Clonal Analysis of *Staphylococcus epidermidis* Isolates Carrying or Lacking Biofilm-Mediating Genes by Multilocus Sequence Typing

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Staphylococcus epidermidis is part of the normal microflora of the human skin but is also a leading cause of device-associated infections in critically ill patients. Commensal and clinical *S. epidermidis* isolates differ in their ability to form biofilms on medical devices; the synthesis of biofilms is mediated by the *icaADBC* operon. Currently, the epidemiological relatedness between *ica*-positive and -negative isolates is not known; neither is it known whether the *ica* genes can spread to biofilm-negative strains through horizontal gene transfer. In this study, multilocus sequence typing (MLST) was employed for the clonal analysis of 118 *S. epidermidis* *ica*-positive and -negative strains. MLST revealed that the majority of *ica*-positive and -negative strains were closely related and formed a single clonal complex. Within this complex one sequence type (ST27) was identified which contained exclusively *ica*-positive isolates and represented the majority of clinical strains tested. ST27 and related *ica*-positive clones carried different *SCCmec* cassettes (conferring methicillin resistance) and the insertion sequence IS256. The findings suggest that the *S. epidermidis* infections analyzed in this report are mainly caused by a single clone (ST27) which occurs preferentially in hospitals and differs from clones in the community. It is hypothesized that the successful establishment of ST27 within nosocomial environments has been facilitated by the presence of genes encoding biofilm and resistance traits.

Staphylococcus epidermidis is a bacterium that constitutes a major component of the normal skin and mucosal microfloras of humans. It is a leading cause of hospital-acquired infections, mostly associated with the use of medical devices in seriously ill or immunocompromised patients. *Staphylococcus epidermidis* pathogenesis relies on the ability of the bacteria to form thick multilayered biofilms on a wide variety of polymer and metal surfaces (12). In general, biofilms consist of bacteria embedded into a polysaccharide matrix which protects bacteria against hostile environments, including antibiotics and the action of the host immune system, and therefore are an important factor in the development of chronic and recurrent infections (13, 30). Although the production of staphylococcal biofilm is dependent upon multiple regulatory proteins, an essential factor is the presence and expression of the four-gene *icaADBC* operon (15). The operon encodes enzymes necessary for production of polysaccharide intercellular adhesin (PIA), which is required for biofilm formation and is involved in hemagglutination and bacterial aggregation (7, 19, 20). In animal models, PIA-negative mutants were significantly less likely to cause catheter-associated infections than their PIA-positive isogenic counterparts (27). Epidemiological studies have clearly shown that the majority of commensal *Staphylococcus epidermidis* isolates lack the *ica* operon, whereas clinical strains obtained from

device-associated infections harbor the genetic information for biofilm formation in addition to certain insertion sequences (IS256) and genes that mediate methicillin and aminoglycoside resistance (1, 9, 10, 17, 36). Currently, the genetic origin of the *ica* genes in *S. epidermidis* is not known, and it is also uncertain how biofilm-forming isolates establish and disseminate within the hospital environment. Occurrence of the *ica* operon in different *S. epidermidis* genetic backgrounds would suggest mobility and horizontal transfer of the biofilm-mediating genes. However, it is also conceivable that the *ica* operon imparted to its host a particular evolutionary advantage which allowed a single *S. epidermidis* clone to spread within multiple hospital environments. Multilocus sequence typing (MLST) has been used as a tool to study the phylogenetic relationships of a variety of bacterial pathogens, including *S. aureus* and *S. epidermidis* (8, 29, 32). It can be used to elucidate evolutionary relationships between strains and to identify ancestral genotypes as well as to predict patterns of divergence within groups of related genotypes. In this report, MLST was used to characterize a collection of 118 *S. epidermidis* isolates. BURST analysis suggested that both *ica*-positive and -negative isolates were derived from a common ancestral clone and that the majority of *ica*-positive isolates shared one specific sequence type (ST). We conclude from these data that the majority of the *S. epidermidis* infections analyzed in this study are caused by a single sequence type which is characterized by its biofilm-forming capacity and antibiotic resistance traits. It is tempting to speculate that this sequence type might represent a suitable

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TABLE 1. MLST profiles of *S. epidermidis* isolates carrying *ica* and *mecA* genes and the IS256 element and distribution of the isolates among the different sequence types

