REVIEW ARTICLE

# Staphylococcus epidermidis biofilms: importance and implications

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The coagulase-negative staphylococci and, in particular, Staphylococcus epidermidis, have emerged as major nosocomial pathogens associated with infections of implanted medical devices. These organisms, which are among the most prevalent bacteria of the human skin and mucous membrane microflora, present unique problems in the diagnosis and treatment of infections involving biofilm formation on implanted biomaterials. Epidemiological data that address whether invasive S. epidermidis strains can be traced to commensal organisms or an endemic occurrence of distinct strains with enhanced virulence have important implications for the implementation of appropriate infection control measures. An extracellular polysaccharide adhesin represents a key virulence determinant in S. epidermidis and is required for biofilm formation. Production of this adhesin, which is encoded by the ica operon, is subject to phase variable regulation (ON  $\leftrightarrow$  OFF switching). Recent advances in understanding the molecular events controlling polysaccharide adhesin synthesis and the potential clinical implications of its phase variable regulation are outlined. Further research in this area may contribute to the development of novel strategies for therapeutic intervention. Finally, in addition to antibiotic prophylaxis, preventive strategies to control S. epidermidis medical devicerelated infections are focusing on the development of improved biomaterials and physical electrical barriers to impede bacterial colonisation.

#### Introduction

Gram-positive cocci and, in particular, Staphylococcus spp., are predominant among the organisms responsible for infective complications following surgical vascular grafts or the implantation of prosthetic devices [1]. Treatment of post-operative infections is further complicated by the emergence of antibiotic-resistant pathogens, which has contributed significantly to the morbidity and mortality of hospitalised patients. Most staphylococcal infections result in acute disease. However, bacterial persistence and recurrent infections are also commonly observed, particularly in patients with indwelling medical devices. The staphylococci, in particular S. aureus, possess a range of virulence factors that contribute to their pathogenesis. S. aureus is the most important pathogen in the genus and is also the most important nosocomial pathogen of surgical wounds [2]. This organism is associated with acute

tissue-related infections in which the production of many exoproteins such as toxic shock syndrome toxin 1, alpha-toxin and tissue degrading enzymes are important virulence factors. Staphylococcal cell-surface proteins play an important role in the staphylococcus—host cell interaction and cell surface proteins such as protein A, collagen-, fibronectin- and fibrinogen-binding proteins are also important virulence factors which promote adhesion to host cells.

### Pathogenesis and epidemiology of S. epidermidis infections

The coagulase-negative staphylococci (CNS) are widely distributed over the surface of the human body, where they constitute the majority of the commensal bacterial microflora. Among the CNS, *S. epidermidis* is the most frequently isolated species and the most common species responsible for infection. In the past, CNS were considered non-pathogenic and their isolation in the laboratory was attributed to specimen contamination. Indeed, these organisms frequently contaminate blood and other clinical specimens, thus presenting

Received 6 July 2000; revised version accepted 12 Jan. 2001.

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difficulties for both laboratory and clinical staff in distinguishing between contaminating and invasive isolates. CNS strains responsible for infection are generally not speciated, while only research studies have characterised relatedness among individual CNS strains. Epidemiological analysis of CNS disease has focused on hospital-acquired infections because of the close relationship between the use of implanted medical devices and the colonisation of these devices by CNS. Community-acquired infections associated with CNS generally involve patients with chronic indwelling catheters, prosthetic joints and other implanted devices. Although CNS have been shown to bind to a range of host matrix proteins (collagen, vitronectin, fibrinogen, fibronectin and laminin) [3, 4], the production of an extracellular polysaccharide adhesin which promotes direct interaction with the surface of inert synthetic medical devices represents their most important adhesin.

Infections caused by S. epidermidis are often persistent and relapsing. Although S. epidermidis and other CNS are generally the causative organisms in the majority of device-related infections [5], the proportions vary depending on the type of infection and centre surveyed. Following central nervous system shunt procedures, CNS are the causative organisms in 48-67% of infective complications [6]; these organisms are also responsible for 50-70% of catheter-related infections [7]. The high rate of intravascular catheterisation among hospitalised patients highlights the clinical impact of these infections. The CNS are also responsible for a high proportion of prosthetic cardiac valve infections (40-50%) [8], joint replacement infections (20-50%) [9] and the majority of infections following neurosurgical procedures [6]. The prevalence of methicillin-resistant S. epidermidis (MRSE) strains [2, 10, 11] and the emergence of vancomycin resistance in this species further complicate treatment of biomaterial infections [12, 13].

