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.*, 2006; McCann *et al.*, 2008; Uçkay *et al.*, 2009).

The virulence factors of *S. epidermidis* have not been fully characterized (Gill *et al.*, 2005; Yao *et al.*, 2005). The main focus has been on the capacity of *S. epidermidis* to adhere to the surfaces of foreign bodies and to matrix proteins of the host, as well as biofilm formation (Von Eiff *et al.*, 2002; Fitzpatrick *et al.*, 2005; Kong *et al.*, 2006; Mack *et al.*, 2007; Otto, 2008).

The surface of the cell wall of *S. epidermidis* expresses a family of proteins that specifically binds to extracellular host matrix proteins (Desvaux *et al.*, 2006), such as fibrinogen, fibronectin, laminin, elastin and collagen. Some of these proteins display a common C-terminal cell wall sorting signal (CWS) for their correct incorporation into peptidoglycans of the cell wall (Mazmanian *et al.*, 2001; Comfort & Clubb, 2004; Bowden *et al.*, 2005; Gill *et al.*, 2005; McCrea *et al.*, 2000). This CWS is referred to as the LPXTG (leucine–proline–variable amino acid (X)–threonine–glycine) motif. These cell-wall-associated surface proteins are covalently linked to the cell-membrane-associated transpeptidases, designated sortases, produced by the bacteria. Among *S. epidermidis*, a sortase named surface protein sorting A (SrtA) has been described (Comfort & Clubb, 2004). SrtA anchors the protein that contains the CWS (Comfort & Clubb, 2004).

So far, at least 11 different *S. epidermidis* cell-wall-associated surface proteins with a sortase signal have been identified (Bjertsjö

Rennermalm, 2005; Bowden *et al.*, 2005) including *S. epidermidis* fibrinogen binding protein (Fbe) (Nilsson *et al.*, 1998), and serine-aspartate repeat protein F (SdrF) (Bowden *et al.*, 2005). Recently, additional cell-wall-associated surface proteins have been reported, denominated *S. epidermidis* surface (Ses) proteins (Bjertsjö 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 (Bjertsjö 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 μ l 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 *sesI* 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

Table 1. Presence of the gene encoding *SesI* 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

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

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 × 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 sequencing ready reaction kit (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 *sesI* gene of *S. epidermidis* strain RP62A (GenBank accession number CP000029.1; base position 1693743–1693355).

The presence of the *sesI* 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 *sesI* 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 *SesI* protein is a virulence factor of *S. epidermidis* or at least a marker of invasive capacity. Since healthy people do not regularly carry *sesI*-positive strains it could be assumed that patients may acquire the *sesI*-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 *sesI* 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 *sesI*-positive, but these *S. epidermidis* isolates were collected from healthy infants and from patients with invasive disease in hospital. In contrast, the prevalence of *sesI* 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, *sesI* 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 *SesI* 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 *SesI*-expressing

isolates compared with *sesI*-negative *S. epidermidis*. In addition, the dynamics of the antibody response against these cell-wall-associated surface proteins of *S. epidermidis* should be outlined.

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