

## Chapter 2

# *Staphylococcus epidermidis* in Biomaterial-Associated Infections

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**Abstract** Coagulase-negative staphylococci, mainly *Staphylococcus epidermidis*, are currently the most frequent cause of hospital acquired infections in the USA. Mostly, but not exclusively, *S. epidermidis* infections are linked to the use of implanted medical devices like central venous catheters, prosthetic joints and heart valves, pacemakers, cardiac assist devices, cerebrospinal fluid shunts, and intraocular lenses. As new molecular techniques reveal that *S. epidermidis* are by no means the most prominent bacteria of the skin and mucous membrane flora, the implication is that *S. epidermidis* has specific virulence factors, which transforms this commensal bacterial species into one of the most successful pathogens in modern medicine. A vast array of specific attachment factors for native and host protein-modified device surfaces and the ability to accumulate in adherent multilayered biofilms appear to be vital for the success of *S. epidermidis* as a pathogen. Biofilm formation contributes to the ability of the organism to withstand the host's innate and acquired immune defense mechanisms and to resist antimicrobial therapy, so that device removal is a regular feature for the treatment of *S. epidermidis* biomaterial-associated infection. Recent developments in the understanding of *S. epidermidis* virulence are reviewed in this chapter.

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## 2.1 Introduction

Medical progress in recent decades is closely linked with the ever-increasing use of implanted biomedical devices, which allow monitoring of vital functions and administration of drugs to critically ill patients, and intermittently or permanently support or replace failing organ functions. These implants usually are highly beneficial to the individual patient; however, their use is linked to complications and one of the most important of these is infection. Biomaterial-associated infections have a number of interesting features, which make them special. First, they are most commonly caused by coagulase-negative staphylococci (mostly *Staphylococcus epidermidis*), *Staphylococcus aureus*, and also other bacteria of low virulence potential for otherwise healthy individuals, which reside on skin and mucous membranes. Second, they frequently take a chronic persistent course indicating that the innate and acquired immune system does not deal with the causative organisms in an effective way. And third, antimicrobial therapy is regularly unsuccessful due to phenotypic resistance of the causative organisms, while the isolated microbes are susceptible when tested under standard planktonic laboratory conditions. This makes removal of the device a frequent necessity. The phenomenon of phenotypic resistance is frequently attributed to impaired penetration of antimicrobials through the biofilm; however, it is more likely to be an altered metabolic state referred to as tolerance, occurring in bacterial persisters [1].

Currently, it is thought that the formation of adherent multilayered biofilms is of genuine importance in the pathogenesis of biomaterial-associated infections. Biofilms are complex consortia of adherent microorganisms encased in a polymeric matrix [2]. Biofilm formation can be separated into four distinct phases. First, primary attachment of cells to a surface; second, accumulation of microorganisms in multiple layers; followed third by maturation of the biofilm; and fourth, detachment of single cells or groups of cells, which may start the whole process of biofilm formation elsewhere [3].

Coagulase-negative staphylococci, in particular *S. epidermidis*, are prototypic bacteria forming biofilms important in infection.

## 2.2 Clinical Importance of Coagulase-Negative Staphylococci

The major risk factor for infection with coagulase-negative staphylococci is the presence of implanted biomedical devices like central venous catheters, prosthetic joints, fracture fixation devices, cardiac pacemakers and heart valves, artificial lenses, vascular grafts, mammary implants, and CSF-shunts [4–6]. In Germany alone more than 2.5 million of these biomedical devices are used annually [5]. A major complication of their use is infection, affecting up to 100,000 patients annually in Germany. Similar figures were reported for other industrialized countries like the USA [6] indicating that millions of patients are at risk worldwide. Additional

patient-related risk factors for infection with coagulase-negative staphylococci are malignancy, chemotherapy, leukopenia, premature birth, care in an ICU, bone marrow transplantation, and immunosuppression for reasons such as polytrauma, HIV infection, and transplantation [4, 5, 7].

According to data reported to the National Health Care Safety Network, coagulase-negative staphylococci today are the most frequent cause of device- or surgery-associated hospital-acquired infection in the USA [8]. Coagulase-negative staphylococci were ranked first for all infections (15.3%) and for central line associated blood stream infections (CLABSI; 34.1%) and second for surgical site infections (13.7%) [8]. Similarly, coagulase-negative staphylococci were the most common cause of CVC-associated blood-stream infection (32.1%) reported from German ICUs to the hospital infection surveillance system KISS [9] and for CLABSI reported from ICUs to the provincial hospital infection surveillance program SPIN in Quebec, Canada (53%) [10]. Incidence of CLABSI varies from 0.9 to 1.9/1,000 central line days [9] and 1.67 to 4.4/1,000 central line days depending on type of ICU reporting [10]. In the 1990s 50,000–120,000 patients were estimated to contract nosocomial catheter-related blood stream infections annually in the USA [11, 12]. More recently CLABSI episodes associated with US ICUs were estimated to have decreased from 47,000 in 2001 to 18,000 in 2009, which was mainly associated with a decrease in *S. aureus* infections and a more modest decrease in infections with Gram-negative rods, *Candida* spp., and *Enterococcus* spp. [13]. In 2009 an estimated 23,000 CLABSIs occurred among patients from inpatient wards and in 2008 an estimated 37,000 CLABSIs occurred in patients receiving outpatient hemodialysis [13], indicating that coagulase-negative staphylococci are pathogens of major importance in CLABSI-associated morbidity. Up to 80% of coagulase-negative staphylococci isolated from blood cultures are *S. epidermidis* while the remainder represent other coagulase-negative staphylococcal species, most frequently *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus capitis*, *Staphylococcus saprophyticus*, *Staphylococcus warneri*, *Staphylococcus cohnii*, *Staphylococcus xylosus*, *Staphylococcus chromogenes*, *Staphylococcus schleiferi*, or *Staphylococcus lugdunensis* [5, 14]. CLABSI is associated with a significant mortality of 12–25% [13].

Coagulase-negative staphylococci (11%) are the third most common cause of infective endocarditis after *S. aureus* (31%) and viridans streptococci (17%) and the second most common cause of prosthetic valve associated infective endocarditis (17%) and other intracardiac devices (26%) after *S. aureus* with 23% and 35%, respectively [15]. Eighty-two percent of coagulase-negative staphylococci causing prosthetic valve infective endocarditis were *S. epidermidis* [16]. However, despite the predilection of coagulase-negative staphylococcal infection for implanted biomaterials it emerges that these organisms also cause severe cases of native valve infective endocarditis particularly in nondrug abusers [15]. Almost 25% of cases of native valve infective endocarditis due to staphylococci were caused by coagulase-negative staphylococci [15], with a mortality (19%) not statistically significantly different from that of *S. aureus* infective endocarditis [17].

Eighty percent of coagulase-negative staphylococcal isolates causing native-valve endocarditis were *S. epidermidis* [18]. *S. lugdunensis* is of particular importance in infective endocarditis with a high associated morbidity and mortality (50%) in excess of *S. aureus* [19].

Cardiac implantable electronic devices—pacemakers and implanted defibrillators—pose a risk of infection, which are caused by coagulase-negative staphylococci in 27% of cases, the second most common cause following *S. aureus* (34%) [20]. *S. epidermidis* (38%) was the cause of bloodstream infection related with cardiac assist devices followed by *S. aureus* (24%) [21, 22], while in another large series *S. aureus* (34%) was more frequent than coagulase-negative staphylococci (27%) [20].

