septic shock, since we detected *sea* more frequently in blood isolates from patients with septic shock than in isolates from patients without septic shock (12). The *sea* and *tst* genes have been detected in several pandemic MRSA clones (CC8 and CC30 and CC5 and CC30, respectively) (11). Recently, Diep et al. demonstrated that horizontal transfer of virulence genes such as *sea* and *tst* was epidemiologically associated with the emergence of new virulent strains of both hospital- and community-acquired MRSA (8). However, mice are less susceptible than humans to superantigenic toxins, and D-galactosamine administration, which enhances mouse susceptibility to these toxins, was not used in our study. Finally, among the *sea*-positive clonal MRSA isolates tested here, three ST8 isolates were nonlethal for mice. Thus, CP5 expression and SEA or TSST-1 production were not sufficient to induce mortality in mice and other still unknown virulence factors may be involved in the pathogenesis of sepsis induced by ST8 and ST5 MRSA clones. This conclusion is supported by our observation that deletion of *tst* in an MRSA ST5 strain enhanced, rather than reduced, its virulence. This in

turn supports the putative role of *tst* as a negative global regulator of other exoproteins, as shown by Vojtov et al. (23). However, our conclusions are not restricted to observations made in one mouse strain; mortality induced with the MRSA ST8 clones was similarly variable in the more susceptible BALB/c strain, where strains that are avirulent in C57BL/6 mice remained weakly virulent.

In summary, SCC*mec* type IVA in the ST8 background was associated with decreased virulence, while conversely SCC*mec* type IV in the ST5 background was associated with increased virulence. Thus, the effect of the SCC*mec* cassette on phenotypic characteristics may depend on the genetic background of the strains and heterogeneity may be linked not only to one specific virulence factor, but to differences in global regulation or to single nucleotide polymorphisms, pathogenicity islands, and endogeneous phages, not detected in our study. Whole-genome sequencing of the clonal isolates with different outcomes will facilitate identification of hitherto unknown genes responsible for high virulence.

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