**Pathogenicity and virulence of Staphylococcus aureus**

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**ABSTRACT**

*Staphylococcus aureus* is one of the most frequent worldwide causes of morbidity and mortality due to an infectious agent. This pathogen can cause a wide variety of diseases, ranging from moderately severe skin infections to fatal pneumonia and sepsis. Treatment of *S. aureus* infections is complicated by antibiotic resistance and a working vaccine is not available. There has been ongoing and increasing interest in the extraordinarily high number of toxins and other virulence determinants that *S. aureus* produces and how they impact disease. In this review, we will give an overview of how *S. aureus* initiates and maintains infection and discuss the main determinants involved. A more in-depth understanding of the function and contribution of *S. aureus* virulence determinants to *S. aureus* infection will enable us to develop anti-virulence strategies to counteract the lack of an anti-*S. aureus* vaccine and the ever-increasing shortage of working antibiotics against this important pathogen.

**Introduction**

*Staphylococcus aureus* is one of the most infamous and widespread bacterial pathogens, causing a hard-to-estimate number of uncomplicated skin infections and probably hundreds of thousands to millions of more severe, invasive infections globally per year [1,2]. It is a leading causative agent in pneumonia and other respiratory tract infections, surgical site, prosthetic joint, and cardiovascular infections, as well as nosocomial bacteremia [3]. A review from 2012 estimated that *S. aureus* bacteremia has an incidence rate ranging from 20 to 50 cases/100,000 per year, and 10% to 30% of these patients will die from the infection [4]. In a more recent study from 2017, the annual number of deaths due to *S. aureus* bacteremia in the U.S. was reported to be 20,000 [5]. *S. aureus* bacteremia has been noted to account for a greater number of deaths than that caused by acquired immune deficiency syndrome (AIDS), tuberculosis, and viral hepatitis combined [1,4]. Other *S. aureus* infections, such as moderately severe skin infections, including furuncles, abscesses, and wound infections, are usually not life-threatening but may be accompanied by significant morbidity and pain. Due to their frequency (several millions annually in the U.S.), they represent a considerable public health burden [6]. Finally, *S. aureus* has also been associated with the development of atopic dermatitis [7].

*S. aureus* infections are particularly problematic due to frequently occurring antibiotic resistance in *S. aureus* isolates, among which methicillin-resistant *S. aureus* (MRSA) are the most important clinically [8]. Infections by MRSA are accompanied by increased mortality, morbidity, and hospital stay, as compared to those caused by methicillin-sensitive *S. aureus* (MSSA) [9]. The rates of methicillin resistance among clinical isolates varies greatly by country, ranging from single-digit rates in Scandinavian countries to over 50% for example in the U.S. and China [10]. While hospital-associated MRSA infections are on the decline in the U.S., Europe, China, and many other countries, likely due to increased hygiene and surveillance measures [5,11], they are still on the rise in poorly developed countries, for example in Africa [12]. In addition, even in the U.S., mortality caused by MRSA remains the highest for any antibiotic-resistant pathogen, reported by the CDC to be at ~20,000 in 2018 [5]. Furthermore, there is increased recognition of the considerable clinical importance of methicillin-sensitive *S. aureus* (MSSA) strains. Some MSSA lineages such as sequence type (ST) 398 can have high virulence, causing fatal infections [13,14]. MSSA infections are not monitored as closely as MRSA infections and recently implemented anti-MRSA measures did not cause a similar decrease in MSSA infections, as reported for example in the U.S. and U.K [15].
Resistance to other antibiotics is also widespread in *S. aureus*. For example, resistance to traditional beta-lactam antibiotics (penicillin and derivates) that are sensitive to beta-lactamase is virtually omnipresent in *S. aureus* [16]. Furthermore, *S. aureus* can show, often in combined form, resistance to almost all available antibiotics. Vancomycin remains the antibiotic of last resort for MRSA infections, with highly vancomycin-resistant strains (VRSA) having occurred but not spread, probably due to the strongly increased fitness cost that is imposed by vancomycin resistance genes [17]. However, there are strains that have acquired intermediate resistance to vancomycin (VISA) [18]. In addition to specific antibiotic resistance, nonspecific antibiotic resistance by biofilm formation plays a role in many *S. aureus* infections that are biofilm-associated [19]. These include prosthetic joint and all other indwelling medical-device-associated infections, endocarditis, osteomyelitis, conjunctivitis, and others. Finally, mastitis is a prominent *S. aureus*-mediated, biofilm-associated infection in cattle that represents a major problem for milk and meat industries [20].

In contrast to many other bacterial pathogens, which often rely on only one or a few toxins to promote disease, *S. aureus* produces an astounding array of virulence factors. These include a plethora of toxins and immune evasion factors, and a vast array of protein and non-protein factors that enable host colonization during infection. While there has always been great interest in *S. aureus* virulence, the development of livestock-associated MRSA (LA-MRSA) has garnered significant attention recently [21]. MRSA strains of livestock origin have been reported to cause a number of infections across the species, including respiratory, skin, and foodborne diseases [22]. LA-MRSA strains are highly virulent and have been associated with a range of human diseases [23,24].

In this review, we will give an overview of *S. aureus* virulence mechanisms. We will follow the definition describing virulence factors as those that promote establishment and maintenance of an infection by colonization and immune evasion mechanisms. Notably, we will not include mechanisms that contribute to asymptomatic colonization of *S. aureus* as a commensal, although – given that *S. aureus* infections commonly arise from this state – such mechanisms can be regarded as an important prerequisite for subsequent infection, and refer to dedicated reviews on the subject.

**Origins of infection**

*S. aureus* infections usually originate from asymptomatic colonization or, probably more rarely and particularly in the hospital setting, from infected fomites or transfer from other individuals [24,25]. Several studies have reported associations of colonization of different body sites with invasive infection [24,26,27]. The nares are traditionally regarded as the main *S. aureus* colonization site, but *S. aureus* can colonize many skin sites in addition to the intestine. Persistent colonization only occurs in a subset of the population, ranging from ~10–30%, dependent on the particular study. Colonization of different body sites is usually highly correlated. This correlation is believed to originate from frequent touching and nose picking and the resulting distribution [28,29]. *S. aureus* may also be acquired from animals, especially in the livestock industry, where the development of livestock-associated MRSA (LA-MRSA) has been of great concern. However, outside of that setting, LA-MRSA strains are not considered major contributors to human MRSA infections [30].

Systemic *S. aureus* infection is always dependent on bacterial breach through the epithelial protective layer. For example, skin infections can develop from minor scratches of the skin and may become invasive [31]. However, *S. aureus* can also actively promote epithelial breach, for which α-toxin has predominantly been made responsible by its activation of the metalloproteinase domain-containing protein 10 (ADAM10) to cleave E-cadherin molecules [32,33]. This mechanism breaks adherens junctions and compromises the actin cytoskeleton [34] (Figure 1).

The contamination of indwelling medical devices represents another route of infection that occurs frequently in the hospital setting. The main mechanism underlying this infection route is the capacity of *S. aureus* to adhere to the devices’ plastic material as well as to the matrix molecules that cover the devices soon after insertion, and to form a biofilm on the device [19]. Biofilm formation is also the suspected cause for menstrual staphylococcal toxic shock syndrome (TSS, in which specific *S. aureus* strains producing toxic shock syndrome toxin-1 (TSST-1) form biofilms on high-absorbency tampons [35].

Food poisoning is a special case of acute *S. aureus* infection in which contaminated foods containing staphylococcal enterotoxins (SEs) are ingested [36]. SEs cause emesis in a not completely understood manner that involves induction of histamine release from intestinal mast cells [37]. Similar to TSST-1, they are superantigenic toxins, which activate T-cells in a predominantly nonspecific manner, resulting in an excessive immune response that includes polyclonal T cell activation and massive cytokine release. Systemic infections originating from acute staphylococcal
food poisoning are very rare. Whether sustained intestinal colonization by *S. aureus* can lead to gastro-intestinal and even systemic disease is not known. Probably, the observed correlation between intestinal *S. aureus* colonization and other forms of *S. aureus* infection rather stems from the intestine representing a reservoir for the distribution of *S. aureus* to other epithelial colonization sites [26].

Finally, *S. aureus* also can make use in an opportunistic fashion of primary harm done by other pathogens or pre-disposing conditions. This occurs, for example, in lung infections that have been initiated by a viral infection such as the flu, in which *S. aureus* secondary infection is often the ultimate cause for death [38,39]. Furthermore, *S. aureus* has been shown to contribute to the development of atopic dermatitis via specific toxins, including δ-toxin or similar cytolytic peptides called phenol-soluble modulinps (PSMs) by activating mast cells [7,40,41]. Moreover, *S. aureus* can complicate skin infections caused by other pathogens. One such example is the exacerbation of Buruli ulcers even after the original pathogen (*Mycobacterium ulcerans* in this case) has been eradicated by antibiotic treatment [42].

**Establishment of infection**

After epithelial breach and systemic invasion (Figure 2), the success of a staphylococcal infection depends on the effective evasion of host defenses. *S. aureus* accomplishes this by leaving the bloodstream with its high concentration of cellular and humoral immune defense mechanisms to invade organs and tissues, where it forms encapsulated abscesses. In the case of skin or lung infections, abscesses can form directly after epithelial breach.

While still in the blood, *S. aureus* uses an astounding series of mechanisms to avoid elimination. These range from toxins that destroy phagocytes (leukocidins) and mechanisms that trigger phagocyte apoptosis to the inhibition of complement factors, in addition to agglutination and the formation of thrombi. We will discuss in detail the *S. aureus* factors underlying these immune evasion mechanisms below.

**Avoidance of killing by phagocytes**

Neutrophils are the most prominent leukocytes in the blood, representing ~60% of the leukocyte population. They play a major role in controlling *S. aureus* infection, as evidenced by the extreme susceptibility to *S. aureus* infection observed in patients with neutrophil defects [43]. In addition, recent research has attributed an important role to liver Kupffer cells in *S. aureus* infection, as pathogen elimination in the liver by those cells has been described as a key initial bottleneck for the development of subsequent *S. aureus* bacteremia and the establishment of infection in other organs [44,45]. Most mechanisms of *S. aureus* evasion of phagocyte killing have, however, been investigated in neutrophils. *S. aureus* avoids being eliminated by neutrophils on many levels that include 1) the inhibition of neutrophil extravasation from the bloodstream into the tissues, neutrophil activation, and chemotaxis, 2) inhibition of
phagocytosis by aggregation, protective surface structures, and biofilm formation, 3) inhibition of opsonization, 4) inhibition of neutrophil killing mechanisms, and 5) direct elimination of neutrophils by cytolytic toxins or triggering of apoptosis [46,47]. It has been argued that particularly the efficacy of the latter two mechanisms, which are independent of whether opsonization by antibodies and other opsonins has been successful, may underlie the difficulties of finding a working vaccine for S. aureus, which still remains elusive [48,49] (Figure 3).