Cerebrospinal fluid (CSF) shunts had a high infection rate of 11.7% per patient and 7.2% per procedure in a recent US survey of 7,071 children over a 5 year period, where the aggregated infection rates per hospital varied widely from 4.1 to 20.5% per patient and 2.5–12.3% per procedure [23]. A similarly wide range of hospital infection rates were reported from a Canadian CSF shunt infection surveillance system with a mean infection rate of 4.1% per procedure ranging from 0 to 11.1% in different hospitals [24]. Coagulase-negative staphylococci remain the most prominent cause of CSF-shunt infection, causing 37% of cases, followed by *S. aureus* (18%) in a series from Switzerland [25].

Prosthetic joint infections represent a major burden as the infected joint usually has to be removed, which causes additional major morbidity for the affected patients. The United Kingdom National Joint Registry reports that numbers of total hip and knee replacement procedures have increased from approximately 130,000 in 2005–2006 to almost 179,000 in 2010–2011 in England and Wales [26]. In 2010–2011 there were 7,833 revision hip arthroplasty procedures: 45% due to aseptic loosening and 13% due to infection; and 5,082 knee revision procedures: 33% due to aseptic loosening and 23% due to infection [26]. Deep infection rates reported from a single center in the UK were 0.57% for hip and 0.86% for knee replacements [27]. The Norwegian Joint Registry found a threefold increased risk for revision hip replacements due to deep infection in the time period from 1987 to 1992 as compared to 2003–2007. This increased risk for deep infections was particularly pronounced in uncemented hip replacements where a 5.3-fold risk was observed for the later time period as compared to the 1987–1992 period [28]. In a Medicare patient population, covered by the US government health insurance for people aged 65 or over, the incidence of infection in hip replacements was 1.63% within 2 years and 0.59% from years 2 to 10 [29], while in total knee replacements it was 1.55% within 2 years and 0.46% between years 2 and 10 [30]. In hip and knee replacement infections in the UK coagulase-negative staphylococci were the most frequently observed causative organisms in 36% and 49% of cases compared to 29% and 13% *S. aureus*, respectively [27, 31]. In another study of periprosthetic joint infections coagulase-negative staphylococci accounted for 54.5% of organisms, while *S. aureus* was third with 10.9% after *Propionibacterium acnes* with 13.6% [32]. In a study evaluating proven staphylococcal prosthetic hip and knee joint infections, 77%

*S. epidermidis*, 23% *S. aureus*, 6.3% *S. capitis*, 5.1% *S. lugdunensis*, and 1.3% *S. haemolyticus* and *S. warneri*, respectively, were isolated [33, 34].

## 2.3 Coagulase-Negative Staphylococci as Skin Colonizers

Culture-based studies have identified coagulase-negative staphylococci as the most frequently found organisms of the normal skin flora [35]. The most prevalent and persistent species are *S. epidermidis* and *S. hominis* [36, 37]. Quantitative studies indicated that staphylococci usually made up 50% of the bacteria isolated from the head, nares and axillae and 10–70% of those isolated from legs and arms [38]. *S. epidermidis* represented 90–100% of the staphylococci isolated from the nares of individuals who were not carriers of *S. aureus*, whilst in *S. aureus* carriers *S. epidermidis* still made up 10–20% [38]. A link was recently found between *S. epidermidis* expressing Esp, a *S. epidermidis* serine protease similar to *S. aureus* V8 serine protease, and *S. aureus* noncarrier state [39]. This is reported to be due to interference through Esp with *S. aureus* biofilm formation [39], which could explain the high carrier rate of *S. epidermidis* in non-*S. aureus* carriers. *S. epidermidis* comprises greater than 75% of staphylococci from axillae and head and usually 10–45% of those isolated from legs and arms [38].

More recently skin microbiota from humans have been studied using 16S rRNA gene sequencing with sampling of different skin sites [40]. Superficial forearm skin samples revealed as leading genera in all six individuals studied *Propionibacterium* (22% of all clones), *Corynebacterium* (19% of all clones), and *Staphylococcus* (11.1% of all clones) [41]. However, *S. epidermidis* represented only 2.9% of all clones, while *P. acnes* was represented by 20.6% of all clones [41]. The most abundant staphylococcal species was *S. caprae* (3.5%), followed by *S. epidermidis* (2.9%) and *S. hominis* (1.6%) [41]. Similarly, analyzing skin samples from the inner elbow of five human subjects *S. epidermidis* and *P. acnes* comprised <5% of all represented 16S rRNA gene sequences [42]. In a larger study of 20 distinct skin sites in ten different individuals *Staphylococcus* spp. represented 16.8% of sequences of 205 genera present with more than three sequences [43]. Dry skin sites, e.g., volar forearms, hypothenar palm, and buttock, comprised 4.8% *Staphylococcus* spp. sequences, sebaceous sites including, e.g., glabella, alar crease, external auditory canal, back of scalp, manubrium and back 16.2% *Staphylococcus* spp. sequences, and moist sites, e.g., nares, axilla, antecubital fossa, inguinal crease, popliteal fossa, and umbilicus 21.2% *Staphylococcus* spp. sequences, respectively [43].

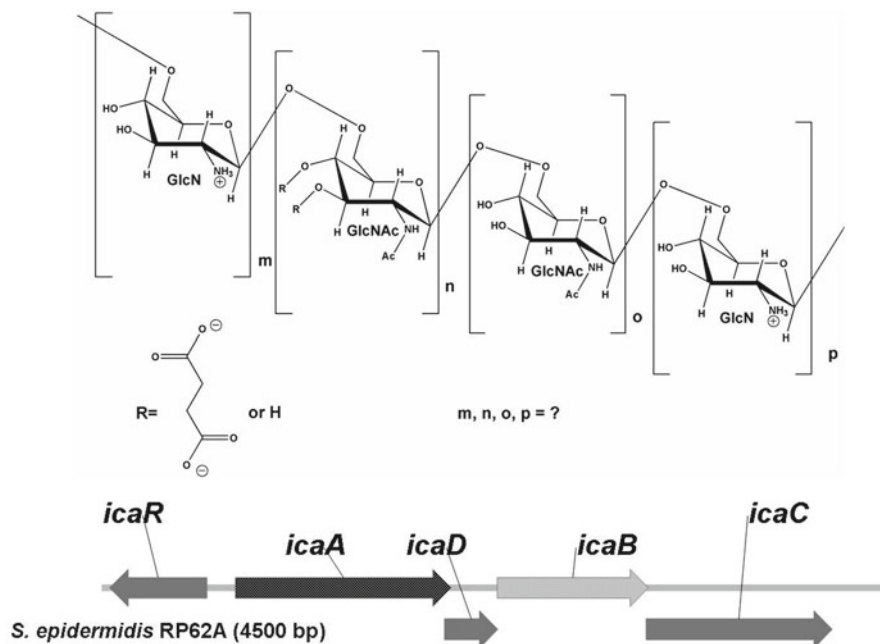
In contrast to the understanding of coagulase-negative staphylococci as accidental pathogens [44] in prosthetic-device associated infections despite low virulence potential, simply owing to their great abundance and frequent contamination of the device during implantation [45–47], the newer in-depth understanding of the skin microbiota would suggest that coagulase-negative staphylococci, in particular *S. epidermidis*, have specific virulence factors which make them successful pathogens in the scenario of biomaterial-associated infections.

## 2.4 Biofilm Accumulation

Biofilm accumulation in *S. epidermidis* is affected by intercellular adhesins, the predominant one being polysaccharide intercellular adhesin (PIA) [48–51] (Fig. 2.1). However, not all *S. epidermidis* strains identified in biomaterial-related infections are able to produce PIA or possess the gene locus *icaADBC* encoding synthetic machinery for PIA synthesis [52, 53]. An alternative, PIA-independent accumulation mechanism was therefore suspected [33, 48–50, 54–56], and eventually discovered in accumulation associated protein, Aap [57–59], biofilm associated protein Bap [60], extracellular matrix binding protein Embp [61, 62], and *S. epidermidis* SesC [63]. Additional proteinaceous intercellular adhesins may also exist [33].

