GENETIC DETERMINANTS AND BIOFILM FORMATION OF CLINICAL *STAPHYLOCOCCUS EPIDERMIDIS* ISOLATES FROM BLOOD CULTURES AND INDWELLING DEVICES

A. Mertens¹ and B. Ghebremedhin^{1,2*}

¹Medical Microbiology, Otto-von-Guericke-University Clinic, Magdeburg, Germany

²Medical Microbiology, Faculty of Medicine, University of Witten/Herdecke, Witten, Germany

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For a long time, *Staphylococcus epidermidis*, as a member of the coagulase-negative staphylococci, was considered as part of the physiological skin flora of the human being with no pathogenic significance. Today, we know that *S. epidermidis* is one of the most prevalent causes for implant-associated and nosocomial infections. We performed pheno- and genotypic analysis (*ica*, IS256, SCCmec types, *agr* groups) of biofilm formation in 200 isolates. Fifty percent were genetically *ica*-positive and produced biofilm. Among all studied isolates, *agr* II and III and SCCmec type I were the most prevalent, whereas within the selected multi-resistant isolates (29%), *agr* I and III and SCCmec type II dominated. SCCmec type I and *mecA*-negative *S. epidermidis* isolates were associated with *agr* II. The majority of the blood culture and biopsy isolates were assigned to *agr* III and SCCmec type I, whereas *agr* II was predominantly detected in *mecA*-negative *S. epidermidis* isolated from catheter and implant materials. MLST analysis revealed the major clonal lineages of ST2, ST5, ST10, and ST242 (total 13 STs). ST2 isolates from blood cultures were *icaA/D*-positive and harbored SCCmec types II and III and IS256, whereas the *icaA/D*- and IS256-positive ST23 isolates were assigned to SCCmec types I and IV.

Keywords: *S. epidermidis*, biofilm, *ica*, antibiotic resistance, *agr*, SCCmec, IS256

Introduction

Among the coagulase-negative staphylococci, *Staphylococcus epidermidis* strains have long been regarded as apathogenic, but their important role as pathogens and their increasing incidence have been recognized and studied in the recent years [1–3]. During the past two decades, *S. epidermidis* has emerged as one of the major pathogens in nosocomial infections. Infections caused by this organism generally occur in newborns, elderly individuals, immunocompromised patients, and especially in patients with indwelling catheters and other implanted devices. Its major pathogenicity lies in its ability to form biofilm on polymeric surfaces [4, 5]. As catheters and implanted devices are increasingly used in medical practice, infection due to *S. epidermidis* is now regarded as among the top five microbial causes of nosocomial infections [6].

Biofilm formation has long been suspected of making a major contribution to *S. epidermidis* infections after implantation of medical devices, in particular catheters [7, 8]. The use of biofilm formation by *S. epidermidis* strains

as a marker of pathogenicity has led to conflicting results because of difficulties in the standardization of measuring biofilm production [7, 8] and phase variation in the expression of biofilm [9, 10]. *S. epidermidis* is believed to produce biofilm in two stages: rapid initial attachment of the bacteria to polymer surfaces via a surface-associated autolysin encoded by the gene *atlE* [11], followed by cell proliferation and the production of polysaccharide intercellular adhesion encoded by the *ica* operon, including four genes: *icaA*, *icaD*, *icaB*, and *icaC* [12]. The *ica* operon was shown to be more prevalent in clinical *S. epidermidis* isolates obtained from catheter-related infections and presumed sepsis than in isolates from normal skin flora of healthy control groups [9, 13]. That means pathogenicity appears to be a complex phenomenon, which could be accounted for not only by large genetic differences but also by small variations in gene contents. This prompted us to perform a detailed comparative pheno- and genotypic analysis of *S. epidermidis* isolates from different sampling sites, complementing the previous approaches used to differentiate pathogenic strains from the non-pathogenic ones [14].