Sequence type (no. of isolates)	MLST profile ^a	No. of isolates carrying ^b			Ratio of clinical vs. commensal isolates	Geographic origin(s) (no. of isolates)	No. of geographic locations
		<i>icaADBC</i>	IS256	<i>mecA</i>			
ST2 (30)	1-1-3-1-1-1-1	7	1	1 (NT)	10:20	Germany (24); U.S. ^c (5); Ireland (1)	5
ST5 (10)	1-1-3-2-1-1-1	1	2	2 (IV)	3:7	Germany (8); U.S. (1); Norway (1)	5
ST7 (2)	1-1-3-6-2-1-1	2	1	1 (II)	1:1	Germany (1); U.S. (1)	2
ST8 (5)	1-2-3-1-1-1-1	2	0	0	2:3	Germany (5)	3
ST12 (2)	1-6-3-1-1-1-1	2	0	0	0:2	Germany (2)	1
ST18 (3)	2-1-3-1-1-1-1	1	1	1 (IV)	0:3	Germany (2); <i>S. epidermidis</i> ATCC 12228	2
ST27 (35)	7-1-3-1-1-1-1	35	28	27 (II, III, IV, NT)	32:3	Germany (24); Norway (7); U.S. (4)	9
ST28 (2)	7-1-3-1-1-3-1	2	2	2 (III)	2:0	U.S. (2)	1
ST29 (6)	7-1-3-6-2-4-1	6	6	4 (IV)	6:0	Germany (6)	2
ST33 (1)	12-1-5-6-2-5-12	1	1	1 (IV)	0:1	Germany (1)	1
ST34 (3)	1-1-3-2-2-1-1	2	3	3 (IV)	1:2	Germany (3)	2
ST35 (1)	12-1-3-1-2-5-12	0	0	0	1:0	Germany (1)	1
ST36 (1)	1-1-3-1-1-5-1	0	0	0	1:0	Germany (1)	1
ST40 (1)	1-1-13-11-1-1-11	0	0	0	1:0	Germany (1)	1
ST43 (1)	2-1-3-2-1-1-1	0	0	0	1:0	Germany (1)	1
ST44 (3)	1-5-3-1-1-1-10	0	0	0	1:2	Germany (3)	3
ST45 (1)	1-2-3-2-1-1-1	0	0	0	1:0	Germany (1)	1
ST46 (1)	1-1-3-12-1-1-1	0	0	0	0:1	Germany (1)	1
ST47 (1)	1-1-3-1-1-1-9	0	0	0	0:1	Germany (1)	1
ST48 (1)	1-1-3-1-1-1-8	0	0	0	0:1	Germany (1)	1
ST49 (1)	1-2-3-6-10-1-1	0	0	0	0:1	Germany (1)	1
ST50 (1)	1-1-3-1-11-5-10	0	0	0	0:1	Germany (1)	1
ST51 (1)	7-1-3-1-2-1-7	1	0	0	1:0	U.S. (1)	1
ST52 (1)	13-5-3-1-1-1-10	0	0	0	0:1	U.S. (1)	1
ST53 (1)	1-1-3-1-1-1-4	0	0	1 (IV)	1:0	U.S. (1)	1
ST54 (1)	12-10-5-7-2-5-6	0	0	0	0:1	U.S. (1)	1
ST55 (1)	1-1-3-1-1-1-13	0	0	0	0:1	U.S. (1)	1
ST56 (1)	1-1-15-1-1-1-8	0	0	0	0:1	U.S. (1)	1

^a Allelic profiles of the sequence types consisting of the allele numbers of the genes analyzed (from left to right: carbamate kinase [*arcC*], shikimate 5-dehydrogenase [*aroE*], glycerol kinase [*glpK*], guanylate kinase [*gmk*], phosphate acetyltransferase [*pta*], triosephosphate isomerase [*tpiA*], and acetyl coenzyme A acetyltransferase [*yqi*]).

^b SCCmec type(s) is in parentheses.

^c U.S., United States.

genetic background for the acquisition of these genes, resulting in a well-adapted clone which spreads in hospital environments.

MATERIALS AND METHODS

Bacterial isolates. A total of 118 *S. epidermidis* strains, 62 *ica*-positive and 56 *ica*-negative isolates, were analyzed in this study. The collection consisted of 53 isolates of commensal origin and 65 clinical isolates collected between 1991 and 2004 in six different areas of Germany (89 isolates), one hospital in Ireland (1 isolate), one hospital in Norway (8 isolates), and five different areas in North America (18 isolates) (Table 1). *S. epidermidis* ATCC12228 and *S. epidermidis* RP62A, two reference strains of which the full-genome nucleotide sequences have recently been published, were also included in the study (11, 34). Clinical strains originated from blood cultures of patients with venous access ($n = 31$), prosthetic and native valve endocarditis ($n = 3$ and $n = 5$, respectively), cerebrospinal fluid ($n = 1$), and catheter-related urinary tract infections ($n = 25$). Commensal isolates were obtained from nasal swabs of healthy volunteers ($n = 41$); 11 commensal isolates were taken from nasal swabs of health care workers. Detailed strain information and references are available on the www.mlst.net site in the *S. epidermidis* database. Isolates were identified to the species level by biochemical characterization using the API-20-Staph system (bioMérieux, Marcy l'Etoile, France). Isolates that exhibited ambiguous results by this method were retested by amplification and direct nucleotide sequencing of PCR fragments coding for 16S rRNA genes using the primers 5'-TACGGCTACCTTGTTACG ACTT-3' and 5'-GAGTTTGATCCTGGCTCA-3' (18).