### 2.4.1 Polysaccharide Intercellular Adhesin

PIA synthesis is probably a general mechanism employed by a diverse group of Gram-positive and Gram-negative eubacteria for biofilm formation [64]. It is



**Fig. 2.1** Schematic representation of polysaccharide intercellular-adhesin (PIA) and *icaADBC* [64]. The backbone of the unbranched polysaccharide consists of  $\beta$ -1,6-linked *N*-acetylglucosamine residues.  $m$ ,  $n$ ,  $o$ ,  $p$  indicate numerous sugar residues of the respective type. Chromosomal region containing *icaADBC* involved in PIA synthesis of *S. epidermidis* RP62A (4,500 bp). IcaAD have glycosyltransferase activity, IcaC is necessary for synthesis of full length PIA, and IcaB is the deacetylase [53, 116]. IcaR is a transcriptional regulator controlling in part *icaADBC* transcription [94]

certainly the major functional component for intercellular adhesion in *S. epidermidis* biofilms [48–50]. The *icaADBC* locus encodes enzymes for PIA synthesis in *S. epidermidis* [52, 53] and is also found in *S. aureus* [65, 66], *S. caprae* [67], and *S. lugdunensis* [68]: homologous DNA sequences have been detected in other coagulase-negative staphylococci [65, 69]. Other human pathogens such as *Escherichia coli* [70], *Aggregatibacter actinomycetemcomitans* [71], *Actinobacillus pleuropneumoniae* [71], *Yersinia pestis* [72–75], *Acinetobacter baumannii* [76], and *Bordetella* spp. [77, 78] have all been found to possess gene loci (*pgaABCD* or *hmsHFRS*) orthologous to *icaADBC*, which are involved in synthesis of polysaccharides used in biofilm formation.

#### 2.4.1.1 Structure of PIA and Related Polysaccharides

The structural analysis of polysaccharides involved in biofilm formation of *S. epidermidis* was facilitated by the isolation of isogenic biofilm-negative transposon insertion mutants from biofilm-positive wild-type strains. Prior to that, difficulties had arisen with components from media used for bacterial culture being purified and analyzed, with misleading results ([79], for a review see also [80, 81]): however, comparison of extracts of wild-type and mutant bacteria circumvented this problem [49, 82]. The structural analysis of PIA and related polysaccharides from staphylococci and other bacteria like *Escherichia coli* has been recently reviewed [64, 83].

The structure of PIA was first described for biofilm-forming *S. epidermidis* 1457 and RP62A. PIA was extracted from the cells by sonication after the strains had been cultured in trypticase soy broth, which revealed the existence of both a major polysaccharide I (>80%), and a minor polysaccharide II (<20%) [82]. Polysaccharide I did not bind to Q-Sepharose, whilst polysaccharide II was moderately anionic. Chemical analyses and NMR spectroscopy have demonstrated that polysaccharide I is a linear homoglycan of  $\beta$ -1, 6-linked 2-amino-2-deoxy-D-glucopyranosyl residues. Approximately 80–85% of them are *N*-acetylated; the rest are non-*N*-acetylated and carry a positive charge. Cation exchange chromatography was used to identify molecular species with non-*N*-acetylated glucosaminyl residue content ranging between 2 and 26%. These were shown by chain cleavage by deamination with  $\text{HNO}_2$  to be randomly distributed: glucosaminyl-rich sequences were somewhat predominant. Polysaccharide II, whilst structurally related to polysaccharide I, has a lower proportion of non-*N*-acetylated D-glucosaminyl residues. It was shown by colorimetric assay to contain ester-linked succinate and phosphate (see below), and is therefore anionic [49, 82]. Despite a high apparent molecular weight indicated by elution in the void volume of Sephadex G200 [49, 82] or Sephacryl S300 columns (C. Fischer and D. Mack, unpublished results), the ratio of reducing terminal sugar residues to total sugar residues was shown by methylation analysis to be 1:130, implying an average  $M_r$  of 30,000 for PIA polysaccharide chains [82]. This implies aggregation of PIA polysaccharide chains in solution. PIA was shown to function also as the hemagglutinin of *S. epidermidis* [84–86].



NMR spectroscopy and chemical analysis have been used for detailed analysis of polysaccharide produced by biofilm-producing *S. aureus* MN8m, which was referred to by Joyce et al. as *Staphylococcus aureus* exopolysaccharide (SAE) [87]. That study confirmed a basic structure of  $\beta$ -1,6-linked *N*-acetylglucosamine (GlcNAc) homopolymer, with presence of *O*-succinylation at the 3- and 4-hydroxyl groups of the GlcNAc residues being revealed for the first time by means of NMR spectroscopy [87]. However, no phosphate was detected in SAE [87]. In comparison to PIA preparations from *S. epidermidis* 1457, which contained only 15–20% non-*N*-acetylated glucosamine residues, SAE from *S. aureus* MN8m had a higher proportion at 43% [82, 87]. The content of ester-linked succinate was comparable, at 8–9% for the *S. aureus* MN8m polysaccharide versus 6% for the *S. epidermidis* 1457 polysaccharide. Size-exclusion chromatography was used to reveal an apparent molecular weight of 346,000 for SAE. SAE was active in *in vitro* hemagglutination assays [87]. In conclusion, these studies demonstrate that *S. aureus* MN8m produces a polymer that is closely related both chemically and biologically to *S. epidermidis* PIA [82, 87].

The structure of PIA polysaccharide produced by *S. epidermidis* RP62A and *S. aureus* MN8m has now been independently investigated using a variety of different culture conditions prior to sonication for extraction of the polysaccharide [88]. Purification and NMR spectroscopy confirmed the basic structure of PIA and direct NMR-evidence for the presence of *O*-succinylation in PIA from *S. epidermidis* RP62A was obtained [88]. The ratios of neutral and negatively charged PIA I and PIA II were similar to those previously observed for *S. epidermidis* 1457 [82]. It is worth noting, in particular, that Sadovskaya and colleagues compared the PIA and SAE extracted from *S. epidermidis* 1457 and *S. aureus* MN8m using identical conditions and NMR spectroscopy [88]. As well as similar elution profiles this revealed virtually identical  $^1\text{H}$  NMR spectra. A higher proportion of charged groups, succinoyl residues and free amino groups in SAE was indicated by more pronounced peaks at 5.1, 2.7, and 3.0 ppm, in comparison to PIA from *S. epidermidis* RP62A [88]. No evidence for the presence of polysaccharide-bound phosphate was obtained when 0.9% NaCl was used instead of phosphate buffered saline in the purification process, with the implication that the initial observation of the presence of phosphate may have been due to carryover of buffer [82, 88].

PIA has also been referred to by other names including PS/A [89, 90], PNSG [66], PNAG [91], and SAE [87]. To summarize, PIA is a homoglycan of  $\beta$ -1,6-linked *N*-acetylglucosamine, with a fraction of free 2-amino groups (no *N*-acetylation) conferring positive charges and *O*-succinoyl ester residues conferring negative charges. In spite of variations in the degree of nonacetylated, free amino groups, *O*-succinylation, and possibly molecular size, PIA, P/SA, SAE, and PNAG are now generally accepted to represent the same chemical entity [64, 81, 92, 93].

