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Heterogeneity of Methicillin-Susceptible *Staphylococcus aureus* Strains at a German University Hospital Implicates the Circulating-Strain Pool as a Potential Source of Emerging Methicillin-Resistant *S. aureus* Clones

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Recently, we demonstrated rapid dissemination of different methicillin-resistant *Staphylococcus aureus* (MRSA) clones at the Institute for Microbiology at the University of Magdeburg (B. Ghebremedhin, W. König, and B. König, Eur. J. Clin. Microbiol. Infect. Dis. 24:388–398, 2005). The majority of them harbored the readily transmissible *mec* cassette type IV. Thus, theoretically, methicillin-susceptible *Staphylococcus aureus* (MSSA) might capture the *mecA* gene from circulating MRSA, or MRSA strains might catch mobile toxin genes from MSSA. Therefore, we characterized MSSA strains circulating at the University Hospital in Magdeburg. Among a total of 84 MSSA strains under study, about 40% possessed the *tst* (toxic shock syndrome toxin) gene and up to four additional enterotoxin genes. *tst*-positive MSSA strains belonged to all known *agr* groups (I to IV) and to 14 different *spa* types (t008, t012, t015, t019, t024, t056, t065, t127, t133, t162, t271, t287, t399, and t400), and they were classified by multilocus sequence typing (MLST) as ST1, ST8, ST30, ST39, ST45, ST101, ST121, ST395, and ST426. In contrast, simultaneously circulating MRSA strains ($n = 24$) harbored in general two or three genes of the enterotoxin gene cluster, and the *tst*-positive MRSA isolates belonged to the well-known epidemic types ST22, ST45, and ST228 and were classified as *spa* types t001, t028, and t032. From our results, one may conclude that the pool of circulating MSSA strains is an important parameter with regard to the epidemiology of hospital- and community-acquired MRSA clones and their potential virulence.

The emergence of *Staphylococcus aureus* strains resistant to methicillin (MRSA) and other antimicrobial agents has become a major concern, especially in the hospital environment, because of the higher mortality due to systemic MRSA infections (45). Methicillin resistance is conferred by carriage of the *mecA* gene (3), which is located on a genetic element called the staphylococcal cassette chromosome (SCC) in *S. aureus* (16, 21). The mechanism(s) responsible for *mecA* transfer is not known, but evidence supports horizontal transfer of the *mecA* gene between different staphylococcal species (14) as well as different gram-positive bacteria (2).

Analysis of the natural population dynamics and expansion of pathogenic clones of *S. aureus* provided evidence that essentially any *S. aureus* genotype carried by humans can transform into a life-threatening human pathogen but that certain clones are more virulent than others (28).

Many *S. aureus* strains produce one or more specific staphylococcal exotoxins, including staphylococcal enterotoxins (SEs), staphylococcal exfoliative toxins, and toxic shock syndrome toxin 1 (TSST-1). These toxins cause infections ranging from relatively mild involvement of the skin and soft tissue to life-threatening sepsis, necrotizing pneumonia, and toxic shock syndrome (TSS) (13, 24, 26, 27, 33).

SEs have been classified as members of the pyrogenic toxin superantigen family because of their biological activities and structural relatedness. SEs have been divided into five serological types (*sea* through *see*) on the basis of their antigenicity. In recent years, the existence of new types of SE genes (*seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, and *seo*), which belong to the operon of the enterotoxin gene cluster (*egc*), has been reported (19, 20, 32, 34).

These toxins cause TSS and related illnesses through their capacity to induce massive cytokine release from both macrophages and T cells by direct binding rather than classical antigen presentation to the major histocompatibility complex class II molecules and the Vβ region of specific T cells (39, 40).

TSST-1 is a potent superantigen and the most common cause of TSS. It is produced exclusively by *S. aureus*, and approximately 20% of natural isolates are producers. Lindsay et al. suggested that *tst* is carried by a family of closely related pathogenicity islands that interact in a highly specific way with certain staphylococcal phages, and they stated that this interaction may be responsible for the spread of *tst* among staphylococcal strains (25).

