REVIEW

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Staphylococcal adaptation to diverse physiologic niches: an overview of transcriptomic and phenotypic changes in different biological environments

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Host niches can differ strongly regarding, for example, oxygen tension, pH or nutrient availability. *Staphylococcus aureus* and other staphylococci are common colonizers of human epithelia as well as important human pathogens. The phenotypes that they show in different host environments, and the corresponding bacterial transcriptomes and proteomes, are currently under intense investigation. In this review, we examine the available literature describing staphylococcal phenotypes, such as expression of virulence factors, gross morphologic characteristics and growth patterns, in various physiological environments. Going forward, these studies will help researchers and clinicians to form an enhanced and more detailed picture of the interactions existing between the host and staphylococci as some of its most frequent colonizers and invaders.

First draft submitted: 6 July 2015; Accepted: 21 September 2015; Online: 20 November 2015

The study of effects that the host environment has on bacteria is crucial to understanding pathogenesis and discovering new avenues for the treatment of infection. *Staphylococcus aureus*, a Gram-positive cocci species, permanently or transiently colonizes a large portion (up to 60%) of the human population and is thus generally considered part of normal human microbiota. However, it has the potential to cause many diseases, including chronic and severe infections such as persisting skin and soft tissue infections (SSTIs), infectious endocarditis, infections of prostheses, septic arthritis and osteomyelitis [1]. Environmental factors that can vary widely within the host, such as pH, nutrient availability or oxygen tension, can induce *S. aureus* to express a variety of different phenotypes [2–4]. Host environmental factors that contribute to the pathogenesis of staphylococcal disease are generally acknowledged to be of great importance, but are at the moment poorly characterized [1].

Presently, heavy emphasis is placed on researching genetic cues within staphylococci that are crucial to pathogenesis. For the purpose of fully understanding mechanisms of pathogenesis, researchers commonly examine phenotypes that result from the artificial perturbation of bacterial genes. *In vitro* investigation of molecular pathways using such strains is performed under a highly controlled environment and with laboratory media. Most *in vivo* studies, while more clinically relevant, typically evaluate outcomes of bacterial infection using gene deletion strains (e.g., the ability of an infection to thrive with or without expression of key virulence genes), or assess strains that are isolated from clinical samples, cultured in laboratory media and tested for the presence or absence of genes associated with virulence.

KEYWORDS

• gene expression • host–pathogen interaction • niche adaptation • staphylococci

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Further information can be derived from the bacterial genome composition; and certain clones of *S. aureus* show stronger propensities than others for causing specific infections, such as SSTIs [5]. However, since bacterial behavior depends strongly on the environment, gene presence or absence alone only gives limited information about host–pathogen interaction. In the past, genome-wide analysis of gene expression in samples obtained from clinical or experimental infections has been challenging due to difficulties in obtaining sufficient amounts of RNA at high purity [6]. However, recent technical advances have allowed researchers to take a closer look at the impact of the host environment on bacterial gene expression and *in vivo* phenotypic characteristics. Furthermore, proteomic analyses are increasingly being employed to decipher protein expression under different conditions and *in vivo* environments. In this review, we present and discuss current knowledge about the effect of different *in vivo* or simulated *in vivo* environments on the behavior, expression of virulence factors, growth, antibiotic susceptibilities and gross morphological characteristics of staphylococci, focusing on *S. aureus* as the most important human pathogen.

S. aureus virulence factors

Virulence is defined as the ability of a pathogen to reduce host fitness as well as the ability of an organism to establish an infection and cause disease in a host. *S. aureus* produces a vast array of virulence factors, some of which we briefly present in the following, which are involved with diverse virulence mechanisms, such as immune evasion, colonization, immune stimulation or lysis of host cells. Notably, researchers have put much emphasis on the search for genes necessary for staphylococcal virulence, but much less consideration has been afforded to elucidating the specific effects of the host environment on these virulence-associated genes.

S. aureus produces a series of toxins that subvert mechanisms of innate host defense [7], and many different toxins that can be classified as cytotoxins, pyrogenic (fever-producing) toxins, enterotoxins and exfoliative toxins [8]. Studies that were performed using gene deletion strains have shown that several staphylococcal toxins are instrumental in causing massive tissue damage. Among the most potent cytotoxins is the phenol-soluble modulins (PSM) family of peptides [9]. Expression of PSMs is controlled by

the *agr* locus, which also controls many other toxins such as hemolysins (e.g., α -toxin) and leukotoxins [10,11]. Often, toxins are encoded on mobile genetic elements; and thus, in those cases, the analysis of gene presence alone may give important information on strain toxicity [8]. This is the case for many of the toxins that block mechanism of innate host defense as well as some cytotoxins (e.g., Pantone–Valentine and other leukocidins) [12]. In contrast, several toxin genes such as those coding for PSMs or α -toxin are invariably present in *S. aureus* isolates. *S. aureus* isolates may differ strongly in the capacity to produce those latter toxins, which directly correlates with virulence in animal models [13,14].

Biofilm formation is known to be key to the pathogenesis of persistent staphylococcal infection, such as infective endocarditis and prosthetic joint infection. During biofilm-associated infection, the bacteria form sessile communities on surfaces or in aggregates with host tissue [15]. This phenotype contributes to immune evasion and recalcitrance to antibiotic treatment [16]. Genetic manipulation has frequently been used to decipher which genes are needed for staphylococcal biofilm formation [17]. An extracellular biofilm matrix forms to serve the dual purpose of containing the staphylococci and shielding the bacteria from immune cell surveillance, all while simultaneously permitting diffusion of nutrients into the biofilm. One very important biofilm extracellular matrix molecule is the exopolysaccharide polysaccharide intercellular adhesin, PIA, which is also known as poly-N-acetyl-glucosamine, or PNAG [18,19]. The PIA/PNAG biosynthetic operon *ica* comprises the *icaA*, *icaB*, *icaC* and *icaD* genes [20]. Together, IcaA, IcaB, IcaC and IcaD produce, modify and export PIA into the extracellular space, where it adheres to bacterial surfaces and provides scaffolding for the accumulation of other biofilm factors [21,22]. Biofilm structuring, dispersal and dissemination of biofilm-associated infection are dependent upon factors that are controlled by the Agr quorum-sensing system, most notably PSMs [23].

In many *S. aureus* strains, immune evasion is enhanced by a capsule, consisting primarily of hexosaminuronic acid polymers, that provides protection from phagocytosis. This capsule is formed by gene products of the *cap* gene locus, of which several variants exist, the most frequent of which produce capsules of serotypes 5 and 8 [24]. Capsule synthesis is a demonstrated virulence

determinant in *S. aureus* infection [25], but it is absent from several clinically relevant strains such as USA300 [26]. It is subject to regulation by a variety of environmental factors and regulatory systems [24].

Surface proteins, such as accumulation-associated protein (Aap), extracellular matrix binding protein (Embp), protein A (SpA), the fibronectin-binding proteins (FnbpA and FnbpB) and other similar proteins, in addition to establishing initial contact with host matrix proteins, contribute to strengthening cell–cell adhesion [27]. The difference in staphylococcal gene expression between the biofilm and planktonic states is quite marked [28–30]. In an *in vitro* analysis of global gene expression in *S. aureus* biofilms, 48 genes were induced at least twofold in biofilm cultures compared with planktonic cultures. Additionally, 84 genes were repressed by a factor of two or more (e.g., *spa* and *agr* genes) [28]. This may be due to the change in the microenvironment that is known to exist once bacteria form biofilms; in mature biofilms, the pH decreases and anaerobic respiration increases. Similar observations have been made in other staphylococci, including *S. epidermidis* [30].

It is crucial to consider the varying conditions that may exist within each host niche in order to determine what effects the host niche may impact on the ability of staphylococci to effectively colonize and cause damage to the host.

Main regulatory pathways in staphylococci

S. aureus has a number of regulatory circuits, which allow control of virulence determinants such as adhesion proteins and toxins, in addition to metabolic targets, in response to the host environment. Among the most intensely studied are the quorum-sensing system Agr [31], the small transcriptional regulator proteins of the SarA family [32], the components of the alternative sigma factor SigB [33] and several two-component systems such as Arl and Srr [34,35]. Agr is tightly controlled by bacterial density. When sufficient cell density is reached, Agr is activated by a secreted autoinducing peptide (AIP), which through signal transduction via the AgrAC two-component system, the regulatory RNAIII and the repressor of toxins (Rot) DNA-binding protein generally induces production of toxins and exoenzymes and decreases production of several surface proteins [31]. SarA is the prototype of the Sar regulator family [32]. It controls

many of the extracellular and cell wall associated virulence proteins and is known to serve as a direct activator of the *agr* operon [36]. The SigB regulatory pathway, which is also a regulator of SarA expression, modulates the expression of surface proteins such as clumping factors [37–39]. The Srr pathway comprises a 2-component system that is responsive to oxygen tension [40]. The ArlRS two-component system is involved in the downregulation of some virulence genes such as α -toxin and protein A, is important for cell growth, division and colonization of polymer surfaces [34,41]. All of these pathways are activated/repressed in a temporal and environment-dependent manner and are interconnected in a way to permit bacterial infection in the host.