#### 2.4.1.2 Biosynthesis of PIA and Related Polysaccharides

The production of PIA is controlled by the *icaADBC* locus of *S. epidermidis* [52], an operon with four open reading frames: *icaA*, *icaD*, *icaB*, and *icaC* [53]. *icaR* is



found upstream of the *icaA* start codon and encodes a 185 amino acid (aa) *tetR*-type transcriptional regulator which is proposed to have a helix-turn-helix DNA binding motif at the *N*-terminus [94]. IcaR negatively controls *icaADBC* transcription, which is also influenced by TcaR [94, 95]. It is part of a regulatory network governing transcription of *icaADBC* including the alternative sigma factor  $\sigma^B$ , *sarA*, *sarX*, *sarZ*, *ygs*, *gpdS*, *spx*, ClpP, and quorum sensing regulator *luxS* [94, 96–108]. Regulation of *icaADBC* expression is beyond the scope of this article but is discussed in several reviews [44, 109–113]. It is known to differ significantly between *S. aureus* and *S. epidermidis* [114].

Production of a functionally active PIA molecule requires expression of all four *icaADBC* genes [93]. The process has been the subject of detailed study in recombinant strains of *S. carnosus* which expressed different combinations of the *icaADBC* genes and with UDP-GlcNAc as a sugar donor [53]. IcaA belongs to the glycosyltransferase 2 family. It is an integral membrane protein with 412 aa and 4 predicted transmembrane domains [52, 53, 115], and directs the synthesis of  $\beta$ -1,6-linked GlcNAc oligosaccharides of up to 20 GlcNAc units. IcaD is required for full activity of IcaA *in vitro*. It is a 101 aa integral membrane protein with two potential membrane spanning domains: it may be a chaperone directing folding and membrane insertion of IcaA and may act as a link between IcaA and IcaC [53]. Also essential for the synthesis of fully functioning PIA is IcaC, a 355 aa integral membrane protein with ten predicted transmembrane domains, which may be involved in externalization and elongation of the growing polysaccharide [53].

IcaB is a member of the polysaccharide deacetylase family, including, for example, chitin deacetylases or the chitooligosaccharide deacetylase NodB of *Rhizobium melioli*. In its mature form it is a 259 aa secreted protein with a predicted signal sequence, responsible for the de-*N*-acetylation of PIA, and crucial for PIA activity in biofilm formation and for virulence in *S. epidermidis* [116]. In  $\Delta$ *icaB*-mutants, where the *icaB* gene has been deleted, PIA is poorly retained on the cell surface as it does not contain non-*N*-acetylated GlcNAc [116]. Similar results have been obtained for *S. aureus* [117].

*In vitro* membrane preparations of *icaADBC*-positive *S. epidermidis* 1457 grown in TSB lacking glucose generated production of immunoreactive PIA upon addition of UDP-GlcNAc. Bacteria grown *in vivo* under the same conditions did not synthesize PIA—an additional unknown glucose-dependent protein factor is required [118]. The biosynthetic pathway for *O*-succinylation of PIA is currently uncertain.

## 2.4.2 Proteinaceous Intercellular Adhesins in Biofilm Accumulation

### 2.4.2.1 Accumulation Associated Protein

Accumulation associated protein Aap, previously described in RP62A derived biofilm negative mutant M7 [57], was discovered as an intercellular adhesin

through analysis of a clinical *S. epidermidis* isolate 5179 from a patient with chronic ventriculoatrial CSF-shunt infection, who was bacteremic over a period of almost 2 months [119]. Despite clear clinical significance, all isolates of *S. epidermidis* from this patient were biofilm-negative *in vitro*, which was due to an insertion of IS257 in *icaA* of *S. epidermidis* 5179 [119]. Reports about precise excision of IS256 from *icaADBC* with restoration of biofilm formation [96] initiated long-term biofilm cultures of *S. epidermidis* 5179, which led to the isolation of stable biofilm-forming revertants [58]. However, IS257 was still in place in the revertants, which did not produce any PIA but still formed biofilms. Comparison of surface proteins of wild-type 5179 and biofilm-producing revertant 5179-R1 led to identification of an additional 140 kDa protein of the revertant as Aap [58]. Aap is anchored via a LPXTG motif and comprises two major domains: domain A, which may itself contain a lectin-like domain [120], and a repetitive domain B, composed of a variable number of 128 amino acid repeats [58]. In *S. epidermidis* removal of Aap N-terminal domain A through proteolytic processing leads to exposure of domain B, which gives Aap intercellular adhesive properties causing biofilm accumulation [58]. The proteolytic activation may be mediated either by staphylococcal exoproteases or by the host [58]. Aap has significant homology with cell wall protein SasG of *S. aureus* [121, 122], in which SasG-mediated biofilm accumulation has been recently demonstrated using the same mechanisms; however, a hypothesis of self-activation of SasG was put forward [123, 124]. Domain B repeats contain “G5 domains,” which hypothetically were related to *N*-acetylglucosamine binding activity [120]. Recently, however, it was demonstrated that G5 domains of Aap are zinc ( $\text{Zn}^{2+}$ )-dependent adhesion molecules incorporating 2–3  $\text{Zn}^{2+}$  ions in the dimer interface [125]. Zinc chelation inhibits biofilm formation by *S. epidermidis* and *S. aureus* [125]. Tandem G5-domains associate in a modular fashion suggesting a Zinc-zipper mechanism for G5 domain-based intercellular adhesion. Clearly the ability to respond to host proteases by forming a biofilm is in the organism’s favor and enables it to evade phagocytic clearance, as indicated by the less efficient killing of Aap-aggregated *S. epidermidis* 5179-R1 as compared to its biofilm-negative, nonaggregated wild-type [58, 126].

Aap is the essential component of fibrillar structures visible on *S. epidermidis* NCTC 11047 by electron microscopy [59], which resemble *S. epidermidis* surface proteins Ssp1 and Ssp2 [44, 127, 128]. Ssp1 and Ssp2 were discovered as proteins mediating adhesion to polystyrene, which was inhibited by Ssp1-specific monoclonal antibodies [127, 128].

Aap domain A mediates adhesion of *S. epidermidis* NCTC 11047 to human skin corneocytes, and may represent the primary function of Aap in colonizing *S. epidermidis* strains [129]. Monoclonal anti-Aap specific antibodies have been reported to inhibit biofilm formation of *S. epidermidis* RP62A, a prime example of a PIA-dependent biofilm forming *S. epidermidis* strain [130]. This is in line with the original observation of impaired Aap expression in biofilm-negative mutant M7 of RP62A [57]. Recently it was shown that three mAbs against the Aap C-terminal single B-repeat construct followed by the 79-aa half repeat (AapBrpt1.5) had differential effects on RP62A biofilm formation, depending on the specific epitopes

[131]. Biofilm inhibition and increased biofilm formation were observed with the respective mAbs, which also influenced exopolysaccharide synthesis including eDNA and PIA [131]. Proteases as contained in medicinal maggots excretion/secretions may specifically disperse Aap-dependent biofilms [132].

Aap is common in clinical isolates [33, 54, 133, 134], and Aap-dependent biofilm formation can be directly demonstrated in clinical isolates [33, 58], so it seems not unlikely that it is, like PIA, an important virulence factor.

#### 2.4.2.2 Extracellular Matrix Binding Protein Embp

The intercellular adhesive properties of Embp were discovered in a clinically significant *S. epidermidis* 1585 isolate from blood cultures in a port-catheter infection, which was *icaADBC*- and *aap*-negative and did not form biofilm *in vitro* [62]. Long-term biofilm culture led to the isolation of a stable biofilm-positive variant 1585v, which was used to isolate isogenic biofilm-negative transposon mutants. Tn917 insertions were located in the distal gene of the giant 1 MDa Embp, which had been previously described as a fibronectin binding protein [61]. A translocation in 1585v led to overexpression of a 460 kDa truncated isoform of Embp necessary for biofilm formation [62]. Embp is a giant fibronectin-binding protein harboring 59 found in various architectures (FIVAR) and 38 protein G-related albumin-binding (GA) domains. The FIVAR domains of Embp mediate binding of *S. epidermidis* to solid-phase attached fibronectin, constituting the first step of biofilm formation on conditioned surfaces [62]. The binding site in fibronectin was assigned to the fibronectin domain type III12 [62]. Embp-mediated biofilm formation also protects *S. epidermidis* from phagocytosis by macrophages [62]. More detailed assessment of the antiphagocytic properties of Embp of *S. epidermidis* indicated that the aggregative state leads to decreased uptake and a diminished inflammatory response by J774A.1 macrophages [126]. Interestingly, culture in goat serum induced expression of Embp in biofilm-negative wild-type *S. epidermidis* 1585 and rendered it biofilm-positive, which probably explains its virulence in the initial *in vivo* infection [62].