The Panton-Valentine leukocidin (Luk-PVL) belongs to the family of bicomponent toxins (37). Luk-PVL is associated with skin and soft-tissue infections as well as with more serious infections, e.g., severe necrotizing pneumonia (8, 24).

The aim of this work was to characterize the methicillin-susceptible *S. aureus* (MSSA) strains at the University Hospital in Magdeburg and the nearby rehabilitation and chronic care facility (RCCF) as a potential source for newly emerging MRSA strains by horizontal genetic exchange and to compare these with the simultaneously circulating MRSA clones. To serve this purpose, we determined their antibiotic resistance

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phenotypes and used a combination of different molecular typing methods, including multilocus sequence typing (MLST), *spa* typing, *agr* specificity, and analysis of their pathogenicity profiles.

MATERIALS AND METHODS

Bacterial isolates, isolate identification, and antibiotic susceptibility testing. *S. aureus* strains were isolated and identified from various clinical specimens sent to the Institute for Microbiology at the University of Magdeburg. In this regard, we obtained swabs ($n = 70$) from different locations (e.g., wound, skin, ear, throat, and peritoneum) as well as secretions from the lung, stomach, peritoneum, and abscesses ($n = 12$). Colonies that were isolated from the respective specimens and that were yellow on mannitol salt agar (mannitol fermenters) were plated to purity on blood agar and incubated at 37°C in air for 24 h. The isolates were identified as *S. aureus* on the basis of positive catalase, coagulase, and DNase tests. Final identification and antimicrobial resistance testing were performed with the Phoenix, an automated bacteriology system that performs bacterial identification and susceptibility testing analyses (Becton Dickinson). The results were interpreted in accordance with the Clinical and Laboratory Standards Institute (http://www.clsi.org). In addition, resistance to methicillin was detected on oxacillin resistance screening agar medium (Mueller-Hinton oxacillin [bioMérieux]) and was confirmed by screening for PBP2a (penicillin-binding protein 2a) (Slidex MRSA detection; Denka Seiken). The *mecA* gene was detected by PCR as described by Murakami et al. (31).

DNA extraction. Strains were grown on brain heart infusion agar or in the same broth at 37°C overnight. Genomic DNA used as a target for all molecular methods was extracted by using the QIAGEN DNA extraction kit according to the manufacturer's suggestions, with the modification that 20 μl of lysostaphin (1 mg/ml) and 20 μl of lysozyme (100 mg/ml) were added at the cell lysis step. The concentration of DNA was estimated spectrophotometrically.

Multilocus sequence typing (MLST). MLST was carried out by the methodology described by Enright et al. (10). The allelic profile of *S. aureus* isolates was obtained by sequencing (using BigDye fluorescent terminators) internal fragments of seven "housekeeping" genes (*arcC* [carbamate kinase], *aroE* [shikimate dehydrogenase], *glpF* [glycerol kinase], *gmk* [guanylate kinase], *pta* [phosphate acetyltransferase], *tpi* [triosephosphate isomerase], and *yqiL* [acetyl coenzyme A acetyltransferase]) and submitted to the MLST home page (http://www.mlst.net), where seven numbers depicting the allelic profile were assigned that defined the MLST type. For phylogenetic analysis, the relatedness of lineages was displayed as a dendrogram constructed from the matrix of pair-wise differences in allelic profiles by using the unweighted pair-group method with arithmetic averages.

***spa* typing.** The *spa* type was received by single-locus DNA sequencing (using BigDye fluorescent terminators) of repeat regions of the *Staphylococcus* protein A gene (*spa*) as described by Harmsen et al. (15). Repeats were assigned a numerical code, and the *spa* type was deduced from the order of specific repeats.