* Corresponding author: B. Ghebremedhin; Medical Microbiology Helios Wuppertal, Faculty of Medicine, University of Witten/Herdecke, Witten, Germany; E-mail: beniam.ghebremedhin@med.ovgu.de

Other research groups have shown that staphylococcal biofilm formation is a highly variable factor, which is influenced by both regulatory processes and genetic mechanisms, such as phase variations, mutations, and chromosomal rearrangements [15–18]. The observation that some of these genetic processes are mediated by the action of insertion sequence (IS) elements prompted us to investigate the distribution of common staphylococcal IS elements among *S. epidermidis* strains of clinical and commensal origin. Previous investigations demonstrated that IS256 can be involved in phase variation of biofilm formation in *S. epidermidis*. Biofilm formation is mainly mediated by the expression of the *icaADBC* operon, which encodes enzymes for the production of the polysaccharide intercellular adhesin PIA [10]. To investigate a possible association between the *ica* genes, biofilm formation, and IS256, all strains were tested for the presence of the *ica* genes and biofilm formation as described previously [9]. *Staphylococcus* accessory gene regulator (*agr*) locus influences the expression of many virulence genes in staphylococci. Three allelic groups of *agr*, which generally inhibit the regulatory activity of each other, are described within the *S. epidermidis* [19]. The gene structure and sequences of the *S. epidermidis* *agr* system are very similar to those of *S. aureus*, and the locus may play a comparable role. The *agr* system, in both *S. aureus* and *S. epidermidis*, is approximately 3.5 kb in size with 68% similarity and comprises the genes *agrA*, *agrB*, *agrC*, and *agrD*, which are co-transcribed (RNAII) [19]. In this study, we focused on the *agr* group specificity and SCCmec types of the respective strains, besides the biofilm production, the antibiotic resistance profile, and the presence of IS256 and *ica* operon. SCCmec is a mobile genetic element that carries the *mecA* gene, which is responsible for methicillin resistance in staphylococci. We studied the correlation between the antibiotic resistance, including resistance to macrolides and lincosamides, IS256 presence, biofilm formation, and *agr* group specificity as well as the SCCmec types in nosocomial *S. epidermidis* isolates.

Materials and methods

Bacterial isolates, identification, and antibiotic susceptibility testing

In this study, a total of 200 *S. epidermidis* isolates (blood culture, catheter tips, drain catheters, implants, and biopsies) from hospitalized patients at the University Clinic Magdeburg, Germany, were analyzed.

The identification and susceptibility testing of the *S. epidermidis* isolates (penicillin, oxacillin, trimethoprim/sulfamethoxazole, erythromycin, clindamycin, gentamicin, ciprofloxacin, levofloxacin, tetracycline, vancomycin, teicoplanin, and linezolid) were performed by the automated VITEK 2® system (*bioMérieux*, Marcy-l'Etoile, France) [20]. The results were interpreted in accordance

to Clinical Laboratory Standards Institute (CLSI) [21] guidelines.

Biofilm production on Congo Red Agar (CRA)

Biofilm formation by *S. epidermidis* was screened according to the Freeman et al. [22] method for *Staphylococcus* isolates, which requires the use of a specially prepared solid medium brain heart infusion (BHI) broth supplemented with 5% sucrose and Congo red. The medium was composed of BHI (37 g/l), sucrose (50 g/l), agar (10 g/l), and Congo red stain (0.8 g/l). Congo red was prepared as a concentrated aqueous solution and autoclaved at 121 °C for 15 min separately from other medium constituents and was then added when the agar had cooled to 55 °C. Plates were inoculated and incubated aerobically for 24–48 h at 37 °C. Positive result was indicated by black colonies with a dry crystalline consistency.

DNA extraction

Chromosomal DNA was isolated from overnight cultures grown on blood agar at 37 °C. Genomic DNA was extracted by using the Qiagen® DNA extraction kit according to the manufacturer's suggestions (Qiagen, Hilden, Germany) with the modification that 20 µl of lysostaphin (Sigma; 1 mg/ml) and 20 µl lysozyme (Qiagen; 100 mg/ml) were added at the cell lysis step. The concentration of the DNA was assessed spectrophotometrically [20].

