Detection of Virulence-Associated Genes Not Useful for Discriminating between Invasive and Commensal Staphylococcus epidermidis Strains from a Bone Marrow Transplant Unit

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Because of their biofilm-forming capacity, invasive Staphylococcus epidermidis isolates, which cause the majority of nosocomial catheter-related bloodstream infections (BSIs), are thought to be selected at the time of catheter insertion from a population of less virulent commensal strains. This fact allows the prediction that invasive and contaminating strains can be differentiated via detection of virulence-associated genes. However, the hospital environment may pave the way for catheter-related infections by promoting a shift in the commensal bacterial population toward strains with enhanced virulence. The distribution of virulence-associated genes (icaADBC, aap, atIE, bhp, fbe, embp, mecA, IS256, and IS257), polysaccharide intercellular adhesin synthesis, and biofilm formation were investigated in S. epidermidis strains from independent episodes of catheter-related BSIs in individuals who have received bone marrow transplantation (BMT). The results were compared with those obtained for commensal S. epidermidis isolates from hospitalized patients after BMT and from healthy individuals, respectively. The clonal relationships of the strains were investigated by pulsed-field gel electrophoresis. icaADBC, mecA, and IS256 were significantly more prevalent in BSI isolates than in commensal isolates from healthy individuals. However, the prevalence of any of the genes in clonally independent, endogenous commensal strains from BMT patients did not differ from that in invasive BSI strains. icaADBC and methicillin resistance, factors important for the establishment of catheter-related infections, already ensure survival of the organisms in their physiological habitat in the hospital environment, resulting in a higher probability of contamination of indwelling medical devices with virulent S. epidermidis strains. The dynamics of S. epidermidis populations reveal that detection of icaADBC and mecA is not suitable for discriminating invasive from contaminating S. epidermidis strains.

Nosocomial bloodstream infections (BSIs) are a common and severe complication after bone marrow transplantation (BMT) (19). Coagulase-negative staphylococci, especially Staphylococcus epidermidis, which is a normal commensal organism of the skin, are encountered most frequently (19, 27). S. epidermidis BSIs typically occur in the context of implanted central venous, Hickman, or Quinton catheters. The reason for this tight pathogenic relationship is the ability of S. epidermidis to establish foreign body-adherent biofilms, which consist of multilayered bacterial communities stabilized by intercellular adhesive mechanisms (2, 8, 20). S. epidermidis biofilm formation depends on the production of the polysaccharide intercellular adhesin (PIA), synthesized by icaADBC-encoded proteins (7, 10). The important roles of icaADBC, PIA synthesis, and biofilm formation in the pathogenesis of a S. epidermidis biofilm are well known. Other factors involved in foreign body colonization, like the autolysin AtIE, the fibrinogen binding protein Fbe, and the accumulation-associated protein Aap, displayed no differential distribution in invasive and commensal S. epidermidis isolates (5, 6, 31). However, in those studies data for invasive strains were compared with data for commensal strains from healthy individuals with no contact with the hospital environment. Therefore, as S. epidermidis infections are typically endogenous in character (20), the S. epidermidis populations from which invasive strains are recruited, i.e., the commensal strains of hospitalized patients, were neglected, until now. Conse-
quently, the aim of the present study was to investigate the ecology of invasive and commensal *S. epidermidis* populations in patients after BMT by analyzing the distribution of icaADBC as well as those of atle (9), fbe (25), and aap (13), all of which represent additional factors involved in the pathogenesis of *S. epidermidis*. Furthermore, the presence of the newly identified *S. epidermidis* homologue of the biofilm-associated protein Bap (called bhp) from *Staphylococcus aureus* (3) and the fibronectin binding protein Embp (32) and the presence of insertion sequences IS256 and IS257 were characterized. Both populations of hospital isolates were compared with commensal *S. epidermidis* isolates from healthy individuals with no contact with the hospital environment in order to tag the genetic virulence markers relevant in this specific clinical context and to characterize the course of selection events leading to a shift in the genetic population structure.

