Staphylococcus epidermidis surface protein I (SesI): a marker of the invasive capacity of S. epidermidis?

Staphylococcus epidermidis is a commensal that comprises a substantial part of the normal skin flora of humans. The pathogenic potential of this bacterium is, however, low. Nevertheless, when the skin barrier is breached, serious infections can occur, and S. epidermidis has emerged as a major cause of foreign body infections, as well as infections in immunocompromised patients and neonates (Von Eiff et al., 2002; Ziebuhr et al., 2008; Uc¸kay et al., 2009).

The virulence factors of S. epidermidis have not been fully characterized (Gill et al., 2008; Uc¸kay et al., 2009). Among the virulence factors described (Comfort & Clubb, 2004), SrtA protein sorting A (SrtA) has been identified (Bjertsjø et al., 2005; Bowden et al., 2005). SrtA is a sortase named surface (Ses) proteins (Bjertsjø Rennermalm, 2005; Bowden et al., 2005; Gill et al., 2005). One of these, SesI, consists of 201 amino acids and displays the LPETG motif as a sortase substrate. This protein elicits an immune response with antibody production if the patient is infected with an S. epidermidis isolate expressing SesI during infection (Bowden et al., 2005). These antibodies have also been found to be opsonic, and macrophage phagocytosis is enhanced by antibodies against SesI (Bjertsjø Rennermalm, 2005).

The present work determined the presence of the gene encoding SesI, using a real-time LightCycler PCR system detecting 408 of its 606 bp DNA sequence, among S. epidermidis isolates obtained from several patient groups that commonly experienced S. epidermidis infections. Thirty-two S. epidermidis isolates obtained during revision operations (multiple tissue biopsies, usually ≥5 samples) for prosthetic joint infections (hip, n=24; knee, n=7; elbow, n=1) with extraction or exchange were analysed.

Fifty-one S. epidermidis blood isolates collected from patients with haematological malignancies, which had been previously phenotypically and genotypically characterized, were investigated (Persson et al., 2006). The findings of S. epidermidis isolates in blood cultures were classified into two groups. Growth of S. epidermidis in both sets of two blood cultures (i.e. four positive cultures), comprising 29 S. epidermidis isolates, was taken to indicate true bacteraemia, while growth in only one of four culture bottles, comprising 22 isolates, was taken to indicate contamination. In addition, 26 S. epidermidis isolates obtained intraoperatively from cardiac surgery patients included in the LOGIP (local gentamicin for sternal wound infection prophylaxis) trial (Friberg et al., 2005), and previously phenotypically characterized (Olsson et al., 2007), were analysed. Nineteen isolates were from multiple samples (i.e. ≥2 cultures) obtained at reoperation of patients with postoperative sternal wound infection and were regarded as clinically significant isolates. Seven S. epidermidis isolates, interpreted as contaminants, were obtained from cultures from patients who underwent reoperations for indications other than postoperative sternal wound infection, e.g. postoperative tamponade or bleeding, or from a single culture where multiple cultures were negative or displayed the presence of other pathogens. Finally, 13 S. epidermidis isolates from the anterior nares and 11 isolates from the skin of the wrists of 24 healthy individuals without any association with healthcare were also analysed as being representative of commensals.

All isolates were characterized to the species level using the 32 ID API Staph system (bioMérieux). DNA isolation from the reference strain, S. epidermidis RP62A (ATCC 35984), was performed using the MagNA Pure compact system (Roche Molecular Biochemicals) according to the instructions of the manufacturer. Genomic DNA of the clinical isolates was extracted by denaturing one to five colonies suspended in 100 μl sterile water at 98 °C for 15 min and then centrifuging at 12 000 g for 5 min. The non-diluted supernatant fraction was used as a template for amplification in the real-time PCR.

