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## ***Staphylococcus epidermidis* device-related infections: pathogenesis and clinical management**

Maureen T. McCann, Brendan F. Gilmore and Sean P. Gorman

### **Abstract**

*Staphylococcus epidermidis*, the most frequently isolated coagulase-negative staphylococcus, is the leading cause of infection related to implanted medical devices (IMDs). This is directly related to its capability to establish multilayered, highly structured biofilms on artificial surfaces. At present, conventional systemic therapies using standard antimicrobial agents represent the main strategy to treat and prevent medical device-associated infections. However, device-related infections are notoriously difficult to treat and bacteria within biofilm communities on the surface of IMDs frequently outlive treatment, and removal of the medical device is often required for successful therapy. Importantly, major advances in this research area have been made, leading to a greater understanding of the complexities of biofilm formation of *S. epidermidis* and resulting in significant developments in the treatment and prevention of infections related to this member of the coagulase-negative group of staphylococci. This review will examine the pathogenesis of the clinically significant *S. epidermidis* and provide an overview of the conventional and emerging antibiofilm approaches in the management of medical device-associated infections related to this important nosocomial pathogen.

### **Introduction**

Previously regarded as relatively innocuous, *Staphylococcus epidermidis* has gained significant interest in recent years and has become one of the most important pathogens in nosocomial infections (Vuong & Otto 2002), especially among immunocompromised, immunosuppressed, long-term hospitalised and critically ill patients (Domingo & Fontanet 2001; Ziebuhr 2001)

*S. epidermidis*, the most frequently isolated species of coagulase-negative staphylococci (CONS), is the leading cause of infections related to implanted medical devices (IMDs) (Rupp & Archer 1994). This is directly related to its capability to establish multilayered, highly structured biofilms on artificial surfaces (Figure 1).

Device-associated biofilms are notoriously difficult to eradicate (Gilbert et al 1997; Costerton et al 1999), with sessile populations being up to 1000-fold more resistant than their planktonic (free-floating) counterparts (Gilbert et al 1997). This is attributable to a number of factors observed in biofilm populations (Table 1), including restricted penetration, decreased growth rate, a distinct genetic phenotype (Handke et al 2004; Harrison et al 2004; Fitzpatrick et al 2005; O’Gara 2007), the expression of resistance genes (Maira-Litrán et al 2000) and the presence of biofilm persister cells (Lewis 2001, 2005; Sporing & Lewis 2001; Roberts & Stewart 2005).

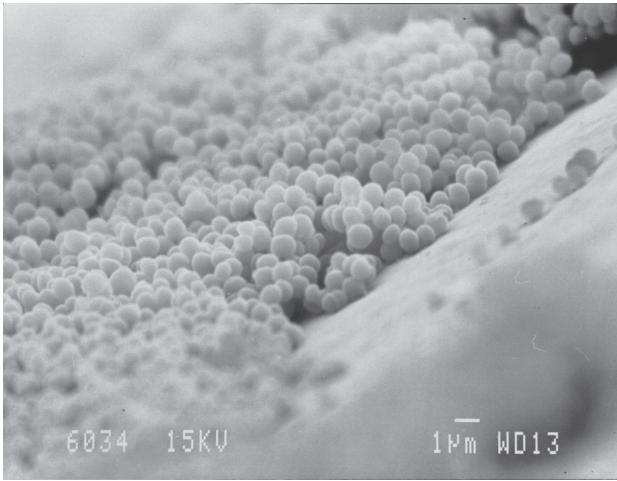
The adherence of pathogenic bacteria to medical devices and their subsequent colonisation and biofilm formation results in infection and often device dysfunction. Furthermore, infectious agents can disperse from the original site of colonisation and cause infection in other suitable niches. As antimicrobial treatment has little or no effect against biofilm populations on colonised medical devices, surgical removal and replacement of the device is often necessary and in cases where this is not a viable option, patients require intermittent antibiotic therapy for the remainder of their lives (Costerton et al 2003), leading to a significant morbidity and mortality (Rohde et al 2006).

Infecting organisms can originate from a number of sources, including the skin at the insertion site, colonisation of the medical device before implant, airborne contamination and microorganisms shed from theatre staff and other healthcare workers. Other factors that

Queens University of Belfast,  
School of Pharmacy, 97 Lisburn  
Road, Belfast BT9 7BL, UK

Maureen McCann, Brendan  
Gilmore, Sean Gorman

**Correspondence:** S. Gorman,  
Queens University of Belfast,  
School of Pharmacy, 97 Lisburn  
Road, Belfast BT9 7BL, UK.  
E-mail: s.gorman@qub.ac.uk



**Figure 1** Scanning electron microscope image of *S. epidermidis* biofilm grown onto the surface of polystyrene pegs.

increase the risk of prosthesis infection include prolonged hospitalisation, multiple surgical procedures at the time of implant, remote infections in other body parts, surgery duration and the amount of tissue devitalisation (Choong & Whitfield 2000). The increased use of IMDs and the growing number of immunocompromised and critically ill patients have also contributed to the rising number of medical device-related infections over the past number of years (Raad et al 1998).

The intent of this review is to discuss the pathogenesis of the clinically significant *S. epidermidis* and provide an overview of the conventional and emerging approaches in the management of medical device-associated infections related to this important nosocomial pathogen.

## Spectrum of Infection

*S. epidermidis*, primarily considered as non-pathogenic, normally colonises human epithelium and mucous membranes and rarely causes infection in immunocompetent patients (Ziebuhr et al 2006), except in native valve endocarditis (Caputo et al 1987). However, in recent years, the bacterium has emerged as a major causative organism in nosocomial infections (Vuong & Otto 2002). This member of the coagulase-negative group of staphylococci can cause severe infection following penetration of the protective epithelial barriers of the human body (Kocianova et al 2005). Medical device-related infections represent the main type of infection associated with *S. epidermidis* (Vuong & Otto 2002). Clinical presentation and infection syndrome are dependent upon the insertion site and type of medical device used (Heilmann & Peters 2001). Examples of device-related infections associated with *S. epidermidis* include prosthetic valve endocarditis following prosthetic valve implantation (Verhoef & Fleer 1983), keratitis due to contact lens use (Elder et al 1995), delayed onset post-operative endophthalmitis associated with intraocular lens implantation (Jansen et al 1991), bacteriuria following the use of urinary catheters (Warren 2001), intravascular catheter-associated infection (Rupp & Archer 1994; Rupp & Hamer 1998; Rupp et al 1999) and prosthesis-related infection including the septic loosening of joint prostheses after total joint arthroplasty (Gallo et al 2003; Ip et al 2005). Such infections can have potentially devastating consequences.

## Pathogenesis of *S. epidermidis* Infections

The pathogenesis of medical device-related infections associated with *S. epidermidis* is characterised by the

**Table 1** Factors contributing to biofilm resistance

Restricted penetration	Biofilms are enclosed within a protective extrapolymeric substance matrix (composed of a variety of components including exopolysaccharide, proteins and nucleic acids). This acts as a physical barrier that restricts access and penetration of some antimicrobial agents.
Decreased growth rate	Antimicrobials designed to target metabolic pathways are more effective in killing rapidly growing cells. Slow growth of biofilm cells significantly contributes to decreased susceptibility of sessile populations to growth-rate-dependent antimicrobials.
Distinct phenotype including expression of resistance genes	Gene expression in biofilms characterises a distinct physiological status that confers resistance to antimicrobials and the host immune defence, thus enabling bacteria to persist through infection. Furthermore, acquisition of resistant traits through lateral transfer of genetic material occurs more efficiently in biofilm cells as compared with their planktonic counterparts.
Persister cells	The presence of persister cells and their contribution to biofilm resistance is a relatively new phenomenon. Persisters are a unique class of inactive but highly protected cells that withstand a wide range of antimicrobial agents (Brooun et al 2000; Spoering & Lewis 2001), providing greater antimicrobial protection in a biofilm compared with a growing planktonic population (Roberts & Stewart 2005).
Altered chemical microenvironment	The extreme microenvironmental conditions present within a biofilm, including altered pH, pO <sub>2</sub> , pCO <sub>2</sub> , divalent cation concentration, hydration level and pyrimidine concentration, compromises the activity of certain antimicrobials, including aminoglycosides, macrolides and tetracyclines (Dunne 2002).

microorganism's ability to colonise the surface of IMDs by the formation of highly resistant biofilms. *S. epidermidis* biofilms are characterised by the reduction in basic cell processes and the induction of protective factors (Kong et al 2006). Physiological changes in *S. epidermidis* biofilms protect the bacteria from the host immune defence system by lowering the sensitivity toward harmful molecules, including antibiotics, cytokines and antibacterial peptides, and by causing a shift to a non-aggressive state reducing inflammation and the chemotaxis of immune cells to the site of infection (Yao et al 2005b). Such immune-evasion tactics enable the bacteria to persist during infection. Yao et al (2005b) described a global change in gene expression in *S. epidermidis* biofilms, including low metabolism, decreased transcription and translation and a shift from aerobic production of energy to fermentation, resulting in a non-aggressive and protected mode of growth that is less sensitive to antibiotics and the host immune defence and optimally suited to guarantee long-term survival during chronic infection.

In addition to its ability to form intrinsically resistant biofilms on the surface of polymers, *S. epidermidis*, is also one of the leading nosocomial pathogens that demonstrate a characteristic multidrug resistant pattern, including resistance to methicillin, quinolone and glycopeptide antibiotics (Raad et al 1998; Ziebuhr et al 2006). The extensive use of antimicrobial agents and disinfectants in the hospital environment provides an ideal setting for the development of resistant bacteria, further compounding this problem.

The success of *S. epidermidis* as a pathogen within the hospital environment can also be explained by its highly adaptive nature, inherent genetic variability (Yao et al 2005a) and intrinsic genetic flexibility, all of which enable it to withstand hostile external environments. Staphylococci demonstrate pronounced phenotypic variability and, as a result, specific properties such as colony morphology, growth rate, haemolysis and biofilm formation differ significantly among variants of the same parent strain (Ziebuhr 2001). *S. epidermidis* possesses superb recombination potential and the ability to transfer genetic material, resulting in the spread and evolution of resistance traits within nosocomial bacterial communities (Ziebuhr et al 2006). The evolving nature of *S. epidermidis* and its adaptation to the dynamic nosocomial environment has recently been highlighted by Kozitskaya et al (2005) who reported that *S. epidermidis* infections were mainly caused by a single intercellular adhesin (*ica*)-positive clone of *S. epidermidis* (Sequence type 27) whose presence was widespread within hospital settings in Germany. The ST27 clone was rarely found outside medical facilities and differed from commensal strains in the community and it was hypothesised that the successful establishment of ST27 within nosocomial environments was facilitated by the presence of genes encoding biofilms and resistance traits.

The lethal combination of the ability of *S. epidermidis* to form resilient biofilms, its multidrug resistance and genetic flexibility and the high selective forces operating in the hospital environment results in the survival, success and predominance of *S. epidermidis* as a nosocomial pathogen (Ziebuhr et al 2006).

## Mechanism of Biofilm Formation of *S. epidermidis*

Costerton et al (1987) defined a biofilm as the accumulation of microorganisms and their extracellular products to form a highly structured bacterial community on a surface. Biofilm formation of *S. epidermidis*, as with other bacteria, is a multistep process and occurs in four distinct phases (detailed in the following section) — attachment (adhesion), accumulation, maturation and detachment.

Planktonic microorganisms grow preferentially on surfaces and once attached, microbial adhesion and anchorage to the surface takes place. Accumulation of cellular aggregates rapidly follows, resulting in small adherent microcolonies coalescing to form large coherent bacterial biofilms (Choong & Whitfield 2000). The proliferation stage is facilitated by the upregulation of specific adhesion genes and the production of exopolysaccharide substances (e.g. polysaccharide intercellular adhesin (PIA)), which cement the bacterial cells to the surface and to other cells within the biofilm resulting in a defined structure (Choong & Whitfield 2000). Growth, multiplication and maturation of the bacterial cell consortium occur, establishing a complex biofilm architecture. Dissemination of microorganisms from the biofilm enables bacteria to colonise new surfaces and renew the biofilm formation cycle elsewhere.

The behaviour of *S. epidermidis* during biofilm development and within the sessile community is greatly influenced by cell-to-cell communication, a process known as quorum sensing (QS) (discussed later), which appears to influence biofilm formation at each of the stages of biofilm formation (Kong et al 2006).

### Surface conditioning

Before biofilm formation, surface conditioning of the medical device occurs upon implantation within the body. Proteinaceous, macromolecular components present in body fluids, such as urine, blood, saliva or mucus, adsorb onto the device immediately to form a conditioning film (Choong & Whitfield 2000). The proteinaceous molecules present within the conditioning film play an important role in bacterial adhesion (Choong & Whitfield 2000). Indeed, several of the host proteins present in the conditioning film can serve as receptors for bacterial attachment (Fitzpatrick et al 2005).

### Attachment

Initial bacterial adhesion is the first critical step in the development of implant-associated infection. Wang et al (1993) demonstrated that even low levels of adherent bacteria can be sufficient to potentiate an infection and noted that a biomaterial that mediates the adhesion of as little as 1% of the bacterial flux to the surface may amass a critical concentration of surface bacteria.

The initial adherence and attachment of *S. epidermidis* is mediated through a complicated interplay of virulence, device and host factors. Biomaterial surface chemistry



dictates initial adhesion, but also has long-term effects on the biofilm development process, including aggregation, viability, biofilm thickness and slime production in the presence of serum proteins (Patel et al 2007). When the role of material surface chemistry was studied in the formation of biofilms of *S. epidermidis* on polyurethanes modified with polyethylene oxide, adhesion, colonisation and biofilm formation were significantly impeded (Patel et al 2007).

Attachment can be based on direct binding to the native polymer surface of a medical device or via interaction with host extracellular matrix proteins present in the conditioning film that forms rapidly on the medical device surface following implantation. Both mechanisms are believed to be of importance during the crucial stage of initial colonisation, although there is debate as to which mechanism takes precedence. Wang et al (1993) observed that activated platelets mediated the adhesion of *S. epidermidis* RP62A to a hydrophobic polyethylene surface at a statistically significant level while adsorbed proteins did not. This observation is very important as *S. epidermidis* can adhere strongly to platelets, an integral part of the blood-contacting surface of the IMD, and as a result the susceptibility of the implant to infection remains high, years after the IMD is inserted (Wang et al 1993). In agreement with Wang et al (1993), Patel et al (2007) also reported that initial adhesion of *S. epidermidis* is suppressed in the presence of adsorbed serum proteins. As surface conditioning by host matrix proteins occurs within seconds following implantation of medical devices, attachment modulated by the presence of serum proteins may offer a more accurate representation of the in-vivo situation. Regardless of the exact mechanism of initial attachment, the end result is successful bacterial adhesion, which is an essential prerequisite in the pathogenicity of *S. epidermidis* infections and is rapidly followed by colonisation of the IMD.

#### Attachment to abiotic surfaces

Initial attachment of the planktonic microorganism to the unmodified surface of biomaterials is dependent upon a number of nonspecific, physicochemical variables such as electrostatic and hydrophobic interactions, van der Waals forces, surface tension, stearic hindrance and temperature (Dunne 2002).

The involvement of specific protein factors in the initial attachment of *S. epidermidis* to abiotic surfaces has also been noted, including the staphylococcal surface proteins, SSP-1 (280kDa) and SSP-2 (250kDa), which are organised in fimbria-like structures and mediate the adherence of *S. epidermidis* to polystyrene surfaces (Veenestra et al 1996). The major autolysin, AtlE, is also thought to play a dual role in adherence of *S. epidermidis* (Rohde et al 2006); it is active in attachment to both unconditioned and conditioned polymer surfaces.