***agr* group-specific multiplex PCR and toxin gene detection.** Extracted genomic DNA was used as a template to amplify specific *agr* alleles (GenBank accession numbers X52543, AF001782, AF001783, AF288215, Z49220, AF346724, and AF346725). For multiplex PCR, one primer set was prepared to amplify the four specific *S. aureus agr* alleles using the primers described by Lina et al. (23). Amplification was carried out under the following conditions: an initial 5-min denaturation step at 95°C, then 25 stringent cycles (1 min of denaturation at 94°C, 1 min of annealing at 55°C, and 1 min of extension at 72°C), and a final extension step at 72°C for 10 min.

Sequences specific for *sea-see*, *seg-sej*, *tst*, and *lukS-lukF* were detected by PCR on a PE-9600 thermocycler (Perkin-Elmer). The primers used to detect *sea* to *see*, *seg* to *sej*, and *tst* were described by Becker et al. (4, 5). *luk-PV* genes were detected as described by Lina et al. (24). PCR products were analyzed by electrophoresis through 1.5% agarose gels.

RESULTS

Distribution of MSSA isolates among the different departments. In total, 82 consecutive MSSA strains were under study. The highest proportions of MSSA clones have been isolated from the department of dermatology (35.37%) and the intensive care units (50.01%). The remaining MSSA strains (14.62%) were distributed among eight different departments equally.

TABLE 1. Resistance phenotypes of MSSA strains during the study period at the Otto-von-Guericke University and the RCCF in Flechtingen, Germany[a]

Resistance phenotype	No. (%) of MSSA strains positive for phenotype
No resistance	18 (23)
PEN	32 (39)
GEN	1 (1.22)
CIP	1 (1.22)
PEN-CIP	7 (8.54)
PEN-GEN	1 (1.22)
TET-CIP	2 (2.44)
PEN-TET	4 (4.88)
ERY-CLI	1 (1.22)
PEN-TET-CIP	3 (3.66)
PEN-ERY-CLI	5 (6.1)
ERY-CIP-CLI	1 (1.22)
PEN-TET-ERY-CLI	1 (1.22)
PEN-ERY-CIP-CLI	4 (3.7)
PEN-ERY-CIP-CLI-GEN	1 (1.22)
Total	82 (100)

[a] Antibiotics given: penicillin (PEN), oxacillin (OXA), ciprofloxacin (CIP), erythromycin (ERY), clindamycin (CLI), gentamicin (GEN), and tetracycline (TET).

Antimicrobial resistance phenotypes of the MSSA strains. We primarily examined the resistance of the MSSA isolates ($n = 82$) to antibiotics of different classes. Our data indicate that 58 (70.7%), 19 (23.2%), 13 (15.9%), 13 (15.9%), and 3 (3.7%) MSSA strains were resistant to penicillin (PEN), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), and gentamicin (GEN), respectively (data not shown). Eighteen (23%) MSSA isolates showed no resistance to the tested antibiotics.

We next analyzed the resistance phenotypes of the respective MSSA strains ($n = 82$). As is apparent from Table 1, the majority of MSSA strains ($n = 32$; 39%) possessed resistance to only PEN. Besides resistance to penicillin, 12 (14.6%) strains were resistant to only one additional antibiotic, 8 (9.75%) strains were resistant to two additional antibiotics, and 5 strains (6.1%) were resistant to three additional antibiotics. One MSSA strain showed a wide resistance pattern to PEN, CIP, CLI, ERY, and GEN. Only six strains were resistant to antibiotics (GEN, CIP, TET-CIP, ERY-CLI, or ERY-CIP-CLI) other than PEN.