Traditional typing methods for CNS such as biotyping, antibiotic resistance profiling and plasmid analysis are limited by poor discrimination and reproducibility. The application of molecular techniques such as pulsedfield gel electrophoresis (PFGE) and PCR-based random amplification of polymorphic DNA, combined with phenotypic analysis, is more effective. Indeed, most recent investigations have used PFGE, alone or in combination with other phenotypic and genotypic methods to type isolates of S. epidermidis. As S. epidermidis is predominant among CNS strains responsible for infection, most studies have focused on this species. An analysis of S. epidermidis strains in the nares of healthy adults indicated that there are multiple types of this organism in each individual [14]. A key question addressed in epidemiological analysis of CNS infections within individual units is whether the responsible strains originate from random carriage on the skin of infected patients and healthcare workers or are the result of the endemic presence of distinct strains with enhanced virulence.

The emergence of *S. epidermidis* strains with enhanced capacity for colonising implanted biomaterials has important implications for the development and implementation of therapeutic strategies and effective infection control measures. However, the epidemiological data do not always support such a pattern. A survey of the literature reveals that while some studies have suggested that individual clones are responsible for multiple infections in individual units [13, 15–21], others have found no relationship between CNS isolates responsible for infections of multiple patients [22–25].

Many *S. epidermidis* infections can be caused by multiple strains. However, a recent study has indicated that multiple *S. epidermidis* strains isolated from infections of individual joint prostheses, as determined by colony morphology and antibiotic resistance profiles, may be explained by genomic instability of a single infectious clone rather than infection by the presence of a mixture of infecting strains [26].

#### S. epidermidis biofilms

Most bacteria in natural environments are organised in biofilms [27-29]. The recognition that bacteria exist in such altruistic multicellular populations and that these sessile bacterial communities (attached to a surface) constitute a major component of global bacterial biomass has become the focus of considerable investigation. The development of a biofilm is initiated when bacterial cells attach to a surface and begin to excrete slimy, glue-like substances, which serve to anchor the cells. The formation of Pseudomonas aeruginosa biofilms on an abiotic (non-living) surface involves the formation of a monolayer of cells followed by the appearance of microcolonies which appear to form by aggregation of cells present in the monolayer [30]. Channels between these microcolonies may facilitate the diffusion of nutrients into, and waste products away from, the biofilm. The development of bacterial biofilms has important economic and medical consequences. Most industrial biofouling problems are caused by biofilms [27], as are many of the infections treated by clinicians.

Compromised individuals are particularly at risk from infections that involve biofilms. The organisms responsible for these infections often have ecological niches commensal with the human body or occur in environments with which we frequently interact, e.g., water. In some infections, more than one bacterial species or mixtures of fungi and bacteria can be involved in biofilm formation. In addition to device-related infections, many biofilm-related infections involve colonisation of host tissues, e.g., viridans group streptococci in endocarditis and *P. aeruginosa* in cystic fibrosis pneumonia. However, the majority of biofilms form on inert

surfaces or on dead tissue [28]. Advances in our understanding of biofilm formation can assist in the development of novel strategies for the prevention and treatment of biofilm-related infections.

The emergence of S. epidermidis as a pathogen has been synonymous with the now widespread use of intravascular catheters in modern medicine. The inherent capacity of this organism to cause infection derives primarily from its ability to form mucoid biofilms on the inert synthetic surfaces of indwelling medical devices. This has serious clinical consequences, giving rise to many persistent and chronic infections. The bacterial cells within the biofilm are embedded in an exopolysaccharide matrix previously referred to as slime, which affords the bacterial population protection from host defence mechanisms and antimicrobial agents [28, 31]. Furthermore, the altered physiology of the sessile cells results in altered growth rates which impair the effectiveness of growth-ratedependent antibiotics. The clinical symptoms that result from biofilm-related infections are consistent with the establishment of a host immune response to antigens released from the biofilm. However, not only does the host response fail to eradicate the biofilm, but it may also result in damage to surrounding tissues. In contrast, antibiotic therapy or the action of the host immune response, or both, is generally effective against individual cells released from the biofilm [32]. Nevertheless, in terms of overall persistence, planktonic or free-floating cells, which were previously part of the biofilm, may play a role in the establishment of a new focus of infection. Thus, biofilm infections can often show recurring symptoms until the source of the infection is removed surgically.

Although the formation of biofilms on indwelling medical devices is generally associated with CNS, particularly *S. epidermidis*, *S. aureus* strains are also capable of biofilm formation [33, 34]. Thus, in addition to their ability to interact with the host-derived proteinaceous conditioning film which quickly coats inserted medical devices, some *S. aureus* strains are also capable of direct adhesion to plastic surfaces.