Important environmental cues

Perhaps the most frequently considered environmental factors of the various host niches are oxygen tension, pH and nutrient starvation. *S. aureus* is facultative and responds to oxygen limitation through the induction of anaerobic respiration and direct changes in regulatory gene expression, mediated by the SrrAB (Staphylococcal respiratory response) pathway [42]. SrrAB is an upstream effector of *ldh*, the TCA cycle enzymes, *agr* and *ica* [35]. The switch from aerobic to anaerobic respiration is associated with profound changes in the transcription patterns of approximately 9% of all *S. aureus* genes [42]. Decreased oxygen tension activates SrrAB and directly influences SrrA binding to the promoter region of the *agr* P2 promoter, which controls cytolytic proteins and quorum sensing [43]. Conversely, SrrAB activation under decreased oxygen tension directly promotes the production of *ica* transcripts, leading to increased biofilm formation [44]. These effects, however, have only been shown in laboratory media, and further research is necessary to determine whether similar changes are observed in physiological fluids. *In vivo*, hypoxic conditions have been reported in abscesses, the urogenital tract and within synovial joints and the spine (disc) [42,45,46].

Altered gene expression can also be due to shifts in pH. The pH values of host environments vary widely. Most important for staphylococci is the shift from the slightly acid conditions on human skin as their premier habitat to higher pH values encountered during infection, such as a pH of approximately 7.4 in human blood. During *in vitro* growth of *S. aureus*, the accumulation of metabolites can cause a 2-unit decrease

in pH, which causes over 400 transcripts to change significantly, independent of nutrient availability. Notably, *agr*-controlled transcripts, many of which are virulence-associated genes, are increased at lower pH, as present in the skin, urogenital tract, lung, mouth and within abscesses [47]. There are conflicting results about the effect of pH on biofilm formation. In one *in vitro* study, decreased pH (5.5) was reported to induce staphylococcal biofilm formation [47], whereas in another study, a decrease to pH 4.5 did not appear to induce biofilm/PIA formation, induce *agr*-controlled virulence factors or promote enterotoxin production [2]. Clearly, more comprehensive *in vitro* and *in vivo* studies will be necessary to better determine the impact of the pH on staphylococcal phenotypes.

Nutrient availability and osmolarity of the growth environment strongly influence staphylococcal gene expression. For example, high NaCl concentration (1 M) decreases the growth rate of *S. aureus* and the transcription of protein A (*spa*), α -toxin (*hla*) and toxic shock syndrome toxin 1 (*tst*)-encoding genes by at least tenfold, while only a moderate effect of high salt concentration on *sarA* and *agr* expression was reported [48]. Similarly, varying concentrations of sucrose, from 0.2 to 1 M, reduced expression of *spa*, *hla*, *tst* and *sarA*, but had no effect of *agr* expression [48]. In contrast, increased concentrations of glucose repress *agr* expression in multiple strains of *S. aureus* [49]. High osmolyte levels have been reported to repress the expression of some *agr*-controlled exoproteins such as Eta and staphylococcal enterotoxins B and C [48,50–52]. Furthermore, the availability of metal ions such as Mg^{2+} , Ca^{2+} , Fe^{2+} and Zn^{2+} is crucial to the expression of several virulence genes, for example, *tst* [48,53,54]. Finally, analysis of *in vitro* and *in vivo* transcriptional changes under low-iron conditions showed an impact of Fe^{2+} concentration on *agr*-controlled transcripts [55].

In vivo, there are a variety of compounds, either produced endogenously (antimicrobial peptides [AMPs], reactive oxygen species, etc.), or introduced exogenously (e.g., antibiotics), that can alter the host environment and may cause a change in staphylococcal gene expression. *In vitro* experiments have been performed to determine the effects of these antibacterial agents on gene expression in staphylococci. AMPs form an important part of innate host defense on human epithelia [56]. Several studies have investigated the impact that AMPs have on

gene expression of staphylococci [57–60]. Central to the transcriptional adaptations of staphylococci to the presence of the usually membrane-damaging AMPs is the increased expression of bacterial defense mechanisms, which include increased expression of the *mprF*, *dlt* and *vraFG* transcripts [58,59]. MprF is an enzyme that produces lysyl-phosphatidylglycerol, whose incorporation in the cytoplasmic membrane decreases interaction with the commonly cationic AMPs [61]. The *dlt* operon is responsible for the D-alanylation of teichoic acids, decreasing attraction of cationic AMPs to the bacterial surface [62]. Finally, the ABC transporter VraFG may be directly involved in AMP resistance by AMP expulsion [59], but it may also form an integral part of the staphylococcal AMP sensing mechanism [63], which is based on binding of AMPs to the ApsS (GraS) histidine kinase and the ApsRSX (GraRSX) three-component signal transduction system [58].

Another method that the host uses to combat infection is the production of reactive oxygen species. Macrophages and other lymphocytes use reactive oxygen species (such as hydrogen peroxide and superoxides) to kill *S. aureus*. A microarray study performed to assess the effects of H_2O_2 on *S. aureus* gene expression showed a notable decrease in the expression of the *ica* locus, indicating that H_2O_2 production inhibits the ability of *S. aureus* to produce biofilm [64]. Finally, there are multiple studies that have shown an impact of subinhibitory antibiotic concentrations of antibiotics on the expression of virulence genes, such as *hla*, PSMs and toxic shock syndrome toxin (TSST-1) [65–67].

Host niches may influence host–pathogen interaction not only depending on their physico-chemical composition, but also depending on the interactions between host and pathogen proteomes. For example, tissue adherence and colonization are facilitated by the interaction of *S. aureus* with host matrix proteins, which are present in different amounts in the various host niches. *Staphylococcus aureus* expresses a family of surface proteins, called ‘microbial surface components recognizing adhesive matrix molecules’ (MSCRAMMs), which binds host matrix proteins such as collagen, fibronectin, fibrin and fibrinogen [27,68]. In addition to impacting attachment to host tissue, MSCRAMMs contribute to bacterial aggregation and biofilm formation. Different strains of *S. aureus* have varying expression of MSCRAMMs [69]. Notably,

Table 1. Major *Staphylococcus aureus* adaptations in response to environmental cues

Host niche	Potential environmental cue(s)	Ref.	Reported adaptation phenotype and factors up/down-regulated	Ref.
Skin and soft tissues	Varying humidity, pH, temperature, microbiota	[76,78,136]	Upregulated <i>hlg</i> , <i>spa</i> , <i>hla</i> , <i>lukS-PV</i> , <i>lukE</i> Downregulated <i>agr</i> , <i>psm</i> β	[43,87–89]
Urinary tract	Varying pH	[47,137]	Slow growth Biofilm formation	[130,131]
Nares	Low pH Low nutrient availability AMPs Mucociliary interactions Competing microflora	[101,102,138]	Dispersed phenotype rather than biofilm (?) Downregulation of <i>agr</i>	[3,101]
Reproductive tract	Varying oxygen tension and pH	[139]	Biofilm formation Low production of virulence factors	[133]
Lungs	Antecedent viral infection Varying pH	[104,140]	Biofilm formation, PIA expression Downregulation of <i>agr</i> Upregulation of <i>hld</i> , <i>psm</i>	[105]
Blood/vasculature	Presence of serum proteins Presence of red and white blood cells, platelets Flow/shear stress	[141–145]	Aggregation Spread/dissemination Differential expression of <i>agr</i> , <i>psm</i> (conflicting results) Increased production of surface proteins	[43,107,111,112,117,146]
Bone/joints	High mineral content (bone) Fibrous tissues High viscosity, varying oxygen tension and cellularity, protein composition (synovial fluid)	[46,147–150]	Aggregation/biofilm formation intracellular persistence (osteoblasts/chondrocytes) Antibiotic recalcitrance Decrease in <i>agr</i> , <i>psm</i> Increase in surface protein (FnbpA, ClfA) gene expression	[118,124,151]
CSF	Ultra-filtrate of serum with CSF-specific proteins (e.g., cystatin), low cellularity	[152]	N/A	N/A

many MSCRAMMs are under, usually negative, regulation by Agr [11,69]; and thus, environmental cues that impact Agr expression will also impact the expression of MSCRAMMs.