**MATERIALS AND METHODS**

**Bacterial strains.** All *S. epidermidis* isolates were identified as *S. epidermidis* by negativity for the clumping factor and with the ID32Staph system (bioMérieux, Mary l’Etoile, France). Reference strains *S. epidermidis* 1457 and biofilm-negative transposon mutant 1457-M10 have been described previously (21). The negative for the clumping factor and with the ID32Staph system (bioMérieux, Mary l’Etoile, France). Reference strains *S. epidermidis* 1457 and biofilm-negative transposon mutant 1457-M10 have been described previously (21). The

**TABLE 1. Distribution of virulence-associated genes in different *S. epidermidis* populations**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Invasive strains (n = 16)</th>
<th>Commensal strains, BMT patients (n = 25)</th>
<th>Commensal strains, healthy subjects (n = 15)</th>
<th>Primers</th>
<th>Reference or conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>icaA</td>
<td>15 (93.8)</td>
<td>20 (80)</td>
<td>2 (13)</td>
<td>Forward, 5'-TCG ATG CGA TTT GTT CAA ACA T-3'; reverse, 5'-CTG TTT CAT GGA AAC TCC-3'</td>
<td>15</td>
</tr>
<tr>
<td>aap</td>
<td>15 (93.8)</td>
<td>23 (92)</td>
<td>13 (86.7)</td>
<td>Forward, 5'-AAA CGG TGG TAT CTT ACG TGA A-3'; reverse, 5'-CAA TGT TGG ACC ATC TAA ATC AGC T-3'</td>
<td>5 min, 94°C; 30 cycles of 30 s, 94°C; 30 s, 60°C; 50 s, 72°C; final extension, 4 min, 72°C</td>
</tr>
<tr>
<td>bhp</td>
<td>3 (18.8)</td>
<td>4 (16)</td>
<td>2 (26.7)</td>
<td>Forward, 5'-ATG GTA TTA GCA AGC TCT CAG CTG G-3'; reverse, 5'-AGG GTT TTC ATC TGG ATC CG-3'</td>
<td>5 min, 94°C; 30 cycles of 30 s, 94°C; 30 s, 61°C; 90 s, 72°C; final extension, 4 min, 72°C</td>
</tr>
<tr>
<td>fbe</td>
<td>16 (100)</td>
<td>25 (100)</td>
<td>15 (100)</td>
<td>Forward, 5'-CTA CAA GTT CAG GTC AAG GAC AAG G-3'; reverse, 5'-GGG TCG GCC TAT TTC CTG CAG-3'</td>
<td>5 min, 94°C; 30 cycles of 30 s, 94°C; 30 s, 60°C; 50 s, 72°C; final extension, 4 min, 72°C</td>
</tr>
<tr>
<td>embp</td>
<td>14 (87.5)</td>
<td>19 (76)</td>
<td>15 (100)</td>
<td>Forward, 5'-AGG GGT ACA AAT GTC AAT-3'; reverse, 5'-AGT AGT GCT CTAG CAT CAT CC-3'</td>
<td>32</td>
</tr>
<tr>
<td>atle</td>
<td>16 (100)</td>
<td>25 (100)</td>
<td>15 (100)</td>
<td>Forward, 5'-CAA CTG CTC AAC CGA GAA CA-3'; reverse, 5'-TTT GGA GAT GGT GTG CCC CA-3'</td>
<td>5 min, 94°C; 30 cycles of 30 s, 94°C; 30 s, 55°C; 30 s, 72°C; final extension, 4 min, 72°C</td>
</tr>
<tr>
<td>mecA</td>
<td>14 (87.5)</td>
<td>24 (96)</td>
<td>1 (6.7)</td>
<td>Forward, 5'-GAA ATG ACT GAA CGT CCG AT-3'; reverse, 5'-GCC ATC AAT GTC ACC GTA GT-3'</td>
<td>5 min, 94°C; 30 cycles of 30 s, 94°C; 30 s, 55°C; 30 s, 72°C; final extension, 4 min, 72°C</td>
</tr>
<tr>
<td>IS256</td>
<td>15 (93.8)</td>
<td>19 (76)</td>
<td>0</td>
<td>Forward, 5'-TGA AAA GCG AAG AGA TTC AAA GC-3'; reverse, 5'-ATG TAG GTC CAT AAG AAC GGC-3'</td>
<td>35</td>
</tr>
<tr>
<td>IS257</td>
<td>16 (100)</td>
<td>25 (100)</td>
<td>15 (100)</td>
<td>Forward, 5'-ACG TTC ATC ATG CAA CGG TC-3'; reverse, 5'-AGT GTT CGC TTA ACT TGC TAG-3'</td>
<td>5 min, 94°C; 30 cycles of 30 s, 94°C; 30 s, 55°C; 30 s, 72°C; final extension, 4 min, 72°C</td>
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</tr>
<tr>
<td>Forward, 5'-ATG GTA TTA GCA AGC TCT CAG CTG G-3'; reverse, 5'-AGG GTT TTC ATC TGG ATC CG-3'</td>
</tr>
<tr>
<td>Forward, 5'-CTA CAA GTT CAG GTC AAG GAC AAG G-3'; reverse, 5'-GGG TCG GCC TAT TTC CTG CAG-3'</td>
</tr>
<tr>
<td>Forward, 5'-AGG GGT ACA AAT GTC AAT-3'; reverse, 5'-AGT AGT GCT CTAG CAT CAT CC-3'</td>
</tr>
<tr>
<td>Forward, 5'-CAA CTG CTC AAC CGA GAA CA-3'; reverse, 5'-TTT GTA GAT GTT GTG CCC CA-3'</td>
</tr>
<tr>
<td>Forward, 5'-GAA ATG ACT GAA CGT CCG AT-3'; reverse, 5'-GCC ATC AAT GTC ACC GTA GT-3'</td>
</tr>
<tr>
<td>Forward, 5'-TGA AAA GCG AAG AGA TTC AAA GC-3'; reverse, 5'-ATG TAG GTC CAT AAG AAC GGC-3'</td>
</tr>
<tr>
<td>Forward, 5'-ACG TTC ATC ATG CAA CGG TC-3'; reverse, 5'-AGT GTT CGC TTA ACT TGC TAG-3'</td>
</tr>
</tbody>
</table>