**Inhibition of neutrophil extravasation, activation, and chemotaxis**

Unless neutrophils directly interact with pathogens in the bloodstream, they need to migrate from the blood to the site of infection, which is a complex process that involves neutrophil rolling and adhesion to the endothelium, penetration through the endothelium (diapedesis), as well as activation and migration to the pathogen along a gradient of chemoattractants in a process called chemotaxis [50]. Activation occurs via primary stimulation (“priming”) by specific cytokines such as IL-8, CSF, and IFN-γ or the C3a and C5a complement factors [51]. Chemoattractants can be host-derived, such as leukotriene B4 or IL-8, or produced by the bacteria. In Gram-positive bacteria, neutrophil activators/chemoattractants include the specific N-terminal lipoylated structure of lipoproteins [52], peptidoglycan [53], unmethylated CpG sequences in DNA [54], and formylated peptides, which derive from the N-formylation of methionine during protein synthesis [55]. Lipoteichoic acid is also pro-
inflammatory, but its role may have been considerably overestimated as a result of contamination with lipopeptides [56]. Importantly, all these structures are specific for bacteria and thus enable the immune system to recognize bacterial invaders. They have been termed “pathogen-associated molecular patterns” (PAMPs) and usually activate Toll-like receptors (TLRs) [57], which function as homo- or heterodimers. Diacylated lipoproteins activate TLR2/TLR6, while triacylated lipoproteins activate TLR2/TLR1 and CpG sequences TLR9. In *S. aureus*, the PSMs, which include the δ-toxin, form a large part of the secreted protein amount and, being secreted without a signal peptide, retain their pro-inflammatory N-formyl methionine part [58,59]. However, PSMs are also chemotactic for neutrophils without N-formylation and both the N-formylated and non-N-formylated forms predominantly activate the formyl peptide receptor 2 (FPR2) [60]. Classical PAMPs are not secreted and require direct surface interaction or shedding from the surface to act as activators or chemoattractants. Interestingly, PSMs facilitate shedding of lipoproteins, which are
possibly the most important *S. aureus* chemoattractants [56], thus having a double direct and indirect impact on neutrophil attraction and activation [61].

*S. aureus* inhibits neutrophil extravasation, activation, and chemotaxis using a plethora of factors and mechanisms. The staphyloccocal superantigen-like protein 5 (SSL5) and SelX, which bind to P-selectin glycoprotein ligand-1 (PSGL-1) on the leukocyte surface, inhibit extravasation via the inhibition of neutrophil adhesion via PSGL-1 to the P-selectin anchor on endothelial cells [62]. SelX has been found to significantly contribute to pathogenesis in a rabbit pneumonia model via neutrophil inhibition rather than its superantigenic activity [63]. Activation and chemotaxis are inhibited by many members of the SSL family including SSL3, SSL4, SSL5, and SSL10, which bind to G protein-coupled receptors (GPCRs) and TLRs. For example, SSL3 and SSL4 bind to TLR2 [64,65], SSL10 to CXCR4 [46], and SSL5 generally to GPCRs [67]. CHIPS (chemotaxis-inhibitory protein of *Staphylococcus*) binds and inhibits FPR1 and C5aR [68,69], while FLIPr and FLIPr-like inhibit FPR1 and FPR2 [70,71]. The secreted *S. aureus* staphopain protease and lipase also contribute to the inhibition of chemotaxis. Staphopain degrades CXCR2, thereby inhibiting neutrophil migration toward cytokines recognized by that receptor [72]. The Geh lipase has recently been discovered to degrade lipoproteins by removing the pro-inflammatory lipoylated N-terminus, preventing recognition of these PAMPs by neutrophils [73].

While the importance of pathogen recognition for example via TLR2 is reflected by the increased disease manifestations found in TLR2 knockout mice infected with *S. aureus* [74], the contribution to pathogenesis of the many neutrophil chemotaxis and activation-inhibitory factors is hard to monitor in mouse models due to their functional redundancy and specificity for humans. In many cases, their relative contribution to *S. aureus* virulence is therefore not known.

**Inhibition of phagocytosis by aggregation, protective surface structures, and biofilm formation**

Many invasive microorganisms produce capsular exopolysaccharides whose main function is to protect the pathogen from phagocytosis. Several clinical strains of *S. aureus* produce capsules, commonly of serotypes 5 or 8 with the structure \((-\rightarrow4)\)-3-O-Ac-β-\(-\rightarrow6\))-β-d-ManNAcA-(1→4)-α-L-FucNAc-(1→3)-β-d-FucNAc-(1→)_n \(\text{and}\ \((-\rightarrow3)\)-4-O-Ac-β-\(-\rightarrow6\)-β,d-ManNAcA-(1→3)-α-L-FucNAc-(1→3)-β-d-FucNAc-(1→)_m \) respectively [75,76]. However, the USA300 lineage, which has become the main source of hospital- and community-associated infections in the U.S [1,77], does not produce a capsule, indicating that other immune evasion mechanisms can substitute for the protective function of capsule [78]. This situation is particularly important for the design of anti-*S. aureus* vaccines, which in the past have frequently relied on capsular polysaccharides 5 and 8 as components [79]. Interestingly, capsule formation is only associated with increased virulence in some infection types, such as bacteremia, while it appears counterproductive in situations where it may shield necessary adhesins, such as endocarditis [80,81].

Polysaccharide intercellular adhesin (PIA, also called PNAG for poly-N-acetyl glucosamine according to its structure) is a cell surface-located homopolymeric exopolysaccharide of *S. aureus* and other staphylococci that is made of partially deacetylated GlcNAc units [82,83]. The positive charge that is introduced by deacetylation attracts the molecule to the negatively charged bacterial surface [84,85]. PIA/PNAG is a major component of the staphylococcal biofilm matrix [19]. With biofilm formation by itself representing an efficacious mechanism to inhibit phagocytosis, PIA/PNAG prevents phagocytosis by two mechanisms: (i) shielding the cellular surface from phagocyte attacks and (ii) contributing to the biofilm network [85,86]. Similar to capsule, PIA/PNAG is not produced in all *S. aureus* strains, emphasizing the multi-factorial character of phagocyte evasion mechanisms [87]. Of note, PIA/PNAG has also been investigated as a vaccine component [88].

While biofilm formation most frequently occurs on indwelling medical devices and refers to surface-associated bacterial agglomerations, *S. aureus* uses other aggregation mechanisms in the blood to escape ingestion by phagocytes. Namely, it produces thrombi by the combined, non-redundant activity of coagulase and von Willebrand factor-binding protein (vWbp), which bind prothrombin (factor II of the coagulation cascade), forming a complex called staphylothrombin [89,90]. Staphylothrombin can cleave fibrinogen to fibrin clots in the absence of the vascular damage signal that is normally necessary for this step. *S. aureus* then uses fibrinogen-binding proteins such as clumping factor A (ClfA) to adhere to the fibrin clots and form fibrin-containing bacterial aggregates [91]. The fibronectin-binding proteins FnBPA and FnBPP also activate aggregation of platelets [92].

**Inhibition of opsonization**

Efficient phagocytosis requires opsonization of the bacterial targets by antibodies (immunoglobulins, Igs) or complement. Igs bind to pathogens via their F\(_{\text{ab}}\)
segments and to phagocytes via their Fc regions. There are differences in opsonization efficacy depending on the Ig subclass, with IgM having the highest due to its polymeric structure. In addition to being opsonic, the presence of IgGs on the bacterial cell surface stimulates the classical pathway of complement fixation.

*S. aureus* produces three proteins that interfere with Ig deposition. The best-known is surface protein A (SpA), which produces a “camouflage coat” of non-specific IgGs on the *S. aureus* surface via nonspecific binding to the Fc regions of IgG [93]. It also binds to the Fcα region of IgM, serving as a B cell superantigen and causing B cell apoptosis. Furthermore, it skews the immune response away from other *S. aureus* virulence factors by triggering the production of plasma B cells that recognize almost exclusively protein A [94,95]. Sbi (*S. aureus* binder of IgG) binds exclusively to the Fc region of IgG, but also to the serum component apolipoprotein H, in addition to complement factors H and C3 [96,97]. SSL10, a protein with multiple further functions in immune evasion, also binds to the IgG Fc region, preventing receptor-mediated phagocytosis [98].

The primary role of the complement system during infection with Gram-positive bacteria such as *S. aureus* is the deposition of C3b on the bacterial surface for opsonization, which happens when C3 is cleaved via C3 convertases from one of three independent pathways (the lectin classical, and alternative pathways) [99]. Further complement factors, such as C5a that is formed upon C3-C3b interaction, act as chemoattractants for additional immune cells, a mechanism reported to also matter during *S. aureus* systemic infection [100].

*S. aureus* produces a plethora of proteins that inhibit the complement system. The most central and versatile one, staphylococcal complement inhibitor (SCIN), inhibits all three pathways by a multi-pronged approach that leads to the inhibition of C3 convertases, diminishing C3b deposition and C5a chemoattractant formation [101]. Many further *S. aureus* virulence factors with other previously identified roles in pathogenesis also inhibit complement. For example, the extracellular fibrinogen-binding protein Efb binds C3 via its C-terminus and fibrinogen via its N-terminus, thereby covering bacteria in a surface layer of fibrinogen that inhibits recognition of surface-bound C3b [102]. The homologous extracellular complement-binding protein EcB lacks fibrinogen-binding activity but inhibits the alternative pathway C3 convertase and all C5 convertases [103]. Several further *S. aureus* proteins inhibit specific pathways: the collagen adhesin (Cna) the classical pathway [104], SdrE the alternative pathway [105,106], and the extracellular adherence protein (Eap) the lectin and classical pathway [107]. Finally, SSL7 inhibits complement in two ways, via inhibition of IgA recognition by binding to the Fc region of IgA and by binding to C5 [108].

In addition to these very specific opsonization inhibition mechanisms, opsonization can be inhibited via the proteolytic activity of secreted *S. aureus* proteases. *S. aureus* produces a series of secreted proteases with relatively low substrate specificity, the most important of which are staphylococcal serine protease (V8 protease; SpA), cysteine protease (SspB), metalloprotease (aureolysin; Aur), and staphopain (Scp) [109]. While the main function of these proteases may consist in nutrient acquisition, it is likely that they also destroy many immune defense proteins. This has specifically been shown in the case of complement for aureolysin, which cleaves C3 [110]. Furthermore, the V8 protease has been shown to cleave IgGs [111]. However, whether this mechanism contributes to pathogenesis is largely speculative [112].

Of note, many complement inhibitory factors of *S. aureus* are human-specific; therefore, their contribution to virulence is hard to model in mouse infection models [46]. Furthermore, for many of the abovementioned factors – such as the proteases – the specific contribution to complement-related pathogenesis is hard to determine due to their multi-functional nature. However, where such a measurement is possible and has been performed, for example measuring in-vivo mortality and neutrophil influx using an efb/ecb double mutant, it showed a key role of complement inhibition in *S. aureus* virulence that adds to the evolution biology argument that many such mechanisms are conserved in clinical *S. aureus* isolates [113].