Table 1 summarizes possible environmental cues in each host niche, as well as the corresponding reported *S. aureus* adaptations. The following is a summary of data collected from studies on *S. aureus* asymptomatic colonization or infections of various host niches.

Skin colonization & infection

Coagulase-negative staphylococci (CoNS) such as *S. epidermidis* are common colonizers of human skin [70,71]. *Staphylococcus aureus* permanently colonizes the nares and ano-genital areas of at least a third of the human population [72,73]. Furthermore, *S. aureus* is a common cause of SSTIs [74,75]. The milieu on human skin can differ strongly, not only from human to human, depending on lifestyle and hygiene, but also from one anatomic location to the next [76,77].

Temperature, humidity, pH, lipid composition and production of AMPs vary considerably over time and location [76,78]. Thus, it is difficult to simulate the conditions staphylococci encounter during SSTIs and even more so during asymptomatic skin colonization. Abscess models with subcutaneous or intradermal injection of bacteria may give a valuable representation of human SSTI, but there are virtually no models that mimic staphylococcal skin colonization with acceptable accuracy. Some researchers have characterized the interactions of *S. aureus* with skin through the use of skin equivalents. In skin equivalents, collagen and fibroblasts are combined to form a dermal matrix; and keratinocytes are subsequently seeded onto the matrix and permitted to differentiate, thus simulating a skin-like substrate [78].

While *S. aureus* is not primarily thought of as an intracellular organism, internalization of *S. aureus* by a variety of cell types is believed to contribute to persistence and the phenomenon of

recurring infection [79,80]. In addition to surviving in macrophages [81], *S. aureus* is known to persist intracellularly in epithelial cells [82]; both mechanisms likely matter during SSTI. In one study that examined gene expression of *S. aureus* after internalization in human epithelial cells, a significant downregulation of *agr* in the internalized bacteria was shown [83]. Specifically, AgrA was shown to be downregulated by approximately tenfold by microarray analysis and 40-fold by qPCR validation as compared with noninternalized bacteria cultured in the presence of cells. In the same study, the virulence regulator *sarS* and the gene encoding superoxide dismutase (*sodA*), an enzyme detoxifying reactive oxygen species, were strongly induced by internalization in an *in vitro* simulation of epithelial cell colonization [83]. However, in another study, phagosomal escape and survival in epithelial cells were attributed to the Agr-controlled PSMs [84], which is in line with similar findings in macrophages and neutrophils [84,85], and suggest upregulation of Agr and PSMs after internalization. In a microarray study specifically addressing gene expression changes after neutrophil phagocytosis, genes involved in capsule synthesis, oxidative stress and virulence, were upregulated following ingestion. Agr showed slight down-, while the virulence regulator Sae showed slight upregulation [86]. Overall, gene expression changes after internalization appear to be dependent strongly on the experimental setup and will need to be verified in *in vivo* settings.

There is also an increasing number of *in vivo* studies examining gene expression in human or experimental SSTI. In a subcutaneous infection model in rabbits, Yarwood *et al.*, using subgenomic DNA microarrays, found a 17-fold decrease in RNAPIII levels, representative of Agr expression, as compared with expression in Todd–Hewitt medium [43]. Another study focused on virulence gene expression during human cutaneous *S. aureus* infection. Using material obtained directly from 40 clinical cutaneous abscesses, a relative upregulation of cytolytic toxin gene transcripts, such as *hla*, *lukS-PV*, *lukE* and *hlgB*, and a relative decrease in RNAPIII, bacteriocin (*bsa*) and protein A (*spa*) transcripts, as compared with laboratory broth, was found using qRT-PCR [87]. Researchers also monitored transcriptomic changes by microarray in a rabbit model of skin infection with the community-associated MRSA strain USA300 and noted that virulence factors such as leukocidins,

as well as extracellular matrix binding protein expression increased dramatically (e.g., between five- and eightfold for leukocidins, and 12-fold for fibronectin-binding protein) within 1 day of infection [88].

Recently, Date *et al.* showed that in a human cutaneous abscess, the expression levels of 262 genes were significantly altered. Using microarray analysis of three clinical samples, these authors reported that 10.1% of genes were upregulated, and 7.3% of genes were downregulated, as compared with a 4-h culture grown in tryptic soy broth [89]. While some toxins, such as the Pantone–Valentine leukocidin (PVL) and γ -hemolysin, were among the most highly upregulated in these cutaneous abscesses, others such as PSMs, were significantly downregulated. Interestingly, the transcription profile in human cutaneous abscesses was remarkably similar to that in infected mouse kidneys [89]. These results may reflect the importance of certain toxins in SSTI, but they also show the limitations of an approach that only measures overall transcriptional changes of bacteria in abscesses, disregarding specific micro-environments and interaction with cells. PSMs, for example, are known to be upregulated after internalization in professional and nonprofessional phagocytes [84,85]. Thus, changes observed in the entire abscess material may not necessarily mirror well-established roles of virulence factors in the pathogenesis of SSTI that are specific to the interaction with a subset of cells.

Nasal & respiratory tract colonization & infection

The nares as the predominant niche of *S. aureus* colonization present a unique environment; and several staphylococcal factors influencing nasal colonization have been analyzed, such as teichoic acids and specific surface proteins [90,91]. Animal models of nasal colonization use cotton rats or mice [92,93]. Results from such models have shown that there is a distinct pattern of gene expression in the nasal ecological niche. For example, virulence factors controlled by the *agr* locus are weakly expressed, as assessed by qRT-PCR of samples collected from four persistently colonized individuals [94]. Nasal colonization with staphylococci, particularly *S. aureus* [95], has long been associated with subsequent infection (see below), although approximately 30% of the general population carries *S. aureus*

asymptotically [72]. Nevertheless, this association has driven decolonization efforts, particularly for the purpose of prophylaxis prior to many cardiothoracic and orthopedic procedures [96,97]. *S. aureus* in the nasal niche is therefore routinely eradicated with antibiotics and does not appear to be recalcitrant to antibiotics [98–100]. This, and the finding that swabs of the nares only contain low amounts of *S. aureus*, have led researchers to believe that *S. aureus* in the nares prefer a dispersed rather than a biofilm phenotype [101]. In one study, human nasal secretions were analyzed by metabolomics and found to contain nutrients in rather low amounts, which led to the creation of a growth medium for the *in vitro* simulation of the nasal environment, called synthetic nasal medium (SNM) [3]. Bacterial culture of *S. aureus* USA300 in laboratory medium and SNM showed that in SNM, there was no increase in transcript levels of RNAIII, *psm* β or the clumping factor gene *clfB*, as measured by qRT-PCR [3]. These *in vitro* findings are in accordance with those of an *in vivo* study, which was performed using swabs taken directly from nasal *S. aureus* carriers (all asymptomatic) and showed by qRT-PCR that there was no activation of *agr* in the nasal niche [94].

Respiratory infections commonly originate from nasal colonization and traumatic injury or underlying conditions such as influenza or immune deficiencies. Certain clones are better suited to persist in the respiratory tract, indicating that the immune environment in the respiratory tract is greatly important to the pathogenesis of *S. aureus* respiratory tract infections and supporting the notion that a compromised immune system, as in the case of postviral pneumonia or in hosts with immunodeficiency syndromes such as chronic granulomatous disease (CGD) [102,103], permits *S. aureus* to take hold in the respiratory tract. In a postinfluenza model of *S. aureus* pneumonia, expression of α -toxin, a factor known to be crucial for *S. aureus* lung infection, was greatly decreased, and antecedent viral infections caused significant alterations in bacterial gene expression by qRT-PCR analysis [104]. In a murine study of early lung infection, the transcriptome of *S. aureus* at 6 h post infection was analyzed using microarrays. Principal component analysis revealed an overall downregulation of most virulence factors controlled by *agr*. Interestingly, however, transcript levels of all PSMs were greatly increased (between eight and 13-fold) [105].

Blood & vascular infection

Staphylococcal dissemination in the blood, known as bacteremia, is a life-threatening condition, and can result in sepsis and acute shock, as well as long-term colonization of the vasculature, including cardiac valves, as seen in endocarditis [106]. Endocarditis is largely associated with intravenous drug users, who introduce *S. aureus* directly into the blood stream through contaminated needles or poor sterilization of the injection site [107,108]. There is a shortage of data available concerning gene expression of *S. aureus* in human cardiac vegetations; however, one study has shown that there is reduced expression of *hla* *in vivo* [109].

A study of infective endocarditis in a diabetes mellitus model assessed the *S. aureus* MRSA strain COL transcriptome within endocardial vegetations compared with *in vitro* growth. Microarray analyses showed that 61 genes, mainly associated with metabolism, were upregulated. Among toxins and toxin-related genes, no significant difference was detected in the expression of *agrA*, *hld* or *hla*. However, there was a significant increase in the expression of the leukocidin *lukF* and *lukS* genes in cardiac vegetations compared with controls [110].