Analysis of toxin genes. The ability of *S. aureus* to cause a variety of diseases in humans and animals may be attributed to its ability to produce a plethora of virulence factors. Therefore, we analyzed the MSSA strains for the presence of *tst*, *sea* to *sej*, and the Panton-Valentine leukocidin gene (*luk-PV*). Among the 82 MSSA strains, 8 expressed no enterotoxin gene under study. Eighteen strains were positive for only a single gene of the staphylococcal enterotoxin cluster. Thirty strains harbored two genes simultaneously, 19 strains harbored three genes simultaneously, and 6 strains harbored four genes simultaneously. Only one strain possessed five genes of the enterotoxin gene cluster (Table 2). The latter strain was isolated from an outpatient at the department of dermatology and possessed the *luk-PV* gene as well. With regard to the enterotoxin genes, the *sea* gene was detected nine times and the *seb* gene was detected four times. The *sea* and *seb* genes were never detected

TABLE 2. Pathogenicity profiles of MSSA strains under study at the Otto-von-Guericke University and the RCCF in Flechtingen, Germany

Toxin gene(s)	No. of MSSA (n = 82) strains positive for toxin gene(s)
None	8
sea	3
sed	1
sei	13
sej	1
sec, *sei*	4
sed, *sei*	4
seg, *sei*	18
seh, *sei*	1
sei, *sej*	3
sea, *seg*, *sei*	4
seb, *seg*, *sei*	1
sec, *seg*, *sei*	5
sed, *sei*, *sej*	5
seg, *sei*, *sej*	4
sea, *seg*, *sei*, *sej*	2
seb, *sed*, *sei*, *sej*	2
sec, *seg*, *sei*, *sej*	1
sec, *seh*, *sei*, *sej*	1
seb, *sed*, *seg*, *sei*, *sej*	1

together. The individual results of the PCR analysis are summarized in Table 2. We next analyzed the presence of another classical superantigen, the *tst* gene, among the MSSA strains under study. About 40% of MSSA strains (n = 33) possessed the *tst* gene. All *tst*-positive MSSA strains were also positive for up to four additional genes of the staphylococcal enterotoxin gene cluster (Table 3). Twenty-one of the 33 *tst*-positive MSSA strains were predominantly found in the departments of dermatology and in the intensive care units. Due to the fact that most *tst*-positive MSSA strains were isolated from departments at high risk for MRSA, we performed a detailed analysis of the *tst*-positive *S. aureus* strains.

Clonal relatedness of *tst*-positive *S. aureus* isolates. (i) Multilocus sequence typing (MLST). We determined the genetic backgrounds of *tst*-positive MSSA strains (n = 21) by multilocus sequence typing (MLST). MLST revealed nine different sequence types (STs) among the 21 MSSA isolates. In this regard, we detected 1, 6, 1, 2, 5, 2, 1, 2, and 1 strain of ST1, ST8, ST30, ST39, ST45, ST101, ST121, ST395, and ST426, respectively. These results indicate a high heterogeneity among the MSSA isolates with regard to their MLST types.

TABLE 3. Pathogenicity profiles of *tst*-positive MSSA strains under study at the Otto-von-Guericke University and the RCCF in Flechtingen, Germany

Toxin genes	No. of MSSA (n = 33) positive for toxin genes
sei, *tst*	5
sec, *sei*, *tst*	4
sed, *sei*, *tst*	1
seg, *sei*, *tst*	6
seh, *sei*, *tst*	1
sei, *sej*, *tst*	1
sea, *seg*, *sei*, *tst*	4
sec, *seg*, *sei*, *tst*	1
sec, *seg*, *sej*, *tst*	1
sed, *sei*, *sej*, *tst*	4
seg, *sei*, *sej*, *tst*	1
sea, *seg*, *sei*, *sej*, *tst*	1
seb, *sed*, *sei*, *sej*, *tst*	2
sec, *seg*, *sei*, *sej*, *tst*	1