#### Formation of S. epidermidis biofilms

The ability of *S. epidermidis* to colonise biomaterial implants depends on the composition of the biomaterial and organism characteristics such as production of adhesin. Formation of *S. epidermidis* biofilms is proposed to occur in a two-step manner and much investigation has been directed towards dissecting the biochemical and molecular basis of this process. A

cellular accumulation process to form the mature biofilm follows rapid initial attachment to an inert plastic surface. At the biochemical level extracellular polysaccharide adhesins play an essential role in initial bacterial adherence and intercellular adhesion (biofilm formation). Two major polysaccharides produced by S. epidermidis have been examined, i.e., capsular polysaccharide adhesin (PSA) and polysaccharide intercellular adhesin (PIA). In the two-step model proposed by Mack et al. [35, 36] initial adherence is mediated by PSA or one of several proteins (including autolysin [37]), or both, and accumulation of cells is due to production of PIA. The PIA is encoded by the ica (intercellular adhesin) operon [38]. However, it has been reported recently that this operon also encodes PSA and that PSA and PIA are closely related chemically [39]. Recent investigations have also indicated that the purified polysaccharide responsible for haemagglutination (S. epidermidis strains have the ability to haemagglutinate erythrocytes) and PIA are also closely related if not identical [40]. PIA/PSA produced by S. epidermidis is composed primarily of N-acetyl-glucosamine in  $\beta$ -1,6-glycosidic linkages containing deacetylated amino groups and succinate and phosphate substitutions [35, 39, 41].

The ica gene cluster, which contains all the genes necessary for production of polysaccharide adhesin, was identified by transposon mutagenesis to isolate mutant S. epidermidis strains deficient in biofilm formation [35, 38, 42, 43]. The ica locus contains an operon, *icaADBC* (Fig. 1), which appears to contain the structural genes required for PIA synthesis. The IcaA gene product is a transmembrane protein with homology to N-acetyl-glucosaminyltransferases [44]. The functions of IcaB and IcaC are less well defined. However, IcaB is likely to be secreted while IcaC is predicted to be an integral membrane protein [38]. Expression of the small icaD gene appears to be necessary for optimal N-acetylglucosaminyltransferase activity [44]. A fifth gene, icaR, is located upstream of the icaA gene and is transcribed divergently from the icaADBC operon. The product of the icaR gene has homology to DNA binding transcriptional regulatory proteins and may be involved in the regulation of the ica structural genes.

The significance of the *ica* gene cluster in *S. epidermidis* infection has been demonstrated in a number of studies. Ziebuhr *et al.* [45] reported that 85% of *S. epidermidis* blood culture isolates contained the *ica* genes, compared with 6% of saprophytic isolates. However, a more recent study indicated a higher incidence (37.5%) of the *ica* gene cluster among



Fig. 1. Genetic organisation of the ica gene cluster from S. epidermidis.

carriage strains of S. epidermidis [46]. Nevertheless, these data indicate that saprophytic strains of S. epidermidis that contain the ica gene cluster have a competitive advantage in terms of colonising indwelling medical devices. Indeed, a multiplex PCR assay developed to distinguish between invasive and contaminating S. epidermidis strains found that targeting the ica and mecA genes detected significantly more infecting than contaminating isolates [46]. The ica operon was also found to be the only genetic marker capable of discriminating between commensal S. epidermidis strains and invasive isolates responsible for infections of joint prostheses [47]. Rupp et al. have recently demonstrated in animal models the essential requirement for an intact ica operon and production of polysaccharide adhesin in the pathogenesis of S. epidermidis catheter-related infection [48, 49]. In two recent reports, the polysaccharide adhesin encoded by the ica operon of S. aureus was used successfully to immunise mice against S. aureus kidney infection [50, 51]. It is significant that these investigators also reported that while few clinical isolates of S. aureus produce polysaccharide adhesin in vitro, it is elaborated during human and animal infection.

## Phase variation of polysaccharide adhesin production

Production of PIA, which represents the key virulence factor of S. epidermidis, is subject to  $ON \leftrightarrow OFF$ switching (phase variation). Altered regulation of important virulence genes is a favoured mechanism employed by many bacterial species to achieve the rapid and reversible changes characteristic of phase variable phenotypes. These alterations can be achieved by local genomic re-arrangements, altered activity of regulatory proteins or modulation of transcription or translation of the appropriate gene through strand slippage mechanisms [52]. Little is known about the molecular basis of phase variation of polysaccharide adhesin production in S. epidermidis. However, recent evidence has demonstrated that reversible insertion and excision of an insertion sequence element is responsible for ON ↔ OFF switching of the ica operon in approximately one-third of phase variants under laboratory conditions [53].