The PCR for detection of the sesl gene and screening of clinical isolates was performed in a LightCycler 2.0 system (Roche Molecular Biochemicals) using SYBR Green I fluorescence melting curve analysis to detect the specific amplicon. The PCR
mixture contained 0.5 μM each primer (5'-GCT GAT TAT GTA AAT GAC TCA AAT-3', 5'-AGC TTT TGT TGT TTG AGC TTC-3') (Scandinavian Gene Synthesis), 3 mM MgCl₂, 1 x LightCycler FastStart DNA master SYBR Green I (Roche Molecular Biochemicals) and PCR-grade H₂O. The PCR programs started with a pre-incubation at 95 °C for 10 min, followed by 35 cycles of amplification (10 s denaturation at 95 °C, 10 s annealing at 57 °C and 16 s extension at 72 °C); the programs ended with melting curve analysis. In each PCR run, the reference strain was included as a positive control and water was used instead of the DNA template for the negative control.

Initial confirmation of the PCR product was made by means of 2% agarose gel electrophoresis and DNA sequencing using the ABI BigDye terminator v 1.1 cycle analyzer (Applied Biosystems). The ABI Prism 310 genetic analyzer (Applied Biosystems) and the ABI Prism 310 genetic analyzer (Applied Biosystems). The sequence of a 389 bp amplification product was found to be 100% identical with the sesl gene of S. epidermidis strain RP62A (GenBank accession number CP000029.1; base position 1693743–1693355).

The presence of the sesl gene was more prevalent, although this was not statistically significant, in isolates from all three patient groups than among comparable and representative contaminants (Table 1). However, none of the S. epidermidis isolated from the skin or the nares of healthy individuals representing commensals carried the sesl gene. Comparing S. epidermidis isolates from the three groups of clinically infected patients with the commensals, highly significant differences were found (P<0.0001, Fisher’s exact test).

This may indicate that the Sesl protein is a virulence factor of S. epidermidis or at least a marker of invasive capacity. Since healthy people do not regularly carry sesl-positive strains it could be assumed that patients may acquire the sesl-positive strains from the hospital environment after admission and thus rapidly become colonized. This emphasizes the importance of a short preoperative stay in hospital and also appropriate infection control procedures.

Our results regarding the prevalence of the sesl gene among blood culture isolates are in accordance with those of Bowden et al. (2005). However, in that study 29% of isolates representing normal flora were also found to be sesl-positive, but these S. epidermidis isolates were collected from healthy infants and from patients with invasive disease in hospital. In contrast, the prevalence of sesl reported by Bjertsjö Rennermalm (2005) was much higher. However, the number of isolates analysed was limited and the origins of the skin isolates were not specified.

In conclusion, sesl was not found among the normal S. epidermidis flora of healthy individuals without any healthcare association, but was found in approximately 50% of clinical isolates causing invasive infections. The pathogenic significance of the Sesl protein could be further investigated both by using knockout mutants of S. epidermidis in various models and by studying the clinical presentation, course and outcome of infections caused by Sesl-expressing isolates compared with sesl-negative S. epidermidis. In addition, the dynamics of the antibody response against these cell-wall-associated surface proteins of S. epidermidis should be outlined.

**Table 1. Presence of the gene encoding Sesl among 133 S. epidermidis isolates obtained from patients with prosthetic joint infections, from blood culture isolates obtained from patients with haematological malignancies, from isolates obtained during reoperation of cardiac surgery patients, and from commensals obtained from the nares and the skin of the wrists of healthy controls**

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of sesl-positive isolates (%)</th>
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<tbody>
<tr>
<td>Prosthetic joint infections (n=32)</td>
<td>21 (66%)</td>
</tr>
<tr>
<td>Haematological malignancies, bacteraemia (n=29)</td>
<td>13 (45%)</td>
</tr>
<tr>
<td>Haematological malignancies, contamination (n=22)</td>
<td>6 (27%)</td>
</tr>
<tr>
<td>Cardiac surgery, postoperative, infection (n=19)</td>
<td>10 (53%)</td>
</tr>
<tr>
<td>Cardiac surgery, postoperative, contamination (n=7)</td>
<td>2 (29%)</td>
</tr>
<tr>
<td>Nares (n=13)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Skin flora (n=11)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

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**Fitzpatrick, F., Humphreys, H. & O’Gara, J. P. (2005).** The genetics of staphylococcal biofilm formation – will a greater understanding of pathogenesis lead to better management of device-related infection? *Clin Microbiol Infect* 11, 967–973.