***ica*-, *mecA*-, and IS256-specific PCRs and Southern hybridizations.** Isolation of chromosomal DNA, restriction enzyme cleavage, and Southern blotting were done as described previously (36). The *ica* operon and IS256 were detected by Southern hybridization. For this purpose *icaA*- and IS256-specific gene probes were amplified using the primers *IcaA*-forward (5'-GAC CTC GAA GTC AAT AGA GGT-3') and *IcaA*-reverse (5'-CCC AGT ATA ACG TTG GAT ACC-3') and the primers IS256-forward (5'-TGA AAA GCG AAG AGA TTC AAA

GC-3') and IS256-reverse (5'-ATG TAG GTC CAT AAG AAC GGC-3'), respectively, with *S. epidermidis* RP62A chromosomal DNA as the template and conditions as described previously (17, 37). Bacterial strains were tested for the presence of the *mecA* gene by PCR using the primers 5'-AAA ATC GAT GGT AAA GGT TGG C-3' and 5'-AGT TCT GCA GTA CCG GAT TTG C-3' (accession no. X52593) and the following conditions: 1 min at 95°C, 30 s at 52°C, and 45 s at 72°C for 30 cycles. The resulting 532-bp PCR fragment was visualized by agarose gel electrophoresis and ethidium bromide staining. *S. epidermidis* RP62A and *S. carnosus* TM300 served as positive and negative controls, respectively.

Determination of SCCmec types. SCCmec typing was performed by a multiplex PCR approach with subsequent visualization of the amplified DNA fragment patterns by agarose gel electrophoresis and ethidium bromide staining (24). To distinguish between SCCmec types I to IV, primers and conditions identical to those described by Oliveira et al. (24) were used, with modifications of primers PlsF (5'-GGG GTG GTT AAT GGT ATG AAT AAA-3') and PlsR (5'-CGG AAT GTT GCT CTT GGT TGT GCG TTT TC-3') (23).

MLST. Internal PCR fragments of seven housekeeping genes were amplified using previously described primers (32): for carbamate kinase (*arcC*), *arcC*-F (5'-TGT GAT GAG CAC GCT ACC GTT AG-3') and *arcC*-R (5'-TCC AAG TAA ACC CAT CGG TC TG-3'); for shikimate 5-dehydrogenase (*aroE*), *aroE*-F (5'-CAT TGG ATT ACC TCT TTG TTC AGC-3') and *aroE*-R (5'-CAA GCG AAA TCT GTT GGG G-3'); for glycerol kinase (*glpK*), *glpK*-F (5'-CATCACCACGGTCAAAACATGC-3') and *glpK*-R (5'-CAG GTC GTC CAA TCT ATC ACG C-3'); for guanylate kinase (*gmk*), *gmk*-F (5'-TCG ATT CTT AGC GAG TTC AAC C-3') and *gmk*-R (5'-CCT TCA GGT GTT GGA AAG GG-3'); for phosphate acetyltransferase (*pta*), *pta*-F (5'-TAC TGC ATC GTA TCC ACC TAA ACG-3') and *pta*-R (5'-TGG TGC TGC ACA TTC TAC TGG AG-3'); for triosephosphate isomerase (*tpiA*), *tpiA*-F (5'-CCA CCA TAT TGA ATA CGT GTA GCG-3') and *tpiA*-R (5'-GCT TAC TTT GAA GAA AGC GGT G-3'); and for acetyl coenzyme A acetyltransferase (*yqi*), *yqi*-F (5'-TGC TGG ACG GAG TTG TGC TAA C-3') and *yqi*-R (5'-ATC CTG CTC GTA TTG CTG CG-3'). Nucleotide sequences were determined for both

FIG. 1. Proportion of *ica*-positive and -negative *S. epidermidis* isolates (A) and distribution of different sequence types (B) among clinical *S. epidermidis* strains.

strands by direct, automated sequencing of the PCR products by use of an ABI Prism 310 DNA sequencer (Applied Biosystems, Darmstadt, Germany) or a LiCor model 4000L DNA sequencer (LiCor Biosciences, Lincoln, NE). Nucleotide sequences were compared to known alleles for each locus via the MLST website (<http://www.mlst.net>). For each isolate a seven-digit allelic profile was established which defined a sequence type (ST). Clonal analysis of the STs was performed using BURST, a Web-implemented clustering algorithm (<http://www.mlst.net>), which divides MLST data sets into groups of related isolates and predicts the founding genotype of each clonal complex (28). STs with at least five of seven identical alleles were defined as a clonal group.

Recombination analysis. Recombination rates were estimated by pairwise comparison of the nucleotide sequence of each single-locus variant (SLV; i.e., an ST which differs from another ST in only one locus and remains unchanged in the other six) with that of its predicted clonal ancestor as described previously (6). Briefly, BURST was used to identify SLVs and their associated ancestor STs. The nucleotide sequences of the various alleles were compared to each other using Sequence Output, a Macintosh program available from the MLST website, which allows for displaying polymorphic sites. An allele was determined to have resulted from point mutation when two criteria were fulfilled: first, when the allele differed only in one nucleotide site from its putative ancestor allele, and second, when the allele was unique within the data set of this study and within the data deposited in the mlst.net database. Recombination was determined to have occurred when an allele differed in more than one nucleotide site or when the allele varied in a single site but corresponded to a known allele found elsewhere in the database.