Clearly the process of polysaccharide adhesin phase variation in *S. epidermidis* is complex and involves more than one mechanism. Moreover, by contributing to the release of planktonic cells from mature biofilms, this property may have important implications in the pathogenesis of persistent and recurrent *S. epidermidis* biomaterial infections. Furthermore, an interesting association between the levels of resistance to methicillin, oxacillin and penicillin and the ability to form biofilms has been observed [54, 55], suggesting that phenotypic or genotypic change(s) which affect phase variation of polysaccharide adhesin production may

also impact on other properties. The global regulatory systems, Agr and Sar, which have been implicated in methicillin resistance in *S. aureus* [56], have also been identified in *S. epidermidis* [57, 58] and a recent report has indicated that an *S. epidermidis* agr deletion mutant is affected in its ability to form biofilms [59].

In biofilm-forming *S. aureus* strains the *ica* gene cluster, which is required for biofilm formation [34], is also subject to phase variable regulation [33]. However, it has recently been reported that while the majority of clinical *S. aureus* strains possess the *ica* structural genes, polysaccharide adhesin in *S. aureus* is expressed predominantly under in-vivo rather than in-vitro conditions [50, 51].

### Preventive strategies

The inherent resistance of bacterial biofilms to antimicrobial agents, together with the increasing number of antibiotic-resistant strains, highlights the need for effective preventive strategies. Prophylactic antibiotic therapy to cover surgical insertion of most biomaterials, apart from temporory intravascular devices, is now common practice. Nevertheless, infective complications often arise [60] and a number of reports have advised against the use of antibiotic prophylaxis, particularly vancomycin. Sieradzki and colleagues reported on the emergence of an S. epidermidis strain with elevated vancomycin tolerance following vancomycin prophylaxis in a dialysis patient [61]. In terms of catheterrelated infections, precautionary methods - including the use of aseptic techniques to prevent bacterial contamination from the insertion site and from catheter hubs during insertion – are recommended [60].

Alternative strategies to inhibit bacterial attachment or colonisation, or both, of implanted biomaterials are now the focus of many investigations. These strategies focus on the establishment of physical electrical barriers to colonisation and the use of biomaterials impregnated with antimicrobial agents. One approach involves combining antibiotic therapy with the passage of a low voltage electric current (or low frequency ultrasound [62]) through the implanted biomaterial. The generation of electrolytes such as protons, hydroxyl ions, reactive oxygen intermediates, oxygen and hydrogen appears to enhance antibiotic killing of bacterial cells attached to the device surface and is termed the bioelectric effect [63, 64]. A number of studies have demonstrated the effectiveness of a low voltage electric current and antibiotic prophylaxis against the establishment of S. epidermidis biofilms [65, 66]. However, these approaches have yet to be tested in a clinical setting.

A more established approach is the use of biomaterials impregnated with antimicrobial agents such as antibiotics or silver ions. Strategies to develop biomaterials with resistance to bacterial colonisation focus on achieving an optimum concentration of the antimicrobial agent in the biomaterial in order to deliver either a short-term high- concentration or a long-term constant concentration of the particular agent. The effectiveness of silver and silver-antibiotic combinations, incorporated into a range of biomaterials, against the development of *S. epidemidis* biofilms has been demonstrated *in vitro* [67], in animal models [68, 69] and in clinical trials [70]. In addition to reducing the rate of infection, the use of antiseptic-impregnated catheters has economic benefits and can significantly reduce hospitalisation costs [71]. It remains to be seen whether the routine use of such catheters is both cost-effective and associated with reduced infection rates.

#### Conclusions and future outlook

The prophylactic antibiotic treatment of patients with indwelling catheters and other biomaterials can often fail to protect against infection and can contribute to the emergence of antibiotic-resistant pathogens. Alternative strategies involving antiseptic bonded biomaterials are now in common use and future work on such biomaterials is likely to yield improved devices. At a molecular level, studies on the genetics and biochemistry of adhesion to and colonisation of biomaterials by S. epidermidis are identifying new targets for antimicrobial chemotherapy. Therapeutic strategies which interfere with the expression or activity of genes and gene products involved in S. epidermidis biofilm formation are likely to provide novel and potentially beneficial alternatives to current therapies. Finally, evidence of the dissemination of individual clones of S. epidermidis among hospital wards and units highlights the importance of implementing appropriate infection control practices to reduce infection rates.

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