GenBank accession numbers are given in parentheses.

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d) P = 0.04 for invasive strains versus commensal strains from BMT patients; P = 0.0002 for commensal strains from BMT patients versus commensal strains from healthy subjects; P < 0.0001 for comparison of all three groups of strains.

e) P = 0.68 for invasive strains versus commensal strains from BMT patients; P = 1.0 for commensal strains from BMT patients versus commensal strains from healthy subjects; P = 0.95 for comparison of all three groups of strains.

f) P = 0.84 for invasive strains versus commensal strains from BMT patients; P = 0.68 for commensal strains from BMT patients versus commensal strains from healthy subjects; P = 0.92 for comparison of all three groups of strains.

g) Statistical analysis is not applicable.

h) P = 0.61 for invasive strains versus commensal strains from BMT patients; P = 0.1 for commensal strains from BMT patients versus commensal strains from healthy subjects; P = 0.49 for comparison of all three groups of strains.

i) P = 0.49 for comparison of all three groups of strains.

j) P = 0.68 for invasive strains versus commensal strains from BMT patients; P < 0.0001 for commensal strains from BMT patients versus commensal strains from healthy subjects; P < 0.0001 for comparison of all three groups of strains.

k) P = 0.28 for invasive strains versus commensal strains from BMT patients; P < 0.0001 for commensal strains from BMT patients versus commensal strains from healthy subjects; P < 0.0001 for comparison of all three groups of strains.