RESULTS

MLST profiles and *ica* presence in clinical and commensal isolates. MLST revealed a total of 28 different sequence types (STs) among the 118 *S. epidermidis* isolates (Table 1). Sequence types ST2 to ST29 corresponded to known allelic profiles in the mlst.net *S. epidermidis* database, whereas ST33 to ST56 represented novel sequence types. Entire nucleotide sequences of all alleles are available via the mlst.net database. The majority of the clinical strains (58 of 65; 89.2%) were *ica* positive (Fig. 1A) and displayed nine different STs (Fig. 1B). In this respect, ST27 was a remarkable clone. It harbored almost half of all clinical isolates tested (32 of 65; 49.2%) and the bulk of the *ica*-positive isolates (35 of 62; 56.4%) (Fig. 1B). Among commensal strains of healthy volunteers, the *ica* operon was found in only 4 of 41 isolates (9.7%); three of these belonged

to ST27 and one to ST12. Commensal strains from hospital staff, however, carried the *ica* operon in 8 of 11 isolates (72.7%). These isolates were grouped into ST2 ($n = 3$), ST7 ($n = 1$), ST12 ($n = 1$), ST18 ($n = 1$), ST33 ($n = 1$), and ST34 ($n = 1$).

Clonal relationship of the STs. BURST was used to divide the 118 isolates into clonal complexes by comparison of their allelic profiles (Fig. 2). This analysis indicated that the majority of isolates are closely related to each other and form a single major group or clonal complex (Fig. 2). ST2, which was the sequence type that generated the most abundant single-locus variants (SLVs), was considered the common ancestor clone. A total of 30 isolates, both *ica* positives and negatives (7 versus 23), belonged to ST2. ST27, the clone that represented the majority of the *ica*-positive isolates (56.4%) and 49.2% of the clinical isolates (Fig. 1), is a single-locus variant of ST2 differing only in the *arcC* allele. Interestingly, this clone is a source of isolates which carry, concomitantly with the *ica* operon, genes that mediate methicillin resistance (*mecA*) and the insertion sequence IS256 (see below).

Detection of the *ica* genes in an unrelated genetic background. BURST analysis identified four additional STs which were unrelated to the major clonal group (Fig. 2). Each of these STs was represented by one isolate. ST33 and ST35 shared five identical alleles and were therefore regarded as a minor group (Table 1 and Fig. 2). ST40 and ST54 are singletons and are unrelated to each other and to any other ST identified in this study. Interestingly, ST33 represented an *ica*-positive strain, indicating that the *ica* operon can also be present in *S. epidermidis* lineages that differ from the ST2-derived clonal complex.

SCCmec typing and IS256 detection. In previous studies we have shown a correlation between the presence of the *ica* operon, methicillin resistance, and detection of the insertion sequence IS256 in clinical *S. epidermidis* isolates (17). Methicillin resistance in staphylococci is mediated by the *mecA* gene, which is located on a large, mobile DNA element termed SCCmec (16). Four major SCCmec types have been identified for which mobility and repeated transfer into different genetic backgrounds of *S. aureus* and coagulase-negative staphylococci has been demonstrated (5, 8, 32). IS256 is an insertion sequence element which is part of the aminoglycoside resistance-mediating transposon Tn4001 but also occurs independently in multiple copies in the genomes of staphylococci and enterococci. IS256 has the capacity to influence expression of the *ica* operon and subsequent biofilm formation by reversible insertion into the *ica* operon and its regulatory genes as well as by chromosomal rearrangements (2, 14, 35, 37). To investigate whether SCCmec cassettes and IS256 occur in specific STs, all isolates were tested for the presence of these genes. Of the 118 *S. epidermidis* strains tested, 43 (37 clinical isolates and 6 commensal isolates) were *mecA* positive. Four of the commensal isolates originated from hospital staff members. SCCmecII was detected in 13 strains, SCCmecIII in 14 strains, and SCCmecIV in 13 strains. Three *mecA*-positive isolates were nontypeable, and none of the strains carried an SCCmecI cassette (Table 1). ST27, the most prevalent *ica*-positive clone, contained 27 (62.8%) of the 43 methicillin-resistant isolates. Four different SCCmec cassettes were found in these 27 *mecA*-positive isolates: SCCmecII ($n = 12$), SCCmecIII ($n = 12$), SCCmecIV

FIG. 2. BURST analysis was used to identify clonal complexes. The predicted clonal ancestor is indicated in the central ring; SLVs are shown in the middle and double-locus variants in the outer ring. Red labels mark the number of *ica*-positive isolates, blue numbers indicate IS256-positive strains, and green labels designate the number and the type of the SCCmec cassettes identified. I, SCCmecI; II, SCCmecII; III, SCCmecIII; IV, SCCmecIV; NT, none typeable; MS, methicillin susceptible. The blue dotted line marks the dominant *ica*-positive sequence type ST27.