**Inhibition of neutrophil killing mechanisms**

Once neutrophils have managed to ingest *S. aureus* despite the many mechanisms *S. aureus* has to evade phagocytosis, *S. aureus* cells are attacked by the very efficient bactericidal activities that are present in the neutrophil phagosome. Myeloperoxidase (MPO), which is released from primary granules, produces reactive oxygen species (ROS). Primary granules also release antimicrobial peptides (AMPs) such as defensins. Secondary granules release further antimicrobial proteins, such as lysozyme. The bactericidal mechanisms of a neutrophil are commonly categorized as oxygen-dependent (myeloperoxidase, MPO) and -independent (antimicrobial peptides and proteins) [114]. *S. aureus* has developed many mechanisms to interfere with both.

The oxygen-dependent mechanisms consist of NADPH oxidase, which produces superoxide from
O2. Superoxide (O2−) is then spontaneously converted to hydrogen peroxide (H2O2). MPO produces hypo-chlorous acid (HOCI) from the reaction of H2O2 with chloride. HOCI is the major ROS effector molecule [115]. S. aureus has several mechanisms providing resistance to ROS and inhibiting ROS production enzymes. Staphyloxanthin is the orange pigment that has given S. aureus its name (“aureus,” golden). This molecule contains a series of conjugated double bonds that scavenge radicals originating from ROS activity [116,117]. Additionally, S. aureus produces superoxide dismutase, which converts superoxide to the less toxic H2O2 [118,119], as well as catalase (KatA) and alkyl hydroperoxide reductase C (AhpC), which further detoxify H2O2 by turning it into oxygen and water [119,120]. Furthermore, S. aureus produces a lactate dehydrogenase, which is inducible by nitric oxide (NO), another ROS, and contributes to maintaining redox homeostasis in the phagosome [121]. Also, S. aureus can inhibit the oxidative burst by converting ADP and AMP to adenosine [122] and resist the toxicity of copper, which is imported into macrophage phagosomes and contributes to ROS production, via a mobile genetic element-encoded copper hypotolerance system [123]. Lastly, staphylococcal peroxidase inhibitor (SPIN) directly inhibits MPO [124].

Defensins and other AMPs are commonly positively charged and many function as pore formers in the bacterial membrane. With AMPs being attracted to the bacterial surface by electrostatic interaction, staphylococcal resistance mechanisms consist in reducing the negative charge of the membrane and cell wall [125]. The dlt operon esterifies hydroxyl groups in teichoic acids with alanyl residues, introducing one positive charge per alanine into this cell surface polymer, increasing the net charge of the cell surface [126]. The MprF (Multiple peptide resistance factor) membrane enzyme is a lysyl-phosphatidylglycerol (Lys-PG) synthase and Lys-PG flippase that introduces Lys-PG in the outer layer of the cytoplasmic membrane, also reducing attraction of AMPs [127,128]. Both the mprF and dlt genes are regulated by a system termed antimicrobial peptide-sensing system (Aps)RSX, also called (Gra)RSX (for gramicidin resistance), that responds to binding of cationic AMPs [129]. ApsRS/GraRS form a two-component system. ApsX/GraX and the ApsRSX/GraRSX-controlled VraFEG transport system are also involved in signal transduction by that system in a way that is not completely understood [130–132]. AMPs are also subject to proteolytic degradation by secreted S. aureus proteases, a mechanism that is also induced by and affects the negatively charged AMP, dermcidin [133]. Among the many antibacterial proteins neutrophils secrete into the phagosome, lysozyme is knowingly very efficient against Gram-positive bacteria. However, its efficacy toward S. aureus is limited due to the enzymatic activity of an enzyme called OatA, which acetylates the muramic acid parts of peptidoglycan [134]. On the other hand, S. aureus subverts the activity of antimicrobial proteases secreted into the neutrophil phagosome via the extracellular adherence protein (Eap) [135].

Neutrophil extracellular traps (NETs), another, unconventional mechanism of pathogen killing that neutrophils use after pathogen-induced lysis (“NETosis”) [136], is also believed to contribute to host defense against S. aureus, although this is controversial [137]. The finding that secreted S. aureus nucleo-protects from NET-mediated killing in vitro and contributes to infection in vivo indicates that there may be a role for NETs in fighting S. aureus infections [138], although this is difficult to demonstrate directly. In addition, S. aureus produces an enzyme called AdsA that produces deoxyadenosine from nuclelease-digested NET DNA [139]. Deoxyadenosine triggers caspase-3-mediated death of other immune cells, which has been shown to remove macrophages from the centers of S. aureus abscesses [139].

**Toxin-driven elimination of neutrophils**

The mechanisms S. aureus uses to avoid killing by neutrophils that we have discussed so far are of what one could call “passive” in character. However, S. aureus also synthesizes a series of toxins that directly eliminate neutrophils and other leukocytes. These mainly consist of a-toxin (Hla) [140], the bicomponent leukocidins [141], and the PSMs [142].

Alpha-toxin is probably the most famous and also most important toxin of S. aureus in terms of contribution to pathogenesis (Figure 1). It has multiple functions; we have already discussed the role in epithelial breach in the previous section. Most notably, α-toxin is a major cytolsin for many cell types including leukocytes [140]. It is a 33 kDa protein in its mature form and oligomerizes into a heptameric structure that forms a stable membrane-spanning pore [143]. Pore formation is a receptor-dependent process that uses the ADAM10 protein as a receptor, explaining the differences in cytolytic capacity toward different cell types from different species that have been reported for α-toxin [32,140]. Alpha-toxin induces a series of inflammatory events in the target cell and induces the NLRP3 inflammasome, generally at lytic concentrations and likely forming part of the events leading to cell death by pyroptosis [140,144]; but some are also observed at
sublytic concentrations [145]. The contribution of α-toxin to *S. aureus* infection has been demonstrated using isogenic mutants in many animal infection models, including pneumonia [146,147], skin infection [148], and sepsis [149], to name but a few.

The bicomponent leukocidins include Panton-Valentine leucocidin (PVL, encoded by the *lukS-PV* and *lukF-PV* genes), LukDE, LukAB (LukGH), and the HlgAB and HlgCB combinations of the HlgA, HlgB, and HlgC proteins (also called γ-toxin) [141]. All these toxins require assembly of a *lukF* and a *lukS* moiety. The S subunit recognizes a membrane protein receptor [a chemokine receptor for PVL and LukED [150,151] and an integrin for LukAB/GH [152]] and then recruits the F subunit, leading first to dimerization and ultimately, after considerable structural changes that facilitate membrane insertion, to the formation of an octameric pore [153]. All strains of *S. aureus* are capable of producing at least three (γ-toxin and LukAB/GH) leukocidins [141]. Highly virulent strains produce five, with PVL – which is only produced by an overall 2–3% of *S. aureus* isolates – having been in the center of attention due to its association with CA-MRSA isolates [154]. PVL has a considerable impact on CA-MRSA lung infection in a rabbit model [155], while its contribution to skin infection, the main manifestation of CA-MRSA disease, has remained controversial [148,156]. Notably, analysis of most leukocidins, with the noticeable exception of LukDE, is not possible in mouse models due to the species specificity of the leukocidin receptors [141], unless humanized mice are used [157]. The different receptors also underlie cell specificity, a likely reason for the fact that *S. aureus* produces several different leukocidins. All bicomponent leukocidins are quite specific for leukocytes, while γ-toxin also efficiently lysed erythrocytes by a receptor-mediated mechanism [158]. Of note, LukAB/GH is less related in sequence to the other leukocidins and appears to have specific functions. For example, it has been reported to facilitate lysis of target cells after phagocytosis [159]. Finally, similar to α-toxin, leukocidins appear to have pro-inflammatory functions at sublytic concentrations, as shown particularly for PVL [160,161].

PMSs are a family of amphiphatic, α-helical peptides that include the early-described δ-toxin [142]. The α-type PMSs (~20–25 amino acids) have considerable cytolytic activity against neutrophils, which is most pronounced in the PSMα3 peptide [59]. The activity of PMSs is believed to be receptor-independent, leading to detergent-like membrane perturbation, and has been observed with many other cell types. At sublytic concentrations, PMSs have strong pro-inflammatory and chemotactic effects on neutrophils and keratinocytes [59,60,162]. PSMs have been reported to significantly contribute to blood [59] and lung infections [163], but there is epidemiological and experimental evidence indicating that their most pronounced relative contribution is to skin infections [59,164]. Of note, PSMs are – except possibly for LukAB/GH – the only leukocyte toxins that are believed to exert their main function after phagocytosis, and they have been shown to facilitate escape from the phagolysosome [165,166].

A more indirect way to eliminate neutrophils is to accelerate their natural self-destruction via apoptosis. Triggering of apoptotic events by *S. aureus* has been described for many toxins and cell types, such as by AdsA in macrophages [167], but specifically for neutrophils only in the case of PVL and the staphopain B protease [168,169].

**Tissue invasion**

In systemic murine infection, *S. aureus* cells arrive soon (after 1–3 h) in the organs, where microscopically discernable lesions become visible after ~48 h [170,171]. Invasion by *S. aureus* of organs and tissues from the bloodstream not only requires immune evasion, as discussed above, but also adhesion and further structural processes. Many of these steps are facilitated by surface-anchored proteins that belong to the MSCRAMM (microbial surface components recognizing adhesive matrix molecules) family [172]. These proteins are secreted by the general secretion pathway and then tethered to peptidoglycan via sortase A-catalyzed reaction that links the threonine of a conserved N-terminal structure (LPXTG motif) to the amino group of the terminal glycine residue of the pentaglycine branch in peptidoglycan [173]. MSCRAMMs characteristically contain repeat sequences that allow spanning through the cell wall and exposed matrix protein-binding domains. Among the many MSCRAMMs with divergent functions, of which we discussed some involved in immune evasion already, the collagen-binding protein Cna and the fibronectin-binding proteins FnBP A and FnBP B have key functions in tissue adherence [174–176]. Providing evidence for the function of an MSCRAMM in its natural strain background is difficult due to functional redundancy. Such evidence has therefore in several cases been produced using knock-in (often by heterologous expression in *Lactococcus lactis*) rather than knock-out approaches [172].
Maintenance of infection

Abscess formation

Once an abscess is established, bacterial proliferation ensues in addition to the infiltration of a large number of leukocytes. Interestingly, in this situation, the main chemottractants for leukocytes were shown to be lipopeptides, using a mutant in the lgt gene that codes for an essential lipoprotein synthesis enzyme. This mutant showed bacterial proliferation without typical abscess formation [177].