#### Attachment to biotic surfaces

Microbial adherence of *S. epidermidis* is also mediated by specific protein factors. *S. epidermidis* produces surface proteins that are involved in the interaction with host proteins of the extracellular matrix present in the host-derived

conditioning film, and therefore in biofilm formation (von Eiff et al 2002). Examples include the surface associated autolysin AtlE (Heilmann et al 1997), which promotes binding to polystyrene surfaces and is also involved in vitronectin adhesion, the fibrinogen-binding Fbe (Nilsson et al 1998) and SdrG, SdrF and SdrH cell-surface-associated proteins (McCrea et al 2000). Fbe, SdrG, SdrF and SdrH are all members of the recently identified protein family, the serine-aspartate (SD)-repeat-containing (Sdr) family of cell-wall-anchored surface proteins (von Eiff et al 2002), which interact with extracellular matrix proteins. Embp, a fibronectin-binding protein (Williams et al 2002) and GehD, which binds to collagen (Bowden et al 2002), also specifically bind to extracellular matrix proteins and mediate adherence of *S. epidermidis* cells. In addition, the non-protein molecule teichoic acid, found in the cell wall, interacts with immobilised fibronectin, mediating binding of *S. epidermidis* (Hussain et al 2001).

The combined effect of specific and nonspecific factors results in the direct attachment of *S. epidermidis* to the IMD.

#### Accumulation

Following microbial adherence to the IMD, bacteria proliferate and accumulate in multilayered cell clusters (von Eiff et al 1999), resulting in an extensive network of accumulated bacteria. Despite the initial suppression of adherence of *S. epidermidis* in the presence of adsorbed proteins, interbacterial adhesion involved in the accumulation phase of intercellular aggregation and proliferation is increased dramatically in the presence of host serum proteins, resulting in the formation of a robust mature biofilm and therefore emphasising the importance of adsorbed proteins in biofilm development (Patel et al 2007).

The biofilm accumulation and development phase is characterised by the production of factors that mediate intercellular adhesion, including the extracellular polysaccharide, polysaccharide intercellular adhesin (PIA) and the biofilm-associated proteins – Aap (accumulation associated protein) and Bhp (Bap homologue protein).

#### Polysaccharide intercellular adhesin (PIA)

PIA is a major component of the extracellular staphylococcal carbohydrate matrix of *S. epidermidis* (Ziebuhr et al 2006). Mack et al (1992, 1994) identified PIA as the major functional component involved in intercellular adhesion, essential for the accumulation of multilayered *S. epidermidis* biofilms. PIA is a linear  $\beta$ -1,6-linked glucosaminoglycan, composed of  $\beta$ -1,6-linked *N*-acetylglucosamine residues containing up to 15% de-*N*-acetylated amino groups and substituted succinate and phosphate residues, which confer simultaneously positive and negative charges on the extracellular polysaccharide (Mack et al 1996). The unbranched structure facilitates long-range contacts and interactions between adjacent polysaccharide strands and the cell wall or lectins (or both), leading to intercellular adhesion and biofilm accumulation (Mack et al 1996). The exopolysaccharide PIA has also been implicated in the haemagglutinating activity of *S. epidermidis* (Fey et al 1999; Mack et al 1999). PIA is



**Figure 2** Organisation of the *icaADBC* gene cluster from *S. epidermidis* (adapted from O’Gara & Humphreys (2001)).

synthesised by enzymes encoded by the intercellular adhesin (*ica*) operon (Mack et al 1994, 1996; Heilmann et al 1996), which is significantly more prevalent in colonising *S. epidermidis* isolates (Vandecasteele et al 2003). Interestingly, PIA and biofilm expression are influenced by a variety of environmental stress stimuli, including the presence of ethanol, oxygen, salt, nitrite and several antibiotics (Schlag et al 2007).

The *ica* locus is composed of an operon, *icaADBC*, which encodes the structural genes required for PIA synthesis (Figure 2). The *ica* operon is composed of four open reading frames, *icaA*, *icaD*, *icaB* and *icaC* (Rohde et al 2006). A fifth gene, the divergently transcribed *icaR* gene, responsible for the transcription of *icaADBC* is located upstream of the *icaA* start codon. Conlon et al (2002) reported that the *icaR* gene encodes a transcriptional repressor involved in environmental regulation of the *ica* operon expression and biofilm formation in *S. epidermidis*. The opening frame *icaD* is located between *icaA* and *icaB*, overlapping with both genes. IcaA is responsible for *N*-acetylglucosaminyltransferase activity during PIA synthesis; the presence of IcaD is required for optimal transferase activity (Gerke et al 1998). IcaB, a deacetylase, is responsible for the deacetylation of the poly-*N*-acetylglucosamine molecule (Vuong et al 2004c). The transmembrane protein IcaC plays a putative role in externalisation, elongation and translocation of the growing polysaccharide to the cell surface (Rohde et al 2006) and is responsible for the production of the full-length PIA molecule, which is able to react with anti-PIA antisera. PIA production is regulated by the regulatory elements, SigmaB ( $\sigma^B$ ) and SarA, which operate independently of one another.  $\sigma^B$  and SarA regulate virulence factors in CONS and are involved in the transcriptional regulation of the *icaADBC* operon (Handke et al 2007). SarA, the global stress response regulator, and the *rsbU* gene (a positive regulator of  $\sigma^B$ ) both have important functions in the regulation of *ica* operon expression and the subsequent PIA synthesis of *S. epidermidis* (Conlon et al 2004; Knobloch et al 2004; Handke et al 2007). However, the exact role of  $\sigma^B$  in the regulation of *ica* operon expression requires further investigation due to the lack of an identifiable consensus binding site for this sigma factor (Conlon et al 2004).

The role of the *ica* operon, PIA production and the correlation with biofilm formation in infectious staphylococci has been extensively investigated. Ziebuhr et al (1997) demonstrated the significance of the *ica* gene cluster in the pathogenesis and biofilm formation of *S. epidermidis* in patients with device-associated septicaemia; 85% of *S. epidermidis* blood culture isolates contained the intercellular adhesin gene cluster, compared with 6% of saprophytic strains. Frebourg et al (2000) detected the *ica* gene using a PCR-based assay in 77% of blood culture isolates compared with 38% in commensals. Epidemiological

evidence also illustrated the correlation between pathogenicity and the presence of the *ica* genes in virulent strains of *S. epidermidis*, whereby the *ica* operon was present in 81.5% of infectious strains of which 62.9% formed biofilms (Galdbart et al 2000). The *ica* locus and biofilm formation are important parameters for staphylococcal colonisation and survival on IMDs (Fluckiger et al 2005); however, recent publications have revealed the emergence of biofilm-positive and *ica*-negative staphylococcal clinical isolates (Qin et al 2007). Recently, Ninin et al (2006) observed an increase in the subpopulation of biofilm-positive, intercellular adhesin (*ica*)-negative *S. epidermidis* isolates to 9.17% of the total 109 clinical isolates that caused bacteraemia in bone-marrow transplant recipients. Chokr et al (2006) also noted that PIA synthesis alone is not sufficient to produce a biofilm and that staphylococci can form a biofilm independent of PIA production, which was consistent with the findings that the presence of the *ica* locus alone is not sufficient for biofilm formation (Fitzpatrick et al 2002). Qin et al (2007) described two *ica*-negative *S. epidermidis* clinical strains, SE1 and SE4, that exhibited heterogeneity in biofilm architecture compared with the well-characterised *ica*-positive biofilm producer, RP62A *S. epidermidis* strain. A study carried out by Hennig et al (2007) reported a spontaneous switch in an *ica* insertion mutant to an alternative type of biofilm with an extracellular matrix composed of proteins rather than polysaccharide. Biofilm formation in the clinically significant *S. epidermidis* is a multifactorial process, ensured by more than one mechanism other than that which is dependent upon PIA expression (Hennig et al 2007).

#### *Accumulation-associated protein (Aap)*

In addition to exopolysaccharide involvement, recent evidence suggests that surface proteins play a leading role during the development of bacterial biofilm communities (Latasa et al 2006). Specifically, in *S. epidermidis*, the Accumulation associated protein (Aap) is essential to biofilm development and is involved in the second, accumulation phase of biofilm formation (Rohde et al 2005). Hussain et al (1997) first described Aap as a 140 kDa extracellular protein necessary for the accumulation of *S. epidermidis* strains onto polymer surfaces. Initially Aap was thought to act as a putative cell-wall receptor for PIA (Mack 1999), although it has subsequently been demonstrated that Aap is capable of mediating intercellular adhesion and biofilm formation in a completely polysaccharide-independent background (Rohde et al 2005). Transcription of the *aap* gene is upregulated in biofilm formation (Hennig et al 2007), whereby transcript analysis revealed an enhanced expression of *aap* in a PIA-independent biofilm producer. Aap is prevalent in clinical isolates of *S. epidermidis*, with *aap* encoding genes significantly more prevalent in catheter-colonising isolates than in invasive or skin isolates (Vandecasteele et al 2003).

The importance of this gene to biofilm production has recently been highlighted in a study by de Araujo et al (2006) in which 82% of methicillin resistant *S. epidermidis* isolates harboured the *aap* gene.

Aap is a cell-wall anchored (CWA) protein that is also secreted into extracellular fluids by biofilm-bound cells (Sun et al 2005). As with other CWA proteins, Aap is organised into several discrete domains; an N-terminal domain composed of 16 amino acid repeats, an all- $\beta$  A region, a B repeat region of 13 repeats of 128 amino acids and 19 proline-rich, tandem repeats of 6 amino acids, a sortase-recognition sequence LPDTG used as a Gram-positive cell-wall anchor, and a hydrophobic transmembrane region followed by a positively charged cytoplasmic tail at the C-terminus (Bowden et al 2005). B-repeat numbers are subject to variation, with the number of repeats varying between isolates (Rohde et al 2004; Bowden et al 2005; Monk & Archer 2007). Domain B also contains several G5 domains (named after the presence of conserved glycine residues), a common feature of which is *N*-acetylglucosamine binding (Bateman et al 2005). The major component of the  $\beta$ -1,6-linked glucosaminoglycan PIA is a ~28 kDa soluble linear, *N*-acetylglucosamine (Maira-Litrán et al 2002). It is therefore a distinct possibility that the G5 domain of Aap may be a putative binding site for PIA. This is in agreement with the initial proposal that lectin-like cross-linking of PIA by the 140 kDa protein could lead to aggregation or binding of PIA to the cell surface via Aap (Mack 1999). Although Aap can mediate biofilm formation in the absence of PIA (Rohde et al 2005), the multicellular behaviour of *S. epidermidis* is both complex and multifactorial. Aap could therefore play a co-operative role in biofilm formation with PIA whenever it is expressed.

Aap can be found in different forms on the *S. epidermidis* cell surface – as the full length (220 kDa) or truncated isoform (140 kDa); the truncated Aap is mainly composed of the repetitive B domain and is functional in biofilm accumulation in a PIA-independent manner. The N-terminal domain B is responsible for determining the aggregative properties of Aap as biofilm formation can be inhibited by purified domain B but not domain A (Rohde et al 2005). The latter authors illustrated that truncation of Aap is necessary for biofilm formation as expression of the truncated Aap isoform (comprising the repetitive B domain) in the Aap-negative *S. epidermidis* 1585 resulted in biofilm formation, but remained biofilm negative upon expression of the complete Aap. This provides further evidence that domain A of the protein is not involved and that biofilm formation occurs if Aap is truncated following the loss of the terminal A domain. Rohde et al (2005) also demonstrated, by inhibition of biofilm formation by addition of  $\alpha$ -2-macroglobulin, a broad-spectrum protease inhibitor, that Aap requires proteolytical processing by staphylococcal proteases to gain adhesive function.

More recently, Banner et al (2007) proposed that full-length Aap is expressed on cells of *S. epidermidis* NCTC 11047 (ATCC 14990) as tufts or short fibrils, the expression of which may be subject to phase variation and which contribute to the overall cell surface hydrophobicity.

Interestingly, not all biofilm-positive *S. epidermidis* strains produce the Aap protein (Hussain et al 1997) and this PIA-independent mechanism cannot be used solely to explain biofilm formation in PIA-negative strains.

#### *Biofilm-associated protein (Bap)*

A second surface protein, the Biofilm-associated protein (Bap), also plays an important role in protein-mediated, PIA-independent biofilm development of *S. epidermidis*. First described as a CWA protein inducing biofilm formation in an *Staphylococcus aureus* bovine mastitis isolate (Cucarella et al 2001), the homologue of Bap has subsequently been identified and found to promote biofilm formation in bovine *S. epidermidis* C533, as disruption of the *bap* gene abolished the capacity for *S. epidermidis* to form a biofilm (Tormo et al 2005). The gene encoding Bap has also been detected in several other CONS species of mastitis origin, including *Staphylococcus chromogenes*, *Staphylococcus xylosum*, *Staphylococcus simulans* and *Staphylococcus hycus*, all of which were found to be strong biofilm producers despite the fact that the *icaADBC* operon was absent (Tormo et al 2005). Tormo et al (2005) concluded that Bap orthologues present in CONS mastitis isolates induce an alternative, polysaccharide-independent mechanism of biofilm formation. Cucarella et al (2004) concurred that the presence of Bap may facilitate biofilm formation and suggested that Bap may compensate for the deficiency of the biofilm matrix polysaccharide PIA, encoded by the *ica* operon. To date, the function of Bap has been extensively investigated in *S. epidermidis* mastitis isolates: given the diversity between isolates from different hosts, further studies are required to determine the role of this protein in the biofilm formation process of human *S. epidermidis* strains.

Bap-related proteins share common structural features – they are present on the bacterial cell surface, have a high molecular weight, contain a core of tandem repeats and play a significant role in the pathogenesis of bacterial infection (Lasa & Penadés 2006). Two members of the Bap protein family have been described in *S. epidermidis*, Bap and Bhp (Bap homologue protein), both of which share striking structural similarities typical of CWA proteins present in Gram-positive bacteria, as revealed by amino-acid sequence.

Like Aap, and most CWA proteins, Bap has a multi-domain architecture (A, B, C and D) and is composed of an N-terminal signal sequence for extracellular secretion (43 amino acids), 16 identical 258nt tandem repeat units encoding reiterations of an 86 amino-acid sequence (C repeats) and a C-terminal segment containing a consensus LPXTG motif (Tormo et al 2005). Bhp also contains the structural characteristics of CWA proteins (N-terminal signal sequence, carboxy-terminal segment containing an LPXTG motif followed by a series of positively charged residues) and exhibits striking structural identity and similarity with the Bap protein of *S. epidermidis* (Tormo et al 2005). Despite these remarkable structural similarities and the suggestion that Bhp-related proteins may too be involved in biofilm formation (Tormo et al 2005), it now appears that this may not be the case. Bhp is found in only a minority of isolates from human infections (Rohde et al 2004) and is therefore of

**Table 2** Summary of the major factors operative in the primary attachment and bacterial accumulation phases of *S. epidermidis* biofilm formation

Primary attachment	Accumulation
Biomaterial surface chemistry	Polysaccharide intercellular adhesin (PIA)
Hydrophobic interactions	Accumulation associated protein (Aap)
Staphylococcal surface proteins (SSP-1 and SSP-2)	Biofilm associated protein (Bap/Bhp)?
Matrix protein binding (via Embp, Fbe, GehD, SdrF, SdrG, SdrH)	
Autolysin AtlE	

little significance in biofilm formation. Transcript analysis revealed diminished expression and repression of the *bhp* gene in an *S. epidermidis* variant producing proteinaceous biofilm (Hennig et al 2007). Hennig and co-workers also noted that higher expression levels of Bhp did not account for enhanced biofilm formation in a PIA-negative mutant. Furthermore, Qin et al (2007) observed that the two genes encoding Aap and Bhp were notably absent in two *ica*-negative, biofilm-positive *S. epidermidis* clinical strains, SE1 and SE4, and concluded that various novel molecular mechanisms operate in biofilm formation of *S. epidermidis*.