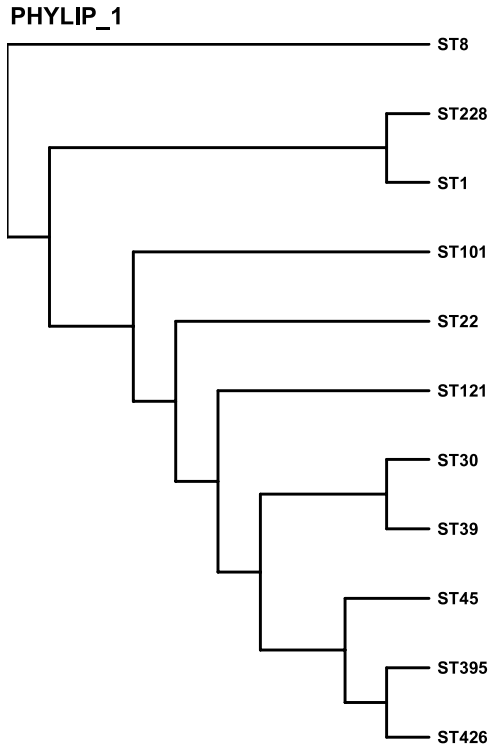

FIG. 1. Dendrogram constructed to analyze the clonal relatedness of the *tst*-positive MSSA strains.

(ii) Phylogenetic analysis of and relationship between strains with different ST patterns. In order to analyze the relationship among strains with different ST patterns, a dendrogram (Fig. 1) was constructed; zero genetic distance corresponds to identical ST patterns. Each terminal branch of the dendrogram represents a sequence type (ST). For comparison, we added MRSA clones of MLST ST22, ST45, and ST228, which are the major MRSA clones at the University Hospital in Magdeburg (12). As the *S. aureus* isolates were compared in a dendrogram tree, three major clusters were identified. ST8 belongs to cluster I, while ST1 and ST228 belong to cluster II. The third cluster is heterogenous and accounts for ST101, ST22, ST121, ST30, ST39, ST45, ST395, and ST426. According to the denodrogram, ST426, ST395, and ST45 clones of cluster III are more closely related to each other than to other cluster III clones, whereas ST30 and ST39 clones, cluster more closely. ST121 clones seem to be not directly related to the other members of cluster III.

(iii) *spa* typing. The *spa* gene of *S. aureus* encodes protein A and was used for the characterization of MSSA isolates. The analysis of the *spa* gene sequences revealed different repeats (Ridom StaphType). Among the 21 MSSA isolates we detected, 14 different *spa* types were detected: t008, t012, t015, t019, t024, t056, t065, t127, t133, t162, t271, t287, t399, and t400. The results are presented in Table 4. It is apparent from Table 4 that a distinct MLST type could also cover different *spa*

TABLE 4. Analysis of *spa* types and the corresponding MLST types for the different MSSA strains

spa type (*n*)	No. of *S. aureus* strains of MLST type:								
	ST1	ST8	ST30	ST39	ST45	ST101	ST121	ST395	ST426
t008 (2)	0	2	0	0	0	0	0	0	0
t012 (1)	0	0	1	0	0	0	0	0	0
t015 (2)	0	0	0	0	2	0	0	0	0
t019 (1)	0	0	0	0	0	0	0	1	0
t024 (2)	0	2	0	0	0	0	0	0	0
t056 (2)	0	0	0	0	0	2	0	0	0
t065 (2)	0	0	0	0	2	0	0	0	0
t127 (1)	1	0	0	0	0	0	0	0	0
t133 (1)	0	0	0	0	1	0	0	0	0
t162 (1)	0	0	0	0	0	0	1	0	0
t271 (1)	0	0	0	0	0	0	0	0	1
t287 (1)	0	0	0	0	0	0	0	1	0
t399 (2)	0	0	0	2	0	0	0	0	0
t400 (2)	0	2	0	0	0	0	0	0	0

TABLE 5. Analysis of MLST types and the corresponding accessory gene regulator (*agr*) types for the different MSSA strains

MLST (*n*)	No. of *S. aureus* isolates of *agr* type:			
	I	II	III	IV
ST1 (1)	0	0	1	0
ST8 (6)	6	0	0	0
ST30 (1)	0	0	1	0
ST39 (2)	0	0	2	0
ST45 (5)	4	0	0	1
ST101 (2)	2	0	0	0
ST121 (1)	0	0	0	1
ST395 (2)	2	0	0	0
ST426 (1)	0	1	0	0

types. In this regard, ST8 covered *spa* types t008, t024, and t400, and ST45 belonged to *spa* types t015, t065, and t133. The remaining MLST types—ST1, ST30, ST39, ST101, ST121, ST395, and ST426—were each assigned to one *spa* type only: t127, t012, t399, t056, t162, t287, and t271, respectively.