The altered scenario of high bacterial numbers surrounded by a high number of leukocytes requires considerable adaptation of bacterial physiology compared to the initiation of infection. First and foremost, mechanisms to evade killing by leukocytes, in addition to those discussed above, include the formation of an encapsulated abscess that inhibits additional infiltration of leukocytes [170]. One study used mutants in the cell wall-anchored proteins of strain Newman to monitor which factors promote which state of kidney abscess formation in a mouse systemic infection model. With the caveat that this strain does not produce or properly express some of the factors probably involved (such as Cna, FnBPA and FnBPB), it still gave key insight in abscess formation processes [171]. According to that study, iron (heme) acquisition facilitated by the IsdA and IsdB proteins is vital during the initial stage of abscess formation. The next stage of mature abscess formation is characterized by layers of necrotic and intact neutrophils surrounding the abscess center, which contains the proliferating S. aureus cells. In this stage, the coagulases Coa and vWbp produce fibrin clots to inhibit leukocyte infiltration. Another main contributor was protein A, although it is not known to which of protein A’s many functions the phenotype was due to [171]. Results from a more recent study indicate that the pro-inflammatory function of protein A is important for proper skin abscess formation and healing [178], which reflects, like many other studies, the importance of an adequate level of host response to S. aureus infection.

Furthermore, high bacterial density means that nutrients become scarce. S. aureus produces a series of cytolysins that can lyse cells and enzymes to digest the released nutrient macromolecules. In addition to the cytolytic properties of leukocidins toward leukocytes, we already mentioned the general cytolytic properties of PSMs, which may underlie the strong contribution those cytolysins have to the formation of subcutaneous, lung, and kidney abscesses [59,179,180]. Some S. aureus cytolysins synergize to achieve extremely strong hemolysis. This activity is known from the in-vitro effect known as CAMP reaction and occurs between β-toxin, a sphingomyelinase, and δ-toxin or other PSMs [181,182]. The main function of degradative exoenzymes, which include proteases, lipases, and nucleases, is assumed to be nutrient acquisition, but direct evidence is not available due to functional redundancy and the fact that many of these enzymes have additional functions, for example, in immune evasion.

Biofilms

Another important way in which S. aureus maintains an infection is by the formation of biofilms [19]. These can form on abiotic material of indwelling medical devices, but also on tissue surfaces, such as on heart valves in the case of endocarditis. Biofilm formation develops in three main stages: adhesion, maturation/proliferation, and detachment. Adhesion in vivo occurs to human matrix proteins via cell-wall anchored and other surface proteins, many of which belong to the MSCRAMM family [172]. In the second stage, a biofilm matrix is produced that connects cells and which in S. aureus consists of the PIA/PNAG exopolysaccharide [83], extracellular DNA [183], teichoic acids [184] and – often amyloid-forming – proteins such as SasG [185]. Biofilms have a distinct three-dimensional structure with channels that are formed by the surfactant activity of PSMs and degradative exoenzymes such as proteases [186–188]. Detachment of cells clusters from the biofilm occurs by extensive activity of these biofilm-structuring factors.

The main role of biofilm formation during infection is to protect the bacteria from phagocyte attacks [189]. Some have argued that biofilm formation and abscesses have similar characteristics. However, there are certainly key differences. For example, biofilms are not usually surrounded by large layers of neutrophils as abscesses are, which is likely due to the fact that in biofilms, S. aureus lives in a comparatively less aggressive state [190] and does not produce or shed through the biofilm matrix a large amount of chemoattractant molecules. In addition, S. aureus biofilms have been shown to skew the host immune response toward an anti-inflammatory state [189,191].

Internalization, persistence, and distribution of infection

A further way to hide from attacks of the immune system that S. aureus employs is to shelter within host cells. This has been shown for phagocytes such as neutrophils and monocytes [192–194] as well as a series of non-phagocytic cells, including epithelial and endothelial cells, keratinocytes, and osteoblasts [195]. S. aureus that persists in
neutrophils is infectious and this mechanism is believed to contribute to the spread of *S. aureus* during an infection [193]. Most likely, its main role is in persistence during passage through the bloodstream. Invasion of non-phagocytic cells contributes to chronicity of infection [195] and is mediated in part by FnBPs [196], but also other factors such as Eap [197]. FnBPs bind fibronectin on the cell surface via a tandem-β-zipper mechanism [198]. After internalization by phagocytosis or via FnBPs, *S. aureus* escapes from the phagosome, involving the above-mentioned activity of PSMs [199]. The events that lead to intracellular persistence versus target cell lysis are incompletely understood. Formation of so-called small-colony variants (SCVs), which are cells with reduced metabolism that do not express cytolysins, are likely involved [200]. It is interesting that SCVs express high amounts of FnBPs, facilitating invasion of neighboring cells upon cell lysis [201].

While persistence in phagocytes may have a role in spreading through the bloodstream, escape from an established infection site, such as an abscess or a biofilm, requires specific further mechanisms. Factors that rupture an abscess and lead to local dissemination include staphylokinase, an enzyme that forms a complex with plasmin, catalyzing further plasminogen activation, which ultimately leads to increased proteolysis and fibrinolysis [202]. Of note, the activity of staphylokinase is specific for human plasminogen. Systemic spread from an established in-vivo biofilm infection is mediated by PSMs, which facilitate biofilm detachment [186]. It may hypothetically also occur via biofilm-degrading enzymes [188]; yet this has not been established in vivo.

While initiation and maintenance of infection during the first days is largely due to efficient evasion of innate immune defenses, the interaction of *S. aureus* with the acquired immune defense becomes defining once this arm of the immune system is activated, after about one to two weeks post-infection. We already mentioned the crucial importance of protein A in that aspect and the many ways by which *S. aureus* interferes with opsonization. Another way by which *S. aureus* interferes with the adaptive immune system is by subverting T-cell responses, particularly of IL-17-producing T-cells, which many lines of evidence suggest have a key role in host defense against *S. aureus* [203,204]. *S. aureus* may induce a status of adaptive tolerance in T-cells (in-vivo energy), for example, via enterotoxin B [205], and superantigens, in general, may reduce protective T-cell responses [206]. Furthermore, the inhibition of T-cell responses that is observed during murine *S. aureus* infection [207] occurs via myeloid-derived suppressor cells (MDSCs), and to a minor extent regulatory T-cells (Tregs) [208], but it is not known how *S. aureus* induces this mechanism.

### Genetics of virulence

Virulence factors of *S. aureus* are often encoded on the pathogen’s accessory genome that differs from the core genome, which predominantly encodes “housekeeping” functions. The accessory genome contains mobile genetic elements (MGEs) like plasmids, transposons, insertion sequences, prophages, and pathogenicity islands, which in addition to virulence factors also contain antibiotic resistance determinants [209,210]. The large family of staphylococcal pathogenicity islands (SaPIs), while not containing transfer machinery like plasmids or phages, rely on helper phages for transduction [211]. The accessory genome also contains genomic islands (νSAα, νSAβ, νSAγ), which encode a series of virulence factors and appear to have originated from MGEs, but lost the ability to be transferred other than by non-MGE-specific modes of transfer. They are thus quite stable and so widespread that their contents can be considered characteristic for the entire species, although specific subtypes are associated with different lineages [212]. This contrasts the isolate-specific MGEs, which are often linked to specific diseases (“toxinooses”) due to the encoding of the respective responsible toxins, such as TSST-1 or the food poisoning enterotoxins [210,211].

Plasmids and transposons typically contain antibiotic resistance genes, while phage-related and pathogenicity islands contain most *S. aureus* toxins and other virulence determinants [210]. Important *S. aureus* toxins encoded on prophages include PVL, the immune evasion proteins CHIPS and SCIN, the exfoliative toxins A and B, as well as staphylokinase and a series of enterotoxins. Of note, the gene encoding β-toxin (β-hemolysin), *hlb*, which has been associated with virulence functions [213], is rendered nonfunctional in many *S. aureus* strains by insertion of the phage that carries CHIPS, SCIN, and staphylokinase [214], a process generally called “negative conversion.” There is evidence that *hlb* may be “repaired” by phage excision and is important for infectious colonization [215]. SaPIs are mostly known for enterotoxins and TSST. Toxins that are encoded on genomic islands and usually only vary in expression between different isolates include α-toxin, PSM peptides, SSLs, the lipoprotein-like toxins (LPLs), the leukocidin LukDE, and some enterotoxins [210,216,217]. Interestingly, the genomic island νSAβ also contains what appears to be an intact biosynthesis cluster for the production of a lantibiotic, but its expression and potential role in bacterial interference has never been directly shown.
Virulence regulation in S. aureus is highly complex and mediated by a large amount of regulatory systems. Only the most important are depicted here. Many virulence factors are impacted by the alternative sigma factor SigB, the Sar family of DNA-binding proteins (of which SarA is shown), the Agr quorum-sensing system, and further regulators such as SaeRS. Except for Agr, the exact triggering mechanisms of these systems remain largely elusive. Oxygen is sensed by SrrAB, which has an important role in ROS resistance, and Fur is a DNA-binding repressor that regulates iron uptake, but also virulence genes.

Regulation of virulence

The expression of S. aureus virulence determinants is subject to a wide variety of regulatory influences (Figure 4). These include regulation by locus-specific regulatory factors, such as the icaR gene adjacent to the ica operon [219], which is itself subject to many regulatory impacts [220], or the PSM-sensing PmtR protein controlling the PSM exporter Pmt operon [58,221], as well as global regulators that regulate a series of virulence genes and which are often driven by specific environmental conditions. Here, we will focus on some selected, key global regulators.

The most extensively studied staphylococcal virulence regulator is Agr (accessory gene regulator), which is a quorum-sensing system that up-regulates many toxins and virulence determinants when cell density reaches a certain threshold [222,223]. This regulation is believed to link the expression of virulence determinants to the state of infection when they are needed for nutrient acquisition and immune evasion, while delaying their expression during early infection to prevent the triggering of immune responses. Furthermore, within a phagosome, Agr-controlled virulence factors are expressed presumably because the confined environment activates the quorum-sensing system by a mechanism called “diffusion sensing” [224]. Historically, Agr has been described to generally down-regulate adhesion factors, whose main function was supposed to be during early infection, but this notion has mostly been reversed due to the recognition of many additional functions of MSCRAMMs and the finding that Agr does not regulate most MSCRAMMs in such fashion in clinical strains [225]. Most stringently controlled by Agr are secreted proteases and the PSMs. PSMs are directly controlled by the response regulator of the Agr two-component system, AgrA, while other Agr targets are regulated in an indirect fashion via the regulatory RNAIII, which forms part of Agr and represents its main intracellular effector molecule [226,227]. RNAIII-dependent gene regulation occurs in most cases via inhibition of a DNA-binding repressor protein called Rot [228]. The extracellular quorum-sensing signal of the Agr system is a post-translationally modified short thiolactone-containing autoinducing peptide (AIP) [229]. S. aureus has at least four Agr subgroups that differ in the sequence of the AIP, its modifying enzyme AgrB and the membrane histidine kinase AgrC, to which it binds and which upon activation triggers phosphorylation of AgrA [230]. Most AIPs of non-self, including those from other staphylococcal species, are inhibitory, which may play a role in interbacterial interaction in vivo.
As expected from the regulation of many toxins and other virulence factors, agr mutants show significant defects in many animal infection models, such as in infective endocarditis [233], skin and soft tissue infections [225,234], pneumonia [235,236], septic arthritis [237], osteomyelitis [238], and atopic dermatitis [239].