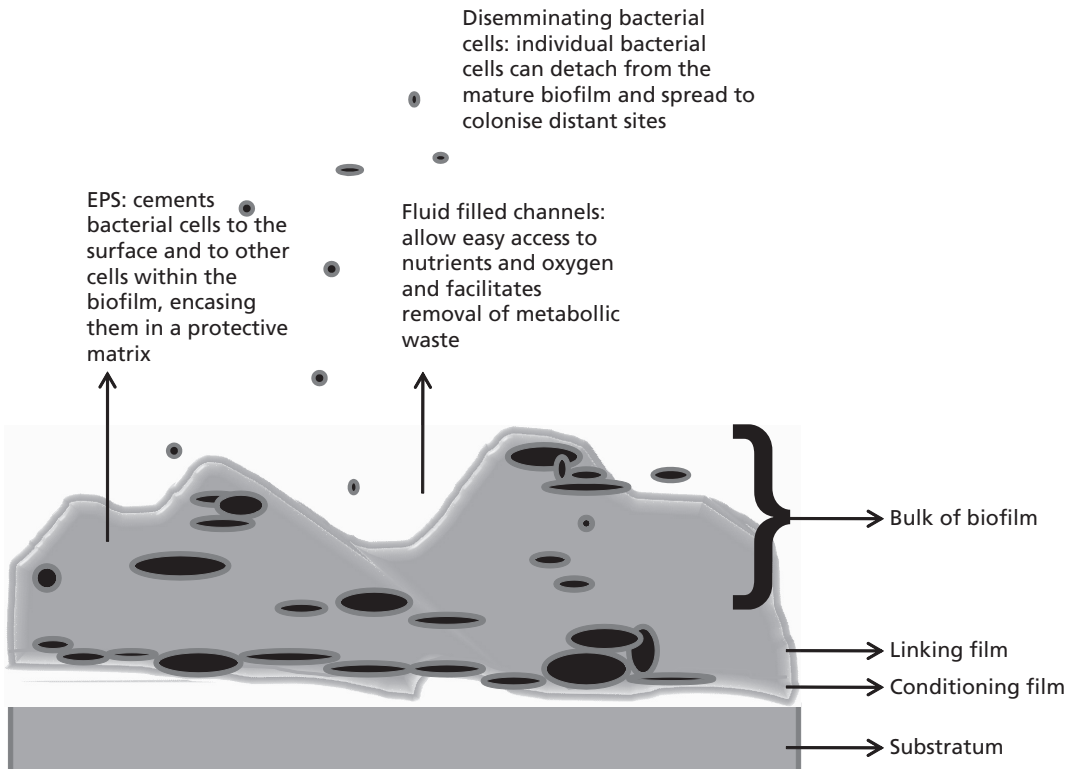
Table 2 provides a summary of the major factors involved in the primary attachment and accumulation stages of *S. epidermidis* biofilm formation.

**Maturation and detachment**

Maturation of the *S. epidermidis* biofilm is characterised by the generation of a slime glycocalyx, which encases surface-bound organisms in a gelatinous matrix (Dunne 2002).

Although the slime exopolysaccharide is not essential to the overall process of surface colonisation, it is thought to increase the stability of the biofilm architecture, leading to a more robust structure and making IMDs colonised with slime-positive strains more difficult to treat (Dunne 2002). It may also act as a barrier to the host response and administered antibiotics (Patel et al 2007). A mature biofilm structure comprises several layers, including the main bulk of the biofilm, a linking film, a conditioning film and the substratum to which the biofilm is attached (Habash & Reid 1999). The mature structure (Figure 3) reveals groups of microcolonies, often in mushroom-like forms separated by fluid-filled channels that are thought to deliver nutrients and oxygen to all cells in the biofilm and facilitate the removal of metabolic waste (Habash & Reid 1999).

Finally, individual bacterial cells, capable of actively leaving a biofilm, can arise and spread from the surface film on the outer side of the mature biofilm to colonise distant sites. Dissemination of bacterial cells and the establishment of additional infecting sites are of fundamental importance



**Figure 3** Mature biofilm structure (adapted from Habash & Reid (1999)). EPS, extrapolymeric substances.



for the spread of biofilm-associated infection (Kong et al 2006). The dispersion of virulent staphylococci has important implications for *Staphylococcus* biofilm infections; cells actively detaching from the biofilm not only may colonise alternative body sites but also may contribute to the toxemia associated with acute staphylococcal infections (Yarwood & Schlievert 2003). Expression of surface-associated adhesins, localised shear stress, cell viability and growth patterns may all contribute to the overall detachment process (Yarwood et al 2004). Recent studies have also suggested that the accessory gene regulator (*agr*) QS system (see below) plays a key role in the detachment process (Vuong et al 2004a; Yarwood et al 2004). When time-lapse confocal scanning laser microscopy was performed on staphylococcal biofilm populations it emerged that cells that expressed *agr* and, most probably, *agr*-dependent virulence factors appeared to be released from the biofilm (Yarwood et al 2004). Vuong et al (2004a) observed that *agr* expression was confined to the upper, most exposed, layers of the biofilm and expression of *agr* in the deeper cell layers was not detectable, and concluded that *agr* is most likely involved in promoting biofilm detachment.

At the biofilm interface, where *agr* is expressed, a layer of amphipathic peptides, known as phenol soluble modulins (PSMs), can exist (Vuong et al 2004a). PSM is a complex of amphiphilic peptides with inflammatory properties, the production of which is widespread throughout many CONS and contributes significantly to their virulence capacity (Otto 2004b). Delta-toxin, which is encoded by RNA-III, is one of the components of PSM and has been identified in *S. epidermidis*, *S. aureus*, *Staphylococcus saprophyticus* and *Staphylococcus haemolyticus* (McKevitt et al 1990; Mehlin et al 1999). Not only can the detergent-like peptide delta-toxin inhibit biofilm formation of *S. epidermidis* in-vitro (Vuong et al 2003) but it also plays a role in detachment from surfaces. Delta-toxin molecules inhibit hydrophobic interactions between bacterial cell surfaces, lowering the surface tension at the biofilm interface and causing the separation and detachment of bacterial cells from the biofilm.

### Quorum Sensing — Regulation of Biofilm Formation

Bacteria possess global regulatory systems that adapt virulence gene expression to changing environmental conditions during infection (Kong et al 2006). Among these global regulatory systems, cell-cell communication, also known as quorum sensing, has come to the forefront over recent years. QS, or cell-cell communication, describes the regulation of gene expression in response to increasing cell density and thereby enabling the bacteria to adapt to changing environmental conditions, such as a change in nutrient supply, altered oxygen levels and the switch from planktonic to biofilm growth (Otto 2004a, b). QS plays an essential role in synchronising gene expression and functional co-ordination among bacterial communities (Dong & Zhang 2005) and is crucial to establishing a well-ordered surface community. Specifically, QS systems play a central role in staphylococcal

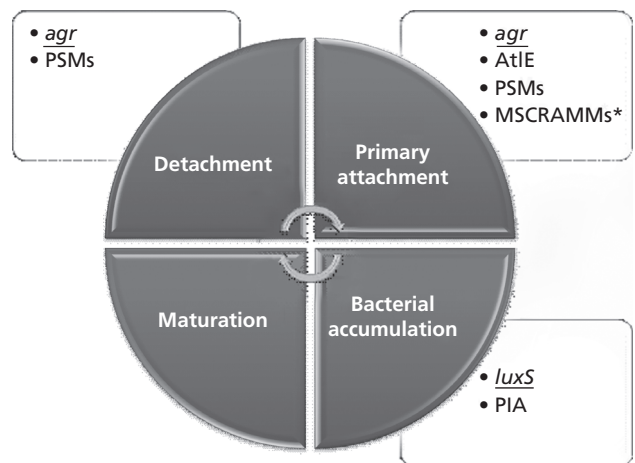
pathogenesis and appear to influence biofilm development at many of the distinct stages of biofilm formation (Figure 4). QS systems use small signalling molecules known as autoinducers (AIs). Once the AIs accumulate to a certain threshold level, activation of the QS system occurs and triggers the direct/indirect transcription of target genes (Xu et al 2006), often including a series of virulence factors.

Two QS systems have been identified and characterised in staphylococci, the *luxS* QS system and the accessory gene regulator (*agr*) system, both of which regulate several of the biofilm-associated factors of *S. aureus* and *S. epidermidis* at various stages of biofilm formation (Figure 4).

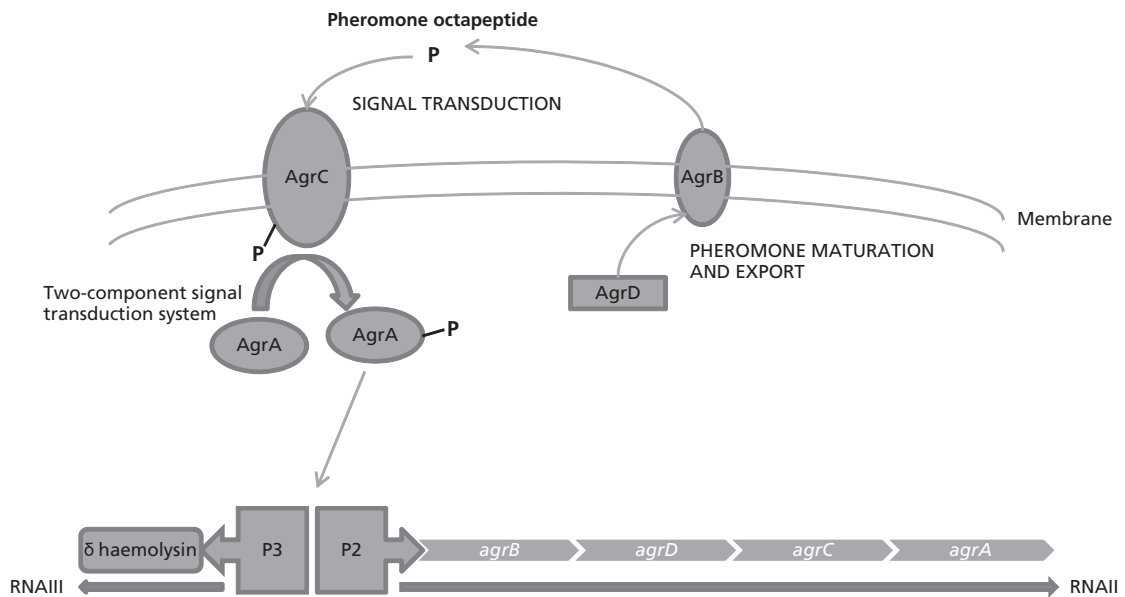
#### *agr* system

The first of the staphylococcal QS systems is the accessory gene regulator (*agr*) system, which has been characterised in great detail and assigned a key role in the pathogenesis of staphylococcal infections. The density-dependent, autoinducing *agr* circuit is crucial for invasiveness of *S. epidermidis* and greatly influences its capacity to cause biofilm-associated infections (Kong et al 2006).

The *agr* locus consists of two divergent transcription units, RNAII and RNAIII, controlled by the two promoters P2 and P3, respectively (Ji et al 1995). RNAII contains four genes (*agrA*, *agrB*, *agrC* and *agrD*) that are transcribed monocistronically from the promoter P2 (Mack 2007) while P3 drives the transcription of RNAIII, the effector molecule of the *agr* system (Yarwood et al 2004). RNAIII controls the transcription of target genes including several secreted virulence factors. In general, *agr* upregulates the expression of exoenzymes and toxins and downregulates the expression of surface proteins (Otto 2004a). The RNAIII region also encodes the peptide toxin delta-haemolysin (via *hld*), which is not involved in regulation (Figure 5).



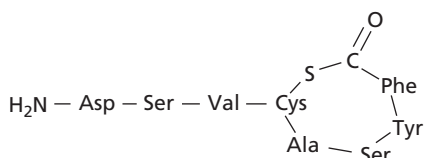
**Figure 4** QS regulation of biofilm-associated factors in *S. epidermidis*. \*Regulation of the microbial surface components recognising adhesive matrix molecules (MSCRAMMs) in *S. epidermidis* by *agr* is speculative. Results suggest that MSCRAMMs do not follow the classical pattern of *agr* downregulation (Otto 2008).



**Figure 5** The staphylococcal *agr* system. AgrD, the autoinducing pro-peptide, encoded by the *agrD* gene is post-translationally modified by the endopeptidase, AgrB. The modified pheromone octapeptide binds to the transmembrane receptor, AgrC, activating the response regulator AgrA. This, in turn, induces transcription of RNAII and RNAIII via the P2 and P3 promoters. Adapted from Otto (2004a).

The *agr* circuit comprises a two-component signal transduction system (AgrA and AgrC), AgrD, the prepheromone protein, and AgrB, which presumably is responsible for the post-translational modification and maturation of the pre-pheromone peptide to the mature peptide pheromone (Otto 2004a). The modified octapeptide pheromone (Figure 6) is the autoinductive signalling molecule of the *agr* system and contains a thiolactone ring structure, which is essential for biological activity (Mayville et al 1999). Ring size, central cysteine and thiolactone structure are conserved in staphylococcal pheromones; however, amino-acid sequence and length of *N*-terminal peptidyl sequence is subject to interspecies variation (Otto 2004a). The AI pheromone binds to histidine kinase AgrC and activates the response regulator AgrA, inducing the transcription of RNAII and RNAIII (Ji et al 1995).

The involvement of the *agr* system in biofilm formation has been demonstrated by a number of research groups. Vuong et al (2003) investigated the molecular basis of the influence of the *agr* QS system on biofilm formation in *S. epidermidis*. They constructed an *agr* deletion mutant and determined *agr*-dependent regulation of biofilm factors. The *agr*-negative phenotype enhanced biofilm formation on polymer surfaces compared with the *agr* wild type. Both primary attachment and AtlE expression were significantly



**Figure 6** *agr* pheromone structure. Adapted from Otto (2004a).

increased in the *agr* mutant, highlighting the regulatory effect exerted by the *agr* system on biofilm formation. Interestingly, PIA synthesis was not regulated by the *agr* system.

In another study carried out by Vuong et al (2004a), a QS *agr* *S. epidermidis* mutant formed a significantly thicker biofilm than the wild type suggesting that biofilm formation is controlled by *agr*. Vuong et al (2004a) proposed that biofilm formation involves *agr* activation at the biofilm–fluid interface and *agr*-dependent regulation of factors promote cell detachment from the biofilm.

In addition to its involvement in biofilm formation, the *agr* system also facilitates growth and survival in infected hosts through regulating the production of virulence factors of *S. epidermidis*. PSM, a pro-inflammatory peptide produced in abundance by *S. epidermidis*, is one of the first well-defined virulence factors of this nosocomial pathogen, with its production highly regulated and adapted to different types and stages of infection to ensure bacterial survival (Vuong et al 2004b). Novel liquid chromatography mass-spectrometry was used to quantify production of PSM components from 76 *S. epidermidis* strains (Vuong et al 2004b). Notably, production of the PSM components was completely abolished in an *agr* mutant, indicating that the *agr* QS system strictly regulates the production of the PSM peptides. Additionally, the *agr* mutant significantly reduced the activation of the innate immune response, including the activation of HIV-1-LTR, production of  $\text{TFN}\alpha$  and chemotaxis of neutrophils, underscoring the importance of this regulatory system on the pro-inflammatory capacity of *S. epidermidis*. Mehlin et al (1999) also confirmed that the inflammatory PSM peptide complex has a strong capacity to activate the human innate immune response and can contribute to the systemic manifestations of Gram-positive sepsis.

Interestingly, staphylococcal pheromones, the signalling molecules of the *agr* system, show cross-inhibiting properties whereby pheromones of self induce the *agr* system in contrast to pheromones of non-self, which suppress the *agr* response (Otto et al 2001).

Importantly, and unlike many QS systems described in Gram-negative bacteria, the QS systems of staphylococci reduce (rather than induce) biofilm formation (Kong et al 2006). This effect can primarily be related to an increase in protease expression and the production of PSMs and, secondly, to a decrease in adhesin production upon *agr* activation. Consequently, the activation of *agr* might facilitate seeding and metastasis of the staphylococcal cells. Permanent elimination of the QS system used by *S. epidermidis* seems to be advantageous to survival and establishment of infection, enhancing the success of *S. epidermidis* as a pathogen through the increased production of colonisation factors, biofilm formation and reduced activation of the innate immune response.