(iv) Analysis of *agr*. Variation in *agr* specificity type has been proposed as a possible influence on population dynamics of *S. aureus* (23). Therefore, we investigated the *agr* specificity groups of the different *tst*-positive MSSA strains by PCR using *agr* group-specific primers.

As is apparent from Table 5, the *tst*-positive MSSA strains belonged to *agr* types I, II, III, and IV. All ST8, ST101, and ST395 strains expressed *agr* type I. Among the five strains of ST45, the *agr* type of one stain was detected as type IV, while the other four strains belonged to *agr* type I. The *agr* type of the ST121 isolate was detected as type IV, and the ST426 isolate expressed *agr* type II. ST1 and ST30 isolates belonged to the new emerging American clones, which were detected among community-acquired MRSA (CA-MRSA) strains, harboring *agr* type III. The ST39 strains also expressed *agr* type III.

DISCUSSION

MRSA is still a dominant hospital-associated pathogen (h-MRSA). However, there are ongoing changes in the epidemiology of MRSA. In former times, MRSA strains were clonal and there were only a few epidemic strains; MRSA strains are now more heterogenous. In addition, there is an evolution of so-called community-acquired MRSA (CA-MRSA) with characteristics distinct from those of the traditional h-MRSA.

Genetically, methicillin-resistant *S. aureus* (MRSA) is produced when methicillin-susceptible *S. aureus* (MSSA) acquires a mobile genetic element, staphylococcal cassette chromosome *mec* (SCC*mec*). Toxin-producing MSSA may also alter the pathogenicity of established MRSA by the transfer of virulence factors via plasmids or mobile elements. It is hypothesized that the evolution of CA-MRSA is a recent event due to the acquisition of *mec* DNA by previously methicillin-susceptible strains that circulated in the community. Thus, besides the tracking of MRSA dissemination, we need precise knowledge about the circulating MSSA strains and have to monitor the pathogenicity profiles of MRSA and MSSA strains. This study investigated the heterogeneity of MSSA at the University Hospital in Magdeburg, Germany.

It is well known that MRSA prevalence varies almost 100-fold around the world. Quite recently we described that among the *S. aureus* isolates collected over a 1-year period at the University Hospital of Magdeburg, Germany, approximately 7.3% were classified as MRSA by laboratory analysis (12). The highest prevalence of MRSA has been in the departments of dermatology and the intensive care units of anesthesiology and surgery (12). In accordance with this finding, in this study the highest levels of MSSA isolated from clinical specimens were from the respective departments as well.