($n = 1$), and SCCmec NT ($n = 2$). IS256 was detected in 45 of 118 *S. epidermidis* strains: 40 clinical strains and 5 commensals. Four of the five IS256-positive commensals were obtained from health care workers. IS256-carrying strains were detected in nine STs which contained both *ica*-positive and SCCmec-positive isolates, and ST27 again represented the clone containing the most IS256-positive isolates (28 of 45; 62.2%). Taken together, these data suggest that SCCmec and IS256 occur jointly in specific *S. epidermidis* clones which harbor the *ica* operon and occur preferentially in the hospital environment.

Geographic distribution of *ica*-positive clones. Table 1 summarizes the properties of each ST with respect to *ica* and IS256 presence, including SCCmec typing as well as its geographic distribution. *ica*-positive STs were preferentially found in medical facilities. Interestingly, these clones were not restricted to a specific geographic area. *ica*-positive ST27 isolates were identified in hospitals from four regions in Germany, in one hospital in Norway, and at four locations in the United States. Except for ST12, ST28, ST33, and ST51, which represent only one or two isolates, respectively, all *ica*-positive STs were detected in more than one hospital (Table 1).

Estimates of recombination rates. To address the question of whether *S. epidermidis* strains diverge by point mutation or by recombination, a pairwise comparison of single-locus variants (SLV) was done by analysis of their polymorphic sites (data not shown). An allele was considered as likely to have arisen by point mutation when (i) it differed only in one nucleotide from the corresponding ancestor allele and (ii) the allele was unique in the data set and the database on the *S. epidermidis* mlst.net site. In contrast, recombination was assumed when the allele differed in more than one nucleotide and/or was already present in the MLST database (6). Table 2 shows that 12 of 15 SLVs likely evolved by recombination and only 3 of 15 by point mutation, suggesting that recombination is the major mechanism for divergence of *S. epidermidis* strains.

DISCUSSION

Prevalence of ST27 and other *ica*-positive clones in hospital settings. Employing a recently established MLST scheme, this study demonstrated that the majority of the *S. epidermidis* infections analyzed in this report are caused by a single clone

TABLE 2. Variant alleles of SLVs of the common ancestor and estimates of recombination rates

ST of clonal ancestor	ST of SLV	Variant locus in SLV	Ancestral allele	SLV allele	No. of nucleotide differences	Other STs containing SLV allele	SLV allele origin
ST2	ST5	<i>gmk</i>	1	2	3	ST34; ST43; ST45	Recombination
ST2	ST8	<i>aroE</i>	1	2	1	ST9; ST10; ST16; ST45; ST49	Recombination
ST2	ST12	<i>aroE</i>	1	6	1	ST13; ST19; ST26	Recombination
ST2	ST18	<i>arcC</i>	1	2	1	ST17; ST19; ST43;	Recombination
ST2	ST27	<i>arcC</i>	1	7	13	ST28; ST29; ST51	Recombination
ST2	ST36	<i>tpi</i>	1	5	1	ST23; ST35; ST50; ST54	Recombination
ST2	ST46	<i>gmk</i>	1	12	1	None	Mutation
ST2	ST47	<i>yqi</i>	1	9	7	None	Recombination
ST2	ST48	<i>yqi</i>	1	8	7	ST56	Recombination
ST2	ST53	<i>yqi</i>	1	4	1	ST6; ST16; ST24	Recombination
ST2	ST55	<i>yqi</i>	1	13	1	None	Mutation
ST5	ST34	<i>pta</i>	1	2	10	ST3; ST7; ST20; ST21; ST23; ST29; ST30; ST33; ST35; ST51; ST54	Recombination
ST5	ST43	<i>arcC</i>	1	2	1	ST17; ST18; ST19	Recombination
ST5	ST45	<i>aroE</i>	1	2	1	ST8; ST9; ST10; ST16; ST49	Recombination
ST44	ST52	<i>arcC</i>	1	13	1	None	Mutation

(i.e., ST27) whose presence is widespread within hospital settings in Germany. Interestingly, this sequence type was also detected in a Norwegian hospital and in medical facilities in the United States. In addition to ST27, 11 other *ica*-positive clones with similar properties were identified. The presence of these clones in the hospital environment is in agreement with a recent MLST study on the distribution of SCCmec cassettes in clinical *S. epidermidis* isolates (32). Wisplinghoff and co-workers analyzed a North American collection of 67 methicillin-resistant and -susceptible *S. epidermidis* isolates from blood cultures and prosthetic valve endocarditis. Nine of the 32 MLST profiles identified in the SCCmec study coincide with *ica*-positive STs identified in our report, i.e., ST2, ST5, ST7, ST8, ST12, ST18, ST27, ST28, and ST29. Moreover, there was good agreement with respect to the SCCmec types identified in the STs of both reports. At present we do not know whether the isolates analyzed by Wisplinghoff et al. are also *ica* and IS256 positive, but their origination from device-associated infections makes it very likely that they are also biofilm formers.