PCR method for amplification of ica operon

The presence of the entire *ica* operon in biofilm-producing strains and in non-producing *S. epidermidis* was checked by amplification of the gene loci, encompassing a region of the *icaADBC* locus, as described [23].

PCR method for detection of IS256 insertion element

PCR detection of the insertion element IS256 was performed as described before [24].

agr group-specific multiplex PCR

For analysis of the *agr* group specificity, PCR was carried with specific primers to amplify different *agr* groups according to the modified protocol of Li et al. [25]. The A primers were selected from conserved sequences to amplify a 1022-bp fragment of *agr* that is common to *agr* groups I, II, and III. The B primers were selected from the hypervariable region common to *agr* groups II and III. The PCR result for *agr* group I was negative but that of groups II and III was positive. With the C primers, the typing was

specific for *agr* group II, whereas *agr* groups I and III were negative.

Determination of SCCmec types

SCCmec types I to IV were determined by a multiplex PCR with specific primers and subsequent visualization of the amplified DNA fragment patterns by agarose gel electrophoresis as described by Oliveira et al. [26] and Kozitskaya et al. [27].

Multilocus sequence typing (MLST)

All isolates were analyzed by three MLST protocols, as previously described by Wisplinghoff et al. [28]. The sequences of both strands of all PCR products were resolved with an ABI 3700 automated sequencer (PE Applied Biosystems) with BigDye fluorescent terminators and the primers used in the initial PCR amplification. The alleles at each locus were distinguished by using sequence software (available from <http://www.mlst.net>). Allelic profiles, consisting of the allele numbers at each of the seven loci, were assigned to sequence types (STs).

Results

Two hundred *Staphylococcus epidermidis* isolates from hospitalized patients at the University Clinic of Magdeburg were collected and analyzed. Approximately one third was recovered from blood culture, whereas the other specimens were catheter tips, implant materials, and biopsies/punctures. The catheter tips were sub-grouped into central venous catheters, drain catheters, and other catheter types. Specimens like punctures, abscesses, and intra-surgical materials were grouped into biopsies/punctures. Few isolates were recovered from implants, total joint arthroplasty, pacemaker, and intrauterine devices.

Antibiotic resistance of *S. epidermidis*

Over 91% of the isolates were penicillin (PEN)-resistant and 76% oxacillin (OXA)-resistant. High resistance rates were detected against erythromycin (ERY, 70.5%), clindamycin (CLI, 67.5%), and ciprofloxacin (CIP, 57%). Resistance toward gentamicin (GEN) was observed in 46% of the isolates and toward trimethoprim-sulfamethoxazole (SXT) in 25% of the isolates. All isolates were susceptible toward vancomycin, teicoplanin, and linezolid. One hundred and fifty-two isolates were PEN- and OXA-resistant, whereas the antibiotic resistance profile PEN–OXA–CIP was observed in 103 isolates. Additional GEN resistance was detected in 71 of the isolates.

PEN–OXA–CIP–GEN–ERY resistance profile shared 62 (31%) isolates.

Multi-resistant profile (PEN–OXA–CIP–GEN–ERY–CLI) shared 41 (29.5%) isolates, and broader antibiotic resistance profile (PEN–OXA–CIP–GEN–ERY–CLI–SXT) was detected in 15.5% of the *S. epidermidis* isolates. Only 4% of the *S. epidermidis* isolates were susceptible to all tested antibiotics.

Biofilm formation

Thirty-two percent of the *S. epidermidis* isolates produced black colonies on the Congo red agar (CRA) and were therefore classified as biofilm producers. *S. epidermidis* isolates, which were gained from implants, produced biofilms most predominantly (46.2%). One third of the isolates from drain catheters and biopsies produced biofilm phenotypically. Seventy-five percent of the *S. epidermidis* isolates from blood culture and central venous catheter tips produced no biofilm phenotypically.