Until 1995, most MRSA strains from around the world, including Germany, exhibited multiresistance phenotypes (45). Meanwhile, the resistance phenotypes of MRSA strains have changed, and we also described a narrowing of the resistance pattern in epidemic MRSA strains quite recently at the University Hospital in Magdeburg (12, 43). In this regard, we also detected Barnim (ST22) and southern German (ST228) epidemic MRSA strains which were resistant to only one, two, or no further antibiotics beside penicillin and oxacillin. The Berlin (ST45) epidemic MRSA showed a minor resistance pattern as well (12). A rising number of CA-MRSA strains show low-level resistances as well (44). Among the 82 MSSA strains in this study, most isolates possessed resistance to only penicillin, followed by strains with resistance to one or two more antimicrobial substances. Thus, the capture of the *mecA* gene, probably through SCC*mec* cassette type IV, will lead to MRSA strains with narrowed susceptibility profile. In our study, we show a penicillin resistance of 70.7%. These data are in good concordance with the data from the GENARS (German Network for Antimicrobial Resistance Surveillance) project (http://www.genars.de). From 2002 up to now, approximately 73% of all clinical *Staphylococcus aureus* isolates analyzed during the GENARS project (n = 4,200) exhibited penicillin resistance. However, surveillance data may differ in one country. In this regard, the data from the Antimicrobial Surveillance Study of the Paul Ehrlich Society for Chemotherapy (http://www.p-e-g.org) from 2004 showed a resistance of 76.7% against penicillin. However, only 841 *Staphylococcus aureus* strains were under study. With regard to the levels of susceptibility to gentamicin, erythromycin, clindamycin, and ciprofloxacin, our data are similar to those obtained by the Paul Ehrlich Society for Chemotherapy in 2004. In summary, with the exception of methicillin

resistance, both MRSA and MSSA strains are heterogenous in their susceptibility patterns.

The relative virulence of MRSA and fully methicillin-susceptible *S. aureus* has been scrutinized. The majority of studies support the concept that MSSA and MRSA strains have equivalent potentials for colonization and causing disease. In general, bacteremia isolates of *S. aureus* often contain these classical members of the superantigen family, isolates from patients with diarrhea carry *seb*, and isolates from wound infections harbor the *sec* gene (40). A large number of *S. aureus* strains isolated from furuncles and carbuncles produce Panton-Valentine leukocidin. In addition to the enterotoxins, toxic shock syndrome toxin 1 (TSST-1) of *S. aureus* is associated with septic shock and toxic shock syndrome (39).

In the present study, we detected only one *luk-PV*-positive MSSA strain. Only a small percentage of MSSA strains harbored the gene *sea*, *seb*, or *sec*, the classical staphylococcal enterotoxin genes. In contrast, we detected in nearly all MSSA strains the *sei* gene, which belongs to the *egc* cluster. Surprisingly, about 40% of the MSSA strains under study possessed the *tst* gene. In contrast to other reports, we detected two *tst*-positive strains ($n = 22$) which possessed the *seb* gene as well (6). Interestingly, the *tst*-positive MSSA strains were recovered from the departments with the highest MRSA rates (dermatology and intensive care units of anesthesiology and surgery).

Up to now, we rarely detected *tst*-positive MRSA strains, mainly ST22 and ST228, at the University Hospital in Magdeburg. *tst*-positive MRSA strains belonged to the well-known epidemic MLST types ST22, ST45, and ST228 and were classified as *spa* types t001, t028, and t032 (data not shown). From the literature, it is known that in Europe the epidemic MRSA strain EMSRA-16 sometimes harbored the *tst* gene. Other reports about TSST-1 production by MRSA strains exist as well (38), although there are no available MLST data for the respective strains.

In this context, understanding the epidemiology of TSST-1-producing MSSA is clinically important because of the rare but potentially devastating symptoms caused by toxic shock syndrome toxin 1 (TSST-1). Thus, one has to keep in mind the emergence of CA-MRSA harboring the *tst* gene. There has been considerable speculation about the origin and evolution of the MRSA strains. According to Fitzgerald et al., MRSA strains have arisen multiple independent times by lateral transfer of the *mec* elements into methicillin-susceptible precursors (11). Up to now, the *mec* gene has been found to be present in up to eight distinct *S. aureus* lineages that are highly differentiated in terms of overall chromosomal gene content. Presently there are further changes occurring in the emergence and spread of epidemic MRSA in German hospitals (22, 44, 45, 46). MLST has been used to study the evolution of pandemic clones of MRSA (9, 10, 36). We recently described for the University Hospital in Magdeburg that the most abundant types were two of the newly emerging MRSA clones, the Barnim epidemic MRSA (ST22) and the southern German epidemic MRSA (ST228). In contrast to other parts of Germany, the Berlin epidemic MRSA (ST45) was less abundant in Magdeburg (12).