Mutants in agr arise frequently in a process known as “quorum cheating,” which describes the situation that specific members of a population mutate agr to save energy and benefit from the maintained Agr function of other cells in the population [240–242]. Populations only or predominantly consisting of Agr-dysfunctional cells have benefits in biofilm-associated infections due to the enlarged biofilms that agr mutants form and the concomitant increased resistance toward neutrophil attacks [242]. This is believed to explain the increased frequency of agr mutants isolated from chronic infection and bacteremia [243], which commonly originate from biofilm-associated infection of indwelling medical devices. Of special note, spontaneous mutation in agr occurs frequently in the laboratory, probably due to the absence of selective pressure for virulence factor expression, which – together with a lack of thorough genetic analysis – can lead to erroneous attribution of regulatory functions to other proteins and systems [244–246].

Further important global regulators for which the inducing environmental cues are known include Fur, which responds to low iron availability [247], and SrrAB, which is an oxygen-responsive regulator [248]. Fur not only regulates iron uptake, but also a series of virulence factors such as toxins and immune evasion proteins [249–251]. The role of Fur is believed to consist in coordinating the pathogen’s attack on the host, with iron restriction signaling entry into the body and consequent need for those factors. Accordingly, fur mutants show significant reduction in virulence in animal infection models [250,251]. SrrAB is an oxygen-sensitive two-component system that relies on redox-sensitive cysteines [252] to promote resistance to oxidative stress [253]. Under anaerobic conditions, it down-regulates agr while up-regulating ica expression, with the consequence of increased resistance to neutrophil attacks [254].

The Sar family comprises several short proteins (~120 amino acids) with helix-turn-helix DNA-binding sequences [255]. They are all homologous to the SarA prototype and believed to form one or two-domain dimeric winged helix structures [256]. Sar family proteins have multiple virulence factor targets and interact with a multitude of other regulatory systems [255]. Often the impact of one Sar homolog on a given virulence factor gene can be opposite to that of another. SarA is mostly known for its strong impact on protease expression, which is achieved in part but not entirely through regulation of Agr [257]. Although it is believed that the reason for the existence of multiple Sar homologs is to fine-tune virulence factor expression according to different environmental conditions, similar to many other regulators with pronounced and divergent impact on the expression of S. aureus virulence determinants, such as SaRS [258] and ArfRS [259], the molecular or environmental triggers of many Sar family regulators are not known [255]. A noticeable exception is MgrA, which has multifold effects on virulence [260–263], contains redox-sensitive cysteines and thus reacts to oxygen and reactive oxygen species [264]. MgrA is similar to SarZ, which also works as an oxidation sensor using thiol-based oxidation sensing [265].

Similar to many other bacteria, S. aureus has an alternative sigma factor called SigB, whose gene is embedded in a locus also harboring a series of anti-sigma factor genes, which together are believed to react to a multitude of environmental conditions such as growth phase and heat shock [266]. SigB interacts with many other regulators, such as Agr and SarA, and has a profound impact on virulence gene expression [267,268]. It is believed to adapt S. aureus physiology to chronic infection [269].

**Anti-virulence therapeutic strategies**

Driven by the concerning global spread of antibiotic resistance, and in the case of S. aureus the additional, ongoing difficulties of finding a working vaccine, there is increased recent interest in the development of alternative treatment strategies for bacterial infections. Anti-virulence strategies, which represent the translational arm of bacterial pathogenesis research, are often claimed to have a smaller risk for the development of resistance, but in S. aureus face the considerable problem of multiple and often functionally redundant virulence factors [270]. Therefore, anti-virulence approaches in S. aureus either target a virulence determinant with established widespread and extraordinarily important impact on pathogenicity, or aim to eliminate several virulence factors at a time. Three main S. aureus anti-virulence approaches that are currently being investigated follow one or both of these strategies. There is first the development of monoclonal antibodies against α-toxin, which in many isolates is a predominant virulence determinant, such as MED14893 (suvratoxumab) developed by MedImmune [271,272]. Second, several approaches
aim to target all *S. aureus* leukocidins, often in addition to the somewhat similar α-toxin. This approach has been taken for example by the Austrian company ArsaniS, who developed a mAb with cross-reactivity against all leukocidins and α-toxin [273,274]; however, clinical trials have failed. Third, quorum-sensing blockers targeting Agr have been promoted by many researchers [275]. They mainly comprise two classes: those that interfere with AIP binding to AgrC from the extracellular space, such as AIP analogs [276] and several natural compounds including fengycins [277], and those that need to penetrate into the cytoplasm to inhibit the response regulator AgrA, and are commonly more hydrophobic, such as savirin or apicidin [278,279]. Notably, Agr inhibitors have not yet been analyzed using rigorous testing with systemic application in models of the types of systemic disease for which anti-virulence drugs would be most desirable. Furthermore, they would likely have to be limited to acute types of infection, while for chronic and especially biofilm infection they may be counterproductive [275]. Moreover, as for all anti-virulence compounds, it needs to be established that their in-vivo efficacy is in fact due to their anti-virulence rather than bactericidal effects, which many of especially the more hydrophobic compounds show at only slightly higher concentrations.

**Outlook**

There has been a considerable recent increase in our knowledge about *S. aureus* pathogenesis and *S. aureus* virulence determinants. In addition to many open mechanistic questions, main current challenges are (i) how to use the gained understanding for the development of anti-virulence drugs, and (ii) how to integrate those findings into those from the newly developing field of bacterial interactions in the human microbiome. A particular technical problem arises from the host specificity of several *S. aureus* virulence factors, such as the bicomponent leukocidins, calling for the increased use of humanized mice and/or inclusion of other species in *S. aureus* virulence research.

As for the translational use of virulence findings, it will be crucial to rank the importance of virulence factors depending on strain and disease type in order to select those against which a multi-pronged therapeutic should be developed. Most previous virulence studies focus on one factor, for which a significant role in a given animal infection model is presented; but only very few studies have tried to directly compare different factors using experimental approaches [148,280], especially for the purpose of drug development [281]. Such endeavors have already been taken comparing the impact of different cytolysins on the virulence of important CA-MRSA lineages [179,280], but they need to be considerably expanded in order to base drug development strategies, such as for the development of cross-reactive mAbs or mAb cocktails, on research findings rather than educated guesses. Genetic tools to produce deletion mutants in the virulence genes of interest in clinical strains have been developed [282,283] and this should therefore not represent a bottleneck for such research anymore. Except for mAbs against leukocidins and α-toxin, all anti-virulence approaches are still at the investigational state. Quorum-sensing blockers and other investigational anti-virulence compounds need to undergo more rigorous pre-clinical testing using the established standards for drug development.

*S. aureus* pathogenesis research is also expected to receive substantial stimulation in the years to come from the field of microbial interactions, which has experienced a recent revival due to the ability of in-depth microbiome analyses. Already, interactions have been identified that alter *S. aureus* virulence factor expression, particularly via inhibition of the Agr system, by co-colonizers [232,277]. These findings may lead to the discovery of new drugs or even probiotic approaches for anti-virulence strategies against *S. aureus*.

**Acknowledgments**

This work was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases (NIAID), U.S. National Institutes of Health (NIH).

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases [1 ZIA AI000904].

**References**

[1] Kleven RM, Morrison MA, Nadle J, et al. Active Bacterial Core surveillance MI. Invasive Methicillin-resistant Staphylococcus Aureus Infections in the United States JAMA. 2007;298(15):1763–1771.
[2] Rasigade JP, Dumitrescu O, Lina G. New epidemiology of *Staphylococcus aureus* infections. Clin Microbiol Infect. 2014;20(7):587–588.
[3] Tong SY, Davis JS, Eichenberger E, et al. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3):603–661.
[4] van Hal SJ, Jensen SO, Vaska VL, et al. Predictors of mortality in *Staphylococcus aureus* Bacteremia. Clin Microbiol Rev. 2012;25(2):362–386.

[5] Kourtis AP, Hatfield K, Baggs J, et al. Emerging Infections Program Mag. Ham D, Capers C, Ewing H, Coffin N, McDonald LC, Jernigan J, Cardo D. Vital Signs: Epidemiology and Recent Trends in Methicillin-Resistant and in Methicillin-Susceptible Staphylococcus Aureus Bloodstream Infections - United States MMWR Morb Mortal Wkly Rep. 2019;68(9):214–219.

[6] McCaig LF, McDonald LC, Mandal S, et al. *Staphylococcus aureus*-associated skin and soft tissue infections in ambulatory care. Emerg Infect Dis. 2006;12(11):1715–1723.

[7] Nakamura Y, Oscherwitz J, Cease KB, et al. *Staphylococcus* delta-toxin induces allergic skin disease by activating mast cells. Nature. 2013;503(7476):397–401.

[8] Turner NA, Sharma-Kuinkel BK, Maskarinec SA, et al. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. Nat Rev Microbiol. 2019;17(4):203–218.

[9] Ippolito G, Leone S, Lauria FN, et al. Methicillin-resistant *Staphylococcus aureus*: the superbug. Int J Infect Dis. 2010;14(Suppl 4):S7–11.

[10] Stefani S, Chung DR, Lindsay JA, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents. 2012;39(4):273–282.

[11] Hu FP, Guo Y, Zhu DM, et al. Resistance trends among clinical isolates in China reported from CHINET surveillance of bacterial resistance, 2005–2014. Clin Microbiol Infect. 2016;22(Suppl 1):S9–14.

[12] Falagas ME, Karageorgopoulos DE, Leptidis J, et al. MRSA in Africa: filling the global map of antimicrobial resistance. PLoS One. 2013;8(7):e68024.

[13] Bonesso MF, Yeh AJ, Villaruz AE, et al. Key Role of alpha-Toxin in Fatal Pneumonia Caused by *Staphylococcus aureus* Sequence Type 398. Am J Respir Crit Care Med. 2016;193(2):217–220.

[14] Bouiller K, Bertrand X, Hocquet D, et al. Human Infection of Methicillin-Susceptible *Staphylococcus aureus* CC398: a Review. Microorganisms. 2020;8:11.

[15] Kavanagh KT. Control of MSSA and MRSA in the United States: protocols, policies, risk adjustment and excuses. Antimicrob Resist Infect Control. 2019;8:103.

[16] Lowy FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. J Clin Invest. 2003;111(9):1265–1273.

[17] McGuinness WA, Malachowa N, DeLeo FR. Vancomycin Resistance in *Staphylococcus aureus*. Yale J Biol Med. 2017;90(2):269–281.

[18] Hiramatsu K, Kayayama Y, Matsuo M, et al. Vancomycin-intermediate resistance in *Staphylococcus aureus*. J Glob Antimicrob Resist. 2014;2(4):213–224.

[19] Staphyloccocal Biofilms. Microbiol Spectr. 2018;6(4).

[20] Fluit AC. Livestock-associated *Staphylococcus aureus*. Clin Microbiol Infect. 2012;18(8):735–744.

[21] DeLeo FR, Otto M, Kreiswirth BN, et al. Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet. 2010;375(9725):1557–1568.