The expression of the bacterial Clp proteases (caseinolytic proteases), a class of ATP-dependent proteases involved in bacterial adaptation to stress and associated with the virulence of pathogenic bacteria (Porankiewicz et al 1999; Butler et al 2006), is also negatively regulated by *agr* (Wang et al 2007). The Clp proteases are divided into two distinct domains – an ATPase specificity factor and a proteolytic domain (ClpP) that contains a consensus serine protease active site (Michel et al 2006). Wang et al (2007) recently studied the peptidase function of *S. epidermidis clpP* and investigated its role in the biofilm formation of this important nosocomial pathogen. They showed that not only did deletion of *clpP* diminish biofilm formation, but it also decreased virulence and pathogen success during biofilm formation of *S. epidermidis*. To study the regulation of *clpP* and assess the regulatory involvement of *agr*, *clpP* expression in *S. epidermidis* wild type and an isogenic *agr* mutant strain were examined over a 24-h period. The level of *clpP* expression was significantly higher in the *agr*-mutant than in the wild type and it was concluded that *clpP* expression is down-regulated by the quorum-sensing *agr* system (Wang et al 2007).

### **luxS system**

The second of the QS systems observed in staphylococci is the *luxS* system, which is found in a variety of Gram-negative and Gram-positive species (Kong et al 2006). The *luxS* QS system uses the AI-2 signal as the AI molecule. Xu et al (2006) recently characterised the *luxS* QS system in *S. epidermidis* and its function in gene expression. Using an animal model of device-associated infection, Xu and colleagues showed that *luxS* limited biofilm formation and virulence of *S. epidermidis*. SEM analysis revealed that the *luxS* mutant generated a more compact and thicker biofilm compared with the wild-type strain and semiquantitative biofilm assays showed a significantly increased biomass in the *luxS* mutant compared with the wild type. *luxS* regulates the transcription of *ica* genes and the subsequent production of PIA. When transcription of the *icaC* gene was analysed by quantitative real-time PCR, *icaC* expression was significantly

higher in the *luxS* mutant strain than in the wild type, indicating that the *luxS* system negatively regulates expression of the *ica* gene at the transcriptional level (Xu et al 2006). PIA production was also higher in the *luxS* mutant, confirming that *luxS* also regulates PIA synthesis.

Although both the *agr* and *luxS* QS systems of *S. epidermidis* are involved in the regulation of different factors of biofilm formation, it would appear that both share the same overall effect on biofilm development – activation represses biofilm growth while disabling the regulatory system encourages biofilm formation.

## **Clinical Management of Device-related Infections Associated with *S. epidermidis***

Traditional treatment of *S. epidermidis* infections, including those caused by biofilms on IMDs, involves the use of conventional antibiotic therapy directed against the known or likely causative strain, the final choice depending on the microbiological, pharmacological and toxicological properties of the antibacterial agent. Antimicrobial substances are classified into two main groups – bactericidal or bacteriostatic agents. Bacteriostatic agents halt bacterial growth and reproduction but do not kill the cell; bactericidal agents (including disinfectants, antiseptics and antibiotics) kill bacterial cells. At the surface level, antibiotics kill or inhibit bacterial growth and can negatively affect the adhesion of microorganisms by interfering with bacterial adhesions resulting in the killing or prevention of binding of planktonic bacteria (Habash & Reid 1999).

Accepted clinical practice often includes combination therapy in which two or more antimicrobials are used to treat biofilm-associated infections (Saginur et al 2006). This approach comes from standard clinical practice, such that a broader spectrum of activity is achieved and lower concentrations of the antimicrobial are required, resulting in more effective therapy and decreased resistance (Gorman & Jones 2002).

Administration of prophylactic antibiotic therapy to prevent colonisation is also common practice during surgical insertion of most biomaterials (O’Gara & Humphreys 2001). However, infective complications often arise and it has been shown that even in the presence of antibiotics, adherence, colonisation and the establishment of infection can occur on the surface of IMDs.

Unfortunately, implant-associated infections are recalcitrant to typical antimicrobial therapy and host defences; these bacterial infections tend to be very difficult to eradicate and relapses occur frequently. A number of factors conspire to render medical device-related infections resistant to standard antimicrobial treatment, including the distinct mode of growth displayed by biofilm populations, multi-drug bacterial resistance and the increasing prevalence of *S. epidermidis* as a nosocomial pathogen. In addition, antibiotics currently in use are developed and assessed for activity against planktonic (free-growing) experimental models and are therefore ineffective against biofilm populations. Likewise,

the bacterial samples taken for microbiological culture and sensitivity testing from patients are extrapolated from planktonic free-floating bacteria, which are very different from bacteria in the biofilm mode of growth contributing to the clinical failure rate of treating chronic biofilm-associated infections (Choong & Whitfield 2000). Furthermore, antimicrobials administered systemically or orally often fail to reach the site of infection, again decreasing the success of antimicrobial chemotherapy.

### Antimicrobial resistance

Treatment of *S. epidermidis* infection is very difficult because of the increasing resistance to antibacterial agents. The development of antibiotic-resistant bacteria has increased at a frightening rate since the introduction of antibiotics in the 1940s. The frequent over-use of antibiotics, incorrect diagnosis, inappropriate prescribing, the preferential management of patients with antibiotics with broad-spectrum cover, non-compliance with antibiotic therapy by patients and the misuse of antibiotics in livestock and agriculture have all promoted the rapid spread of resistance even to modern antibacterials (Otto 2004b). The emergence of resistance among nosocomial pathogens can also be attributed to the increasing number of immunocompromised patients, the use of invasive procedures and devices and the breakdown of infection- and disease-control practises within the hospital environment. Antimicrobial resistance has a significant impact on patient outcome by enhancing virulence, delaying the administration of appropriate therapy, limiting available therapy and increasing hospitalisation time and subsequent recovery, leading to increased morbidity and mortality (Cosgrove & Carmeli 2003).

A recent study carried out on antibiotic resistance in exopolysaccharide-forming *S. epidermidis* strains from orthopaedic implant infections found only 10% of the 342 clinical isolates tested to be sensitive to all screened antibiotics (Arciola et al 2005). Up to 80% of the isolates were  $\beta$ -lactam resistant, 37% were methicillin resistant (MRSE) and 38% were resistant to imipenem. Aminoglycoside resistance was also observed in the clinical isolates with a frequency of 31–32%. Although resistance of *S. epidermidis* to vancomycin was not observed in this particular study, it has been reported elsewhere (Sanyal et al 1991; Nunes et al 2002, 2006, 2007). The glycopeptide antibiotics vancomycin and teicoplanin are normally reserved for use against multi-resistant staphylococci; however, due to increasing reliance on these agents, there are reports of reduced susceptibility of both enterococci and staphylococci to glycopeptides. The emergence of vancomycin-resistant CONS is alarming, yet not surprising considering the genetic versatility of staphylococci and the overuse of growth-inhibitory compounds that unavoidably select for the development of resistant organisms (Sritharan & Sritharan 2004). Most of the reports regarding the mechanism of glycopeptide resistance have focused on *S. aureus* and it appears that it is intrinsic and deriving from the accumulation of mutations and not due to genetic exchange (Pfeltz et al 2000). Cell-wall thickening associated with vancomycin resistance in *S. aureus* has been reported by a number of groups and it is thought to be a

pre-requisite for vancomycin resistance in staphylococci (Daum et al 1992; Hanaki et al 1998; Pfeltz et al 2000). Nunes et al (2006) recently characterised the glycopeptide susceptibility profiles and cell-wall ultrastructure of three clinical strains of CONS with reduced susceptibility to glycopeptides, including *S. epidermidis*. They highlighted that changes in cell-wall thickness were related to vancomycin minimum inhibitory concentrations (MICs), indicating that the bacterial cell ultrastructure plays an important role in glycopeptide resistance.

### Resistance of bacteria in biofilms

The intrinsic and acquired resistance of biofilm populations to antimicrobials (see Table 1) has been well documented. The mechanism of biofilm resistance is multifactorial and includes impaired penetration, reduced growth rate and a distinct phenotype exhibited by biofilm bacteria including expression of resistance genes. The biofilm environment promotes genetic exchange of antimicrobial resistance genes, increasing bacterial virulence and contributing to the development of multiresistance phenotypes (de Araujo et al 2006). Saginur et al (2006) recently confirmed the increase in resistance of biofilm-associated staphylococci and demonstrated that biofilm populations are much more resistant to inhibitory and bactericidal effects of antibiotics than planktonic cultures.

Treatment with antibiotics may kill planktonic bacteria shed from the biofilm surface; however, they fail to eradicate those embedded within the biofilm, which can then subsequently act as a nidus for recurrent infection (Stewart & Costerton 2001). Following standard antibiotic treatment, a minority of drug-resistant bacteria exist that repopulate the biofilm. Subsequent retreatment of the repopulated biofilm results only in a modest reduction in bacterial numbers, indicating that the repopulated biofilm is much more resistant to treatment (Ehrlich et al 2004).

At present, conventional systemic therapies, using standard antimicrobial agents, represent the main strategy for the treatment and prevention of medical device-associated infection. However, as detailed above, the available antibiotic therapies are usually ineffective because of the phenomenon of multidrug resistance and the resilient nature of adherent biofilm bacteria. As a result, effective eradication of the infection often necessitates the removal of the implant and its substitution. To address this problem, research efforts have focused over recent years to prevent biofilm formation on the surface of medical devices. Importantly, major advances in this research area have been made, leading to a greater understanding of the complexities of biofilm formation of *S. epidermidis* and resulting in significant developments in the treatment and prevention of infections related to this member of the coagulase-negative group of staphylococci.

### Current approaches – bactericidal and bacteriostatic agents

The limitations of conventional chemotherapy in the treatment of medical device-related infections have prompted the



development of novel approaches, complementary to traditional bactericidal or bacteriostatic mechanisms. These approaches, detailed below, focus on the development of bioactive, anti-infective or antimicrobial devices, which inhibit bacterial adherence or growth by the presence or elution of antimicrobial agents. Another frequently utilised option involves covalent surface modification of the medical device, which renders the surface inhospitable to bacterial colonisation.

### Antimicrobial biomaterials

In an effort to combat biofilm-associated infections on the surfaces of IMDs, many research groups and manufacturers have explored surface modification technologies to overcome bacterial colonisation and infection. Various methods have been employed to modify polymer surfaces and load antimicrobial agents into medical devices, with the ultimate aim to produce bacteria-inhibitory and bactericidal surfaces. A bacteria-inhibitory surface discourages or prevents both bacterial colonisation and proliferation, whereby a bactericidal surface elutes bactericides using controlled drug-release mechanisms (Lin et al 2001); this strategy is based on the prophylactic use of antibiotics, preventing microbial contamination from occurring on the surface of the medical device in the first instance and therefore preventing bacterial colonisation and biofilm formation. These surfaces are relatively low cost, have long shelf-lives, are easily sterilised and do not affect the overall function of a device (Lin et al 2001). Examples include immersion, coating, matrix loading and drug-polymer conjugates. Gamma radiation and albumin affinity surfaces have also been used to modify biomaterial surfaces (Gorman & Jones 2002).

The problem with straightforward antimicrobial loading into a device by coating or immersion is the generation of resistance. Several authors have raised concern that prophylaxis of staphylococcal infections using antibiotic-coated medical devices leads to the proliferation of resistance. As release of standard antibacterial agents from the surface of the medical device is not coordinated with exposure to bacteria, leaching of sub-inhibitory levels of the antimicrobial results, which is generally insufficient to prevent infection but increases the risk for selecting antimicrobial resistant strains. Achieving the desired drug-release kinetics also needs careful consideration; drug release can be both uncontrolled and relatively rapid, from a few hours to a few days, and therefore inappropriate for preventing device-related infections and chronic care (Lin et al 2001). Additionally, the mass of drug that can be incorporated is often insufficient for a prolonged bactericidal or bacteriostatic effect (Gorman & Jones 2002) and antimicrobial release often does not last the lifetime of the device.

Matrix loading, in which the antimicrobial agent is directly loaded into the polymeric matrix of the medical device, directing the controlled drug-release approach, is an attractive alternative to overcome such problems. However, this approach may adversely affect the mechanical properties of the medical device.

The formation of drug-polymer conjugates involves the covalent linkage of an agent to a monomer, before

polymerisation, resulting in the production of an extremely resilient drug-polymer material (Gorman & Jones 2002). Drug-polymer conjugates significantly reduce bacterial adherence and encrustation in urinary catheters, indicating the therapeutic potential of this approach for urinary catheter use in a site-specific manner. However, this approach is not without limitations, including increased cost of manufacture and chemical compatibility of therapeutic agents with the synthetic reaction scheme (Gorman & Jones 2002).

Research efforts have also been directed towards the production of biomaterial surfaces that selectively bind host albumin, as albumin-rich surfaces are known to diminish both bacterial adherence and coagulation. Keogh et al (1992) reported a technique for the production of biomaterials that selectively and reversibly bind albumin. The resulting albumin-binding biomaterial, Cibacron blue, diminished platelet adherence, decreased the surface activation of clotting and discouraged the binding of bacteria, namely *S. epidermidis*. However, the bulk derivatisation method used in the initial efforts of Keogh et al (1992) left ~30% of the surface unmodified and therefore still capable of triggering a thrombogenic reaction and causing infection. Consequently, they developed a new derivatisation method, involving surface grafting of blue dextran, that resulted in materials in which ~100% of the surface was capable of the preferential and reversible binding of albumin. Notably, the blue dextran-derivatised surface, upon pre-exposure to albumin, did not permit the adherence of potential pathogens, including *S. epidermidis*. Most importantly, the albumin-affinity surfaces permitted the normal behaviour of the host defence system on the biomaterial surface (Lin et al 2001). They also eradicated infection without releasing chemicals that are harmful to host tissues. This approach is therefore advantageous as cytotoxicity is low and the host's physiological equilibrium remains undisturbed; however, it is thought to be less effective when used in immunocompromised patients whose defensive cells show abnormal bactericidal activities (Lin et al 2001).

Antimicrobial impregnation is not limited exclusively to antibiotics; coatings that release metals, namely silver and copper ions, and nonspecific antiseptic coatings, including chlorhexidine, benzalkonium and nitrofurazone, have been used effectively against staphylococcal infections (Darouiche et al 1998; Moss et al 2000; Lee et al 2004; Presterl et al 2007). Silver has long been acknowledged as having a wide antimicrobial spectrum of activity. Biomaterials coated or impregnated with silver oxide, silver alloy and, more recently, silver nanoparticles have all been used in attempts to reduce infection, with varying degrees of success (Furno et al 2004). A large clinical trial carried out by Riley et al (1995) reported a lack of efficacy of silver-impregnated catheters versus unimpregnated catheters; in fact, further complications associated with the coated catheters were reported. Furno et al (2004) observed more encouraging results following the impregnation of silicone polymers with nanoparticulate silver metal. Here, the impregnated polymer showed good antimicrobial activity with promising release kinetics.

Conflicting studies relating to the efficacy of antiseptic coatings on the surface of medical devices have also been reported. Borschel et al (2006) assessed the clinical effectiveness

of antiseptic-coated catheters for critically ill patients in a real-world study. Here, they carried out a pretest–post-test cohort designed study measuring the primary outcome of catheter-related bloodstream infection rate after an intervention involving the use of chlorhexidine/silver sulfadiazine coated-catheters in six adult intensive care units. The intervention was associated with a 35% relative risk reduction of catheter-related bloodstream infection (CRBSI) and it was concluded that antiseptic-coated catheters appeared to be both clinically effective and economically viable in a real-world setting. A randomised controlled trial, involving 780 patients requiring central venous catheterisation, assessed catheter colonisation and related infection following insertion of chlorhexidine/silver sulfadiazine coated-catheters. Antiseptic catheters showed a substantial decrease in bacterial colonisation compared with standard uncoated triple lumen catheters; however, this study was unable to show a significant decrease in CRBSI, possibly because of the low infection rate due to fastidious aseptic technique used during catheter insertion (Rupp et al 2005). In contrast, Osma et al (2006) found that central venous catheters impregnated with chlorhexidine and silver sulfadiazine had no effect on the incidence of CRBSI or catheter colonisation in critically ill patients.