#### 2.4.2.3 Other Surface Proteins

*S. aureus* strains of bovine origin have been found to produce a surface protein known as biofilm associated protein (Bap) which supports biofilm formation [135]. It remains uncertain whether Bap is important in the attachment or the accumulative phases of biofilm formation. A homologous protein was found in a few (4/38) biofilm positive *S. epidermidis* strains from ovine or caprine mastitis [60], but not in strains from humans [33, 60]. Allelic replacement of Bap in those isolates led to a biofilm-negative phenotype indicating that indeed in these strains Bap was responsible for biofilm formation [60]. Recently, six human *S. epidermidis* isolates were shown to carry *bap*, but all but one of those isolates were also positive for *icaADBC* so that the

role of Bap for a biofilm-positive phenotype in these strains requires further study [136]. Another surface protein, Bhp, which is related to Bap, is present in 10–19% of *S. epidermidis* isolates from human infections [54, 135]. Therefore, the significance of Bhp for *S. epidermidis* biofilm formation and pathogenesis remains to be determined.

SesC is an LPXTG motif-containing 68 kDa surface protein of *S. epidermidis* distantly related to clumping factor A of *S. aureus* and is expressed more strongly in biofilm-associated as compared to planktonic *S. epidermidis* 1457 and 10b cells *in vitro* and *in vivo* [63]. Rabbit anti-SesC inhibited biofilm formation of a number of *S. epidermidis* isolates *in vitro*, which may be related to changes in primary attachment to fibrinogen-coated surfaces in the presence of anti-SesC [63]. All of 105 *S. epidermidis* isolates were in possession of the *sesC* gene [63]. A specific role of SesC as an intercellular adhesin in biofilm accumulation remains to be demonstrated.

### 2.4.3 Importance of Biofilm Formation for Staphylococcal Virulence

Biofilm-positive *S. epidermidis* strains occur frequently in significant clinical infection (reviewed in ref. [137]). Therefore, the role of biofilm in virulence of coagulase-negative staphylococci, particularly *S. epidermidis*, has been widely discussed and studied. Initially, studies on the pathogenicity of clinical *S. epidermidis* isolates using *in vivo* foreign-body infection models, did not appear to demonstrate that biofilm-producing *S. epidermidis* clinical isolates were more virulent (for a review see ref. [5]). However, subsequent studies using isogenic pairs of biofilm-positive wild-type and biofilm-negative mutants were more conclusive. Biofilm-positive, PIA-producing *S. epidermidis* 1457 was more virulent than its isogenic biofilm-negative transductant 1457-M10 (containing a Tn917 insertion in *icaA* [85]) in a subcutaneous catheter infection model in mice and a central venous catheter infection model in rats [138–140]. A similar result was demonstrated with the same models using an independent isogenic strain pair obtained from biofilm-positive *S. epidermidis* O-47 [141]. An *atlE*-deletion mutant of this strain was also attenuated [141]. In a rat central venous catheter model, expression of *icaRADBC* in *icaADBC*-negative *S. epidermidis* strains also led to increased virulence [142]. A *Caenorhabditis elegans* infection model was used to study biofilm-positive *S. epidermidis* 9142. This strain killed the worms more rapidly than its isogenic *icaA*-insertion mutant 9142-M10 and accumulated to higher intestinal concentrations [143]. Virulence was restored to wild-type in the biofilm-negative mutant by complementation with cloned *icaADBC* [143]. In a collection of *S. epidermidis* infective endocarditis isolates PIA expression and pathogenicity for *C. elegans* was not closely associated [144].

The susceptibility of biofilm-positive, PIA-positive *S. epidermidis* 1457 to killing by antimicrobial peptides like LL-37, dermcidin, and human  $\beta$ -defensin 3 has been compared to that of its isogenic *icaA*-insertion mutant 1457-M10 [145]. The biofilm positive strain was less susceptible to killing and also displayed decreased phagocy-

tosis and killing by polymorphonuclear granulocytes (PMNs). When *S. epidermidis* 1457 was grown as a biofilm and planktonically, the organism in a biofilm was less susceptible to phagocytic killing after opsonization with normal human serum. The planktonic cells were killed to the same extent as planktonic cells of the isogenic biofilm-negative *icaA*-insertion mutant [146]. Biofilm-positive wild-type bacteria preopsonized with normal human serum were more resistant to killing than the isogenic biofilm-negative cells [146], and killing was dependent on activation of classic or lectin-mediated complement pathways. Compared to the mutant, biofilm-positive *S. epidermidis* 1457 induced more C3a, but C3b and human IgG surface deposition was decreased. In an experiment which reproduced the previously observed virulence differences in the isogenic strain pair, bacteria from explanted infected catheters from a mouse catheter infection model, displayed *ex vivo*, a lower level of phagocytic killing by PMNs in the biofilm-positive wild-type bacteria probably due to inadequate opsonization of biofilm bacteria *in vivo* [146]. Biofilm-grown cells of *S. epidermidis* 9142 were killed less efficiently than planktonically grown cells opsonized with antibodies raised against de-*N*-acetylated PIA/PNAG [147, 148]. Anti-PIA/PNAG antibodies diffused into *S. epidermidis* biofilms sufficiently to allow opsonization, when observed by confocal microscopy [147, 149]. Irrespective of the mechanism of biofilm accumulation, PIA, Aap, or Embp, aggregated biofilm *S. epidermidis* cells induced a lower inflammatory response in macrophages than the dispersed biofilm forming bacteria or isogenic biofilm-negative mutants [126], which might indicate another mechanism of how *S. epidermidis* remains below the radar of the immune system in chronic infection.

Since *S. epidermidis* is an opportunistic pathogen, mechanisms of pathogenicity which are important in some types of device-related infection might be less crucial in others. For example, in the guinea-pig tissue cage model [150], there was no difference in virulence between a biofilm-positive wild-type *S. epidermidis* 1457 and its isogenic *icaA*-insertion mutant, and no difference between *icaADBC*-positive and -negative clinical isolates [151, 152]. Nonetheless PIA was expressed *in vivo* in the tissue cages, and when animals were infected with both strains at the same time, the wild-type out-competed the mutant [153]. This may be because phagocytes are severely impaired in tissue cages [154], masking the expected advantage of the wild-type. During chronic catheter infection in rats, *icaADBC* transcription is continuously down-regulated. This suggests that once synthesized, PIA might have a long half-time *in vivo*. This is supported by the lack, to date, of any detectable PIA degrading enzymatic activity in staphylococci, a situation unlike that in *A. actinomycetemcomitans* which produces dispersin B [153, 155–157].