[22] Rozgonyi F, Kocsis E, Kristof K, et al. MRSA more virulent than MSSA? Clin Microbiol Infect. 2007;13(9):843–845.

[23] Otto M. Basis of virulence in community-associated methicillin-resistant *Staphylococcus aureus*. Annu Rev Microbiol. 2010;64:143–162.

[24] von Eiff C, Becker K, Machka K, et al. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group N Engl J Med. 2001;344(1):11–16.

[25] Desai R, Pannaraj PS, Agopian J, et al. Survival and transmission of community-associated meticillin-resistant *Staphylococcus aureus* from fomites. Am J Infect Control. 2011;39(3):219–225.

[26] Senn L, Clerc O, Zanetti G, et al. The Stealthy Superbug: the Role of Asymptomatic Enteric Carriage in Maintaining a Long-Term Hospital Outbreak of ST22 Methicillin-Resistant *Staphylococcus aureus*. mBio. 2016;7(1):e02039–15.

[27] Davis KA, Stewart JJ, Crouch HK, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) nares colonization at hospital admission and its effect on subsequent MRSA infection. Clin Infect Dis. 2004;39(6):776–782.

[28] Acton DS, Plat-Sinnige MJ, van Wamel W, et al. Intestinal carriage of *Staphylococcus aureus*: how does its frequency compare with that of nasal carriage and what is its clinical impact? Eur J Clin Microbiol Infect Dis. 2009;28(2):115–127.

[29] Wertheim HF, Melles DC, Vos MC, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis. 2005;5(12):751–762.

[30] Cuny C, Wieler LH, Livestock-Associated WW MRSA: the Impact on Humans. Antibiotics (Basel). 2015;4(4):521–543.

[31] Stryjewski ME, Chambers HF. Skin and soft-tissue infections caused by community-acquired methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis. 2008;46(Suppl 5):S568–77.

[32] Inoshima I, Inoshima N, Wilke GA, et al. A *Staphylococcus aureus* pore-forming toxin subverts the activity of ADAM10 to cause lethal infection in mice. Nat Med. 2011;17(10):1310–1314.

[33] Inoshima N, Wang Y, Bubeck Wardenburg J. Genetic requirement for ADAM10 in severe *Staphylococcus aureus* skin infection. J Invest Dermatol. 2012;132(5):1513–1516.

[34] Popov LM, Marceau CD, Starkl PM, et al. The adherens junctions control susceptibility to *Staphylococcus aureus* alpha-toxin. Proc Natl Acad Sci U S A. 2015;112(46):14337–14342.

[35] Schlievert PM, Davis CC. Device-Associated Menstrual Toxic Shock Syndrome. Clin Microbiol Rev. 2020;33:3.

[36] Fisher EL, Otto M, Cheung GYC. Basis of Virulence in Enterotoxin-Mediated Staphylococcal Food Poisoning. Front Microbiol. 2018;9:436.

[37] Ono HK, Hirose S, Narita K, et al. Histamine release from intestinal mast cells induced by staphylococcal enterotoxin A (SEA) evokes vomiting reflex in common marmoset. PLoS Pathog. 2019;15(5):e1007803.

[38] McCullers JA. The co-pathogenesis of influenza viruses with bacteria in the lung. Nat Rev Microbiol. 2014;12(4):252–262.
[39] Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. J Infect Dis. 2008;198(7):962–970.

[40] Liu H, Archer NK, Dillen CA, et al. *Staphylococcus aureus* Epicutaneous Exposure Drives Skin Inflammation via IL-36-Mediated T Cell Responses. Cell Host Microbe. 2017;22(5):653–66.

[41] Hodille E, Cuerq C, Badiou C, et al. Delta Hemolysin and Phenol-Soluble Modulins, but Not Alpha Hemolysin or Panton-Valentine Leukocidin, Induce Mast Cell Activation. Front Cell Infect Microbiol. 2016;6:180.

[42] Yeboah-Manu D, Kpeli GS, Ruf MT, et al. Secondary bacterial infections of burnul ulcer lesions before and after chemotherapy with streptomycin and rifampicin. PLoS Negl Trop Dis. 2013;7(5):e2191.

[43] Lekstrom-Himes JA, Gallin JJ. Immunodeficiency diseases caused by defects in phagocytes. N Engl J Med. 2000;343(23):1703–1714.

[44] Pollitt EJG, Szkuta PT, Burns N, et al. *Staphylococcus aureus* infection dynamics. PLoS Pathog. 2018;14(6):e1007112.

[45] Surewaard BG, Deniset JF, Zemp FJ, et al. Identification and treatment of the *Staphylococcus aureus* reservoir in vivo. J Exp Med. 2016;213(7):1141–1151.

[46] NWM DJ, KPM VK, van Strijp JAG. Immune Evasion by *Staphylococcus aureus*. Microbiol Spectr. 2019;7:2.

[47] Rigby KM, DeLeo FR. Neutrophils in innate host defense against *Staphylococcus aureus* infections. Semin Immunopathol. 2012;34(2):237–259.

[48] DeLeo FR, Otto M. An antidote for *Staphylococcus aureus* pneumonia? J Exp Med. 2008;205(2):271–274.

[49] Otto M. Novel targeted immunotherapy approaches for staphylococcal infection. Expert Opin Biol Ther. 2010;10(7):1049–1059.

[50] Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol. 2013;13(3):159–175.

[51] El-Benna J, Hurtado-Nedelec M, Marzaoli V, et al. Priming of the neutrophil respiratory burst: role in host defense and inflammation. Immunol Rev. 2016;273(1):180–193.

[52] Nguyen MT, Gotz F. Lipoproteins of Gram-Positive Bacteria: key Players in the Immune Response and Virulence. Microbiol Mol Biol Rev. 2016;80(3):891–903.

[53] Dziarski R, Gupta D. Peptidoglycan recognition in innate immunity. J Endotoxin Res. 2005;11(5):304–310.

[54] Hemmi H, Takeuchi O, Kawai T, et al. Toll-like receptor recognizes bacterial DNA. Nature. 2000;408(6813):740–745.

[55] Krepel SA, Wang JM. Chemotactic Ligands that Activate G-Protein-Coupled Formylpeptide Receptors. Int J Mol Sci. 2019;20:14.

[56] Hashimoto M, Tawaratsumida K, Kariya H, et al. Not lipoteichoic acid but lipoproteins appear to be the dominant immunobiologically active compounds in *Staphylococcus aureus*. J Immunol. 2006;177(5):3162–3169.

[57] Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. Int Rev Immunol. 2011;30(1):16–34.

[58] Chatterjee SS, Joo HS, Duong AC, et al. *Staphylococcus aureus* toxin export system. Nat Med. 2013;19(3):364–367.

[59] Wang R, Braughton KR, Kretschmer D, et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat Med. 2007;13(12):1510–1514.

[60] Kretschmer D, Gleske AK, Rautenberg M, et al. Human formyl peptide receptor 2 senses highly pathogenic *Staphylococcus aureus*. Cell Host Microbe. 2010;7(6):463–473.

[61] Hanzelmann D, Joo HS, Franz-Wachtel M, et al. Toll-like receptor 2 activation depends on lipopeptide shedding by bacterial surfactants. Nat Commun. 2016;7:12304.

[62] Bestebroer J, Poppelier MJ, Ulfman LH, et al. Staphylococcal superantigen-like 5 binds PSGL-1 and inhibits P-selectin-mediated neutrophil rolling. Blood. 2007;109(7):2936–2943.

[63] Tuffi SW, James DBA, Bestebroer J, et al. The *Staphylococcus aureus* superantigen SEIX is a bifunctional toxin that inhibits neutrophil function. PLoS Pathog. 2017;13(9):e1006461.

[64] Bardolow BW, Vos R, Bouman T, et al. Evasion of Toll-like receptor 2 activation by staphylococcal superantigen-like protein 3. J Mol Med (Berl). 2012;90(10):1109–1120.

[65] Yokoyama R, Itoh S, Kamoshida G, et al. Staphylococcal superantigen-like protein 3 binds to the Toll-like receptor 2 extracellular domain and inhibits cytokine production induced by *Staphylococcus aureus*, cell wall component, or lipopeptides in murine macrophages. Infect Immun. 2012;80(8):2816–2825.

[66] Walenkamp AM, Boer IG, Bestebroer J, et al. Staphylococcal superantigen-like 10 inhibits CXCL12-induced human tumor cell migration. Neoplasia. 2009;11(4):333–344.

[67] Bestebroer J, van Kessel KP, Azouagh H, et al. Staphylococcal SSL5 inhibits leukocyte activation by chemokines and anaphylatoxins. Blood. 2009;113(2):328–337.

[68] de Haas CJ, Veldkamp KE, Peschel A, et al. Chemotaxis inhibitory protein of *Staphylococcus aureus*, a bacterial antiinflammatory agent. J Exp Med. 2004;199(5):687–695.

[69] Postma B, Poppelier MJ, van Galen JC, et al. Chemotaxis inhibitory protein of *Staphylococcus aureus* binds specifically to the C5a and formylated peptide receptor. J Immunol. 2004;172(11):6994–7001.

[70] Prat C, Bestebroer J, de Haas CJ, et al. A new staphylococcal anti-inflammatory protein that antagonizes the formyl peptide receptor-like 1. J Immunol. 2006;177(11):8017–8026.

[71] Prat C, Haas PJ, Bestebroer J, et al. A homolog of formyl peptide receptor-like 1 (FPRL1) inhibitor from *Staphylococcus aureus* (FPRL1 inhibitory protein) that inhibits FPRL1 and FPR. J Immunol. 2009;183(10):6569–6578.
[72] Laaman AJ, Mijnheer G, Mootz JM, et al. Staphylococcus aureus Staphopain A inhibits CXCR2-dependent neutrophil activation and chemotaxis. Embo J. 2012;31(17):3607–3619.

[73] Chen X, Alfonzo F 3rd. Bacterial lipolysis of immune-activating ligands promotes evasion of innate defenses. Proc Natl Acad Sci U S A. 2019;116(9):3764–3773.

[74] Takeuchi O, Hoshino K, Akira S. Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to Staphylococcus aureus infection. J Immunol. 2000;165(10):5392–5396.

[75] Fournier JM, Hannon K, Moreau M, et al. Isolation of type 5 capsular polysaccharide from Staphylococcus aureus. Ann Inst Pasteur Microbiol. 1987;138(5):561–567.

[76] Fournier JM, Vann WF, Karakawa WW. Purification and characterization of Staphylococcus aureus type 8 capsular polysaccharide. Infect Immun. 1984;45(1):87–93.

[77] Diekema DJ, Richter SS, Heilmann KP, et al. Continued emergence of USA300 methicillin-resistant Staphylococcus aureus in the United States: results from a nationwide surveillance study. Infect Control Hosp Epidemiol. 2014;35(3):285–292.

[78] 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 cap locus. mBio. 2015;6(2).

[79] Schafer AC, Lee JC. Staphylococcal vaccines and immunotherapies. Infect Dis Clin North Am. 2009;23(1):153–171.