Resistance develops less readily to antiseptics and economically they hold more promise compared with antibiotics, although concern has been raised by several authors about the cytotoxicity of antiseptic coatings; as they are not selective in their toxicity, not only do antiseptics eliminate bacterial cells but other cellular damage may be inflicted in the process (Gorman & Jones 2002). Similar toxicological issues related to systemic metal accumulation also limit the use of coatings that release metal ions (Campoccia et al 2006). In conclusion, although antiseptic-coating and, indeed, the use of antimicrobial agents other than antibiotics have shown promise, certain limitations associated with their use as antibiofilm agents exist and their applications may be restricted only to special circumstances in patient groups at high infection risk.

Conceptually, the use of biomaterials combined with standard antimicrobial agents is a simple and straightforward strategy to reduce the chances of bacterial colonisation and subsequent biofilm formation. However, this approach is not without limitations. Firstly, owing to the hyper-resistant nature of biofilm communities and the high prevalence of antimicrobial resistance, traditional bactericidal or bacteriostatic mechanisms are ineffective in preventing microbial colonisation of medical devices. The release of sub-inhibitory concentrations of antimicrobials from biomaterials into surrounding tissue and fluids can also induce resistance in infecting bacteria (Gorman & Jones 2002). Biocompatibility of bioactive bulk materials is also a major cause for concern, as is the achievement of desirable drug-release kinetics, a critical aspect to ensure full efficacy of the treatment (Montanaro et al 2007).

### Emerging approaches – nonbactericidal antibiofilm approaches

Recent advances in the knowledge of the molecular mechanisms of biofilm formation and the production and

regulation of virulence factors in *S. epidermidis* have opened the way to the development of a number of therapies that are 'antibiofilm' in nature. These strategies target functional molecules, gene systems and regulatory circuits, which control the overall architecture of the biofilm and, subsequently, the aetiology of medical device-associated infection. Table 3 summarises the mode of action and targets of a variety of novel antibiofilm approaches that have been developed recently for the treatment and prevention of *S. epidermidis* device-related infections. Therapeutic strategies are aimed at the disintegration of established biofilms and include QS perturbation, which leads to the down-regulation of molecules stabilising the biofilm architecture, or the use of enzymes to dissolve the biofilm matrix (Rohde et al 2006). Prophylactic measures include the development of effective immunotherapy and vaccination.

### Novel prophylactic approaches

Once established, device-related infections are almost impossible to eradicate; subsequently, the need for prevention of infection is as acute as ever. As with the saying "prevention is better than cure", these strategies focus on the development of novel antibiofilm agents, employing methods to prevent bacterial colonisation and the development of a clinically relevant infection as opposed to treatment of an already established infection.

Immunoprophylaxis is an extremely promising strategy for the prevention of staphylococcal device-related infections. Götz (2004) has summarised the potential targets for vaccine development in staphylococci. Surface-expressed components of *S. epidermidis*, including PIA, teichoic acids, proteinaceous adhesins and CWA proteins, have been identified as promising targets for vaccine development and immunomodulation. Staphylococcal vaccination has been used, with some degree of success, to prevent infections in animals (including bovine mastitis), suggesting that a staphylococcal vaccine is achievable. As mentioned previously, PIA is a linear  $\beta$ -1,6-linked glucosaminoglycan, composed of  $\beta$ -1,6-linked *N*-acetylglucosamine, which is involved in intracellular adhesion and is essential for the accumulation of multilayered *S. epidermidis* biofilms. PIA is an attractive candidate for vaccine development. Immunisation with either highly acetylated (>95%) or poorly acetylated (<15%) PIA conjugated to diphtheria toxoid showed in-vitro opsonic killing of both *S. aureus* and *S. epidermidis* (Maira-Litrán et al 2005). Protection was due to antibodies directed against the non-acetylated PIA antigen (Maira-Litrán et al 2005). A monoclonal antibody against staphylococcal lipoteichoic acids has been developed by Biosynexus Inc. This monoclonal antibody (BSYX-A110), developed for high-risk patients including low birth weight infants, was reported to be protective in animal models and demonstrated efficacy against *S. aureus* and *S. epidermidis* infections (Götz 2004). Although immunoprophylaxis is an attractive approach for the prevention of device-associated infections, it is not without its drawbacks. It is very likely that staphylococci have developed a number of defence mechanisms that threaten the production of an effective vaccine, including the occurrence of allelic variation,

**Table 3** Novel antibiofilm approaches

Approach	Mechanism of action	Target	Reference
<i>QS interference</i>			
RNA III-inhibiting peptide (RIP)	QS interruption	RNAIII synthesis	Cirioni et al 2003; Balaban et al 2004, 2005
<i>Impairing adhesion</i>			
Biosurfactants, including RC14 biosurfactant 'surlactin'	Anti-adhesive activity; interference with initial bacterial attachment	Microbial adhesion	Valraeds et al 1998; Rodrigues et al 2006
Furanone compounds	Reducing adhesion and colonisation	Gene encoding adhesion and slime production?	Baveja et al 2004; Hume et al 2004
Diterpenoids (salvipisone and aethiopinine)	Destabilising biofilm matrix allowing detachment +/- altering bacterial cell surface hydrophobicity	Biofilm matrix +/- bacterial cell surface	Kuźma et al 2007; Walencka et al 2007
<i>Targeting slime formation</i>			
<i>N</i> -acetyl-D-glucosamine-1-phosphate acetyltransferase (GlmU) inhibitors (N-substituted maleimides)	Inhibiting bacterial cell wall synthesis and PIA formation	PIA biosynthetic enzymes; GlmU enzyme	Burton et al., 2006
<i>N</i> -acetylcysteine (NAC)	Reducing production of extracellular polysaccharide matrix and promoting disruption of mature biofilm	Extracellular polymeric matrix	Pérez-Giraldo et al 1997; Aslam et al 2007
Bacteriophage therapy; phage K & bacteriophage 456	Lytic activity on biofilm cells	Biofilm exopolysaccharide and biofilm cells	Curtin & Donlan 2006; Cerca et al 2007
<i>Immunotherapy</i>			
FN binding receptor monoclonal antibodies (MAbs)	Blocking adhesion	FN binding receptor	Bryers & Ratner 2004
Anti-PIA antibodies	Inhibition of PIA formation	PIA	McKenney et al 2000
Surface binding protein/Fbe antibodies	Blocking adhesion	Fbe	Pei et al 1999; Pei & Flock 2001; Rennermalm et al 2004
Anti-Aap domain B antiserum Aap antibodies	Inhibiting accumulation and intercellular adhesion	Aap	Rohde et al 2005; Sun et al 2005; Rohde et al 2007
<i>Enzymatic removal</i>			
Oxidoreductases & polysaccharide-hydrolysing enzymes	Enzymatic removal and disinfection of biofilm	Biofilm matrix	Johansen et al 1997
Lysostaphin (staphylolytic endopeptidase)	Disruption of biofilm matrix and killing of released bacteria	Peptidoglycan pentaglycine interpeptide cross-bridges of staphylococcal cell wall	Wu et al 2003
Dispersin B (DspB)	Enzymatic degradation of cell bound exopolysaccharide adhesin, an essential component of the biofilm polymeric matrix	$\beta$ -1, <i>N</i> -acetyl-D-glucosamine	Kaplan et al 2004; Donelli et al 2007
Serratiopeptidase	Induces biofilm degradation via proteolytic activity, also enhances antibiotic activity	Biofilm slime matrix	Selan et al 1993; Mecikoglu et al 2006
<i>Immunomodulation</i>			
Interferon $\gamma$	Reversal of macrophage deactivation in the vicinity of implanted biomaterial	Macrophages	Boelens et al 2000a, b

redundant protein functions or altered stress-induced expression properties (Projan et al 2006). Furthermore, as multiple determinants are involved in the establishment of *S. epidermidis* biofilm formation, this organism can rely on other compensatory mechanisms to establish colonisation when a single virulence mechanism is impaired. Without question, effective immunisation and vaccination against *S. epidermidis* device-associated infection is an attractive concept; however, given the extraordinary versatility exhibited by this microorganism, further investigations, both pre-clinical

and clinical, are warranted before an optimal approach is achieved.

### Antipathogenic agents – targeting quorum sensing

Targeting bacterial virulence for antimicrobial chemotherapy and the subsequent development of 'antipathogenic' agents is a relatively new concept. The aim of antipathogenic therapeutics is to identify factors essential for virulence of a pathogen and develop compounds to inhibit them

(Marra 2004). Such drugs that attack bacterial virulence are believed to reduce the development of antimicrobial resistance compared with traditional antibacterial drugs, which kill bacteria or inhibit their growth (Alksne & Projan 2000).

As QS is heavily involved in the expression of virulence factors in staphylococci and in biofilm formation, it represents an ideal target for antimicrobial drug therapy and falls into the category of 'antipathogenic' therapeutics.

QS blockers have been proposed as novel therapeutic agents for the treatment of staphylococcal infections. As they suppress only virulence factor expression and do not act as bacteriolytic or bacteriostatic agents, the risk for resistance development is assumed to be minimised (Otto et al 1999). In addition, since the effective working concentrations of QS inhibitors are non-toxic and below the minimal inhibitory concentration, they are not expected to exhibit adverse effects on beneficial bacteria present in the host (Hentzer et al 2002).

Quenching the QS system as a therapeutic target to control infection is not an original concept and has previously been studied in many Gram-negative species. One of the well-characterised QS signals in Gram-negative bacteria is *N*-acylhomoserine lactones (AHLs), which have been identified in a wide range of Gram-negative bacteria and regulate a variety of biological functions, including virulence and biofilm formation. *Pseudomonas aeruginosa* possesses two AHL-mediated QS circuits: the *las* system and the *rhl* system. The *P. aeruginosa* communication system can effectively be blocked by a novel halogenated furanone compound, which competes with the AHL for its binding site and whose structure is based on the naturally occurring biologically active furanones produced by the Australian red macroalga *Delisia pulchra* (Hentzer et al 2003). Hentzer et al (2002) demonstrated that the synthetic halogenated furanone compound is capable of penetrating the *P. aeruginosa* biofilm matrix where it interferes with biofilm maturation and enhances the process of bacterial detachment through interference with the QS system. Interestingly, the AHLs have been shown to antagonise virulence gene expression and QS in *S. aureus*, through interaction with the cytoplasmic membrane in a saturable and specific manner (Qazi et al 2006). The ability of a novel furanone compound, 3-(1'-bromoethyl)-5-dibromomethylene-2(5*H*)-furanone, to inhibit *S. epidermidis* adhesion and slime production was recently assessed by Baveja et al (2004). Bacterial load and slime production were significantly inhibited at 24 h on all biomaterials tested, indicating that physically adsorbed furanones could be used as coatings to prevent staphylococcal device-associated infections.

Recently, several groups of novel and potent AHL-degradation enzymes have been unveiled in Gram-negative bacteria, including AHL-acylases and AHL-lactonases. These potent AHL-degradation enzymes have been shown to efficiently quench the microbial QS signalling systems and block pathogenic infection, demonstrating the feasibility of disease control through interference with the microbial QS signalling system (Dong & Zhang 2005).

Mayville et al (1999) proposed the use of inhibition of *agr* as an approach to novel therapeutics for the treatment of staphylococcal infections. The inhibitory activity of a

synthetic thiolactone peptide, the signalling molecule of the *agr* QS system, was assayed in a mouse model whereby the ability of an *S. aureus* *agr*-positive strain to cause a skin abscess was assessed. A dramatic attenuation, almost to the extent of the entire *agr* region was observed, demonstrating biological activity with respect to the *agr* response in an acute infection. It is important to note that the majority of staphylococcal infections are chronic and persistent, therefore the findings from this study have limited applications.

Exploitation of the QS system in the prevention of pathogenesis of staphylococci has recently been highlighted by Balaban et al (2005), whereby the possibility of using the putative QS inhibitor ribonucleic-acid-III (RNAIII) inhibiting peptide (RIP) was explored. They found that the heptapeptide RIP prevented graft-associated infections in all species of staphylococci tested, including methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE). Balaban et al (2005) proposed that RIP inhibits the target protein of RAP (TRAP) – a component of a third potential QS in staphylococci, the functioning of which is subject to much academic controversy (Novick 2003). RAP, the RNAIII-activating peptide, is proposed to induce phosphorylation of TRAP, resulting in attachment, biofilm formation and toxin production. RIP is thought to displace RAP binding, inhibiting TRAP phosphorylation, cell adhesion and toxin production and therefore preventing biofilm formation in-vivo (Balaban et al 2005). Balaban et al (2005) found that when the target of RNAIII activating protein activity was disrupted, biofilm formation was reduced under static conditions and that genes involved in toxin production and biofilm formation were down-regulated. When combined with a dermaseptin derivative (DD<sub>13</sub>), an agent believed to kill bacteria via membrane disruption, the hybrid peptide RIP-DD<sub>13</sub> was found to eradicate drug-resistant staphylococcal infections through inhibition of pathogenesis (Balaban et al 2004). These findings suggest that inhibition of biofilm formation by RIP is not due to a detergent-like effect exerted by this amphipathic peptide but caused by interference with a regulatory QS process and therefore pathogenesis, indicating that RIP has potential therapeutic properties. However, further elucidation is required about the exact mechanism of action of the RAP-TRAP system for it to be considered as a QS system of staphylococci in its own right.

Blocking the QS response initially appeared to be both an attractive and reasonable approach to staphylococcal infections as proposed by Mayville et al (1999). However, as detailed previously, staphylococci respond in a unique fashion to inhibition of QS activity by enhancing biofilm formation, increasing the production of virulence factors and reducing the activation of the innate immune response, resulting in bacterial survival and the establishment of more persistent infections. The use of QS blockers as therapeutic agents should be restricted to acute infections or avoided entirely as they may in fact transform an acute infection to a chronic staphylococcal infection (Vuong et al 2000). The flipside of this situation would be activation of either the *agr* or *luxS* QS system in staphylococci (would this result in decreased bacterial pathogenicity?), causing decreased colonisation, reduced biofilm formation and activation of the



body's natural defence mechanism, facilitating the elimination of invading bacteria. Biofilm disruption due to *agr* activation for the development of possible therapeutic strategies is an attractive concept; however, bearing in mind the cross-inhibiting properties of the signalling molecules of the *agr* system, the effect on mixed species biofilms must be considered in this situation. As patients normally carry a number of staphylococcal species, activation of the QS system of one species of staphylococci may decrease the invasiveness of that strain; however it could enhance the pathogenic potential of another.

Finally, the viability of targeting virulence for the development of antibacterial agents must be called into question. Many authors remain sceptical about this approach citing reasons such as the lack of appropriate models to analyse potential virulence inhibitors (Alksne & Projan 2004) and whether or not inhibition of virulence factors in-vivo will have a significant effect to clear an infection already in progress (Marra 2004). In conclusion, harnessing the QS system is a challenging proposition and a potential minefield. Careful consideration and extensive investigation into interference with the QS systems is therefore required and it remains to be evaluated as a feasible target for the development of novel anti-virulence and anti-staphylococcal agents.

### Concluding Remarks

As medical device-related infections associated with *S. epidermidis* have a significant impact on morbidity, mortality and both social and economic costs, prevention and management of such infections remains a priority. Many past efforts have focused on basic infection control measures and the use of traditional antimicrobial agents for the prevention and treatment of infection; however, infections associated with IMDs continue to present at an alarming and unacceptably high rate (Darouiche 2007). Since biofilm formation is a critical aspect of infection, emerging novel therapies currently focus on the prevention of infection using antibiofilm agents that inhibit the microbial attachment process. Recent advancements include QS perturbation, immunotherapy, disruption of the protective extrapolymer matrix and disintegration of the biofilm architecture in a bid to prevent implant-related infection. Potentially, the synergistic combination of novel antibiofilm strategies with more traditional bactericidal and bacteriostatic approaches could be utilised to further prevent and control infection.