In human blood, gene expression is altered compared with laboratory medium. Some studies showed that *agr* is suppressed in human serum or whole blood [43,111], while also reporting that expression of the Agr-controlled γ -hemolysin gene was strongly upregulated when measured by USA300-specific oligonucleotide microarrays [111]. During *S. aureus* bacteremia in human patients, surface proteins (e.g., FnbpA and ClfA) were upregulated by more than twofold, and levels of PSM expression were low according to microarray analysis, which is in accordance with suppression of *agr* under those conditions [112]. Downregulation of Agr was assumed to represent a likely reason for the formation of *S. aureus* aggregates in blood, as this results in increased expression of several MSCRAMMs that bind, for example, fibrin and fibrinogen. However, it needs to be stressed that Agr control of MSCRAMMs can be strongly strain-dependent [69]. Based on results from other infection types and *in vitro* investigation, low PSM production likely further contributes to aggregation [113–115]. Notably, *S. aureus* bacteremia isolates often have a dysfunctional Agr system [116], which is in accordance with a beneficial role of Agr downregulation for *S. aureus* survival in human blood. Contrasting

these studies, another study performed using calf serum reported upregulation of *Agr* and *Agr*-controlled virulence determinants including PSMs, as assessed by qRT-PCR [117]. Whether this contradiction is due to species-specific differences or the experimental setup is not clear.

Bone & joint infections

Osteomyelitis, septic arthritis and prosthetic joint infections are notoriously insidious and difficult to treat [118–120]. Staphylococci have been shown to persist in chondrocytes and osteoblasts [121], thereby evading antibiotics, making treatment without surgical intervention nearly impossible. There is no information on gene expression in humans or animals during joint infections, but limited studies on virulence factor expression have been performed in an *ex vivo* synovial environment.

One study by Szafranska *et al.* determined the adaptive response of *S. aureus* during osteomyelitis. Using high-resolution transcriptomic analysis and mouse models of both chronic and

acute osteomyelitis, these authors found that 444 genes were differentially expressed in *S. aureus* comparing *in vivo* acute osteomyelitis and *in vitro* control experiments. Among these, were several virulence factors such as PSMs and α -toxin. Interestingly, levels of *agr* expression were significantly lower under *in vivo* as compared with *in vitro* conditions. Matrix-binding genes such as *ClfA* and *FnbpA* were also found to be significantly upregulated in osteomyelitis (both acute and chronic) [122].

Bacteria in the synovial environment are especially recalcitrant to antibiotic treatment and difficult to detect [123], a situation that has recently been explained by an extraordinary strong propensity of staphylococci, as the most frequent causes of joint infections, to form aggregates in synovial fluid. In that environment, fibrin- and fibronectin-binding proteins on the surface of *S. aureus* contribute to the formation of large, fibrous agglomerations [124] (Figure 1A). *Staphylococcus aureus* undergoes immediate aggregation upon incubation in synovial fluid through the engagement of those MSCRAMMs with fibrous matrix components (Figure 1B) [124]. In general, aggregation behavior in synovial fluid holds many similarities to the behavior of *S. aureus* in blood. However, synovial fluid, a filtrate of serum with added proteins, causes a more pronounced biofilm phenotype as well as larger aggregate formation than serum [124]. In synovial fluid, there is significantly decreased expression of *agr* and PSMs (when assessed by qRT-PCR) compared with serum and laboratory medium, which is believed to be the cause for the exceptionally strong degree of *S. aureus* aggregation during joint infection [113]. Decreased expression of the biofilm-dispersive PSMs means that after initial aggregation has occurred, biofilm adhesion is increasing and disaggregation is inhibited [113] (Figure 2). This leads to the buildup of PIA/PNAG and likely other matrix components, such as teichoic acids and eDNA, within the dense matrix, and results in biofilm-like aggregations that are recalcitrant to antibiotics [113,124].

Figure 1. *Staphylococcus aureus* in joint infections visualized by scanning electron microscopy. (A) Biofilm isolated from a patient with orthopedic infection. (B) Rapid aggregation of *S. aureus* after 20 min incubation in synovial fluid.

Infections of the CNS

Staphylococcal infections of the CNS are less frequent than those of the skin, mucosa, and bone and joints. The few cases that are reported usually mention the formation of abscesses, disseminated disease or cavernous sinus thrombosis, and are believed to result from superficial skin infections, sinusitis or otitis media [125].

Figure 2. Aggregation of bacteria and formation of biofilm in response to host fibrin.

(A) *Staphylococcus aureus* binds to human matrix proteins present in traumatized joints (such as fibrin) using dedicated, surface-located binding proteins (ClfA, ClfB, FnbA, FnbB). (B) The low activity of Agr in fibrin-mediated aggregates causes low production of the surfactant PSMs, with the result of pronounced cell-to-cell attachment and retention of biofilm macromolecules on the cell surface. This ultimately leads to the formation of enormous, macroscopic cellular aggregates with high resistance to antibiotics.

Postsurgically, infections are present in the form of shunt infections and ventricular drain infections. Fortunately, antibiotic penetration into the cerebrospinal fluid is sufficient for antimicrobial activity [126], which is why *S. aureus* infections of the CNS tend to respond positively to antibiotics, suggesting that biofilm formation is likely less pronounced in CSF than in other fluids, such as serum or synovial fluid. No data are available on staphylococcal gene expression during infection of the CNS.

Infections of the urinary & reproductive tract

Among the staphylococci, *Staphylococcus saprophyticus* is the pathogen most frequently involved with urinary tract infections (UTIs) [127]. Specific adhesins and biofilm formation play key roles for the pathogenesis of *S. saprophyticus* UTI, but how the environment in the urinary tract impacts gene expression of *S. saprophyticus* is unknown [128]. In contrast to *S. saprophyticus*, *S. aureus* is not as common a cause of UTI. *S. aureus* only represents <5% of outpatient and 16% of inpatient UTIs [129], and is primarily a cause of UTIs among patients with urinary tract catheterization. In accordance with the notion of a key role of biofilms during UTI, *S. aureus* was shown to form biofilms in the urinary catheter lumen [130]. Interestingly, urine has been reported to be inhibitory for *S. aureus* growth [131]. For *ex vivo* studies, pooled urine is sometimes used to simulate the urinary environment [132]. Nevertheless, no studies have been performed on the transcriptome of *S. aureus* or *S. saprophyticus* in urine.

S. aureus is one of the most prevalent species present in the male and female genital tracts.

However, *S. aureus* overgrowth in the genital tract is normally inhibited and overpowering infection is rarely a concern, except for in the immunocompromised, or where there is the presence of a foreign body (tampons, intrauterine devices, etc.) [133]. Gene expression by *S. aureus* in the reproductive tract has not yet been studied, but biofilm formation by *S. aureus* on tampons and on menses components has been confirmed through fluorescent *in situ* hybridization [133]. Not much is known about the phenotype of staphylococci in the reproductive tract, but there is a link of *S. aureus* colonization to infertility [134]. Specifically, the expression of spermagglutinins by *S. aureus* in the vaginal tract causes aggregation and decreased motility of sperm leading to decreased fertilization [135]. Other than infertility and the commonly reported toxic shock syndrome due to staphylococcal overgrowth on foreign bodies, no other clinical manifestation of *S. aureus* in the vaginal tract has been reported. Likely, the presence of other bacteria, the pH and the relatively low oxygen tension keeps *S. aureus* in check. Similar to other host niches, much work remains to be conducted on staphylococcal gene expression in both the male and female reproductive tracts.

Conclusion

The host environment can have significant effects on staphylococcal gene expression and overall phenotype. The control of gene expression in response to environmental conditions is crucial for effective staphylococcal colonization and infection. Of particular importance is the ability to switch between planktonic and biofilm phenotypes in response to the host environment.

However, our understanding of how staphylococci respond to specific micro-environments in the host is still very limited.

Future perspective

Staphylococcal virulence, growth, colonization and antibiotic susceptibility vary from niche to niche in the human host. These differences require tailoring of therapeutic approaches, or decolonization measures, to specific infection types and locations, taking into account the different patterns of gene expression and phenotypic changes that occur within each environment. Many of the studies cited in this review describe conflicting results. Inconsistencies may be a result of varying qualities and rigors of study, and must be resolved using state-of-the-art methodologies in future studies. It is apparent that our knowledge about staphylococcal phenotypes in most colonization and infection scenarios is still very limited. Going forward, the improvement of techniques to determine transcriptomic and proteomic changes, such as by RNA sequencing or MS-based approaches

to analyze protein composition, will play a key role in those endeavors. Furthermore, genetic and imaging tools have been recently developed that allow us to measure gene expression levels in *in vivo* settings that closely resemble the situations bacteria encounter during human infection and colonization. Thus, it is to be expected that there will soon be much more detailed insight into host niche-dependent differences in staphylococcal phenotypes and pathogenesis strategies, hopefully leading to a significant improvement of therapeutic approaches to combat staphylococcal infections.