Prevalence of *ica* genes

By use of PCR, the *icaA/D* genes were detected in a duplex amplification assay. Sixty-one *S. epidermidis* isolates were *icaA/D*-positive and produced biofilm phenotypically. Three *icaA/D*-negative *S. epidermidis* isolates somehow produced biofilm phenotypically. In total, 136 isolates were phenotypic biofilm-negative by use of Congo red agar. Sixty-three of these were *icaA/D*-positive and 73 *icaA/D*-negative (Table 1).

Of the 124 *icaA/D*-positive isolates, only 49% were able to produce biofilm in vitro. In 76 *S. epidermidis* isolates, the *ica* genes were not detectable. On Congo agar, approximately 96% of the *icaA/D*-negative isolates did not produce biofilm in vitro.

Table 1. Analysis of pheno- and genotypic biofilm production

<i>icaA/D</i>	Biofilm		Total
	Positive	Negative	
Positive	61	63	124
Negative	3	73	76
Total	64	136	200

Insertion element IS256

The PCR analysis revealed that 105 of the total 200 *S. epidermidis* isolates (52.5%) were positive for the insertion element IS256. When the multi-resistance was considered within the total 200 isolates, the positivity for IS256 was 95.1%. In phenotypic biofilm-negative and *icaA/D*-positive isolates, the insertion element IS256 was detected in 63.5%.

agr group specificity

The *accessory gene regulator (agr)* system occurs in commensals and pathogenic *S. epidermidis* isolates and regulates the production of virulence factor genes. Multiplex PCR analysis was performed to group the isolates. Thirty-nine (19.5%) *S. epidermidis* isolates were grouped in *agr* I and 66 (33%) in *agr* II. Another 70 (35%) isolates were categorized in *agr* III. The remaining 25 (12.5%) of the isolates were *agr*-negative.

Among 41 *S. epidermidis* isolates with multi-resistance (PEN–OXA–CIP–GEN–CLI–ERY) and *icaA/D* positivity, *agr* groups I and III were the most prevalent, whereas *agr* group II was infrequent, and nine multi-resistant isolates (22%) were *agr*-negative.

SCCmec typing

SCCmec type I was most prevalent and was detected in 79 (39.5%) of the isolates. SCCmec type II was carried by 25 (12.5%) *S. epidermidis* isolates and SCCmec type III by 14 (7%) isolates. SCCmec type IV was detected in 10 (5%) isolates. Twenty-five isolates (12.5%) were *mecA*-positive but were not SCCmec-typeable. Forty-seven *S. epidermidis* isolates (23.5%) were negative for *mecA*. Among these, 42 isolates were susceptible toward oxacillin.

In 39% of the multi-resistant isolates (PEN–OXA–CIP–GEN–CLI–ERY), SCCmec type II was detected as the most frequent type. SCCmec type I was detectable in 19.5% and SCCmec type III in 12.2% of the multi-resistant isolates. SCCmec type IV was infrequent in among the unselected isolates of our study population, but increased in amount within the multi-resistant isolates up to 14.6%.

Approximately 10% of the multi-resistant *S. epidermidis* isolates were non-typeable, and another 5% were *mecA*-negative.

Correlation between antibiotic resistance profile, SCCmec types and *agr*

SCCmec type II isolates were significantly resistant to all antibiotics, excluding tetracycline, vancomycin, teicoplanin, and linezolid. SCCmec type III isolates indicated less resistance toward clindamycin (CLI) and erythromycin (ERY) but were mostly resistant toward ciprofloxacin (CIP). *S. epidermidis* isolates which carried SCCmec type IV were mostly resistant toward erythromycin (ERY), clindamycin (CLI), and ciprofloxacin (CIP), but demonstrated less resistance rates against tetracycline (TET), gentamicin (GEN), and sulfamethoxazole-trimethoprim (SXT).

Approximately all *agr* II isolates carried either SCCmec type I or were *mecA*-negative and non-typeable, whereas *agr* group I isolates were associated with the different SCCmec types and those which were non-typeable. The *agr* III isolates showed similar correlation. The infrequent *agr*-negative *S. epidermidis* isolates did not associate with a particular SCCmec type as well.