ST27 and other *ica*-positive clones were rarely found outside of medical facilities, suggesting that they are highly adapted to the hospital environment and differ from commensal *S. epidermidis* in the community. It is conceivable that patients who are admitted to a hospital are soon colonized by these biofilm-forming, multiresistant *S. epidermidis* isolates and that this newly acquired endogenous microflora represents the origin for a later infection. This hypothesis is supported by a recent ecological study in bone marrow transplantation patients demonstrating that these individuals carry *ica*-, *mecA*-, and IS256-positive *S. epidermidis* isolates as commensals (26). Colonization of hospital staff by *ica*-positive *S. epidermidis* isolates in our report highlights again the presence of such strains in the hospital environment. However, larger studies are necessary to elucidate whether or not the personnel might function as transmitters of biofilm-forming strains to patients. Moreover, it will be interesting to elucidate the possible impact of other virulence-associated factors such as the phenol-soluble modulins or other biofilm-mediating genes such as *aap* and *bhp* in these clones (4, 25, 33).

Origin and attributes of ST27 isolates. In addition to *ica*, the majority of ST27 isolates carry different SCCmec cassettes and multiple copies of the insertion sequence IS256. It is unknown why ST27 has become the dominant ST within nosocomial environments in Germany; however, the answer may be related to the increased reliance on implanted medical devices within immunocompromised patients. These devices represent a suitable habitat for colonization, and the extended use of antibiotics and disinfectants generates a highly selective pressure for the generation of resistant variants. Occupation of this niche requires specific prerequisites which are obviously met by ST27. The *ica* operon enables the bacteria to stick to polymer and metal surfaces, and the ability to gain resistance determinants helps ST27 isolates withstand hostile external conditions. The presence of different SCCmec cassettes in ST27 suggests repeated, independent transfer of SCCmec DNA into this genetic background and is an indication of the capacity of ST27 to exchange genetic material. The question of whether or not the *ica* genes are also subject to horizontal gene transfer must remain open at this stage of experimental work. However, the detection of the *ica* operon in an unrelated *S. epidermidis* background in our study and the identification of *ica* homologues in pathogenic *Escherichia coli* and a broad range of other bacterial species support the hypothesis of mobility and lateral transfer of biofilm-encoding genes (31). The recent publication of the genome sequences of a methicillin-resistant *ica*-positive *S. epidermidis* clinical strain and an *ica*-negative commensal isolate revealed that most of the genetic variations between the two genomes are attributed to genomic islands, phages, and integrated plasmids (11, 34). In this regard, it was concluded that lateral gene transfer between staphylococci and other low-GC gram-positive bacteria is common and contributes considerably to resistance and virulence development of *S. epidermidis* (11). Interestingly, we observed in studies of the spontaneous emergence of biofilm-negative variants that DNA encompassing the *ica* operon is unstable and can be readily deleted from the *S. epidermidis* genome (P. D. Fey and W. Ziebuhr, unpublished data). Instability of genetic material is often an indication of mobility, and in this respect it is also

conceivable that the *ica* operon represents mobile DNA that has been lost in the commensal *S. epidermidis* isolates. Another finding of our study is that the generation of SLVs within *S. epidermidis* seems to occur generally by recombination rather than by point mutation. This is in contrast to what has been described in *S. aureus* studies, where clonal complexes were shown to expand primarily by point mutation (6). The molecular background for this difference between the two species remains to be elucidated, but it might reflect again the capacity of *S. epidermidis* to acquire and incorporate foreign DNA.

It is tempting to speculate that ST27 isolates represent an ideal genetic background for biofilm and resistance genes, resulting in well-adapted strains which are then selected in the hospital environment. The presence of multiple copies of IS256 in the ST27 genome might support this adaptation process by an ongoing generation of novel phenotypic and genotypic variants. Thus, involvement of IS256 in genome flexibility has been shown for the modulation of biofilm, resistance, and global regulatory gene expression (2, 3, 21, 22, 35, 37). Therefore, the combination of biofilm formation, antibiotic resistance, and genetic flexibility may explain why ST27 has become the dominant ST within medical facilities. The data of this report provide novel insights into the population structure of *S. epidermidis* and may suggest new infection control measures for the management of these infections in the future.