*S. epidermidis* produces a number of proinflammatory peptides called phenol-soluble modulins (PSMs), which are produced in a strictly *agr*-controlled manner [44, 158–160]. PSM  $\delta$  has strong cytolytic activity towards neutrophils, however, is expressed only at low levels by *S. epidermidis* 1457, in line with a low cytolytic activity of *S. epidermidis* [161]. PSM  $\delta$  is expressed only at very low levels in *S. epidermidis* 1457 biofilms as compared to planktonic cells [162]. PSM  $\beta$  peptides promote *S. epidermidis* biofilm structuring and detachments *in vitro* and dissemi-

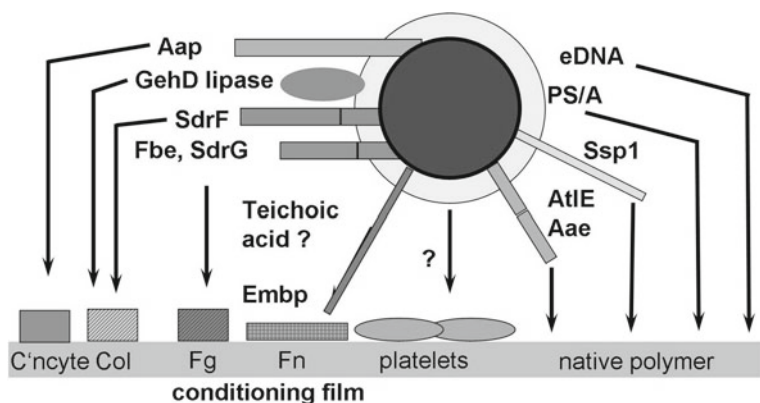
nation of infection during catheter infection *in vivo*, thereby providing the first mechanism of biofilm dispersal in *S. epidermidis* [162].

## 2.5 Primary Attachment to Modified and Unmodified Surfaces

Primary attachment occurs by both specific and nonspecific mechanisms, and to the native surfaces of implanted biomaterials as well as to surfaces primed by adsorption of host-derived plasma proteins, extracellular matrix (ECM) proteins and coagulation products (platelets and thrombi) [163, 164] (Fig. 2.2).

The level of attachment is usually greatest to a native surface, in part aided by the hydrophobicity of the staphylococcal cell surface and the surface of the biomaterial [165–173].

The most important adhesion molecule for native surfaces is *S. epidermidis* autolysin AtlE. This was identified by transposon mutagenesis in a mutant defective in attachment to polystyrene but which could still attach to glass [52, 174]. An AtlE-defective mutant was attenuated in virulence in a rat central venous catheter infection model [141], and in fact AtlE seems to have a dual role in attachment, as it also binds specifically to the ECM protein vitronectin [174, 175]. The high-resolution structure of the amidase domain AmiE of AtlE of *S. epidermidis* has recently been elucidated [176].



**Fig. 2.2** Scheme of attachment mechanisms employed by *S. epidermidis* on native and conditioned polymer surfaces [64]. After implantation biomaterial surfaces are rapidly covered by a conditioning film composed of extracellular matrix proteins, thrombi, and activated platelets. *S. epidermidis* will attach to either native or conditioned surfaces employing a variety of different adhesive molecules. Fbe, SdrG *S. epidermidis* fibrinogen binding protein [182, 183], Embp *S. epidermidis* fibronectin binding protein [61, 62], Aap accumulation associated protein involved in corneocyte adhesion and biofilm accumulation [58, 129], AtlE *S. epidermidis* autolysin, Aae *S. epidermidis* autolysin/adhesin, Ssp1 staphylococcal surface protein 1 [127, 128], GehD *S. epidermidis* lipase, SdrF *S. epidermidis* collagen binding protein [192], PS/A *S. epidermidis* polysaccharide/adhesin, eDNA extracellular DNA, Col collagen, Fg fibrinogen, Fn fibronectin



The 220 kDa cell-wall associated protein Ssp1, which is organized in a fimbria-like structure, mediates attachment to polystyrene in an *S. epidermidis* strain [127, 128] but has not been further studied. However, more recent data raise the possibility that Ssp1 may be related to Aap, which also forms fibrillar appendages on the surface of *S. epidermidis* NCTC11047 [44, 59, 129].

A capsular polysaccharide adhesin, PS/A, of *S. epidermidis* RP62A mediates primary attachment to unmodified silastic catheter surfaces, and was later shown to be structurally very similar if not identical to polysaccharide intercellular adhesin (PIA) of *S. epidermidis* [81, 82, 87–91, 177].

Extracellular DNA (eDNA) released by *S. epidermidis* also contributes to biofilm formation [178, 179]. DNase I can degrade eDNA and thereby inhibit nascent biofilm formation as well as disintegrating preformed biofilms [178, 179]. However, analysis of primary attachment of DNase I treated *S. epidermidis* cells indicated that eDNA acted predominantly in the early attachment phase of biofilm formation [179]. Release of eDNA from the staphylococci involved murein hydrolases like AtlE of *S. epidermidis* [179]. How eDNA release interacts with various other factors involved in *S. epidermidis* biofilm formation remains to be determined.

Specific binding to surface ECM proteins (such as fibronectin, fibrinogen, collagen, thrombospondin, and vitronectin) involves cell-wall associated adhesins known as MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) [180]. As well as the autolysin/adhesin AtlE, mentioned above, another *S. epidermidis* surface-associated autolysin, Aae, has been described, which also binds to vitronectin, though not to polystyrene [181]. The gene encoding the fibrinogen-binding protein Fbe/SdrG [182, 183] is common in clinical *S. epidermidis* isolates [33, 54, 182, 184], but its expression must be highly variable as many strains do not bind avidly to fibrinogen [182]. This may be related to the observation that SdrG/Fbe expression was only observed in *in vivo* grown *S. epidermidis* isolates, but not under *in vitro* conditions simulating *in vivo* conditions, i.e., growth in human serum [185]. Fbe-specific antibodies block adherence of *S. epidermidis* to fibrinogen-coated catheters [186] and attenuate infection in animal models [187, 188]; hence Fbe has potential as a vaccine candidate. In a rat model of intravascular catheter associated infection an *fbe*-deletion mutant was attenuated indicating that Fbe is a virulence factor in device-associated *S. epidermidis* infections [189].

The giant 1 MDa fibronectin-binding protein Embp [61] is also present in the majority of clinical *S. epidermidis* isolates [33, 54] but antibodies directed against it were not sufficiently opsonic to enhance phagocytosis [187]. Binding of *S. epidermidis* to fibronectin-modified surfaces is also enhanced by purified teichoic acids [190].

The lipase GehD is secreted by *S. epidermidis* and specifically binds collagen and promotes attachment to collagen coated surfaces [191]. More recently it was demonstrated that the LPXTG motif-containing *S. epidermidis* surface protein SdrF also mediates specific binding to type I collagen in a heterologous expression system in *Lactococcus lactis* [192]. Collagen binding occurs via the B domain of SdrF and both the  $\alpha 1$  and  $\alpha 2$  chains of type I collagen [192]. It was also found that a single B domain repeat of *S. epidermidis* 9491 retains the capacity to bind to type I collagen [192]. Using the *L. lactis* heterologous expression system and a murine infection model evidence was derived that SdrF may contribute to cardiac assist



device driveline infections [193]. SdrF also mediated binding to unmodified Dacron surfaces covering drivelines [193]. In contrast *L. lactis* expressing GehD bound only weakly to driveline surfaces [193]. Anti-SdrF inhibited *S. epidermidis* 9491 binding in the *in vivo* model only by approx. 50% indicating that additional collagen binding factors may be involved [193].

## 2.6 Molecular Epidemiology of Mechanisms Used in Biofilm Formation in Biomaterial-Related Infection

Despite PIA production being a major mechanism of biofilm accumulation, strains of *S. epidermidis* lacking *icaADBC* may also produce biofilm, and biofilm-positive PIA-negative strains have been isolated in clinical contexts [33, 51, 54–56, 62, 119, 194–196].