Correlation between *agr* group specificity and SCCmec type among the multi-resistant *S. epidermidis* isolates

SCCmec type II and the *agr* groups I and III were most predominantly detected in multi-resistant isolates. Also, a significant correlation between *agr* group I and SCCmec

Table 2. Distribution of *agr* groups in the different specimens (*CV, central venous)

<i>agr</i> Group	Blood culture (%)	CV* catheter (%)	Drain catheters (%)	Biopsies (%)	Implants (%)
I	23.4	21.6	14.3	18.2	7.7
II	21.9	41.2	46.4	22.7	61.5
III	48.4	23.5	32.1	36.4	15.4
Negative	6.3	13.7	7.2	22.7	15.4

Table 3. Distribution of SCCmec types in different specimens (*CV, central venous)

SCCmec type	Blood culture (%)	CV* catheter (%)	Drain catheters (%)	Biopsies (%)	Implants (%)
I	29.7	49.0	50.0	40.9	23.1
II	10.9	13.8	21.4	9.1	7.7
III	15.6	3.9	0	4.5	0
IV	1.6	7.8	7.2	4.5	7.7
Non-typeable	21.9	7.8	0	13.7	7.7
Negative	20.3	17.7	21.4	27.3	53.8

Table 4. MLST analysis of the *S. epidermidis* isolates ($n = 39$) recovered from the different specimen types

Specimen	Sequence type (ST)												
	ST2	ST5	ST6	ST10	ST21	ST23	ST32	ST43	ST48	ST59	ST130	ST135	ST242
Blood culture	6	4	1	2	2	1	1	–	2	3	2	–	1
CV catheter	–	–	–	–	–	1	–	1	–	–	–	1	2
Drain catheter	–	–	–	1	–	–	–	–	–	–	–	1	2
Implants	–	–	–	–	–	1	–	1	–	–	–	–	–
Biopsies	–	–	–	1	–	–	–	–	–	–	1	1	–
Total	6	4	1	4	2	3	1	2	2	3	3	3	5

type II was observed. Furthermore, in multi-resistant isolates, SCCmec type III was exclusively associated with *agr* group III. Between *agr* group III, *agr*-negative isolates, and SCCmec type II, a slight correlation was observed, with no significant preference for SCCmec type II.

SCCmec type and *agr* group specificity in dependency of the clinical samples

In *S. epidermidis*, isolates from central venous catheters, drains, and implants *agr* group II were most prevalent, whereas *agr* group III dominated in the isolates from blood cultures and biopsies (Table 2).

In *S. epidermidis*, isolates from blood cultures, catheters, and biopsies SCCmec I were the frequent type, whereas 50% of the isolates from implants were *mecA*-negative and non-typeable (Table 3).

Multilocus sequence typing (MLST) of selected *S. epidermidis* isolates

Due to economic reasons, 40 *S. epidermidis* isolates were selected for MLST analysis, 14 were isolated from biopsies, catheter tips, implants, and drain catheter and were resistant against penicillin, oxacillin, gentamicin, and ciprofloxacin. Additional 26 *S. epidermidis* isolates were from blood cultures due to clinical significance. One isolate was not assignable to a particular sequence type. The results of the MLST analysis are depicted in Table 4.

In total, 13 different STs were revealed. Approximately 47% of the isolates were assigned to the sequence types ST2, ST5, ST10, and ST242. Among these, ST2 occurred in 15% ($n = 6$ of 40), followed by ST242 (12.5%) and ST5 and ST10 with each 10%. The blood culture isolates were frequently typed as ST2; only a few were assigned to ST5 and ST59. *S. epidermidis* isolates from catheter tips were frequently assigned to ST242.