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REFERENCES

1. Cho, S. H., K. Naber, J. Hacker, and W. Ziebuhr. 2002. Detection of the *icaADBC* gene cluster and biofilm formation in *Staphylococcus epidermidis* isolates from catheter-related urinary tract infections. *Int. J. Antimicrob. Agents* **19**:570–575.
2. Conlon, K. M., H. Humphreys, and J. P. O'Gara. 2004. Inactivations of *rsbU* and *sarA* by IS256 represent novel mechanisms of biofilm phenotypic variation in *Staphylococcus epidermidis*. *J. Bacteriol.* **186**:6208–6219.
3. Couto, I., S. W. Wu, A. Tomasz, and H. de Lencastre. 2003. Development of methicillin resistance in clinical isolates of *Staphylococcus sciuri* by transcriptional activation of the *mecA* homologue. *J. Bacteriol.* **185**:645–653.
4. Cucarella, C., C. Solano, J. Valle, B. Amorena, I. Lasa, and J. R. Penades. 2001. Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. *J. Bacteriol.* **183**:2888–2896.
5. Enright, M. C., D. A. Robinson, G. Randle, E. J. Feil, H. Grundmann, and B. G. Spratt. 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proc. Natl. Acad. Sci. USA* **99**:7687–7692.
6. Feil, E. J., J. E. Cooper, H. Grundmann, D. A. Robinson, M. C. Enright, T. Berendt, S. J. Peacock, J. M. Smith, M. Murphy, B. G. Spratt, C. E. Moore, and N. P. Day. 2003. How clonal is *Staphylococcus aureus*? *J. Bacteriol.* **185**:3307–3316.
7. Fey, P. D., J. S. Ulphani, F. Götz, C. Heilmann, D. Mack, and M. E. Rupp. 1999. Characterization of the relationship between polysaccharide intercellular adhesin and hemagglutination in *Staphylococcus epidermidis*. *J. Infect. Dis.* **179**:1561–1564.
8. Fitzgerald, J. R., D. E. Sturdevant, S. M. Mackie, S. R. Gill, and J. M. Musser. 2001. Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. *Proc. Natl. Acad. Sci. USA* **98**:8821–8826.
9. Frebourg, N. B., S. Lefebvre, S. Baert, and J. F. Lemeland. 2000. PCR-based assay for discrimination between invasive and contaminating *Staphylococcus epidermidis* strains. *J. Clin. Microbiol.* **38**:877–880.
10. Galdhart, J. O., J. Allignet, H. S. Tung, C. Ryden, and N. El Solh. 2000. Screening for *Staphylococcus epidermidis* markers discriminating between skin-flora strains and those responsible for infections of joint prostheses. *J. Infect. Dis.* **182**:351–355.
11. Gill, S. R., D. E. Fouts, G. L. Archer, E. F. Mongodin, R. T. Deboy, J. Ravel, I. T. Paulsen, J. F. Kolonay, L. Brinkac, M. Beanan, R. J. Dodson, S. C. Daugherty, R. Madupu, S. V. Angiuoli, A. S. Durkin, D. H. Haft, J. Vamathevan, H. Khouri, T. Utterback, C. Lee, G. Dimitrov, L. Jiang, H. Qin, J. Weidman, K. Tran, K. Kang, I. R. Hance, K. E. Nelson, and C. M. Fraser. 2005. Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. *J. Bacteriol.* **187**:2426–2438.
12. Götz, F. 2002. *Staphylococcus* and biofilms. *Mol. Microbiol.* **43**:1367–1378.
13. Hall-Stoodley, L., J. W. Costerton, and P. Stoodley. 2004. Bacterial biofilms: from the natural environment to infectious diseases. *Nat. Rev. Microbiol.* **2**:95–108.
14. Handke, L. D., K. M. Conlon, S. R. Slater, S. Elbaruni, F. Fitzpatrick, H. Humphreys, W. P. Giles, M. E. Rupp, P. D. Fey, and J. P. O'Gara. 2004. Genetic and phenotypic analysis of biofilm phenotypic variation in multiple *Staphylococcus epidermidis* isolates. *J. Med. Microbiol.* **53**:367–374.
15. Heilmann, C., O. Schweitzer, C. Gerke, N. Vanittanakom, D. Mack, and F. Götz. 1996. Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. *Mol. Microbiol.* **20**:1083–1091.
16. Ito, T., K. Okuma, X. X. Ma, H. Yuzawa, and K. Hiramatsu. 2003. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. *Drug Resist. Updates* **6**:41–52.
17. Kozitskaya, S., S. H. Cho, K. Dietrich, R. Marre, K. Naber, and W. Ziebuhr. 2004. The bacterial insertion sequence element IS256 occurs preferentially in nosocomial *Staphylococcus epidermidis* isolates: association with biofilm formation and resistance to aminoglycosides. *Infect. Immun.* **72**:1210–1215.
18. Lane, D. J. 1991. 16S/23S rRNA sequencing, p. 115–175. In E. Stackebrandt and M. Goodfellow (ed.), *Nucleic acids techniques in bacterial systematics*. John Wiley & Sons, New York, N.Y.
19. Mack, D., W. Fischer, A. Krokotsch, K. Leopold, R. Hartmann, H. Egge, and R. Laufs. 1996. The intercellular adhesion involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear beta-1,6-linked glucosaminoglycan: purification and structural analysis. *J. Bacteriol.* **178**:175–183.
20. Mack, D., J. Riedewald, H. Rohde, T. Magnus, H. H. Feucht, H. A. Elsner, R. Laufs, and M. E. Rupp. 1999. Essential functional role of the polysaccharide intercellular adhesion of *Staphylococcus epidermidis* in hemagglutination. *Infect. Immun.* **67**:1004–1008.
21. Maki, H., N. McCallum, M. Bischoff, A. Wada, and B. Berger-Bächi. 2004. *tcaA* inactivation increases glycopeptide resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **48**:1953–1959.
22. Maki, H., and K. Murakami. 1997. Formation of potent hybrid promoters of the mutant *lrm* gene by IS256 transposition in methicillin-resistant *Staphylococcus aureus*. *J. Bacteriol.* **179**:6944–6948.
23. O'Brien, F. G., T. T. Lim, F. N. Chong, G. W. Coombs, M. C. Enright, D. A. Robinson, A. Monk, B. Said-Salim, B. N. Kreiswirth, and W. B. Grubb. 2004. Diversity among community isolates of methicillin-resistant *Staphylococcus aureus* in Australia. *J. Clin. Microbiol.* **42**:3185–3190.
24. Oliveira, D. C., and H. de Lencastre. 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **46**:2155–2161.
25. Rohde, H., C. Burdelski, K. Bartscht, M. Hussain, F. Buck, M. A. Horstkotte, J. K. Knobloch, C. Heilmann, M. Herrmann, and D. Mack. 2005. Induction of *Staphylococcus epidermidis* biofilm formation via proteolytic processing of the accumulation-associated protein by staphylococcal and host proteases. *Mol. Microbiol.* **55**:1883–1895.
26. Rohde, H., M. Kalitzky, N. Kroger, S. Scherpe, M. A. Horstkotte, J. K. Knobloch, A. R. Zander, and D. Mack. 2004. Detection of virulence-associated genes not useful for discriminating between invasive and commensal *Staphylococcus epidermidis* strains from a bone marrow transplant unit. *J. Clin. Microbiol.* **42**:5614–5619.
27. Rupp, M. E., P. D. Fey, C. Heilmann, and F. Götz. 2001. Characterization of the importance of *Staphylococcus epidermidis* autolysin and polysaccharide intercellular adhesin in the pathogenesis of intravascular catheter-associated infection in a rat model. *J. Infect. Dis.* **183**:1038–1042.
28. Smith, J. M., N. H. Smith, M. O'Rourke, and B. G. Spratt. 1993. How clonal are bacteria? *Proc. Natl. Acad. Sci. USA* **90**:4384–4388.
29. Urwin, R., and M. C. Maiden. 2003. Multi-locus sequence typing: a tool for global epidemiology. *Trends Microbiol.* **11**:479–487.
30. Vuong, C., J. M. Voyich, E. R. Fischer, K. R. Braughton, A. R. Whitney, F. R. DeLeo, and M. Otto. 2004. Polysaccharide intercellular adhesion (PIA) protects *Staphylococcus epidermidis* against major components of the human innate immune system. *Cell Microbiol.* **6**:269–275.
31. Wang, X., J. F. Preston III, and T. Romeo. 2004. The *pgaABCD* locus of