Financial & competing interests disclosure

This work was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, The National Institutes of Health. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

EXECUTIVE SUMMARY

Staphylococci in diverse physiologic niches

- Each physiologic niche (e.g., the nares, skin, joints, etc.) comprises specific environmental characteristics, which can strongly affect gene expression.
- Bacterial virulence in each niche can be heightened or diminished, depending on the environmental factors present.
- It is crucial to understand the effects of each physiologic niche on the ability of staphylococcal strains to cause infection, or maintain colonization.

Important environmental cues

- Gene expression can be altered by variations in pH, nutrient availability, oxygen pressure and other host factors. Many of those factors impact biofilm production and production of *agr*-controlled virulence factors.
- *Staphylococcus aureus* is more prone to biofilm formation where serum proteins and ECM components are abundant (e.g., in cardiac valves or synovial joints).

Staphylococcus aureus gene expression in various host niches

- Transcriptomic analyses of samples from *in vivo* infection or colonization are very limited.
- *In vitro* transcriptomic analyses closely emulating the *in vivo* environment are currently being developed (e.g., nasal media, *ex vivo* use of physiological fluids, use of physiological pH or oxygen tension).
- Future studies should expand comparisons of gene expression in various physiologic niches, using direct isolation from *in vivo* infections or *ex vivo* simulations adequately representing *in vivo* situations.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

- 1 Gordon RJ, Lowy FD. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. *Clin. Infect. Dis.* 46(Suppl. 5), S350–S359 (2008).
- 2 Bore E, Langsrud S, Langsrud O, Rode TM, Holck A. Acid-shock responses in *Staphylococcus aureus* investigated by global gene expression analysis. *Microbiology* 153(Pt 7), 2289–2303 (2007).
- 3 Krismer B, Liebeck M, Janek D *et al.* Nutrient limitation governs *Staphylococcus aureus* metabolism and niche adaptation in

- the human nose. *PLoS Pathog.* 10(1), e1003862 (2014).
- 4 Resch A, Rosenstein R, Nerz C, Gotz F. Differential gene expression profiling of *Staphylococcus aureus* cultivated under biofilm and planktonic conditions. *Appl. Environ. Microbiol.* 71(5), 2663–2676 (2005).
- 5 King MD. Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft-tissue infections. *Ann. Intern. Med.* 144(5), 309 (2006).
- 6 Conway T, Schoolnik GK. Microarray expression profiling: capturing a genome-wide portrait of the transcriptome. *Mol. Microbiol.* 47(4), 879–889 (2003).
- 7 Rooijackers SH, Van Kessel KP, Van Strijp JA. Staphylococcal innate immune evasion. *Trends Microbiol.* 13(12), 596–601 (2005).
- 8 Otto M. *Staphylococcus aureus* toxins. *Curr. Opin. Microbiol.* 17, 32–37 (2014).
- 9 Peschel A, Otto M. Phenol-soluble modulins and staphylococcal infection. *Nat. Rev. Microbiol.* 11(10), 667–673 (2013).
- 10 Queck SY, Jameson-Lee M, Villaruz AE *et al.* RNAIII-independent target gene control by the agr quorum-sensing system: insight into the evolution of virulence regulation in *Staphylococcus aureus*. *Mol. Cell.* 32(1), 150–158 (2008).
- 11 Recsei P, Kreiswirth B, O'reilly M, Schlievert P, Gruss A, Novick RP. Regulation of exoprotein gene expression in *Staphylococcus aureus* by agr. *Mol. Gen. Genet.* 202(1), 58–61 (1986).
- 12 Malachowa N, DeLeo FR. Mobile genetic elements of *Staphylococcus aureus*. *Cell Mol. Life Sci.* 67(18), 3057–3071 (2010).
- 13 Li M, Diep BA, Villaruz AE *et al.* Evolution of virulence in epidemic community-associated methicillin-resistant *Staphylococcus aureus*. *Proc. Natl Acad. Sci. USA* 106(14), 5883–5888 (2009).
- **Emphasizes the importance of gene expression over gene presence in the evolution of virulence in community-associated methicillin-resistant *Staphylococcus aureus* (MRSA).**
- 14 Wang R, Braughton KR, Kretschmer D *et al.* Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nat. Med.* 13(12), 1510–1514 (2007).
- 15 Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 284(5418), 1318–1322 (1999).
- 16 Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet* 358(9276), 135–138 (2001).
- 17 Otto M. Staphylococcal biofilms. *Curr. Top. Microbiol. Immunol.* 322, 207–228 (2008).
- 18 Mack D, Fischer W, Krokotsch A *et al.* The intercellular adhesion involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear beta-1,6-linked glucosaminoglycan: purification and structural analysis. *J. Bacteriol.* 178(1), 175–183 (1996).
- 19 Cramton SE, Gerke C, Schnell NF, Nichols WW, Gotz F. The intercellular adhesion (ica) locus is present in *Staphylococcus aureus* and is required for biofilm formation. *Infect. Immun.* 67(10), 5427–5433 (1999).
- 20 Heilmann C, Schweitzer O, Gerke C, Vanittanakom N, Mack D, Gotz F. Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. *Mol. Microbiol.* 20(5), 1083–1091 (1996).
- 21 Gerke C, Kraft A, Sussmuth R, Schweitzer O, Gotz F. Characterization of the N-acetylglucosaminyltransferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. *J. Biol. Chem.* 273(29), 18586–18593 (1998).
- 22 Vuong C, Kocianova S, Voyich JM *et al.* A crucial role for exopolysaccharide modification in bacterial biofilm formation, immune evasion, and virulence. *J. Biol. Chem.* 279(52), 54881–54886 (2004).
- 23 Otto M. Staphylococcal infections: mechanisms of biofilm maturation and detachment as critical determinants of pathogenicity. *Annu. Rev. Med.* 64, 175–188 (2013).
- 24 O'Riordan K, Lee JC. *Staphylococcus aureus* capsular polysaccharides. *Clin. Microbiol. Rev.* 17(1), 218–234 (2004).
- 25 Nilsson IM, Lee JC, Bremell T, Ryden C, Tarkowski A. The role of staphylococcal polysaccharide microcapsule expression in septicemia and septic arthritis. *Infect. Immun.* 65(10), 4216–4221 (1997).
- 26 Boyle-Vavra S, Li X, Alam MT *et al.* USA300 and USA500 clonal lineages of *Staphylococcus aureus* do not produce a capsular polysaccharide due to conserved mutations in the cap5 locus. *MBio* 6(2), (2015).
- 27 Foster TJ, Geoghegan JA, Ganesh VK, Hook M. Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*. *Nat. Rev. Microbiol.* 12(1), 49–62 (2014).
- 28 Beenken KE, Dunman PM, Mcaleese F *et al.* Global gene expression in *Staphylococcus aureus* biofilms. *J. Bacteriol.* 186(14), 4665–4684 (2004).
- **First study analyzing genome-wide gene expression in *Staphylococcus aureus* biofilms.**
- 29 Resch A, Leicht S, Saric M *et al.* Comparative proteome analysis of *Staphylococcus aureus* biofilm and planktonic cells and correlation with transcriptome profiling. *Proteomics* 6(6), 1867–1877 (2006).
- **First study analyzing protein production in *S. aureus* biofilms by a proteomic approach.**
- 30 Yao Y, Sturdevant DE, Otto M. 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(2), 289–298 (2005).
- 31 Novick RP, Geisinger E. Quorum sensing in staphylococci. *Annu. Rev. Genet.* 42, 541–564 (2008).
- 32 Cheung AL, Nishina KA, Trotton MP, Tamber S. The SarA protein family of *Staphylococcus aureus*. *Int. J. Biochem. Cell. Biol.* 40(3), 355–361 (2008).
- 33 Hecker M, Reder A, Fuchs S, Pagels M, Engelmann S. Physiological proteomics and stress/starvation responses in *Bacillus subtilis* and *Staphylococcus aureus*. *Res. Microbiol.* 160(4), 245–258 (2009).
- 34 Fournier B, Klier A, Rapoport G. The two-component system ArlS-ArlR is a regulator of virulence gene expression in *Staphylococcus aureus*. *Mol. Microbiol.* 41(1), 247–261 (2001).
- 35 Pragman AA, Yarwood JM, Tripp TJ, Schlievert PM. Characterization of virulence factor regulation by SrrAB, a two-component system in *Staphylococcus aureus*. *J. Bacteriol.* 186(8), 2430–2438 (2004).
- 36 Cheung AL, Bayer MG, Heinrichs JH. sar Genetic determinants necessary for transcription of RNAII and RNAIII in the agr locus of *Staphylococcus aureus*. *J. Bacteriol.* 179(12), 3963–3971 (1997).
- 37 Ziebandt AK, Weber H, Rudolph J *et al.* Extracellular proteins of *Staphylococcus aureus* and the role of SarA and sigma B. *Proteomics* 1(4), 480–493 (2001).
- 38 Nicholas RO, Li T, Mcdevitt D *et al.* Isolation and characterization of a sigB deletion mutant of *Staphylococcus aureus*. *Infect. Immun.* 67(7), 3667–3669 (1999).
- 39 Deora R, Tseng T, Misra TK. Alternative transcription factor sigmaSB of *Staphylococcus aureus*: characterization and role in transcription of the global regulatory