Table 5. Sequence types (frequent STs highlighted in bold) of *S. epidermidis* isolates and their genetic determinants (*agr*, *icaA/D*, SCCmec)

ST (n)	<i>icaA/D</i>		IS256		SCCmec						<i>agr</i>			
	pos	neg	pos	neg	I	II	III	IV	NT	neg	I	II	III	neg
2 (6)	6	–	5	1	–	3	3	–	–	–	2	–	3	1
5 (4)	1	3	1	3	3	–	–	–	1	–	–	3	1	–
6 (1)	1	–	1	–	1	–	–	–	–	–	–	1	–	–
10 (4)	1	3	1	3	4	–	–	–	–	–	2	–	2	–
21 (2)	–	2	–	2	–	–	1	–	–	1	1	1	–	–
23 (3)	3	–	3	–	1	–	–	1	1	–	–	1	2	–
32 (1)	–	1	–	1	–	–	–	–	–	1	–	1	–	–
43 (2)	2	–	2	–	–	1	1	–	–	–	–	–	2	–
48 (2)	2	–	–	2	–	–	–	–	2	–	–	–	2	–
59 (3)	2	1	1	2	–	–	–	–	2	1	–	1	2	–
130 (3)	1	2	1	2	1	–	–	–	1	1	1	–	2	–
135 (3)	3	–	3	–	1	1	1	–	–	–	–	–	3	–
242 (5)	5	–	4	1	1	2	1	1	–	–	1	–	4	–

n , number of isolates; neg, negative; pos, positive; NT, non-typeable

Occurrence of IS256, *icaA/D*, *mecA* and *agr* in sequence typed *S. epidermidis* isolates

A number of the *S. epidermidis* isolates carried the insertion element IS256 and the *icaA/D* genes. Correlation between SCCmec type I and sequence types ST5 and ST10 was observed. All ST2 and ST242 isolates were positive for *icaA/D* genes and the insertion element IS256 but were assigned to different SCCmec types. Table 5 summarizes the results for each sequence type.

Discussion

Approximately 91% of the *S. epidermidis* isolates were penicillin-resistant and 76% oxacillin-resistant. Our population was slightly more resistant compared to the study of Kresken et al. [29]. Their data revealed that 60% were oxacillin-resistant, and only 87% were penicillin-resistant. Our isolates were more resistant toward the other antibiotics as well: erythromycin (70.5% versus 55.3%), ciprofloxacin (57% versus 46.7%), and clindamycin (67.5% versus 34.8%).

Biofilm formation and insertion element IS256

Sixty-two percent of our isolates were *icaA/D*-positive, much more than what was determined in a study by Diamond-Hernández et al. [30] for *S. epidermidis*. Approximately 32% of our isolates were able to produce biofilm phenotypically. Thus, only 50% of these were *ica*-positive and produced biofilm on Congo red agar. Chaieb et al. [31] determined higher proportion of *icaA/D*-positive isolates (72.7%), which phenotypically produced biofilm. Probably, other factors besides the *ica* genes may have an influence in biofilm formation [14]. Other factors, e.g., environment, nutrition subinhibitory concentrations of diverse antibiotics (tetracycline, gentamicin, clindamycin, teicoplanin), and stress (temperature, osmolarity), might have a significant role in biofilm formation and/or repression [17].

In our population, a quarter of the isolates from blood cultures and catheter tips produced biofilm. However, more isolates from implants (46.2%) produced biofilm. The comparison between the different specimens is not equivalent, since 64 isolates were from blood cultures and only 13 isolates were recovered from implants. The isolates in our study and elsewhere, which produced biofilm, were less susceptible toward a number of antibiotics [32–36]. A possible reason for the higher antibiotic resistance may be the reduced growth of the bacteria within the biofilm or the insufficient uptake of antibiotics [37]. Also, Saginur et al. [38] determined a broader antibiotic resistance in biofilm-positive *S. epidermidis* and recommended combinations of antibiotics with rifampicin for sufficient therapy. Three of our isolates were biofilm producers although *ica* genes were not detected. Chaieb et al. [31] described *ica*-

positive *S. epidermidis* isolates which did not produce biofilm and *S. epidermidis* which was *ica*-negative but was somehow able to produce biofilm. Similar results were reported by Kogan et al. [39]. In our study, two of the three biofilm producers were IS256-positive. Possible IS256 insertion within the *ica* gene complex might disrupt the PCR detection of *ica* genes. Ziebuhr et al. [9, 10] described a phase variation process. Upon activation of this operon, a polysaccharide intercellular adhesin (PIA) is synthesized which supports bacterial cell-to-cell contacts and triggers the production of thick, multilayered biofilms. PIA synthesis undergoes a phase variation process. The phase variation and transposition of IS256 into the *ica* gene complex might be the reason for the *icaA/D*-positive isolates which did not phenotypically produce biofilm since 63.5% of the isolates were IS256-positive. Thus, naturally occurring insertion sequence element is actively involved in the modulation of expression of a *Staphylococcus* virulence factor, e.g., biofilm formation.