Since the fundamental molecular basis of *S. epidermidis* biofilm formation has become apparent with the identification of PIA, *icaADBC*, and AtlE [52, 53, 82, 174] numerous studies have been undertaken in *S. epidermidis* strain collections from numerous types of biomaterial related infections, which investigated the presence of *icaADBC* and other genetic factors potentially involved in biofilm accumulation or primary attachment and eventually their correlation with a biofilm forming phenotype *in vitro* (Table 2.1). One of the drivers for these studies is the fact that a majority of clinical isolates of *S. epidermidis* and other coagulase-negative staphylococci represent contaminants, however, recognition of certain genetic traits might aid in resolving this dilemma of distinguishing true pathogens from contaminants. However, the rigor to differentiate contaminating from infecting isolates may have been different in various studies and some of the variation observed may be related to the various frequencies of included contaminants.

As CLABSI is the most frequent infection involving coagulase-negative staphylococci and *S. epidermidis*, many studies have focused on blood culture and catheter tip isolates. In early phenotypic studies it was recognized that biofilm-positive *S. epidermidis* occurs frequently in clinical significant infection, however, and that biofilm-negative strains also can cause infection (reviewed in ref. [137]). However, biofilm formation *in vitro* is due to variability between laboratories. Therefore, the detection of the presence of specific genetic determinants related to biofilm formation and virulence of *S. epidermidis* appears less cumbersome and better comparable between laboratories.

In the first clinical study after discovery of PIA and *icaADBC*, Ziebuhr and coworkers reported that 87% of *S. epidermidis* blood culture isolates were biofilm-positive and 85% were *icaADBC*-positive, while nasal control *S. epidermidis* isolates formed biofilm and were *icaADBC*-positive in 11% and 6%, respectively [51]. In an extension of this study 89.2% of nosocomial clinical isolates were *icaADBC*-positive and almost 50% belonged to multi locus sequence type ST27, whose representatives were all *icaADBC*-positive [197]. In a similar study 77% of clinically significant blood culture *S. epidermidis* isolates as opposed to 28% blood

**Table 2.1**    Molecular epidemiology of *icaADBC* in different biomaterial-related infections

Study	<i>icaADBC</i> -positive infection isolates % (N)	<i>icaADBC</i> -positive contaminant isolates % (N)	<i>icaADBC</i> -positive commensal control isolates % (N)	Comments
<i>Bacteremia</i>				
Ziebuhr 1997 [51]	85% (44/52)		6% (2/36)	Skin and mucosal strains from healthy volunteers
Frebourg 2000 [198]	77% (30/39)	28% (11/39)	38% (6/16)	Contaminants: only one positive BC; commensals: Isolates from hands of healthy volunteers
Vandecasteele 2003 [133]	68% (23/34)	88% (14/16)	52% (15/29)	Contaminants: CVC isolates from patients without bacteremia; commensals: skin isolates from healthy volunteers
<i>CVC infection isolates</i>				
Arciola 2001 [199]	48.5% (33/68)		0% (0/10)	Commensals: Nasal and mucosa from healthy volunteers
Klug 2003 [211]	90% (9/10)		25% (5/25)	Commensals: Hand skin isolates from healthy volunteers
Cafiso 2004 [202]	41% (11/27)			
Petrelli 2006 [201]	70% (47/67)			
<i>Bacteremia in bone-marrow transplant patients</i>				
Rohde 2004 [54]	94% (15/16)		80% (20/25) 13% (2/15)	Nasal isolates from BMT patients Nasal isolates from healthy volunteers No significant difference between multiple or single positive BC
Ninin 2006 [203]	82% (31/38)	85% (60/71)		
<i>Bacteremia in neonatal ICU patients</i>				
de Silva 2002 [204]	42% (5/12)	38% (15/39)	48% (12/25) 37% (17/46)	Sick baby skin isolates Well baby skin isolates Contaminants: BC isolates from babies without infection

(continued)

Table 2.1 (continued)

Study	<i>icaADBC</i> -positive infection isolates % (N)	<i>icaADBC</i> -positive contaminant isolates % (N)	<i>icaADBC</i> -positive commensal control isolates % (N)	Comments
Klingenberg 2005 [205]	61 % (52/85)	52 % (49/95)		Contaminants: BC isolates from babies without clinical signs of sepsis
Bradford 2006 [206]	67 % (4/6)	63 % (5/8)	20 % (2/10)	Contaminants: BC isolates from babies without clinical signs of sepsis; commensals: Nasal strains from healthy adults
<i>Prosthetic joint infections</i>				
Galdart 2000 [208]	81.5 % (44/54)		17 % (4/24)	Commensals: Hand skin flora strains from healthy volunteers
Arciola 2004 [209]	43 % (51/120)			Contaminants: Arthroplasty-associated nonprosthetic joint-infection isolates
Frank 2004 [195]	55 % (17/31)	56 % (5/9)		
Chokr 2006 [55]	82 % (31/38)			All prosthetic joint infection isolates
Rohde 2007 [33]	62 % (32/52)			
	80 % (12/15)			Knee prosthesis infection
	54 % (20/37)			
Koskela 2009 [210]	50 % (16/32)		33 % (8/24)	Hip prosthesis infection
				Commensals: Skin and nasal isolates of healthy volunteers
<i>CSF shunt infection</i>				
Stevens 2008 [134]	79 % (33/42)	31 % (4/13)		Contaminants: CSF isolates deemed not clinical significant
<i>Cardiac pacemaker infection</i>				
Klug 2003 [211]	26 % (7/27)		25 % (5/25)	Commensals: Hand skin isolates from healthy volunteers

culture contaminant isolates carried *icaADBC* [198], while 68% of invasive blood culture *S. epidermidis* isolates, 88% of colonizing catheter tip isolates from asymptomatic patients, and 52% of skin isolates were *icaADBC*-positive, respectively, in a study by Vandecasteele and coworkers [133]. It seems reasonable to conclude that in blood culture isolates potentially PIA producing *S. epidermidis* isolates are highly prevalent highlighting a role of biofilm formation through this mechanism in pathogenesis.

This conclusion is partly supported by studies of *S. epidermidis* isolates from CVC associated infections where 48.5% of isolates were *icaADBC*-positive while all of ten skin isolates did not carry *icaADBC* [199]. In patients with clinical significant CVC-associated infection 70% of *S. epidermidis* isolates from CVC tips (>15 colonies per tip roll culture [200]) were *icaADBC*-positive [201]. In another study only 41% catheter tip isolates (>15 colonies per tip roll culture [200]) were *icaADBC*-positive [202]. The prevalence of *icaADBC*-positive isolates appears lower, however, isolates colonizing CVCs frequently do not cause CVC-associated bacteremia [133, 200] and this may explain the lower prevalence of *icaADBC* in strains from this specific biomaterial.