While much knowledge exists about the molecular mechanisms and regulatory pathways involved in biofilm formation of *S. epidermidis*, it is obvious that much has yet to be learned. Of particular interest is further elucidation of the process of *S. epidermidis* virulence factor expression and regulation. Research is also required to define the effectiveness of pathogenesis suppression in the bid to develop novel antipathogenic agents. The feasibility of targeting bacterial virulence factors must also be considered.

Protection from harmful host conditions, sequestration to a nutrient-rich area and utilisation of cooperative benefits are all attractive incentives that drive bacteria from planktonic to

sessile growth (Jefferson 2004). The preference to exist as a biofilm community combined with the high genomic flexibility and antibiotic resistance exhibited by *S. epidermidis* (Ziebuhr et al 2006) ensures that biofilm-associated infections continue to be extremely difficult to eradicate. This is further confounded by the intrinsic limitations of current in-vitro biofilm models (Jefferson 2004). It is expected that future research will contribute vital new information towards the understanding and clinical management of staphylococcal device-associated infections; however, clinicians should remain prepared to deal with their unique and obstinate nature.

### References

- Alksne, L. E., Projan, S. J. (2000) Bacterial virulence as a target for antimicrobial chemotherapy. *Curr. Opin. Biotechnol.* **11**: 625–636
- Antimicrobial resistance (n.d.) National Institute of Allergy and Infectious Diseases, [http://www3.niaid.nih.gov/about/overview/profile/fy2003/pdf/SSAR\\_ANTIMICROBIAL.pdf](http://www3.niaid.nih.gov/about/overview/profile/fy2003/pdf/SSAR_ANTIMICROBIAL.pdf), accessed 17th January 2008
- Arciola, C. R., Campoccia, D., Gamberini, S., Donati, M. E., Pirini, V., Visai, L., Speziale, P., Montanoro, L. (2005) Antibiotic resistance in exopolysaccharide-forming *Staphylococcus epidermidis* clinical isolates from orthopaedic implant infections. *Biomaterials* **26**: 6530–6535
- Aslam, S., Trautner, B. W., Ramanathan, V., Darouiche, R. O. (2007) Combination of tigecycline and N-acetylcysteine reduces biofilm-embedded bacteria on vascular catheters. *Antimicrob. Agents Chemother.* **51**: 1556–1558
- Balaban, N., Gov, Y., Giacometti, A., Cirioni, O., Ghiselli, R., Mocchegiani, F., Orlando, F., D'Amato, G., Saba, V., Scalise, G., Bernes, S., Mor, A. (2004) A chimeric peptide composed of a dermaseptin derivative and a RNaseIII-inhibiting peptide prevents graft-associated infections by antibiotic resistant staphylococci. *Antimicrob. Agents Chemother.* **48**: 2544–2550
- Balaban, N., Stoodley, P., Fux, C. A., Wilson, S., Costerton, J. W., Dell'Acqua, G. (2005) Prevention of staphylococcal biofilm-associated infections by the quorum sensing inhibitor RIP. *Clin. Orthopaedics Rel. Res.* **437**: 48–54
- Banner, M. A., Cunniffe, J. G., Macintosh, R. L., Foster, T. J., Rohde, H., Mack, D., Hoyes, E., Derrick, J., Upton, M., Handley, L. S. (2007) Localised tufts of fibrils on *Staphylococcus epidermidis* NCTC 11047 are comprised of accumulation association protein (Aap). *J. Bacteriol.* **189**: 2793–2804
- Bateman, A., Holden, M. T. G., Yeats, C. (2005) The G5 domain: a potential N-acetylglucosamine recognition domain involved in biofilm formation. *Bioinformatics* **21**: 1301–1303
- Baveja, J. K., Willcox, M. D., Hume, E. B., Kumar, N., Odell, R., Poole-Warren, L. A. (2004) Furanones as potential anti-bacterial coatings on biomaterials. *Biomaterials* **25**: 5003–5012
- Boelens, J. J., van der Poll, T., Dankert, J., Zaat, S. A. (2000a) Interferon-gamma protects against biomaterial-associated *Staphylococcus epidermidis* infection in mice. *J. Infect. Dis.* **181**: 1167–1171
- Boelens, J. J., Dankert, J., Murk, J. L., Weening, J. J., van der Poll, T., Dingemans, K. P., Koole, L., Laman, J. D., Zaat, S. A. (2000b) Biomaterial-associated persistence of *Staphylococcus epidermidis* in pericatheter macrophages. *J. Infect. Dis.* **181**: 1337–1349
- Borschel, D. M., Chenoweth, C. E., Kaufman, S. R., Hyde, K. V., VanDerElzen, K. A., Raghunathan, T. E., Collins, C. D., Saint, S. (2006) Are antiseptic-coated central venous catheters effective in a real-world setting? *Am. J. Infect. Control* **34**: 388–393

- Bowden, M. G., Visai, L., Longshaw, C. M., Holland, K. T., Speziale, P., Höök, M. (2002) Is the GehD lipase from *Staphylococcus epidermidis* a collagen binding adhesin? *J. Biol. Chem.* **277**: 43017–43023
- Bowden, M. G., Chen, W., Singvall, J., Xu, Y., Peacock, S. J., Valtulina, V., Speziale, P., Höök, M. (2005) Identification and preliminary characterization of cell-wall-anchored proteins of *Staphylococcus epidermidis*. *Microbiology* **151**: 1453–1464
- Brooun, A., Liu, S., Lewis, K. (2000) A dose-response study of antibiotic resistance in *Pseudomonas aeruginosa* biofilms. *Antimicrob. Agents Chemother.* **44**: 640–646
- Bryers, J. D., Ratner, B. D. (2004) Bioinspired implant materials befuddle bacteria. *ASM News* **70**: 232–237
- Burton, E., Gawande, P. V., Yakandawala, N., LoVetri, K., Zhanel, G. G., Romeo, T., Friesen, A. D., Madhyastha, S. (2006) Antibiofilm activity of GlnU enzyme inhibitors against catheter-associated uropathogens. *Antimicrob. Agents Chemother.* **50**: 1835–1840
- Butler, S. M., Festa, R. A., Pearce, M. J., Darwin, K. H. (2006) Self-compartmentalized bacterial proteases and pathogenesis. *Mol. Microbiol.* **60**: 553–562
- Campoccia, D., Montanaro, L., Arciola, C. R. (2006) The significance of infection related to orthopaedic devices and issues of antibiotic resistance. *Biomaterials* **27**: 2331–2339
- Caputo, G. M., Archer, G. L., Calderwood, S. B., DiNubile, M. J., Karchmer, A. W. (1987) Native valve endocarditis due to coagulase negative staphylococci. Clinical and microbiologic features. *Am. J. Med.* **83**: 619–625
- Cerca, N., Oliveira, R., Azeredo, J. (2007) Susceptibility of *Staphylococcus epidermidis* planktonic cells and biofilms to the lytic action of staphylococcus bacteriophage K. *Lett. Appl. Microbiol.* **45**: 313–317
- Chokr, A., Watier, D., Eleaume, H., Pangon, B., Ghnassia, J. C., Mack, D., Jabbouri, S. (2006) Correlation between biofilm formation and production of polysaccharide intercellular adhesin in clinical isolates of coagulase-negative staphylococci. *Int. J. Med. Microbiol.* **296**: 381–388
- Choong, S., Whitfield, H. (2000) Biofilms and their role in infection and urology. *Br. J. Urol.* **86**: 935–941
- Cirioni, O., Giacometti, A., Ghiselli, R., Dell'Acqua, G., Gov, Y., Kamysz, W., Lukasiak, J., Mocchegiani, F., Orlando, F., D'Amato, G., Balaban, N., Saba, V., Scalise, G. (2003) Prophylactic efficacy of topical temporin A and RNAIII-inhibiting peptide in a subcutaneous rat Pouch model of graft infection attributable to staphylococci with intermediate resistance to glycopeptides. *Circulation* **108**: 767–771
- Conlon, K. M., Humphreys, H., O'Gara, J. P. (2002) *icaR* encodes a transcriptional repressor involved in the environmental regulation of the *ica* operon expression and biofilm formation in *Staphylococcus epidermidis*. *J. Bacteriol.* **184**: 4400–4408
- Conlon, K. M., Humphreys, H., O'Gara, J. P. (2004) Inactivations of *rsbU* and *sarA* by IS256 represent novel mechanisms of biofilm phenotypic variation in *Staphylococcus epidermidis*. *J. Bacteriol.* **186**: 6208–6219
- Cosgrove, S. E., Carmeli, Y. (2003) The impact of antimicrobial resistance on health and economic outcomes. *Clin. Infect. Dis.* **36**: 1433–1437
- Costerton, J. W., Cheng, K.-J., Geesey, G. G., Ladd, T. I., Nickel, J. C., Dasgupta, M., Marrie, T. J. (1987) Bacterial biofilms in nature and disease. *Annu. Rev. Microbiol.* **41**: 435–464
- Costerton, J. W., Stewart, P. S., Greenberg, E. P. (1999) Bacterial biofilms: a common cause of persistent infections. *Science* **284**: 1318–1322
- Costerton, W., Veeh, R., Shirtliff, M., Pasmore, M., Post, C., Ehrlich, G. (2003) The application of biofilm science to the study and control of chronic bacterial infections. *J. Clin. Invest.* **112**: 1466–1477
- Curacella, C., Solana, C., Valle, J., Lasa, I., Penades, J. R. (2001) Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. *J. Bacteriol.* **183**: 2888–2896
- Curacella, C., Tormo, M.A., Ubeda, C., Trotonda, M. P., Monzón, M., Critòfol, P., Amorena, B., Lasa, I., Penadés, J. R. (2004) Role of biofilm-associated protein Bap in the pathogenesis of bovine *Staphylococcus aureus*. *Infect. Immun.* **72**: 2177–2185
- Curtin, J. J., Donlan, R. M. (2006) Using bacteriophages to reduce formation of catheter-associated biofilms by *Staphylococcus epidermidis*. *Antimicrob. Agents Chemother.* **50**: 1268–1275
- Darouiche, R. O. (2007) Antimicrobial coating of devices for prevention of infection: principles and protection. *Int. J. Artificial Organs* **30**: 820–827
- Darouiche, R. O., Green, G., Mansouri, M. D. (1998) Antimicrobial activity of antiseptic-coated orthopaedic devices. *Int. J. Antimicrob. Agents* **10**: 83–86
- Daum, R. S., Gupta, S., Sabbagh, R., Milewski, W. M. (1992) Characterization of *Staphylococcus aureus* isolates with decreased susceptibility to vancomycin and teicoplanin: isolation and purification of a constitutively produced protein associated with decreased susceptibility. *J. Infect. Dis.* **166**: 1066–1072
- de Araujo, G. L., Coelho, L. R., de Carvalho, C. B., Maciel, R. M., Coronado, A. Z., Rozebaum, R., Ferreira-Carvalho, B. T., Figueiredo, A. M. S., Teixeira, L. A. (2006) Commensal isolates of methicillin-resistant *Staphylococcus epidermidis* are also well equipped to produce biofilm on polystyrene surfaces. *J. Antimicrob. Chemother.* **57**: 855–864
- Domingo, P., Fontanet, A. (2001) Management of complications associated with totally implantable ports in patients with Aids. *AIDS Patient Care STDS* **15**: 7–13
- Donelli, G., Francolini, I., Romoli, D., Guaglianone, E., Piozzi, A., Raganath, C., Kaplan, J. B. (2007) Synergistic activity of dispersin B and cefamandole nafate in inhibition of staphylococcal biofilm growth on polyurethanes. *Antimicrob. Agents Chemother.* **51**: 2733–2740
- Dong, Y.-H., Zhang, L.-H. (2005) Quorum sensing and quorum quenching enzymes. *J. Microbiol.* **43**: 101–109
- Dunne, W. M. (2002) Bacterial adhesion: seen any good biofilms lately? *Clin. Microbiol. Rev.* **15**: 155–166
- Ehrlich, G. D., Hu, F. Z., Lin, Q., Costerton, W., Post, J. C. (2004) Intelligent implants to battle biofilms. *ASM News* **70**: 127–133
- Elder, M. F., Stapleton, F., Evans, E., Dart, J. K. G. (1995) Biofilm related infections in ophthalmology. *Eye* **9**: 102–109. In: Davey, M. E., O'Toole, G. A. (2000) Microbial biofilms: from ecology to molecular genetics. *Microbiol. Mol. Biol. Rev.* **64**: 847–846
- Fey, P. D., Ulphani, F., Götz, F., Heilmann, C., Mack, D., Rupp, M. E. (1999) Characterisation of the relationship between polysaccharide intercellular adhesin and haemagglutination in *Staphylococcus epidermidis*. *J. Infect. Dis.* **179**: 1561–1564
- Fitzpatrick, F., Humphreys, H., Smyth, E., Kennedy, C. A., O'Gara, J. P. (2002) Environmental regulation of biofilm formation in intensive care unit isolates of *Staphylococcus epidermidis*. *J. Hosp. Infect.* **42**: 212–218
- Fitzpatrick, F., Humphreys, H., O'Gara, J. P. (2005) The genetics of staphylococcal biofilm formation – will a greater understanding of pathogenesis lead to a better management of device-related infection? *Clin. Microbiol. Infect.* **11**: 967–973
- Fluckiger, U., Ulich, M., Steinhuber, A., Döring, G., Mack, D., Landmann, R., Goerke, C., Wolz, C. (2005) Biofilm formation, *icaADBC* transcription and polysaccharide intercellular adhesin synthesis by staphylococci in a device-related infection model. *Infect. Immun.* **73**: 1811–1819
- Frebourg, N., Lefebvre, S., Baert, S., Lumeland, J. F. (2000) PCR-based assay for discrimination between invasive and contaminating *Staphylococcus epidermidis* strains. *J. Clin. Microbiol.* **38**: 877–880