[80] Thakker M, Park JS, Carey V, et al. Staphylococcus aureus serotype 5 capsular polysaccharide is antiphagocytic and enhances bacterial virulence in a murine bacteremia model. Infect Immun. 1998;66(11):5183–5189.

[81] Nemeth J, Lee JC. Antibodies to capsular polysaccharides are not protective against experimental Staphylococcus aureus endocarditis. Infect Immun. 1995;63(2):375–380.

[82] Mack D, Fischer W, Krookotsch 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. 1996;178(1):175–183.

[83] Cranton SE, Gerke C, Schnell NF, et al. The intercellular adhesion (ica) locus is present in Staphylococcus aureus and is required for biofilm formation. Infect Immun. 1999;67(10):5427–5433.

[84] 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. 2004;279(52):54881–54886.

[85] Cerca N, Jefferson KK, Maira-Litran T, et al. Molecular basis for preferential protective efficacy of antibodies directed to the poorly acetylated form of staphylococcal poly-N-acetyl-beta-(1→6)-glucosamine. Infect Immun. 2007;75(7):3406–3413.

[86] Vuong C, Voyich JM, Fischer ER, et al. Polysaccharide intercellular adhesin (PIA) protects Staphylococcus epidermidis against major components of the human innate immune system. Cell Microbiol. 2004;6(3):269–275.

[87] Fitzpatrick F, Humphreys H, O’Gara JP. Evidence for iaCABG-independent biofilm development mechanism in methicillin-resistant Staphylococcus aureus clinical isolates. J Clin Microbiol. 2005;43(4):1973–1976.

[88] Skurnik D, Cywes-Bentley C, Pier GB. The exceptionally broad-based potential of active and passive vaccination targeting the conserved microbial surface polysaccharide PNAg. Expert Rev Vaccines. 2016;15(8):1041–1053.

[89] Cheng AG, McAdow M, Kim HK, et al. Contribution of coagulases towards Staphylococcus aureus disease and protective immunity. PLoS Pathog. 2010;6(8):e1001036.

[90] Friedrich R, Panizi P, Fuentes-Prior P, et al. Staphylocoagulase is a prototype for the mechanism of cofactor-induced zymogen activation. Nature. 2003;425(6957):535–539.

[91] McAdow M, Kim HK, Dedent AC, et al. Preventing Staphylococcus aureus sepsis through the inhibition of its agglutination in blood. PLoS Pathog. 2011;7(10):e1002307.

[92] Fitzgerald JR, Loughman A, Keane F, et al. Fibronectin-binding proteins of Staphylococcus aureus mediate activation of human platelets via fibrinogen and fibronectin bridges to integrin GPIb/IIia and IgG binding to the FgammaR1a receptor. Mol Microbiol. 2006;59(1):212–230.

[93] Forsgren A, Sjoquist J. “Protector A” from S. aureus I Pseudo-immune Reaction with Human Gamma-globulin. J Immunol. 1966;97(6):822–827.

[94] Goodyear CS, Silverman GJ. Death by a B cell super-antigen: in vivo VH-targeted apoptotic supracellular B cell deletion by a Staphylococcal Toxin. J Exp Med. 2003;197(9):1125–1139.

[95] Pauli NT, Kim HK, Falugi F, et al. Staphylococcus aureus infection induces protein A-mediated immune evasion in humans. J Exp Med. 2014;211(12):2331–2339.

[96] Atkins KL, Burman JD, Chamberlain ES, et al. van den Elsen JM. S Aureus IgG-binding Proteins SpA and Sbi: Host Specificity and Mechanisms of Immune Complex Formation Mol Immunol. 2008;45(6):1600–1611.

[97] Zhang L, Jacobsson K, Vasi J, et al. A second IgG-binding protein in Staphylococcus aureus. Microbiology. 1998;144(Pt 4):985–991.

[98] Itoh S, Hamada E, Kamoshida G, et al. Staphylococcal superantigen-like protein 10 (SSL10) binds to human immunoglobulin G (IgG) and inhibits complement activation via the classical pathway. Mol Immunol. 2010;47(4):932–938.

[99] Lambris JD, Ricklin D, Geisbrecht BV. Complement evasion by human pathogens. Nat Rev Microbiol. 2008;6(2):132–142.

[100] von Kockritz-blickwede M, Konrad S, Foster S, et al. Protective Role of Complement C5a in an Experimental Model of Staphylococcus aureus Bacteremia. J Innate Immun. 2010;2(1):87–92.

[101] Rooijakkers SHM, Ruyken M, Roos A, et al. Immune evasion by a staphylococcal complement inhibitor that acts on C3 convertases. Nat Immunol. 2005;6(9):920–927.
[102] Ko Y-P, Kuipers A, Freitag CM, et al. Phagocytosis escape by a *Staphylococcus aureus* protein that connects complement and coagulation proteins at the bacterial surface. PLoS Pathog. 2013;9(12):e1003816.

[103] Jongerius I, Garcia BL, Geisbrecht BV, et al. Convertase inhibitory properties of Staphylococcal extracellular complement-binding protein. J Biol Chem. 2010;285(20):14973–14979.

[104] Kang M, Ko Y-P, Liang X, et al. Collagen-binding microbial surface components recognizing adhesive matrix molecule (MSCRAMM) of Gram-positive bacteria inhibit complement activation via the classical pathway. J Biol Chem. 2013;288(28):20520–20531.

[105] Sharp JA, Echague CG, Hair PS, et al. *Staphylococcus aureus* surface protein SdrE binds complement regulator factor H as an immune evasion tactic. PLoS One. 2012;7(5):e38407.

[106] Zhang Y, Wu M, Hang T, et al. *Staphylococcus aureus* SdrE captures complement factor H’s C-terminus via a novel ‘close, dock, lock and latch’ mechanism for complement evasion. Biochem J. 2017;474(10):1619–1631.

[107] Woehl JL, Stapels DAC, Garcia BL, et al. The Extracellular Adherence Protein from *Staphylococcus aureus* Inhibits the Classical and Lectin Pathways of Complement by Blocking Formation of the C3 Proconvertase. J Immunol. 2014;193(12):6161–6171.

[108] Langley R, Wines B, Willoughby N, et al. The Staphylococcal Superantigen-Like Protein 7 Binds IgA and Complement C5 and Inhibits IgA-FcαRI Binding and Serum Killing of Bacteria. J Immunol. 2005;174(5):2926–2933.

[109] Dubin G. Extracellular Proteases of *Staphylococcus spp*. Biol Chem. 2002;383(7–8):1075–1086.

[110] Laarmann AJ, Ruyken M, Malone CL, et al. *Staphylococcus aureus* metalloproteinase aureolysin cleaves complement C3 to mediate immune evasion. J Immunol. 2011;186(11):6445–6453.

[111] Rousseaux J, Rousseaux-Prevost R, Bazin H, et al. Proteolysis of rat IgG subclasses by *Staphylococcus aureus* V8 protease. Biochim Biophys Acta. 1983;748(2):205–212.

[112] Brezski RJ, Jordan RE. Cleavage of IgGs by proteases associated with invasive diseases: an evasion tactic against host immunity? MABS. 2010;2(3):212–220.

[113] Jongerius I, von Kockritz-blickwede M, Horsburgh MJ, et al. *Staphylococcus aureus* virulence is enhanced by secreted factors that block innate immune defenses. J Innate Immun. 2012;4 (3):301–311.

[114] Kobayashi SD, Malachowa N, DeLeo FR. Neutrophils and Bacterial Immune Evasion. J Innate Immun. 2018;10(5–6):432–441.

[115] Klebanoff SJ, Kettle AJ, Rosen H, et al. Myeloperoxidase: a front-line defender against phagocytosed microorganisms. J Leukoc Biol. 2013;93 (2):185–198.

[116] Clauoldt A, Resch A, Wieland KP, et al. Staphyloxanthin plays a role in the fitness of *Staphylococcus aureus* and its ability to cope with oxidative stress. Infect Immun. 2006;74(8):4950–4953.

[117] Pelz A, Wieland KP, Putzbach K, et al. Structure and biosynthesis of staphyloxanthin from *Staphylococcus aureus*. J Biol Chem. 2005;280(37):32493–32498.

[118] Clements MO, Watson SP, Foster SJ. Characterization of the major superoxide dismutase of *Staphylococcus aureus* and its role in starvation survival, stress resistance, and pathogenicity. J Bacteriol. 1999;181 (13):3898–3903.

[119] Mandell GL. Catalase, superoxide dismutase, and virulence of *Staphylococcus aureus*. In Vitro and in Vivo Studies with Emphasis on Staphylococcal–leukocyte Interaction J Clin Invest. 1975;55(3):561–566.

[120] Cosgrove K, Coutts G, Jonsson IM, et al. Catalase (KatA) and alkyl hydroperoxide reductase (AhpC) have compensatory roles in peroxide stress resistance and are required for survival, persistence, and nasal colonization in *Staphylococcus aureus*. J Bacteriol. 2007;189(3):1025–1035.

[121] Richardson AR, Libby SJ, Fang FC. A nitric oxide-inducible lactate dehydrogenase enables *Staphylococcus aureus* to resist innate immunity. Science. 2008;319(5870):1672–1676.

[122] Thammavongsa V, Kern JW, Missiakas DM, et al. *Staphylococcus aureus* synthesizes adenosine to escape host immune responses. J Exp Med. 2009;206 (11):2417–2427.

[123] Zapotoczna M, Riboldi GP, Moustafa AM, et al. Mobile-Gene-Element-Encoded Hypertolerance to Copper Protects *Staphylococcus aureus* from Killing by Host Phagocytes. mBio. 2018;9:5.

[124] NWM DJ, Ramyar KK, Guerra FE, et al. Immune evasion by a staphylococcal inhibitor of myeloperoxidase. Proc Natl Acad Sci U S A. 2017;114(35):9439–9444.

[125] Joo HS, Otto M Mechanisms of resistance to antimicrobial peptides in staphylococci. Biochim Biophys Acta. 2015;1848(11 Pt B):3055–3061.

[126] Peschel A, Otto M, Jack RW, et al. Inactivation of the dlt operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides. J Biol Chem. 1999;274(13):8405–8410.

[127] Ernst CM, Kuhn S, Slavetinsky CJ, et al. The lipid-modifying multiple peptide resistance factor is an oligomer consisting of distinct interacting synthase and flippase subunits. mBio. 2015;6:1.

[128] Peschel A, Jack RW, Otto M, et al. *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MrpF is based on modification of membrane lipids with l-lysine. J Exp Med. 2001;193(9):1067–1076.

[129] Li M, Cha DJ, Lai Y, et al. The antimicrobial peptide-sensing system.apps of *Staphylococcus aureus*. Mol Microbiol. 2007;66(5):1136–1147.

[130] Falord M, Karimova G, Hiron A, et al. 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. 2012;56(2):1047–1058.

[131] Meehl M, Herbert S, Gotz F, et al. Interaction of the GraRS two-component system with the VraFG ABC transporter to support vancomycin-intermediate resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother. 2007;51(8):2679–2689.