About 52.5% of the *S. epidermidis* isolates were IS256-positive, but 95% of the multi-resistant isolates were IS256-positive. Supporting our data, Gu et al. [24] determined similar results and concluded that IS256 is a good marker gene to differentiate between invasive and non-invasive isolates. We observed the correlation between the IS256 and the *icaA/D* genes. In 79% of the IS256-positive isolates, the *icaA/D* genes were detectable. Same results were reported by Kozitskaya et al. [14]. Moreover, 47% of the IS256- and *ica*-positive *S. epidermidis* isolates shared a multi-resistant profile (PEN–OXA–CIP–GEN–ERY–CLI), and 66% were resistant toward PEN–OXA–GEN. Also, Kozitskaya et al. [14] described the correlation between IS256 and the resistance toward oxacillin and gentamicin. The insertion element IS256 more frequently occurs in multi-resistant and *ica*-positive isolates.

agr group specificity and antibiotic resistance

In contrast to *S. aureus*, in *S. epidermidis*, three *agr* groups have been described [25, 40]. In our population, *agr* II and III were most prevalent with 33% and 35%, respectively. Twenty percent of the isolates were assigned to *agr* I, and 25 isolates were *agr*-negative. Li et al. [25] detected two thirds of their clinical isolates *agr* I, whereas *agr* II and III were prevalent in 19.3% and 12.5% of the isolates from Chinese population. The difference in the distribution may be caused by geographical and environmental factors.

In multi-resistant isolates, the distribution of the *agr* groups shifted in a manner that *agr* I and III dominated with each 34.1%, whereas *agr* II was detectable in 9.8% of the multi-resistant isolates. Twenty-two percent among these were *agr*-negative. The fact that *agr* I was relatively higher in multi-resistant isolates than in the general population could be due to the clinical relevance of such isolates and may emphasize the pathogenic potential. This finding was supported by Li et al. [25]. Among the multi-resistant group, *agr*-negative isolates could be mutants. These *agr*

mutants may reflect high primary adhesion during the first phase of biofilm formation and therefore benefit during colonization [41].

Vuong and co-workers described *S. epidermidis* isolates, which were permanently *agr*-negative [42]. The *agr*-negative isolates were observed more dominantly in implant-associated infections and were more successful in colonization of surfaces [42].

Besides the *agr* system, there is another *quorum-sensing* system *luxS* in *S. epidermidis*. Similar to *agr*, the *luxS* system has an inhibitory influence during biofilm formation [43] and probably plays a particular role in the regulation of biofilm production in *agr*-negative isolates.

SCCmec typing

SCCmec type I was most prevalent in the study isolates (39.5%), whereas 23.5% were not SCCmec typeable. Mombach Pinheiro Machado et al. [44] studied 129 methicillin-resistant coagulase-negative staphylococci isolated from blood cultures and determined SCCmec type I as most prevalent in *S. epidermidis*; similar to our study, SCCmec IV was infrequent, and very few isolates were non-typeable. The difference in distribution of the SCCmec between our study and that of Mombach Pinheiro Machado et al. [44] in Brazil was the number of SCCmec types II and III. They had no SCCmec type II in their population. In our population, the proportion was 12.5% for this type. Possible rationale for this difference may be the variant therapy, the divergent local antibiotic resistance, and the patient population with their environment. Miragaia et al. [45] studied exclusively methicillin-resistant *S. epidermidis*, and SCCmec IV was the most prevalent type, followed by type III. A number of isolates were also non-typeable. Wisplinghoff et al. [28] determined a different distribution of SCCmec types in methicillin-resistant *S. epidermidis* isolates as well. They detected SCCmec IV as the most dominant type followed by types II and III, rarely SCCmec type I.