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famethoxazole, and tobramycin; in addition, all patients received fluconazole or amphotericin B as well as acyclovir and pentamidine) when blood was drawn for culture. These S. epidermidis strains were regarded as invasive strains, as at least two clonally identical or closely related isolates (30) were obtained from cultures of blood drawn at different time points or from independent cultures of blood drawn at one time per patient. In addition, all patients exhibited systemic signs of infection (fever and elevated C-reactive protein levels), and 15 of 16 patients with a vascular access device in situ showed local inflammatory signs at the catheter insertion site.

(ii) Commensal strains from patients after BMT. Twenty-five clonally independent S. epidermidis isolates were recovered from September 2002 to February 2003 from nose, mucous membrane, and skin swabs taken from 17 patients after BMT. All patients received prophylaxis (quinolones, fluconazole, trimethoprim-sulfamethoxazole, and acyclovir) but did not exhibit signs of infection at the time of sampling.

(iii) Commensal strains from healthy individuals. Fifteen clonally independent S. epidermidis isolates from the noses of 15 healthy volunteers were investigated.

PFGE. Pulsed-field gel electrophoresis (PFGE) of SmaI-cleaved DNA was carried out as described previously (22) with a CHEF-DR II system (Bio-Rad, Munich, Germany).

In vitro biofilm formation and detection of PIA synthesis. S. epidermidis biofilm formation on polymeric surfaces was tested by the semiquantitative microtiter plate test (biofilm assay) with Trypticase soy broth (Becton Dickinson, Cockeysville, Md.) and Trypticase soy broth supplemented with 2% (wt/vol) NaCl as described previously (15, 21). To qualitatively and semiquantitatively detect PIA synthesis, a coagglutination assay (21) using a PIA-specific antiserum and an antiserum specific for a variant PIA (PIAv) antisera was performed essentially as described previously (15, 21).

Detection of icaA, B, C, D, E, and F genes and determination of aap repeat polymorphism. DNA preparation and PCR were performed as described previously (26). The primers and PCR conditions are summarized in Table 1. The aap repeat region (Fig. 1) was amplified by use of the Expand Long Template PCR system (Roche, Mannheim, Germany) under the conditions recommended by the manufacturer with a forward primer (5′-GAT TTA GAT GGT GCA ACA TTG ACA T-3′) located at the 5′ end and a reverse primer (5′-TTG ACG ATT TTC ACC TGT ATC AGG T-3′) located at the 3′ end. Gel-Pro Analyzer (version 3.1) software (Media Cybernetics, Silver Spring, Md.) was used to estimate the PCR fragment sizes.

RESULTS

In order to rule out the possibility of infections due to one endemic S. epidermidis strain, all isolates were typed by PFGE. Comparison of the restriction patterns of strains from different BSI episodes revealed that 10 of 16 BSI episodes were due to clonally unrelated S. epidermidis strains. The remaining six BSI episodes were attributable to S. epidermidis strains belonging to two PFGE types (three strains each of PFGE types I and II; Fig. 2A). PFGE patterns that displayed two to three fragment differences were classified as different types. These clonally related strains were isolated over a period of 5 years and could be differentiated by further subtyping (Fig. 2B). Thus, all BSIs investigated were caused by distinct S. epidermidis strains not representing derivatives of an endemic strain.

The commensal S. epidermidis strains isolated from patients after BMT all displayed unique PFGE patterns and were not related to any of the invasive strains according to their PFGE types (data not shown), demonstrating that during the collection period the patients were not colonized with a single dominant S. epidermidis clone. The S. epidermidis strains isolated from healthy volunteers were all clonally independent from the other strains investigated.

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ing those gene loci suitable markers for invasive strains in BSIs after BMT. However, the data for the commensal strains isolated from BMT patients show that the hospital environment already exerts selective pressure on the commensal *S. epidermidis* population, leading to a high prevalence of *icaADBC*-*, mecA*- and IS256-positive strains, making a differentiation between invasive and colonizing strains on the basis of the detection of these genes impossible.