- locus *sa*. *J. Bacteriol.* 179(20), 6355–6359 (1997).
- 40 Yarwood JM, McCormick JK, Schlievert PM. Identification of a novel two-component regulatory system that acts in global regulation of virulence factors of *Staphylococcus aureus*. *J. Bacteriol.* 183(4), 1113–1123 (2001).
- 41 Fournier B, Hooper DC. A new two-component regulatory system involved in adhesion, autolysis, and extracellular proteolytic activity of *Staphylococcus aureus*. *J. Bacteriol.* 182(14), 3955–3964 (2000).
- 42 Fuchs S, Pane-Farre J, Kohler C, Hecker M, Engelmann S. Anaerobic gene expression in *Staphylococcus aureus*. *J. Bacteriol.* 189(11), 4275–4289 (2007).
- 43 Yarwood JM, McCormick JK, Paustian ML, Kapur V, Schlievert PM. Repression of the *Staphylococcus aureus* accessory gene regulator in serum and *in vivo*. *J. Bacteriol.* 184(4), 1095–1101 (2002).
- 44 Ulrich M, Bastian M, Cramton SE *et al.* The staphylococcal respiratory response regulator SrrAB induces *ica* gene transcription and polysaccharide intercellular adhesin expression, protecting *Staphylococcus aureus* from neutrophil killing under anaerobic growth conditions. *Mol. Microbiol.* 65(5), 1276–1287 (2007).
- 45 Park MK, Myers RA, Marzella L. Oxygen tensions and infections: modulation of microbial growth, activity of antimicrobial agents, and immunologic responses. *Clin. Infect. Dis.* 14(3), 720–740 (1992).
- 46 Lund-Olesen K. Oxygen tension in synovial fluids. *Arthritis Rheum.* 13(6), 769–776 (1970).
- 47 Weinrick B, Dunman PM, Mcaleese F *et al.* Effect of mild acid on gene expression in *Staphylococcus aureus*. *J. Bacteriol.* 186(24), 8407–8423 (2004).
- 48 Chan PF, Foster SJ. The role of environmental factors in the regulation of virulence-determinant expression in *Staphylococcus aureus* 8325–4. *Microbiology* 144(Pt 9), 2469–2479 (1998).
- **An early study addressing the impact of different environmental conditions on the expression of specific *S. aureus* virulence factors.**
- 49 Regassa LB, Novick RP, Betley MJ. Glucose and nonmaintained pH decrease expression of the accessory gene regulator (*agr*) in *Staphylococcus aureus*. *Infect. Immun.* 60(8), 3381–3388 (1992).
- 50 Regassa LB, Betley MJ. High sodium chloride concentrations inhibit staphylococcal enterotoxin C gene (*sec*) expression at the level of *sec* mRNA. *Infect. Immun.* 61, 1581–1585 (1993).
- 51 Genigeorgis C, Sadler WW. Effect of sodium chloride and pH on enterotoxin B production. *J. Bacteriol.* 92(5), 1383–1387 (1966).
- 52 Sheehan BJ, Foster TJ, Dorman CJ, Park S, Stewart GS. Osmotic and growth-phase dependent regulation of the *eta* gene of *Staphylococcus aureus*: a role for DNA supercoiling. *Mol. Gen. Genet.* 232(1), 49–57 (1992).
- 53 Mekalanos JJ. Environmental signals controlling expression of virulence determinants in bacteria. *J. Bacteriol.* 174(1), 1–7 (1992).
- 54 Reeves MW. Effect of trace metals on the synthesis of toxic shock syndrome toxin 1. *Rev. Infect. Dis.* 11(Suppl. 1), S145–S149; discussion S149–S150 (1989).
- 55 Allard M, Moisan H, Brouillette É *et al.* Transcriptional modulation of some *Staphylococcus aureus* iron-regulated genes during growth *in vitro* and in a tissue cage model *in vivo*. *Microbes Infect.* 8(7), 1679–1690 (2006).
- 56 Harder J, Schroder JM. Antimicrobial peptides in human skin. *Chem. Immunol. Allergy* 86, 22–41 (2005).
- 57 Herbert S, Bera A, Nerz C *et al.* Molecular basis of resistance to muramidase and cationic antimicrobial peptide activity of lysozyme in staphylococci. *PLoS Pathog.* 3(7), e102 (2007).
- 58 Li M, Lai Y, Villaruz AE, Cha DJ, Sturdevant DE, Otto M. Gram-positive three-component antimicrobial peptide-sensing system. *Proc. Natl Acad. Sci. USA* 104(22), 9469–9474 (2007).
- **Describes genome-wide responses to antimicrobial peptides (AMPs) and the AMP sensing mechanism in staphylococci.**
- 59 Li M, Cha DJ, Lai Y, Villaruz AE, Sturdevant DE, Otto M. The antimicrobial peptide-sensing system *aps* of *Staphylococcus aureus*. *Mol. Microbiol.* 66(5), 1136–1147 (2007).
- 60 Pietiäinen M, Francois P, Hyrylainen HL *et al.* Transcriptome analysis of the responses of *Staphylococcus aureus* to antimicrobial peptides and characterization of the roles of *vraDE* and *vraSR* in antimicrobial resistance. *BMC Genomics* 10, 429 (2009).
- 61 Peschel A, Jack RW, Otto M *et al.* *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with l-lysine. *J. Exp. Med.* 193(9), 1067–1076 (2001).
- 62 Peschel A, Otto M, Jack RW, Kalbacher H, Jung G, Gotz F. Inactivation of the *dlt* operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides. *J. Biol. Chem.* 274(13), 8405–8410 (1999).
- 63 Falord M, Karimova G, Hiron A, Msadek T. GraXSR proteins interact with the VraFG ABC transporter to form a five-component system required for cationic antimicrobial peptide sensing and resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 56(2), 1047–1058 (2012).
- 64 Chang W, Small DA, Toghrol F, Bentley WE. Global transcriptome analysis of *Staphylococcus aureus* response to hydrogen peroxide. *J. Bacteriol.* 188(4), 1648–1659 (2006).
- 65 Stevens DL, Ma Y, Salmi DB, Mcindoo E, Wallace RJ, Bryant AE. Impact of antibiotics on expression of virulence-associated exotoxin genes in methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. *J. Infect. Dis.* 195(2), 202–211 (2007).
- 66 Joo HS, Chan JL, Cheung GY, Otto M. Subinhibitory concentrations of protein synthesis-inhibiting antibiotics promote increased expression of the *agr* virulence regulator and production of phenol-soluble modulins cytotoxins in community-associated methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 54(11), 4942–4944 (2010).
- 67 Ohlsen K, Ziebuhr W, Koller KP, Hell W, Wichelhaus TA, Hacker J. Effects of subinhibitory concentrations of antibiotics on alpha-toxin (*hla*) gene expression of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* isolates. *Antimicrob. Agents Chemother.* 42(11), 2817–2823 (1998).
- 68 Foster TJ, Hook M. Surface protein adhesins of *Staphylococcus aureus*. *Trends Microbiol.* 6(12), 484–488 (1998).
- 69 Cheung GY, Wang R, Khan BA, Sturdevant DE, Otto M. Role of the accessory gene regulator *agr* in community-associated methicillin-resistant *Staphylococcus aureus* pathogenesis. *Infect. Immun.* 79(5), 1927–1935 (2011).
- 70 Otto M. *Staphylococcus epidermidis* – the ‘accidental’ pathogen. *Nat. Rev. Microbiol.* 7(8), 555–567 (2009).
- 71 Otto M. Virulence factors of the coagulase-negative staphylococci. *Front. Biosci.* 9, 841–863 (2004).
- 72 Wertheim HF, Melles DC, Vos MC *et al.* The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect. Dis.* 5(12), 751–762 (2005).