- Furno, F., Morley, K. S., Wong, B., Sharp, B. L., Arnold, P. L., Howdle, S. M., Bayston, R., Brown, P. D., Winship, P. D., Reid, H. J. (2004) Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection? *J. Antimicrob. Chemother.* **54**: 1019–1024
- Galdbart, J. O., Allignet, J., Tung, H. S., Ryden, C., Solh, E. L. (2000) Screening for *Staphylococcus epidermidis* markers discriminating between skin-flora strains and those responsible for infections of joint prostheses. *J. Infect. Dis.* **182**: 351–355
- Gallo, J., Kolar, M., Novotny, R., Rihakova, P., Ticha, V. V. (2003) Pathogenesis of prosthesis-related infection. *Biomedical Papers of the Medical Faculty University of Palacky, Olomouc, Czech Republic* **147**: 27–35
- Gerke, C., Kraft, A., Sassmuth, R., Schweitzer, O., Götz, F. (1998) Characterisation of the N-acetylglucosaminyltransferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. *J. Biol. Chem.* **273**: 18586–18593
- Gilbert, P., Das, J., Foley, I. (1997) Biofilm susceptibility to antimicrobials. *Adv. Dental Res.* **11**: 160–167
- Gorman, S. P., Jones, D. S. (2002) Antimicrobial biomaterials for medical devices. *World Markets Research Centre, Medical Device Manufacturing and Technology* 97–101
- Götz, F. (2004) Staphylococci in colonisation and disease: prospective targets for drugs and vaccines. *Curr. Opin. Microbiol.* **7**: 477–487
- Habash, M., Reid, G. (1999) Microbial biofilms: their development and significance for medical device-related infections. *J. Clin. Pharmacol.* **39**: 887–898
- Hanaki, H., Kuwahara-Arai, K., Boyle-Vavra, S., Daum, R. S., Labischinski, H., Hiramatsu, K. (1998) Activated cell-wall synthesis is associated with vancomycin resistance in methicillin-resistant *Staphylococcus aureus* clinical strains Mu3 and Mu50. *J. Antimicrob. Chemother.* **42**: 199–209
- Handke, L. D., Conlon, K. M., Slater, S. R., Elbaruni, S., Fitzpatrick, F., Humphreys, H., Giles, W. P., Rupp, M. E., Fey, P. D., O’Gara, J. P. (2004) Genetic and phenotypic analysis of biofilm phenotypic variation in multiple *Staphylococcus epidermidis* isolates. *J. Med. Microbiol.* **53**: 367–374
- Handke, L. D., Slater, S. R., Conlon, K. M., O’Donnell, S. T., Olson, M. E., Bryant, K. A., Rupp, M. E., O’Gara, J. P., Fey, P. D. (2007) SigmaB and SarA independently regulate polysaccharide intercellular adhesin production in *Staphylococcus epidermidis*. *Can. J. Microbiol.* **53**: 82–91
- Harrison, J. J., Ceri, H., Stremick, C., Turner, R. J. (2004) Differences in biofilm and planktonic cell mediated reduction of metalloids oxyanions. *FEMS Microbiol. Lett.* **235**: 357–362
- Heilmann, C., Peters, G. (2001) Biology and pathogenicity of *Staphylococcus epidermidis*. In: Fischetti, V. A., Norwick, R. P., Ferretti, J. J., Portnoy, D. A., Rood, J. J. (eds) *Gram positive pathogens*. American Society for Microbiology, Washington, DC, pp. 442–449
- Heilmann, C., Schweitzer, O., Gerke, C., Vanittanakom, N., Mack, D., Götz, F. (1996) Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. *Mol. Microbiol.* **20**: 1083–1091
- Heilmann, C., Hussain, M., Peters, G., Götz, F. (1997) Evidence for autolysin-mediated primary attachment of *Staphylococcus epidermidis* to a polystyrene surface. *Mol. Microbiol.* **24**: 1013–1024
- Hennig, S., Nyunt Wai, S., Ziebuhr, W. (2007) Spontaneous switch to PIA-independent biofilm formation in an *ica*-positive *Staphylococcus epidermidis* isolate. *Int. J. Med. Microbiol.* **297**: 117–122
- Hentzer, M., Riedel, K., Rasmussen, T. B., Heydorn, A., Anderson, J. B., Parsek, M. R., Rice, S. A., Eberl, L., Molin, S., Høiby, N., Kjelleberg, S., Givskov, M. (2002) Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. *Microbiology* **148**: 87–102
- Hentzer, M., Wu, H., Anderson, J. B., Riedel, K., Rasmussen, T. B., Bagge, N., Kumar, N., Schembri, M. A., Song, Z., Kristofferson, P., Manefield, M., Costerton, J. W., Molin, S., Eberl, L., Steinberg, P., Kjelleberg, S., Høiby, N., Givskov, M. (2003) Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *EMBO J.* **22**: 3803–3815
- Hume, E. B., Baveja, J., Muir, B., Schubert, T. L., Kumar, N., Kjelleberg, S., Griesser, H. J., Thissen, H., Read, R., Poole-Warren, L. A., Schindhelm, K., Willcox, M. D. (2004) The control of *Staphylococcus epidermidis* biofilm formation and in vivo infection rates by covalently bound furanones. *Biomaterials* **25**: 5023–5030
- Hussain, M., Herrmann, M., von Eiff, C., Perdreau-Remington, F., Peters, G. (1997) A 140kDa extracellular protein is essential for the accumulation of *Staphylococcus epidermidis* strains on surfaces. *Infect. Immun.* **65**: 519–524
- Hussain, M., Heilmann, C., Peters, G., Herrmann, M. (2001) Teichoic acid enhances adhesion of *Staphylococcus epidermidis* to immobilised fibronectin. *Microb. Pathogenesis* **31**: 261–270
- Ip, D., Yam, S. K., Chen, C. K. (2005) Implications of the changing pattern of bacterial infections following total joint replacements. *J. Orthopaed. Surg.* **13**: 125–130
- Jansen, B., Hartmann, C., Schaumacher-Pedreau, F., Peters, G. (1991) Late onset endophthalmitis associated with intraocular lens: a case of molecularly proved *S. epidermidis* aetiology. *Br. J. Ophthalmol.* **75**: 440–441
- Jefferson, K. K. (2004) What drives bacteria to produce a biofilm? *FEMS Microbiol. Lett.* **236**: 163–173
- Ji, G., Beavis R. C., Novick, R. P. (1995) Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. *Proc. Natl Acad. Sci. USA* **92**: 12055–12059
- Johansen, C., Falholt, P., Gram, L. (1997) Enzymatic removal and disinfection of bacterial biofilms. *Appl. Environ. Microbiol.* **63**: 3724–3728
- Kaplan, J. B., Raganath, C., Velliyagounder, K., Fine, D. H., Ramasubbu, N. (2004) Enzymatic detachment of *Staphylococcus epidermidis* biofilms. *Antimicrob. Agents Chemother.* **48**: 2633–2636
- Keogh, J. R., Velander, F. F., Eaton, J. W. (1992) Albumin-binding surfaces for implantable devices. *J. Biomed. Mater. Res.* **26**: 441–456
- Knobloch, J. K., Jäger, S., Horstkotte, M. A., Rohde, H., Mack, D. (2004) *RsbU*-dependent regulation of *Staphylococcus epidermidis* biofilm formation is mediated via the alternative sigma factor *sigmaB* by repression of the negative regulator gene *icaR*. *Infect. Immun.* **72**: 3838–3848
- Kocianova, S., Vuong, C., Yao, Y., Voyich, J. M., Fischer, E. R., DeLeo, F. R., Otto, M. (2005) Key role of poly- $\gamma$ -DL-glutamic acid in immune evasion and virulence of *Staphylococcus epidermidis*. *J. Clin. Invest.* **115**: 688–694
- Kong, K.-F., Vuong, C., Otto, M. (2006) *Staphylococcus* quorum sensing in biofilm formation and infection. *Int. J. Med. Microbiol.* **296**: 133–139
- Kozitskaya, S., Olson, M. E., Fey, P. D., Witte, W., Ohlsen, K., Ziebuhr, W. (2005) Clonal analysis of *Staphylococcus epidermidis* isolates carrying or lacking biofilm-mediating genes by multilocus sequence typing. *J. Clin. Microbiol.* **43**: 4751–4757
- Kuźma, Ł., Różalski, M., Walencka, E., Różalska, B., Wysokińska, H. (2007) Antimicrobial activity of diterpenoids from hairy roots of *Salvia sclarea* L.: salviposone as a potential anti-biofilm agent active against antibiotic resistant staphylococci. *Phytomedicine* **14**: 31–35
- Lasa, I., Penadés, J. R. (2006) Bap: a family of surface proteins involved in biofilm formation. *Res. Microbiol.* **157**: 99–107
- Latasa, C., Solano, C., Penadés, J. R., Lasa, I. (2006) Biofilm-associated proteins. *Immunology* **329**: 849–857



- Lee, S. J., Kim, S. W., Cho, Y. H., Shin, W. S., Lee, S. E., Kim, C. S., Hong, S. J., Chung, B. H., Kim, J. J., Yoon, M. S. (2004) A comparative multicentre study on the incidence of catheter-associated urinary tract infection between nitrofurazone-coated and silicone catheters. *Int. J. Antimicrob. Agents* **24S**: S65–S69
- Lewis, K. (2001) Riddle of biofilm resistance. *Antimicrob. Agents Chemother.* **45**: 999–1007
- Lewis, K. (2005) Persister cells and the riddle of biofilm survival. *Biochemistry* **70**: 267–274
- Lin, T. L., Lu, F. M., Conroy, S., Sheu, M. S., Su, S. H., Tang, L. (2001) Antimicrobial coatings: a remedy for medical device-related infections. *Med. Device Technol.* **12**: 26–30
- Mack, D. (1999) Molecular mechanisms of *Staphylococcus epidermidis* biofilm formation. *J. Hosp. Infect.* **43** (Suppl.): S113–S125
- Mack, D. (2007) Microbial interactions in *Staphylococcus epidermidis* biofilms. *Anal. Bioanal. Chem.* **387**: 399–408
- Mack, D., Siemssen, N., Laufs, R. (1992) Parallel induction by glucose of adherence and a polysaccharide antigen specific for plastic-adherent *Staphylococcus epidermidis*: evidence for functional relation to intercellular adhesion. *Infect. Immun.* **60**: 2048–2057
- Mack, D., Nedelmann, M., Krottsch, A., Schwarzkopf, A., Heesemann, J., Laufs, R. (1994) Characterisation of transposon mutants of biofilm-producing *Staphylococcus epidermidis* impaired in the accumulative phase of biofilm production: genetic identification of a hexosamine-containing polysaccharide intercellular adhesin. *Infect. Immun.* **62**: 3244–3253
- Mack, D., Fischer, W., Krottsch, A., Leopold, K., Hartmann, R., Egge, H., Laufs, R. (1996) The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear beta-1,6-linked glucosaminoglycan: purification and structural analysis. *J. Bacteriol.* **178**: 175–183
- Mack, D., Riedewald, J., Rohde, H., Magnus, T., Feucht, H. H., Elsner, H.-A., Laufs, R., Rupp, M. E. (1999) Essential functional role of the polysaccharide intercellular adhesin of *Staphylococcus epidermidis* in haemagglutination. *Infect. Immun.* **67**: 1004–1008
- Maira-Litrán, T., Allison, D. G., Gilbert, P. (2000) An evaluation of the potential of the multiple antibiotic resistance operon (*mar*) and the multi-drug efflux pump *acrAB* to moderate resistance towards ciprofloxacin in *Escherichia coli* biofilms. *J. Antimicrob. Chemother.* **45**: 789–795
- Maira-Litrán, T., Kropec, A., Abeygunawardana, C., Joyce, J., Mark III, G., Goldmann, D. A., Pier, G. B. (2002) Immunochemical properties of the staphylococcal poly-N-acetylglucosamine surface polysaccharide. *Infect. Immun.* **70**: 4433–4440
- Maira-Litrán, T., Kropec, A., Goldmann, D. A., Pier, G. B. (2005) Comparative opsonic and protective activities of the *Staphylococcus aureus* conjugate vaccines containing native or deacetylated staphylococcal poly-N-acetyl-beta-(1,6) glucosamine. *Infect. Immun.* **73**: 6752–6762
- Marra, A. (2004) Can virulence factors be viable antibacterial targets? *Expert Rev. Anti-infective Ther.* **2**: 61–72
- Mayville, P., Ji, G., Beavis, R., Yang, H., Goger, M., Novick, R. P., Muir, R. (1999) Structure-activity analysis of synthetic autoinducing thiolactone peptides from *Staphylococcus aureus* responsible for virulence. *Proc. Natl Acad. Sci. USA* **96**: 1218–1223
- McCrea, K. W., Hartford, O., Davis, S., Eidhin, D. N., Lina, G., Speziale, P., Foster, T. J., Höök, M. (2000) The serine-aspartate (Sdr) protein family in *Staphylococcus epidermidis*. *Microbiology* **146**: 1535–1546
- McKenney, D., Pouliot, K., Wang, Y., Murthy, V., Ulrich, M., Döring, G., Lee, J. C., Goldmann, D. A., Pier, G. B. (2000) Vaccine potential of poly-1-6 beta-D-N-succinylglucosamine, an immunoprotective surface polysaccharide of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *J. Biotechnol.* **83**: 37–44
- McKevitt, A. I., Bjornson, G. L., Mauracher, C. A., Scheifele, D. W. (1990) Amino acid sequence of a deltalike toxin from *Staphylococcus epidermidis*. *Infect. Immun.* **58**: 1473–1475
- Mecicoglu, M., Saygi, B., Yildirim, Y., Karadag-Saygi, E., Ramadan, S. S., Esemeli, T. (2006) The effect of proteolytic enzyme serratiopeptidase in the treatment of experimental implant-related infection. *J. Bone Joint Surg. Am.* **88**: 1208–1214
- Mehlin, C., Headley, C. M., Klebanoff, S. J. (1999) An inflammatory polypeptide complex from *Staphylococcus epidermidis*: isolation and characterisation. *J. Exp. Med.* **189**: 907–917
- Michel, A., Agerer, F., Hauck, C. R., Herrmann, M., Ullrich, J., Hacker, J., Ohlsen, K. (2006) Global regulatory impact of *clpp* protease of *Staphylococcus aureus* on regulons involved in virulence, oxidative stress response, autolysis, and DNA repair. *J. Bacteriol.* **188**: 5783–5796
- Monk, A. B., Archer, J. L. (2007) Use of outer surface protein repeat regions for improved genotyping of *Staphylococcus epidermidis*. *J. Clin. Microbiol.* **45**: 730–735
- Montanaro, L., Campoccia, D., Arciola, C. R. (2007) Advancements in molecular epidemiology of implant infections and future perspectives. *Biomaterials* **28**: 5155–5168
- Moss, H. A., Tebbs, S. E., Farouqi, M. H., Herbst, T., Isaac, J. L., Brown, J., Elliott, T. S. (2000) A central venous catheter coated with benzalkonium chloride for the prevention of catheter-related microbial colonization. *Eur. J. Anaesthesiol.* **17**: 680–687
- Nilsson, M., Frykberg, L., Flock, J. I., Pei, L., Lindberg, M., Gus, M. (1998) A fibrinogen-binding protein of *Staphylococcus epidermidis*. *Infect. Immun.* **66**: 2666–2673
- Ninin, E., Caroff, N., Espaze, E., Marailac, J., Lepelletier, D., Milipied, N., Richet, H. (2006) Assessment of the *ica* operon carriage and biofilm production in *Staphylococcus epidermidis* isolates causing bacteraemia in bone marrow transplant recipients. *Clin. Microbiol. Infect.* **12**: 446–452
- Novick, R. P. (2003) Autoinduction and signal transduction in the regulation of staphylococcal virulence. *Mol. Microbiol.* **48**: 1429–1449
- Nunes, F. P. A., Teixeira, L. M., Bastos, C. C. R., Fonseca, L. S., Santos, K. R. N. (2002) Susceptibility of Brazilian staphylococcal strains to glycopeptides evaluated by different testing methods. *Curr. Microbiol.* **44**: 385–390
- Nunes, F. P. A., Teixeira, L. M., Iorio, N. L. P., Bastos, C. C. R., Fonseca, L. S., Souto-Padrón, T., Santos, K. R. N. (2006) Heterogenous resistance to vancomycin in *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus warneri* clinical strains: characterisation of glycopeptide susceptibility profiles and cell wall thickening. *Int. J. Antimicrob. Agents* **27**: 307–315
- Nunes, A. P., Schuenck, R. P., Bastos, C. C., Magnanini, M. M., Long, J. B., Iorio, N. L., Santos, K. R. (2007) Heterogeneous resistance to vancomycin and teicoplanin among *Staphylococcus* spp. isolated from bacteremia. *Braz. J. Infect. Dis.* **11**: 345–350.
- O’Gara, J. P. (2007) *ica* and beyond: biofilm mechanisms and regulation in *Staphylococcus epidermidis* and *Staphylococcus aureus*. *FEMS Microbiol. Lett.* **270**: 179–188
- O’Gara, J. P., Humphreys, H. (2001) *Staphylococcus epidermidis* biofilms: importance and implications. *J. Med. Microbiol.* **50**: 582–587
- Osma, S., Kahveci, S. F., Kaya, F. N., Akalin, H., Ozakin, C., Yilmaz, E., Kutlay, O. (2006) Efficacy of antiseptic-impregnated catheters on catheter colonization and catheter-related blood-stream infections in patients in an intensive care unit. *J. Hosp. Infect.* **62**: 156–162
- Otto, M. (2004a) Quorum-sensing control in *Staphylococci* – a target for antimicrobial drug therapy? *FEMS Microbiol. Lett.* **241**: 135–141