Thus, overall changes in the prevalence and spread of different epidemic MRSA strains are observed, and thus the emergence of new MRSA clones may be expected. In this study, the *tst*-positive MSSA strains were further characterized according to their MLST and *spa* types. In our study, the TSST-1-producing MSSA isolates represent a heterogenous group covering different STs and *spa* types. STs (sequence types) of the dominant MRSA clones at the University Hospital in Magdeburg during the last 3 years (ST22, ST45, and ST228) were scarcely found or were not found among the present MSSA collection. These results are in agreement with the findings described by Aires de Sousa et al. (1). In contrast to Aires de Sousa et al., we do not conclude from our data that the introduction of SCC*mec* into susceptible clones is most likely a relatively infrequent event. In our study, we detected MLST types ST1, ST8, and ST30 among the *tst*-positive MSSA strains. MLST analysis indicated distinct genetic backgrounds for the arising CA-MRSA strains associated with each geographic origin, namely ST80 in Germany and France (17, 42), ST1, ST8, and ST59 in the United States (35, 29), and ST30 in Australia (7). Among the MSSA strains in this study, we have not detected any MSSA sharing the background of the major European CA-MRSA clone, ST80. We detected MSSA isolates of ST1, ST8, ST30, and ST39. ST30 was recently reported to have spread in the community in Europe. ST39 was associated with MSSA and MRSA in Australia (http://www.mlst.net). Thus, we conclude from our results that similar to *luk*-PV-positive MSSA strains, *tst*-positive MSSA strains, at least of MLST types ST1, ST8, and ST30, have the potential to capture the *mecA* gene and are thus a potential source of *tst*-positive CA-MRSA strains. Whether the additional MLST types may acquire the *mecA* gene is not known. Moreover, many *S. aureus* accessory genes carry virulence factors such as *tst*. These genes are often carried on mobile elements, such as phages and pathogenicity islands, which transfer horizontally between strains (*sea*, *tst*, and *eta*) (30). Thus, it is assumed that the transfer of the *tst* gene can occur at an extremely high frequency (25).

agr specificity type has been proposed as a possible influence on population dynamics in *S. aureus* (23). In agreement with Witte (41), we recently determined *agr* group II for the epidemic MRSA ST228 clone (southern German) and *agr* group I for the newly emerging ones, ST22 and ST45 strains, all circulating at the University Hospital in Magdeburg (12). We analyzed the *agr* specificity groups of the different *tst*-positive MSSA strains as a further contribution to the genotypic characterization of the MSSA isolates. Ji et al. argue that the presence of the *tst* gene in *S. aureus* is coupled to *agr* type III (18). In contrast, in our study all *agr* types were distributed among the different *tst*-positive MSSA strains. These results support the findings which were observed by Moore and Lindsay (30). Thus, we provide evidence that the presence of the *tst* gene is not coupled to a specific *agr* type, at least in MSSA. However, one feature of the described newly emerging CA-MRSA ST1 and ST30 clones is the presence of *agr* type III. Indeed, in our study the *tst*-positive MSSA strains of MLST types ST1, ST30, and ST39 belonged to *agr* group III. In contrast to the CA-MRSA strains of MLST type ST8 described in the literature, we detected only *agr* group II in our ST8 isolates ($n = 6$).

Through the multitude of applied methods, our data contribute to a more precise knowledge of the heterogeneity of

MSSA in a clinical setting. In summary, we observed a heterogeneity of *tst*-positive MSSA clones with regard to MLST, to *spa* typing, to toxin profiles, and to antibiotic resistance patterns. Interestingly, *tst*-positive MSSA strains were predominantly found in the areas with a high incidence of MRSA, the department of dermatology and the intensive care units. Although it is not known if horizontal transfer of the *tst* gene occurs in clinical settings, care must be taken, and further investigations on virulence gene transfer must be conducted.

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