- 73 Yang ES, Tan J, Eells S, Rieg G, Tagudar G, Miller LG. Body site colonization in patients with community-associated methicillin-resistant *Staphylococcus aureus* and other types of *S. aureus* skin infections. *Clin. Microbiol. Infect.* 16(5), 425–431 (2010).
- 74 DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated methicillin-resistant *Staphylococcus aureus*. *Lancet* 375(9725), 1557–1568 (2010).
- 75 Lowy FD. *Staphylococcus aureus* infections. *N. Engl. J. Med.* 339(8), 520–532 (1998).
- 76 Grice EA, Segre JA. The skin microbiome. *Nat. Rev. Microbiol.* 9(4), 244–253 (2011).
- 77 Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. *Science* 326(5960), 1694–1697 (2009).
- 78 Chiller K, Selkin BA, Murakawa GJ. Skin microflora and bacterial infections of the skin. *J. Investig. Dermatol. Symp. Proc.* 6(3), 170–174 (2001).
- 79 Garzoni C, Kelley WL. *Staphylococcus aureus*: new evidence for intracellular persistence. *Trends Microbiol.* 17(2), 59–65 (2009).
- 80 Von Eiff C, Becker K, Metze D *et al.* Intracellular persistence of *Staphylococcus aureus* small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with drier's disease. *Clin. Infect. Dis.* 32(11), 1643–1647 (2001).
- 81 Rogers DE, Tompsett R. The survival of staphylococci within human leukocytes. *J. Exp. Med.* 95(2), 209–230 (1952).
- 82 Bayles KW, Wesson CA, Liou LE, Fox LK, Bohach GA, Trumble WR. Intracellular *Staphylococcus aureus* escapes the endosome and induces apoptosis in epithelial cells. *Infect. Immun.* 66(1), 336–342 (1998).
- 83 Garzoni C, Francois P, Huyghe A *et al.* A global view of *Staphylococcus aureus* whole genome expression upon internalization in human epithelial cells. *BMC Genomics* 8, 171 (2007).
- **Transcriptomic analysis of *S. aureus* gene expression after internalization in epithelial cells.**
- 84 Grosz M, Kolter J, Paprotka K *et al.* Cytoplasmic replication of *Staphylococcus aureus* upon phagosomal escape triggered by phenol-soluble modulins. *Cell. Microbiol.* 16(4), 451–465 (2014).
- 85 Surewaard BG, De Haas CJ, Vervoort F *et al.* Staphylococcal alpha-phenol soluble modulins contribute to neutrophil lysis after phagocytosis. *Cell. Microbiol.* 15(8), 1427–1437 (2013).
- 86 Voyich JM, Braughton KR, Sturdevant DE *et al.* Insights into mechanisms used by *Staphylococcus aureus* to avoid destruction by human neutrophils. *J. Immunol.* 175(6), 3907–3919 (2005).
- 87 Loughman JA, Fritz SA, Storch GA, Hunstad DA. Virulence gene expression in human community-acquired *Staphylococcus aureus* infection. *J. Infect. Dis.* 199(3), 294–301 (2009).
- 88 Malachowa N, Kobayashi SD, Sturdevant DE, Scott DP, DeLeo FR. Insights into the *Staphylococcus aureus*-host interface: global changes in host and pathogen gene expression in a rabbit skin infection model. *PLoS ONE* 10(2), e0117713 (2015).
- 89 Date SV, Modrusan Z, Lawrence M *et al.* Global gene expression of methicillin-resistant *Staphylococcus aureus* USA300 during human and mouse infection. *J. Infect. Dis.* 209(10), 1542–1550 (2014).
- **Describes genome-wide *S. aureus* gene expression in samples obtained from human abscesses.**
- 90 Weidenmaier C, Kokai-Kun JF, Kristian SA *et al.* Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. *Nat. Med.* 10(3), 243–245 (2004).
- 91 Weidenmaier C, Kokai-Kun JF, Kulazovic E *et al.* Differential roles of sortase-anchored surface proteins and wall teichoic acid in *Staphylococcus aureus* nasal colonization. *Int. J. Med. Microbiol.* 298(5–6), 505–513 (2008).
- 92 Kokai-Kun JF, Walsh SM, Chanturiya T, Mond JJ. Lysostaphin cream eradicates *Staphylococcus aureus* nasal colonization in a cotton rat model. *Antimicrob. Agents Chemother.* 47(5), 1589–1597 (2003).
- 93 Kiser KB, Cantey-Kiser JM, Lee JC. Development and characterization of a *Staphylococcus aureus* nasal colonization model in mice. *Infect. Immun.* 67(10), 5001–5006 (1999).
- 94 Burian M, Wolz C, Goerke C. Regulatory adaptation of *Staphylococcus aureus* during nasal colonization of humans. *PLoS ONE* 5(4), e10040 (2010).
- 95 Von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. *N. Engl. J. Med.* 344(1), 11–16 (2001).
- 96 Rao N, Jacobs S, Joyce L. Cost-effective eradication of an outbreak of methicillin-resistant *Staphylococcus aureus* in a community teaching hospital. *Infect. Control Hosp. Epidemiol.* 9(6), 255–260 (1988).
- 97 Hacek DM, Paule SM, Thomson RB Jr., Robicsek A, Peterson LR. Implementation of a universal admission surveillance and decolonization program for methicillin-resistant *Staphylococcus aureus* (MRSA) reduces the number of MRSA and total number of *S. aureus* isolates reported by the clinical laboratory. *J. Clin. Microbiol.* 47(11), 3749–3752 (2009).
- 98 Ammerlaan HS, Kluytmans JA, Wertheim HF, Nouwen JL, Bonten MJ. Eradication of methicillin-resistant *Staphylococcus aureus* carriage: a systematic review. *Clin. Infect. Dis.* 48(7), 922–930 (2009).
- 99 Bode LG, Kluytmans JA, Wertheim HF *et al.* Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. *N. Engl. J. Med.* 362(1), 9–17 (2010).
- 100 Van Rijen MM, Bonten M, Wenzel RP, Kluytmans JA. Intranasal mupirocin for reduction of *Staphylococcus aureus* infections in surgical patients with nasal carriage: a systematic review. *J. Antimicrob. Chemother.* 61(2), 254–261 (2008).
- 101 Krismer B, Peschel A. Does *Staphylococcus aureus* nasal colonization involve biofilm formation? *Future Microbiol.* 6(5), 489–493 (2011).
- 102 Rosenzweig SD, Holland SM. Recent insights into the pathobiology of innate immune deficiencies. *Curr. Allergy Asthma Rep.* 11(5), 369–377 (2011).
- 103 Von Bernuth H, Picard C, Jin Z *et al.* Pyogenic bacterial infections in humans with MyD88 deficiency. *Science* 321(5889), 691–696 (2008).
- 104 Lee MH, Arrecubieta C, Martin FJ, Prince A, Borczuk AC, Lowy FD. A postinfluenza model of *Staphylococcus aureus* pneumonia. *J. Infect. Dis.* 201(4), 508–515 (2010).
- 105 Chaffin DO, Taylor D, Skerrett SJ, Rubens CE. Changes in the *Staphylococcus aureus* transcriptome during early adaptation to the lung. *PLoS ONE* 7(8), e41329 (2012).
- 106 Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin. Microbiol. Rev.* 28(3), 603–661 (2015).
- 107 Miro JM, Anguera I, Cabell CH *et al.* *Staphylococcus aureus* native valve infective endocarditis: report of 566 episodes from the International Collaboration on Endocarditis Merged Database. *Clin. Infect. Dis.* 41(4), 507–514 (2005).
- 108 Shrestha NK, Jue J, Hussain ST *et al.* Injection drug use and outcomes after surgical