The different distribution of the SCCmec types in *S. epidermidis* isolates shown in the diverse studies indicates that the genetic element SCCmec is easily being transferred between the different staphylococci [28, 45–48].

SCCmec type II isolates were mostly resistant toward all antibiotics, excluding tetracycline, glycopeptides, and linezolid, whereas SCCmec type IV isolates in comparison to other types showed more resistance toward tetracycline and trimethoprim-sulfamethoxazole. The SCCmec non-typeable isolates shared the resistance profile against clindamycin and erythromycin, in contrast to SCCmec type III isolates with low resistance rates toward both antibiotics. The results for SCCmec types I and III were supported by those of Mombach Pinheiro Machado et al. [44], although they detected for both types higher resistance rates against gentamicin and trimethoprim-sulfamethoxazole and for SCCmec type I lower resistance rates toward clindamycin and erythromycin. For SCCmec type III iso-

lates, Mombach Pinheiro Machado et al. [44] described much higher resistance rates toward clindamycin and erythromycin. The divergent rates may be reflected by the geographical location of the study population, administration, and consumption of different antibiotics.

Multilocus sequence typing

Within our 40 *S. epidermidis* isolates, ST2 (15%), ST242 (12.5%), ST5, and ST10 (each 10%) dominated. Kozitskaya et al. [27] sequenced *S. epidermidis* isolates which were collected from German, Irish, Norwegian, and North American population. ST27 was the dominant sequence type they identified, followed by ST2 and sporadically ST5. Both ST2 and ST5 were mostly *mecA*-negative and were sporadically *ica* gene-positive. Miragaia et al. [45] studied *S. epidermidis* from patients and healthy controls in 17 countries, including Denmark, Island, Portugal, and Bulgaria. They identified 74 different sequence types with ST2 as the most prevalent type. ST2 was assigned to SCCmec types II, III, IV, and non-typeable. Li et al. [49] studied 80 *S. epidermidis* isolates from patients in Shanghai and determined 16 different sequence types. Also, in their study, ST2 was most prevalent. ST2 was *ica*-positive, phenotypically produced biofilm, and was a carrier of IS256 and SCCmec type III.

Thirty-five oxacillin-resistant *S. epidermidis* isolates from Brazil were studied by Iorio et al. [50]. ST2 (45.7%) was the predominant sequence type, followed by ST23 (25.7%). All ST2 isolates were positive for the *ica* genes and carried SCCmec type III or were non-typeable (four isolates). ST23 isolates were also *ica*-positive and were mostly SCCmec type IV carriers.

In our study, ST2 was also the most dominant type and *icaA/D*-positive. About 83.33% of these isolates were carriers of IS256 and equally of SCCmec types II and III. Therefore, our data support the data of Li et al. [49] and Iorio et al. [50]. Our ST23 isolates, similar to those of Iorio et al. [50], were exclusively *ica*-positive. However, Iorio et al. [50] assigned the ST23 isolates to SCCmec type IV, whereas our ST23 isolates were grouped into SCCmec I and IV. Differences were also observed between the studies concerning ST5 and ST10. In our study, both sequence types were carriers of SCCmec I and were up to 75% positive for *icaA/D* but IS256-negative. In contrast, Li et al. [49] detected ST5 as a carrier of SCCmec V and ST10 exclusively as SCCmec IV carrier and, in most cases, IS256-positive. Consistent with our data, they detected low rate of *icaA/D*-positive isolates typed as ST5 and ST10.

An explanation for the divergent results may be the study population; the above-stated groups compared isolates from patients and healthy individuals, whereas our study population and that of Li et al. [49] included only hospitalized patients.

In our study, most of the ST2 isolates were acquired from blood cultures, followed by ST5 and ST59 isolates. ST242 isolates were recovered from catheter tips.

Otherwise, there was no significant correlation between the identified STs and the sampling type or location.

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