- Escherichia coli* promotes the synthesis of a polysaccharide adhesin required for biofilm formation. *J. Bacteriol.* **186**:2724–2734.
32. **Wisplinghoff, H., A. E. Rosato, M. C. Enright, M. Noto, W. Craig, and G. L. Archer.** 2003. Related clones containing SCCmec type IV predominate among clinically significant *Staphylococcus epidermidis* isolates. *Antimicrob. Agents Chemother.* **47**:3574–3579.
 33. **Yao, Y., D. E. Sturdevant, and M. Otto.** 2005. Genomewide analysis of gene expression in *Staphylococcus epidermidis* biofilms: insights into the pathophysiology of *S. epidermidis* biofilms and the role of phenol-soluble modulins in formation of biofilms. *J. Infect. Dis.* **191**:289–298.
 34. **Zhang, Y. Q., S. X. Ren, H. L. Li, Y. X. Wang, G. Fu, J. Yang, Z. Q. Qin, Y. G. Miao, W. Y. Wang, R. S. Chen, Y. Shen, Z. Chen, Z. H. Yuan, G. P. Zhao, D. Qu, A. Danchin, and Y. M. Wen.** 2003. Genome-based analysis of virulence genes in a non-biofilm-forming *Staphylococcus epidermidis* strain (ATCC 12228). *Mol. Microbiol.* **49**:1577–1593.
 35. **Ziebuhr, W., K. Dietrich, M. Trautmann, and M. Wilhelm.** 2000. Chromosomal rearrangements affecting biofilm production and antibiotic resistance in a *Staphylococcus epidermidis* strain causing shunt-associated ventriculitis. *Int. J. Med. Microbiol.* **290**:115–120.
 36. **Ziebuhr, W., C. Heilmann, F. Götz, P. Meyer, K. Wilms, E. Straube, and J. Hacker.** 1997. Detection of the intercellular adhesion gene cluster (*ica*) and phase variation in *Staphylococcus epidermidis* blood culture strains and mucosal isolates. *Infect. Immun.* **65**:890–896.
 37. **Ziebuhr, W., V. Krimmer, S. Rachid, I. Loessner, F. Götz, and J. Hacker.** 1999. A novel mechanism of phase variation of virulence in *Staphylococcus epidermidis*: evidence for control of the polysaccharide intercellular adhesin synthesis by alternating insertion and excision of the insertion sequence element IS256. *Mol. Microbiol.* **32**:345–356.