- intervention for infective endocarditis. *Ann. Thorac. Surg.* (2015).
- 109 Xiong YQ, Willard J, Yeaman MR, Cheung AL, Bayer AS. Regulation of *Staphylococcus aureus* alpha-toxin gene (*bla*) expression by *agr* *sarA* and *sae* *in vitro* and in experimental infective endocarditis. *J. Infect. Dis.* 194(9), 1267–1275 (2006).
- 110 Hanses F, Roux C, Dunman PM, Salzberger B, Lee JC. *Staphylococcus aureus* gene expression in a rat model of infective endocarditis. *Genome Med.* 6(10), 93 (2014).
- 111 Malachowa N, Whitney AR, Kobayashi SD *et al.* Global changes in *Staphylococcus aureus* gene expression in human blood. *PLoS ONE* 6(4), e18617 (2011).
- Describes genome-wide gene expression differences during growth in blood as compared with laboratory media.
- 112 Den Reijer PM, Lemmens-Den Toom N, Kant S *et al.* Characterization of the humoral immune response during *Staphylococcus aureus* bacteremia and global gene expression by *Staphylococcus aureus* in human blood. *PLoS ONE* 8(1), e53391 (2013).
- 113 Dastgheyb SS, Villaruz AE, Le KY *et al.* Role of Phenol-Soluble Modulins in Formation of *Staphylococcus aureus* Biofilms in Synovial Fluid. *Infect. Immun.* 83(7), 2966–2975 (2015).
- 114 Wang R, Khan BA, Cheung GY *et al.* *Staphylococcus epidermidis* surfactant peptides promote biofilm maturation and dissemination of biofilm-associated infection in mice. *J. Clin. Invest.* 121(1), 238–248 (2011).
- 115 Periasamy S, Joo HS, Duong AC *et al.* *Staphylococcus aureus* biofilms develop their characteristic structure. *Proc. Natl Acad. Sci. USA* 109(4), 1281–1286 (2012).
- 116 Fowler VG Jr., Sakoulas G, McIntyre LM *et al.* Persistent bacteremia due to methicillin-resistant *Staphylococcus aureus* infection is associated with *agr* dysfunction and low-level *in vitro* resistance to thrombin-induced platelet microbicidal protein. *J. Infect. Dis.* 190(6), 1140–1149 (2004).
- 117 Oogai Y, Matsuo M, Hashimoto M, Kato F, Sugai M, Komatsuzawa H. Expression of virulence factors by *Staphylococcus aureus* grown in serum. *Appl. Environ. Microbiol.* 77(22), 8097–8105 (2011).
- 118 Shirtliff ME, Mader JT. Acute septic arthritis. *Clin. Microbiol. Rev.* 15(4), 527–544 (2002).
- 119 Fass RJ. Treatment of osteomyelitis and septic arthritis with cefazolin. *Antimicrob. Agents Chemother.* 13(3), 405–411 (1978).
- 120 Zimmerli W, Moser C. Pathogenesis and treatment concepts of orthopaedic biofilm infections. *FEMS Immunol. Med. Microbiol.* 65(2), 158–168 (2012).
- 121 Hudson MC, Ramp WK, Nicholson NC, Williams AS, Nousiainen MT. Internalization of *Staphylococcus aureus* by cultured osteoblasts. *Microb. Pathog.* 19(6), 409–419 (1995).
- 122 Szafranska AK, Oxley AP, Chaves-Moreno D *et al.* High-resolution transcriptomic analysis of the adaptive response of *Staphylococcus aureus* during acute and chronic phases of osteomyelitis. *MBio* 5(6), pii: e01775–14 (2014).
- 123 Parvizi J, Ghanem E, Sharkey P, Aggarwal A, Burnett RS, Barrack RL. Diagnosis of infected total knee: findings of a multicenter database. *Clin. Orthop. Relat. Res.* 466(11), 2628–2633 (2008).
- 124 Dastgheyb S, Parvizi J, Shapiro IM, Hickok NJ, Otto M. Effect of biofilms on recalcitrance of staphylococcal joint infection to antibiotic treatment. *J. Infect. Dis.* 211(4), 641–650 (2015).
- 125 Naesens R, Ronsyn M, Druwe P, Denis O, Ieven M, Jeurissen A. Central nervous system invasion by community-acquired methicillin-resistant *Staphylococcus aureus*. *J. Med. Microbiol.* 58(Pt 9), 1247–1251 (2009).
- 126 Fong IW. Staphylococcal central nervous system infections treated with cloxacillin. *J. Antimicrob. Chemother.* 12(6), 607–612 (1983).
- 127 Jordan PA, Iravani A, Richard GA, Baer H. Urinary tract infection caused by *Staphylococcus saprophyticus*. *J. Infect. Dis.* 142(4), 510–515 (1980).
- 128 Raz R, Colodner R, Kunin CM. Who are you – *Staphylococcus saprophyticus*? *Clin. Infect. Dis.* 40(6), 896–898 (2005).
- 129 Dobrindt U. [Virulence factors of uropathogens]. *Urologe A* 49(5), 598–605 (2010).
- 130 Gad GF, El-Feky MA, El-Rehewy MS, Hassan MA, Abolella H, El-Baky RM. Detection of *icaA* *icaD* genes and biofilm production by *Staphylococcus aureus* and *Staphylococcus epidermidis* isolated from urinary tract catheterized patients. *J. Infect. Dev. Ctries* 3(5), 342–351 (2009).
- 131 Kaye D. Antibacterial activity of human urine. *J. Clin. Invest.* 47(10), 2374–2390 (1968).
- 132 Erdogan-Yildirim Z, Burian A, Manafi M, Zeitlinger M. Impact of pH on bacterial growth and activity of recent fluoroquinolones in pooled urine. *Res. Microbiol.* 162(3), 249–252 (2011).
- 133 Veeh RH, Shirtliff ME, Petik JR *et al.* Detection of *Staphylococcus aureus* biofilm on tampons and menses components. *J. Infect. Dis.* 188(4), 519–530 (2003).
- 134 Kaur S, Prabha V. Infertility as a consequence of spermagglutinating *Staphylococcus aureus* colonization in genital tract of female mice. *PLoS ONE* 7(12), e52325 (2012).
- 135 Ohri M, Prabha V. Isolation of a sperm-agglutinating factor from *Staphylococcus aureus* isolated from a woman with unexplained infertility. *Fertil. Steril.* 84(5), 1539–1541 (2005).
- 136 Ehlers C, Ivens UI, Moller ML, Senderovitz T, Serup J. Females have lower skin surface pH than men. A study on the surface of gender, forearm site variation, right/left difference and time of the day on the skin surface pH. *Skin Res. Technol.* 7(2), 90–94 (2001).
- 137 Raz R, Chazan B, Dan M. Cranberry juice and urinary tract infection. *Clin. Infect. Dis.* 38(10), 1413–1419 (2004).
- 138 England RJ, Homer JJ, Knight LC, Ell SR. Nasal pH measurement: a reliable and repeatable parameter. *Clin. Otolaryngol. Allied Sci.* 24(1), 67–68 (1999).
- 139 Wagner G, Ottesen B. Vaginal physiology during menstruation. *Ann. Intern. Med.* 96(6 Pt 2), 921–923 (1982).
- 140 Cheng DS, Rodriguez RM, Rogers J, Wagster M, Starnes DL, Light RW. Comparison of pleural fluid pH values obtained using blood gas machine, pH meter, and pH indicator strip. *Chest* 114(5), 1368–1372 (1998).
- 141 Robbie LA, Bennett B, Croll AM, Brown PA, Booth NA. Proteins of the fibrinolytic system in human thrombi. *Thrombosis Haemostasis* 75, 127–133 (1996).
- 142 Schultze HE, Heremans JF. Nature and metabolism of extracellular proteins. In: *Molecular Biology Of Human Proteins With Special Reference To Plasma Proteins* (Vol. 1). 473–477 (1966).
- 143 Tirumalai RS, Chan KC, Prieto DA, Issaq HJ, Conrads TP, Veenstra TD. Characterization of the low molecular weight human serum proteome. *Mol. Cell. Proteomics* 2(10), 1096–1103 (2003).
- 144 Charm SE, Kurland. GS. *Blood Flow and Microcirculation*. Wiley (1974).
- 145 Cokelet GR. The rheology of human blood. *Biomechanics* 72, 63–103 (1972).
- 146 Mcadow M, Kim HK, Dedent AC, Hendrickx AP, Schneewind O, Missiakas DM. Preventing *Staphylococcus aureus* sepsis

- through the inhibition of its agglutination in blood. *PLoS Pathog.* 7(10), e1002307 (2011).
- 147 Jebens EH, Monk-Jones ME. On the viscosity and pH of synovial fluid and the pH of blood. *J. Bone Joint Surg. Br.* 41-B(2), 388–400 (1959).
 - 148 Gibson DS, Rooney ME. The human synovial fluid proteome: a key factor in the pathology of joint disease. *Proteomics Clin. Appl.* 1(8), 889–899 (2007).
 - 149 Ropes MW, Bennett GA, Bauer W. The origin and nature of normal synovial fluid. *J. Clin. Invest.* 18(3), 351–372 (1939).
 - 150 Clarke B. Normal bone anatomy and physiology. *Clin. J. Am. Soc. Nephrol.* 3(Suppl. 3), S131–S139 (2008).
 - 151 Dastgheyb SS, Hammoud S, Ketonis C *et al.* Staphylococcal persistence due to biofilm formation in synovial fluid containing prophylactic cefazolin. *Antimicrob. Agents Chemother.* 59(4), 2122–2128 (2015).
 - 152 Romeo MJ, Espina V, Lowenthal M, Espina BH, Iii EFP, Liotta LA. CSF proteome: a protein repository for potential biomarker identification. *Expert Rev. Proteomics* 2(1), 57–70 (2015).