A difference between invasive BSI strains and commensal *S. epidermidis* strains was found by detection of a length polymorphism of domain B of *aap* (Fig. 2; Fig. 2B), which probably resulted from variations in the number of repeats (Fig. 1). In commensal strains from healthy individuals as well as from BMT patients, an amplicon size larger than 4.2 kb, which comprised about 11 copies of the 128-amino-acid (aa) (384-bp) repeat, was found significantly more often than in the invasive strains (Fig. 3). The size of the repeat domain was stable for subsequent isolates of a strain isolated from a given BSI episode. Interestingly, the sizes of the *aap* repeat domains of strains of PFGE types I and II differed (Fig. 2B), allowing further subtyping.

**DISCUSSION**

Biofilm formation plays the key role in *S. epidermidis* foreign body colonization and infection (20). PIA, synthesized by *icaADBC*-encoded proteins, is essential in this process. Until now, studies investigating the distribution of virulence factors in different *S. epidermidis* populations found a much lower prevalence of *icaADBC* in commensal *S. epidermidis* strains than in invasive strains, whereas other genes, like *fbe*, *atlE*, and *aap*, were all widely distributed in both populations (5, 6, 31, 34). Therefore, according to the present understanding of the pathogenesis of foreign body infections, *icaADBC*-positive strains, which usually represent only a small part of the mainly *icaADBC*- and biofilm-negative commensal *S. epidermidis* population, are preferentially selected at the time of foreign body insertion and colonization due to their biofilm-forming capac-
positive *S. epidermidis* strains, a hypothesis that is supported by the finding that commensal *S. epidermidis* strains isolated from patients hospitalized on a surgical intensive care unit displayed a similar high prevalence of *ica*ADBC, *mec*A, and IS256 (H. Rohde, M. Kalitzky, and D. Mack, unpublished results). Additionally, de Silva et al. (4) also failed to find a difference in rates of *ica*ADBC detection between *S. epidermidis* strains from neonates with BSIs and commensal strains from babies hospitalized in the same intensive care unit. Consequently, the detection of *ica*ADBC and *mec*A appears to be by no means suitable for discriminating invasive and colonizing strains. Furthermore, identification of distinct factors promoting the high prevalence of *ica*ADBC- and *mec*A-positive strains in various clinical settings is demanded in order to develop preventive strategies.

The selective pressure exerted by antibiotics reasonably explains the shift toward a *mec*A-positive *S. epidermidis* population (1, 11). The preferential detection of *ica*ADBC- and biofilm-positive *S. epidermidis* strains can be explained by the linkage of oxacillin resistance expression, biofilm formation, and *ica*ADBC transcription (23, 24) Furthermore, *S. epidermidis* isolates organized in a biofilm are naturally more resistant to antibiotics and alcoholic skin disinfectants (15, 23). Therefore, measures intended to prevent infections on a BMT unit would directly pave the way for hard-to-treat biofilm-related *S. epidermidis* foreign body infections caused by methicillin-resistant strains. With respect to catheter-related BSIs, our findings appear to be a plausible molecular rationale for Infectious Diseases Society of America guidelines recommending the restricted use of antibiotic prophylaxis in afebrile neutropenic patients (12).

It still remains an important question whether all *S. epidermidis* strains selected by the hospital environment finally have the ability to cause foreign body infections or if a distinct subpopulation carrying specific determinants exists. Evidence for further changes in the population profile comes from the detection of *aap* repeat length polymorphisms within BSI and commensal strains. In addition, the majority of invasive *ica*ADBC-positive strains were also biofilm positive, whereas most commensal *S. epidermidis* isolates from BMT patients were biofilm negative, despite an *ica*ADBC-positive genotype. de Silva et al. (4) reported similar results, interpreting this as evidence for differences in the expression control of *ica*ADBC in those strains. Further studies by alternative genetic methods like assays with microarray technology and multilocus sequence typing are warranted in order to identify and characterize *S. epidermidis* subpopulations with enhanced virulence.

**ACKNOWLEDGMENTS**

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