- Otto, M. (2004b) Virulence factors of the coagulase negative staphylococci. *Frontiers Biosci.* **9**: 841–863
- Otto, M. (2008) Staphylococcal biofilms. In: Romeo, T. (ed.) *Bacterial biofilms (current topics in microbiology and immunology)*. Springer-Verlag, Berlin, pp. 207–228
- Otto, M., Stübmuth, R., Vuong, C., Jung, G., Götz, F. (1999) Inhibition of virulence factor expression in *Staphylococcus aureus* by the *Staphylococcus epidermidis* agr pheromone and derivatives. *FEBS Lett.* **450**: 257–262
- Otto, M., Echner, H., Voelter, W., Götz, F. (2001) Pheromone cross-inhibition between *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Infect. Immun.* **69**: 1957–1960
- Patel, J. D., Ebert, M., Ward, R., Anderson, J. M. (2007) *S. epidermidis* biofilm formation: effects of biomaterial surface chemistry and serum proteins. *J. Biomed. Mater. Res.* **80A**: 742–751
- Pei, L., Flock, J. I. (2001) Functional study of antibodies against a fibrogenin-binding protein in *Staphylococcus epidermidis* adherence to polyethylene catheters. *J. Infect. Dis.* **184**: 52–55
- Pei, L., Palma, M., Nilsson, M., Guss, B., Flock, J. I. (1999) Functional studies of a fibrinogen binding protein from *Staphylococcus epidermidis*. *Infect. Immun.* **67**: 4525–4530
- Pérez-Giraldo, C., Rodríguez-Benito, A., Morán, F. J., Hurtado, C., Blanco, M. T., Gómez-García A. C. (1997) Influence of N-acetylcysteine on the formation of biofilm by *Staphylococcus epidermidis*. *J. Antimicrob. Chemother.* **39**: 643–646
- Pfelft, R. F., Singh, V. K., Schmidt, J. L., Batten, M. A., Baranyk, C. S., Nadakavukaren, M. J., Jayaswal, R. K., Wilkinson, B. J. (2000) Characterization of passage-selected vancomycin-resistant *Staphylococcus aureus* strains of diverse parental backgrounds. *Antimicrob. Agents Chemother.* **44**: 294–303
- Porankiewicz, J., Wang, J., Clarke, A. K. (1999) New insights into the ATP-dependent Clp protease: *Escherichia coli* and beyond. *Mol. Microbiol.* **32**: 449–458
- Presterl, E., Suchomel, M., Eder, M., Reichmann, S., Lassnigg, A., Graninger, W., Rotter, M. (2007) Effects of alcohols, povidone-iodine and hydrogen peroxide on biofilms of *Staphylococcus epidermidis*. *J. Antimicrob. Chemother.* **60**: 417–420
- Projan, S. J., Neshin, M., Dunman, P. M. (2006) Staphylococcal vaccines and immunotherapy: to dream the impossible dream? *Curr. Opin. Pharmacol.* **6**: 473–479
- Qazi, S., Middleton, B., Muharram, S. H., Cockayne, A., Hill, P., O'Shea, P., Chhabra, S. R., Cámara, M., Williams, P. (2006) N-Acylhomoserine lactones antagonize virulence gene expression and quorum sensing in *Staphylococcus aureus*. *Infect. Immun.* **74**: 910–919
- Qin, Z., Yang, X., Yang, L., Jiang, J., Ou, Y., Molin, S., Qu, D. (2007) Formation and properties of *in vitro* biofilms of ica-negative *Staphylococcus epidermidis* clinical isolates. *J. Med. Microbiol.* **56**: 83–93
- Raad, I., Alrahan, A., Rolston, K. (1998) *Staphylococcus epidermidis*: emerging resistance and need for alternative agents. *Clin. Infect. Dis.* **26**: 1182–1187
- Rennermalm, A., Nilsson, M., Flock, J. I. (2004) The fibrinogen binding protein of *Staphylococcus epidermidis* is a target for opsonic antibodies. *Infect. Immun.* **72**: 3081–3093
- Riley, D. K., Classen, D. C., Stevens, L. E., Burke, J. P. (1995) A large randomised clinical trial of a silver-impregnated urinary catheter: lack of efficacy and staphylococcal superinfection. *Am. J. Med.* **98**: 349–356
- Roberts, M. E., Stewart, P. S. (2005) Modelling protection from antimicrobial agents in biofilms through the formation of persister cells. *Microbiology* **151**: 75–80
- Rodrigues, L. R., Banat, I. M., van der Mei, H. C., Teixeira, J. A., Oliveira, R. (2006) Interference in adhesion of bacteria and yeasts isolated from explanted voice prostheses to silicone rubber by rhamnolipid biosurfactants. *J. Appl. Microbiol.* **100**: 470–480
- Rohde, H., Kalitzky, M., Kröger, N., Scherpe, S., Horstkotte, M. A., Knobloch, J. K. M., Zander, A. R., Mack, D. (2004) Detection of virulence-associated genes not useful for discriminating between invasive and commensal *Staphylococcus epidermidis* strains from a bone marrow transplant unit. *J. Clin. Microbiol.* **42**: 5614–5619
- Rohde, H., Burdelski, C., Bartscht, K., Hussain, M., Buck, F., Horstkotte, M. A., Knobloch, J. K. M., Heilmann, C., Herrmann, M., Mack, D. (2005) Induction of *Staphylococcus epidermidis* biofilm formation via proteolytic processing of the accumulation associated protein by staphylococcal and host proteases. *Mol. Microbiol.* **55**: 1883–1895
- Rohde, H., Mack, D., Christner, M., Burdelski, C., Franke, G., Knobloch, J. K. M. (2006) Pathogenesis of staphylococcal device related infections: from basic science to new diagnostic, therapeutic and prophylactic approaches. *Rev. Med. Microbiol.* **17**: 45–54
- Rohde, H., Burandt, E. C., Siemssen, N., Frommelt, L., Burdelski, C., Wurster, S., Scherpe, S., Davies, A. P., Harris, L. G., Horstkotte, M. A., Knobloch, J. K., Ragunath, C., Kaplan, J. B., Mack, D. (2007) Polysaccharide intercellular adhesin or protein factors in biofilm accumulation of *Staphylococcus epidermidis* and *Staphylococcus aureus* isolated from prosthetic hip and knee joint infections. *Biomaterials* **28**: 1711–1720
- Rupp, M. E., Archer, G. L. (1994) Coagulase-negative staphylococci: pathogens associated with medical progress. *Clin. Infect. Dis.* **19**: 231–245
- Rupp, M. E., Hamer, K. E. (1998) Effect of subinhibitory concentrations of vancomycin, cefazolin, ofloxacin, L-ofloxacin and D-ofloxacin on adherence to intravascular catheters and biofilm formation by *Staphylococcus epidermidis*. *J. Antimicrob. Chemother.* **41**: 155–161
- Rupp, M. E., Ulphani, J. S., Fey, P. D., Mack, D. (1999) Characterisation of *staphylococcus epidermidis* polysaccharide intercellular adhesin/haemagglutinin in the pathogenesis of intravascular catheter associated infection in a rat model. *Infect. Immun.* **67**: 2656–2659
- Rupp, M. E., Lisco, S. J., Lipsett, P. A., Perl, T. M., Keating, K., Civetta, J. M., Mermel, L. A., Lee, D., Dellinger, E. P., Donahoe, M., Giles, D., Pfaller, M. A., Maki, D. G., Sherertz, R. (2005) Effect of a second-generation venous catheter impregnated with chlorhexidine and silver sulfadiazine on central catheter-related infections: a randomized, controlled trial. *Ann. Intern. Med.* **143**: 570–580
- Roberts, M. E., Stewart, P. S. (2005) Modelling protection from antimicrobial agents in biofilm through the formation of persister cells. *Microbiology* **151**: 75–80
- Saginur, R., Denis, M. S., Ferris, W., Aaron, S. D., Chan, F., Lee, C., Ramotar, K. (2006) Multiple combination bactericidal testing of staphylococcal biofilms from implant-associated infections. *Antimicrob. Agents Chemother.* **50**: 55–61
- Sanyal, D., Johnson, A. P., George, R. C., Cookson, B. D., Williams, A. J. (1991) Peritonitis due to vancomycin-resistant *Staphylococcus epidermidis*. *Lancet* **337**: 54
- Schlag, S., Nerz, C., Birkenstock, T. A., Altenberend, F., Götz, F. (2007) Inhibition of staphylococcal biofilm formation by nitrite. *J. Bacteriol.* **189**: 7911–7919
- Selan, L., Berlutti, F., Passariello, C., Comodi-Ballanti, M. R., Thaller, M. C. (1993) Proteolytic enzymes: a new treatment strategy for prosthetic infections? *Antimicrob. Agents Chemother.* **37**: 2618–2621
- Spoering, A. L., Lewis, K. (2001) Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. *J. Bacteriol.* **183**: 6746–6751
- Sritharan, M., Sritharan, V. (2004) Emerging problems in the management of infectious diseases: the biofilms. *Indian J. Med. Microbiol.* **22**: 140–142

- Stewart, P. S., Costerton, J. W. (2001) Antibiotic resistance of bacteria in biofilms. *Lancet* **358**: 135–138
- Sun, D., Accavitti, M. A., Bryers, J. D. (2005) Inhibition of biofilm formation by monoclonal antibodies against *Staphylococcus epidermidis* RP62A accumulation associated protein. *Clin. Diagn. Lab. Immunol.* **12**: 93–100
- Tormo, M. A., Knecht, E., Götz, F., Lasa, I., Penades, J. R. (2005) Bap-dependent biofilm formation by pathogenic species of *Staphylococcus*: evidence of horizontal gene transfer? *Microbiology* **151**: 2465–2475
- Vandecasteele, S. J., Peetermans, W. E., Merckx, R. R., Rijnders, B. J. A., Eldere J. V. (2003) Reliability of the *ica*, *aap* and *atlE* genes in the discrimination between invasive, colonising and contaminant *Staphylococcus epidermidis* isolates in the diagnosis of catheter related infections. *Clin. Microbiol. Infect.* **9**: 114–119
- Veenestra, G. J., Cremers, F. F., van Dijk, H., Fleer, A. (1996) Ultrastructural organisation and regulation of a biomaterial adhesin of *Staphylococcus epidermidis*. *J. Bacteriol.* **178**: 537–541
- Verhoef, J., Fleer, A. (1983) *Staphylococcus epidermidis* endocarditis and *Staphylococcus epidermidis* infection in an intensive care unit. *Scand. J. Infect. Dis. Suppl.* **41**: 56–64
- von Eiff, C., Heilmann, C., Peters, G. (1999) New aspects in the molecular basis of polymer-associated infections due to staphylococci. *Eur. J. Clin. Microbiol. Infect. Dis.* **18**: 843–846
- von Eiff, C., Peters, G., Heilmann, C. (2002) Pathogenesis of infections due to coagulase negative staphylococci. *Lancet Infect. Dis.* **2**: 677–685
- Vuong, C., Otto, M. (2002) *Staphylococcus epidermidis* infections. *Microbes Infect.* **4**: 481–489
- Vuong, C., Saenz, H. L., Götz, F., Otto, M. (2000) Impact of the *agr* quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus*. *J. Infect. Dis.* **182**: 1688–1693
- Vuong, C., Gerke, C., Somerville, G. A., Fischer, E. R., Otto, M. (2003) Quorum-sensing control of biofilm factors in *Staphylococcus epidermidis*. *J. Infect. Dis.* **188**: 706–718
- Vuong, C., Kocianova, S., Yao, Y., Carmody, A. B., Otto, M. (2004a) Increased colonisation of indwelling medical devices by quorum sensing mutants of *Staphylococcus epidermidis* in vivo. *J. Infect. Dis.* **190**: 1498–1505
- Vuong, C., Dürr, M., Camody, A. B., Peschel, A., Klebanoff, S. J., Otto, M. (2004b) Regulated expression of pathogen-associated molecular pattern molecules in *Staphylococcus epidermidis*: quorum-sensing determines pro-inflammatory capacity and production of phenol-soluble modulins. *Cell. Microbiol.* **6**: 753–759
- Vuong, C., Kocianova, S., Voyich, J. M., Yao, Y., Fischer, E. R., DeLeo, F. R., Otto, M. (2004c) A crucial role for the exopolysaccharide modification in bacterial biofilm formation, immune evasion and virulence. *J. Biol. Chem.* **279**: 54881–54886
- Walencka, E., Rozalska, S., Wysokinska, H., Rozalski, M., Kuzma, L., Rozalska, B. (2007) Salvipisone and aethiopinone from *Salvia sclarea* hairy roots modulate staphylococcal antibiotic resistance and express anti-biofilm activity. *Planta Med.* **73**: 545–551
- Wang, L. W., Anderson, J. M., Marchant, R. E. (1993) *Staphylococcus epidermidis* adhesion to hydrophobic biomedical polymer is mediated by platelets. *J. Infect. Dis.* **167**: 329–336
- Wang, C., Li, M., Dong, D., Wang, J., Ren, J., Otto, M., Gao, Q. (2007) Role of ClpP in biofilm formation and virulence of *Staphylococcus epidermidis*. *Microbes Infect.* **9**: 1376–1383
- Warren, J. W. (2001) Catheter-associated urinary tract infection. *Int. J. Antimicrob. Agents* **17**: 299–303
- Williams, R. J., Henderson, B., Sharp, L. J., Nair, S. P. (2002) Identification of a fibronectin-binding protein from *Staphylococcus epidermidis*. *Infect. Immun.* **70**: 6805–6810
- Wu, J. A., Kusuma, C., Mond, J. J., Kokai-Kun, J. F. (2003) Lysostaphin disrupts *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms on artificial surfaces. *Antimicrob. Agents Chemother.* **47**: 3407–3414
- Xu, L., Li, H., Vuong, C., Vadyvaloo, V., Wang, J., Yao, Y., Otto, M., Gao, Q. (2006) Role of the *luxS* quorum-sensing system in biofilm formation and virulence of *Staphylococcus epidermidis*. *Infect. Immun.* **74**: 488–496
- Yao, Y., Sturdevant, D. E., Viallaruz, A., Xu, L., Gao, Q., Otto, M. (2005a) Factors characterising *Staphylococcus epidermidis* invasiveness determined by comparative genomics. *Infect. Immun.* **73**: 1856–1860
- Yao, Y., Sturdevant, D. E., Otto, M. (2005b) Genomewide analysis of gene expression in *Staphylococcus epidermidis* biofilms: insights into the pathophysiology of *S. epidermidis* biofilms and the role of phenol-soluble modulins in formation of biofilms. *J. Infect. Dis.* **191**: 289–298
- Yarwood, J. M., Schlievert, P. M. (2003) Quorum sensing in *Staphylococcus* infections. *J. Clin. Invest.* **112**: 1620–1625
- Yarwood, J. M., Bartels, D. J., Volper, E. M., Greenberg, E. P. (2004) Quorum sensing in *Staphylococcus aureus* biofilms. *J. Bacteriol.* **186**: 1838–1850
- Ziebuhr, W. (2001) *Staphylococcus aureus* and *Staphylococcus epidermidis*: emerging pathogens in nosocomial infections. *Contrib. Microbiol.* **8**: 102–107
- Ziebuhr, W., Heilmann, C., Götz, F., Meyer, P., Wilms, K., Straube, E., Hacker, J. (1997) Detection of the intercellular adhesion gene cluster (*ica*) and phase variation in *Staphylococcus epidermidis* blood culture strains and mucosal isolates. *Infect. Immun.* **65**: 890–896
- Ziebuhr, W., Hennig, S., Eckart, M., Kranzler, H., Batzilla, C., Kozitskaya, S. (2006) Nosocomial infections by *Staphylococcus epidermidis*: how a commensal bacterium turns into a pathogen. *Int. J. Antimicrob. Agents* **28**: 14–20