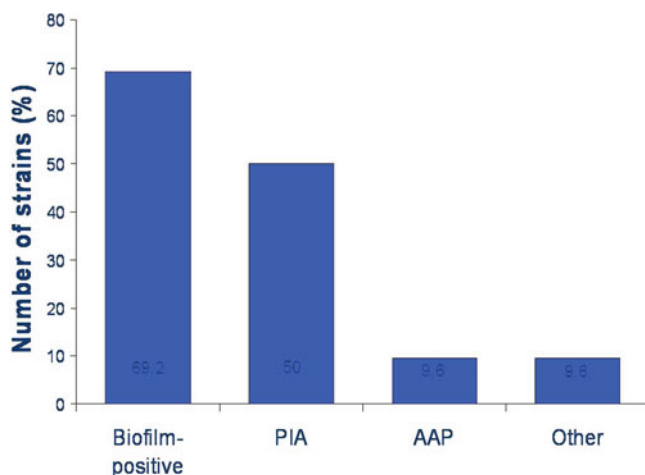
It seems pertinent to speculate that bacteremia isolates from bone marrow transplant patients may represent a different *S. epidermidis* population as many of these patients are severely immunosuppressed and often neutropenic and therefore virulence factors aiding in resistance to phagocytosis like biofilm formation and PIA production may be less important, which may lead to increased representation of *icaADBC*-negative skin isolates amongst infections in this patient population [126, 145, 146]. Eighty-three percent of *S. epidermidis* blood culture isolates from bacteremia in bone marrow transplant patients were *icaADBC*-positive, and there was no significant difference in *S. epidermidis* isolates in this regard from patients with only one positive blood culture (84.5%) as opposed to multiple positive blood cultures (81.6%) [203]. 60.5% of *icaADBC*-positive blood culture isolates produced biofilm, while 59% of *icaADBC*-negative isolates produced biofilm [203]. In a study with strict microbiological inclusion criteria in bone marrow transplant patients —> 2 clonally related or identical *S. epidermidis* isolates from independent blood cultures and signs of infection — all but one bacteremia episode were caused by isolates carrying *icaADBC*, while in commensal skin and nasal *S. epidermidis* isolates from uninfected patients and from healthy subjects were *icaADBC*-positive in 80% and 13%, respectively [54]. This would imply a shift in the commensal *S. epidermidis* populations from predominantly *icaADBC*-negative to predominantly *icaADBC*-positive in bone-marrow transplant patients, which may be caused through selection pressures in hospital. *Aap* was highly prevalent in all isolate groups in this study (87–94%) [54], but varied from 59% in invasive blood culture isolates, 88% in colonizing catheter isolates, and 38% skin isolates in Vandecasteele's study mentioned above [133].

In a study of blood culture isolates from a neonatal ICU 42% of clinical significant blood culture isolates, 38% of blood culture contaminants, 48% of skin isolates from sick babies and 37% of skin isolates from well babies carried *icaADBC* [204]. In a similar setting of a neonatal ICU 61% of clinical significant

blood culture isolates as opposed to 52% of blood cultures contaminants were *icaADBC*-positive [205]. Also in neonatal ICU babies 66% invasive, 63% contaminant, and 20% nasal *S. epidermidis* isolates carried *icaADBC* [206]. Clearly, presence of *icaADBC* cannot be used to assess clinical significance of an isolate in this population. Isolation of *S. epidermidis* in bacteremia of premature neonates is difficult to assess clinically, especially as in many cases only a single positive blood culture is available, due to difficulties of obtaining blood. Therefore, many isolates may represent contaminants similar to *S. epidermidis* isolates from unselected adult blood cultures [207], which could in part explain the lower prevalence of *icaADBC* in *S. epidermidis* in this clinical situation.

In prosthetic joint infection 81.5% of *S. epidermidis* infection isolates carried *icaADBC*, while 16.7% skin flora isolates were positive for the operon [208]. In 120 *S. epidermidis* periprosthetic orthopedic infection isolates only 43% were *icaADBC* positive and 86.3% of those produced biofilm *in vitro* [209]. None of *icaADBC*-negative isolates from this study produced biofilm *in vitro* [209]. Similarly, Frank and coworkers found 55% prosthetic joint infection strains ( $\geq 2$  positive cultures with the same organism) to carry *icaADBC*, a similar prevalence as in nonprosthetic joint infection arthroplasty-associated (only one isolate per episode; therefore possible contaminant) *S. epidermidis* strains (56%) [195]. In another study of isolates from orthopedic periprosthetic infections 81.6% *S. epidermidis* strains carried *icaADBC* [55]. In this study 45.2% of 31 *icaADBC*-positive *S. epidermidis* isolates produced biofilm, while none of the *icaADBC*-negative isolates were biofilm-positive [55]. Prevalence of *icaADBC* in prosthetic joint infection *S. epidermidis* isolates was 50% ( $n=32$ ), while commensal control isolates had *icaADBC*-prevalence of 16.7% ( $n=24$ ) [210].

In a study of clinical significant *S. epidermidis* hip and knee arthroplasty infection isolates prevalence of *icaADBC* was differentially distributed with 54 and 80% of isolates being positive [33]. This study had strict microbiological inclusion criteria for infections which were isolation of two clonally related or identical *S. epidermidis* strains from a joint fluid specimen and from intraoperative biopsies taken weeks to months later during revision surgery [33]. Interestingly, almost 70% of isolates formed biofilm *in vitro*; all biofilm-positive isolates from knee arthroplasty infections carried *icaADBC*, while amongst biofilm-positive hip arthroplasty infection strains only 63% were *icaADBC* positive [33], clearly indicating that other biofilm formation mechanisms in addition to PIA and *icaADBC* are of relevance in these infections. Direct evidence was obtained using specific hexosaminidase DspB and proteases that 50% of all *S. epidermidis* isolates used PIA as intercellular adhesin for biofilm formation, while 19.2% of biofilm-positive isolates used a proteinaceous intercellular adhesin (Fig. 2.3) [33]. For 9.6% of isolates Aap was the relevant intercellular adhesin as determined by biofilm inhibition with anti-Aap, while it is currently unresolved if Embp or other proteinaceous intercellular adhesins are active in the remaining biofilm-positive strains [33, 62]. Importantly, 30% of the clinically significant isolates were *in vitro* biofilm-negative. In four *icaADBC*-negative, *aap*-positive strains biofilm formation could be induced by limited proteolysis with trypsin, which led to Aap activation and Aap-dependent biofilm formation [58].



**Fig. 2.3** Prevalence of biofilm formation and use of different mechanisms of intercellular adhesion in *S. epidermidis* from prosthetic hip and knee joint infections. Data compiled from Rohde et al. [33]

It should be noted that *in vitro* Embp expression is induced only in high concentrations of goat serum leading to biofilm formation of *S. epidermidis* 1585 [62], which could explain why many of the *S. epidermidis* isolates remained biofilm-negative under standard *in vitro* conditions [33].

In shunt-associated meningitis prevalence of *icaADBC* and *aap* in 42 *S. epidermidis* isolates was 78.6 and 47.6% while amongst 13 contaminants 30.8 and 46.2% were *icaADBC*- and *aap*-positive, respectively [134]. In pacemaker-associated infections prevalence of *icaADBC* was low (25.9%;  $n=27$ ) similar to contaminant *S. epidermidis* isolates [211]. Prevalence of adhesion factors AtlE and Fbe was high at 89% in this study [211].

Molecular epidemiologic studies from our laboratory indicate that biofilm accumulation mechanisms are redundantly organized in *S. epidermidis*, the use of various mechanisms is differentially distributed in infection isolates from different types of device associated infections, e.g., CVC-associated bacteremia versus prosthetic hip or knee joint infections [33, 54, 58, 62]. From this work it is also valid to conclude that many *in vitro* biofilm-negative *S. epidermidis* isolates may produce biofilm due to mechanisms, which are not expressed under the respective *in vitro* conditions, but may be expressed *in vivo*. However, it cannot be excluded that some of these isolates do not form biofilm at all and are able to persist in periimplant tissues due to other mechanisms [212–214].

## 2.7 Conclusions

There is wide variation in the prevalence of *icaADBC* and biofilm formation in clinical molecular epidemiologic studies, which would be best explained on the one hand in the varying strictness of identification of clinical significance in dif-

ferent settings and the biologic fact that different biomedical-implants represent different types of infection selecting for *S. epidermidis* strains differentially equipped with virulence factors and molecules functional in biofilm formation. In light of the revolution of molecular epidemiology through third-generation full-genome sequencing it would be particularly important to build collections of clinically well-characterized isolates, which stem from uniform types of implant infections, to learn more about the strategies of these important pathogens of medical progress in the future.

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