[132] Li M, Lai Y, Villaruz AE, et al. Gram-positive three-component antimicrobial peptide-sensing
system. Proc Natl Acad Sci U S A. 2007;104 (22):9469–9474.

[133] Lai Y, Villaruz AE, Li M, et al. The human anionic antimicrobial peptide dermcidin induces proteolytic defence mechanisms in staphylococci. Mol Microbiol. 2007;63(2):497–506.

[134] Bera A, Herbert S, Jakob A, et al. Why are pathogenic staphylococci so lysozyme resistant? The peptidoglycan O-acetyltransferase OatA is the major determinant for lysozyme resistance of Staphylococcus aureus. Mol Microbiol. 2005;55(3):778–787.

[135] Stapels DA, Ramyar KK, Bischoff M, et al. Staphylococcus aureus secretes a unique class of neutrophil serine protease inhibitors. Proc Natl Acad Sci U S A. 2014;111 (36):13187–13192.

[136] Papayannopoulos V, Zychlinsky A. NETs: a new strategy for using old weapons. Trends Immunol. 2009;30 (11):513–521.

[137] Jann NJ, Schmaler M, Kristian SA, et al. Neutrophil antimicrobial defense against Staphylococcus aureus is mediated by phagolysosomal but not phagocytotic trap-associated cathelicidin. J Leukoc Biol. 2009;86 (5):1159–1169.

[138] Berends ET, Horswill AR, Haste NM, et al. Nuclease expression by Staphylococcus aureus facilitates escape from neutrophil extracellular traps. J Innate Immun. 2010;2(6):576–586.

[139] Thammavongsa V, Missiakas DM, Schnewind O. Staphylococcus aureus degrades neutrophil extracellular traps to promote immune cell death. Science. 2013;342(6160):863–866.

[140] Berube BJ, Bubeck Wardenburg J. Staphylococcus aureus alpha-toxin: nearly a century of intrigue. Toxins (Basel). 2013;5(6):1140–1166.

[141] Alonzo F 3rd, Torres VJ. The bicomponent pore-forming leucocidins of Staphylococcus aureus. Microbiol Mol Biol Rev. 2014;78(2):199–230.

[142] Cheung GY, Joo HS, Chatterjee SS, et al. Phenol-soluble modulins–critical determinants of staphylococcal virulence. FEMS Microbiol Rev. 2014;38 (4):698–719.

[143] Song L, Hobough MR, Shustak C, et al. Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. Science. 1996;274(5294):1859–1866.

[144] Craven RR, Gao X, Allen IC, et al. Staphylococcus aureus alpha-hemolysin activates the NLRP3-inflammasome in human and mouse monocytic cells. PLoS One. 2009;4 (10):e7446.

[145] Suttrop N, Habben E. Effect of staphylococcal alpha-toxin on intracellular Ca2+ in polymorphonuclear leukocytes. Infect Immun. 1988;56(9):2228–2234.

[146] Bubeck Wardenburg J, Bae T, Otto M, et al. Poring over pores: alpha-hemolysin and Panton-Valentine leukocidin in Staphylococcus aureus pneumonia. Nat Med. 2007;13(12):1405–1406.

[147] Kitur K, Parker D, Nieto P, et al. Toxin-induced necroptosis is a major mechanism of Staphylococcus aureus lung damage. PLoS Pathog. 2015;11(4):e1004820.

[148] Kobayashi SD, Malachowa N, Whitney AR, et al. Comparative analysis of USA300 virulence determinants in a rabbit model of skin and soft tissue infection. J Infect Dis. 2011;204(6):937–941.

[149] Powers ME, Kim HK, Wang Y, et al. ADAM10 mediates vascular injury induced by Staphylococcus aureus alpha-hemolysin. J Infect Dis. 2012;206(3):352–356.

[150] Alonzo F 3rd, Kozhaya L, Rawlings SA, et al. CCR5 is a receptor for Staphylococcus aureus leukotoxin ED. Nature. 2013;493(7430):51–55.

[151] Spaan AN, Henry T, WJM VR, et al. The staphylococcal toxin Panton-Valentine Leukocidin targets human C5a receptors. Cell Host Microbe. 2013;13 (5):584–594.

[152] DuMont AL, Yoong P, Day CJ, et al. Staphylococcus aureus LukAB cytotoxin kills human neutrophils by targeting the CD11b subunit of the integrin Mac-1. Proc Natl Acad Sci U S A. 2013;110(26):10794–10799.

[153] Yamashita K, Kawai Y, Tanaka Y, et al. Crystal structure of the octameric pore of staphylococcal gamma-hemolysin reveals the beta-barrel pore formation mechanism by two components. Proc Natl Acad Sci U S A. 2011;108(42):17314–17319.

[154] Vandenesch F, Naimi T, Enright MC, et al. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis. 2003;9(8):978–984.

[155] Deep BA, Chan L, Tattevin P, et al. Polymorphonuclear leukocytes mediate Staphylococcus aureus Panton-Valentine leukocidin-induced lung inflammation and injury. Proc Natl Acad Sci U S A. 2010;107(12):5587–5592.

[156] Lipinska U, Hermans K, Meulemans L, et al. Panton-Valentine leukocidin does play a role in the early stage of Staphylococcus aureus skin infections: a rabbit model. PLoS One. 2011;6(8):e22864.

[157] Boguslawski KM, McKeown AN, Day CJ, et al. Exploiting species specificity to understand the tropism of a human-specific toxin. Sci Adv. 2020;6(11):eaax7515.

[158] Spaan AN, Reyes-Robles T, Badiou C, et al. Staphylococcus aureus Targets the Duffy Antigen Receptor for Chemokines (DARC) to Lyse Erythrocytes. Cell Host Microbe. 2015;18(3):363–370.

[159] Ventura CL, Malachowa N, Hammer CH, et al. Identification of a novel Staphylococcus aureus two-component leukotoxin using cell surface proteomics. PLoS One. 2010;5(7):e11634.

[160] Graves SF, Kobayashi SD, Braughton KR, et al. Sublytic concentrations of Staphylococcus aureus Panton-Valentine leukocidin alter human PMN gene expression and enhance bacterial capacity. J Leukoc Biol. 2012;92(2):361–374.

[161] Holzinger D, Gieldon L, Mysore V, et al. Staphylococcus aureus Panton-Valentine leukocidin induces an inflammatory response in human phagocytes via the NLRP3 inflammasome. J Leukoc Biol. 2012;92(5):1069–1081.

[162] Nakagawa S, Matsumoto M, Katayama Y, et al. Staphylococcus aureus Virulent PSMalpha Peptides Induce Keratinocyte Alarmin Release to Orchestrate IL-17-Dependent Skin Inflammation. Cell Host Microbe. 2017;22(5):667–77 e5.
[163] Bloes DA, Haasbach E, Hartmayer C, et al. Peptides Contribute to Influenza A Virus-Associated Staphylococcus aureus Pneumonia. Infect Immun. 2017;85:12.

[164] Berlon NR, Qi R, Sharma-Kuinkel BK, et al. Clinical MRSA isolates from skin and soft tissue infections show increased in vitro production of phenol soluble modulins. J Infect. 2015;71(4):447–457.

[165] Grosz M, Kolter J, Paprotka K, et al. Cytoplasmic replication of Staphylococcus aureus upon phagosomal escape triggered by phenol-soluble modulin alpha. Cell Microbiol. 2014;16(4):451–465.

[166] Munzenmayer L, Geiger T, Daiber E, et al. Influence of Sae-regulated and Agr-regulated factors on the escape of Staphylococcus aureus from human macrophages. Cell Microbiol. 2016;18(8):1172–1183.

[167] Winstel V, Schneewind O, Missiakov D. Staphylococcus aureus Exploits the Host Apoptotic Pathway To Persist during Infection. mBio. 2019;10:6.

[168] Genestier AL, Michallet MC, Prevost G, et al. Staphylococcus aureus Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. J Clin Invest. 2005;115(11):3117–3127.

[169] Smagur J, Guzik K, Magiera L, et al. A new pathway of staphylococcal pathogenesis: apoptosis-like death induced by Staphopain B in human neutrophils and monocytes. J Innate Immun. 2009;1(2):98–108.

[170] Cheng AG, DeDent AC, Schneewind O, et al. A play in four acts: staphylococcus aureus abscess formation. Trends Microbiol. 2011;19(S):225–232.

[171] Cheng AG, Kim HK, Burts ML, et al. Genetic requirements for Staphylococcus aureus abscess formation and persistence in host tissues. Faseb J. 2009;23(10):3393–3404.

[172] Foster TJ. The MSCRAMM Family of Cell-Wall-Anchor Surface Proteins of Gram-Positive Cocci. Trends Microbiol. 2019;27(11):927–941.

[173] Mazmanian SK, Liu G, Ton-That H, et al. Staphylococcus aureus sortase, an enzyme that anchors surface proteins to the cell wall. Science. 1999;285(5428):760–763.

[174] Peacock SJ, Foster TJ, Cameron BJ, et al. Bacterial fibronectin-binding proteins and endothelial cell surface fibronectin mediate adherence of Staphylococcus aureus to resting human endothelial cells. Microbiology. 1999;145(Pt 12):3477–3486.

[175] Gillaspy AF, Lee CY, Sau S, et al. Factors affecting the collagen binding capacity of Staphylococcus aureus. Infect Immun. 1998;66(7):3170–3178.

[176] Xu Y, Rivas JM, Brown EL, et al. Virulence potential of the staphylococcal adhesin CNA in experimental arthritis is determined by its affinity for collagen. J Infect Dis. 2004;189(12):2332–2333.

[177] Bubeck Wardenburg J, Williams WA, Missiakov D. Host defenses against Staphylococcus aureus infection require recognition of bacterial lipoproteins. Proc Natl Acad Sci U S A. 2006;103(37):13831–13836.

[178] Gonzalez CD, Ledo C, Cela E, et al. The good side of inflammation: staphylococcus aureus proteins SpA and Sbi contribute to proper abscess formation and wound healing during skin and soft tissue infections. Biochim Biophys Acta Mol Basis Dis. 2019;1865(10):2657–2670.

[179] Li M, Dai Y, Zhu Y, et al. Virulence determinants associated with the Asian community-associated methicillin-resistant Staphylococcus aureus lineage ST59. Sci Rep. 2016;6:27899.

[180] Cheung GY, Kretschmer D, Duong AC, et al. Production of an attenuated phenol-soluble modulin variant unique to the MRSA clonal complex 30 increases severity of bloodstream infection. PLoS Pathog. 2014;10(8):e1004298.

[181] Cheung GY, Duong AC, Otto M. Direct and synergistic hemolysis caused by Staphylococcus phenol-soluble modulins: implications for diagnosis and pathogenesis. Microbes Infect. 2012;14(4):380–386.

[182] Hebert GA, Hancock GA. Synergistic hemolysis exhibited by species of staphylococci. J Clin Microbiol. 1985;22(3):409–415.

[183] Izano EA, Amarante MA, Kher WB, et al. Differential roles of poly-N-acetylgalcosamine surface polysaccharide and extracellular DNA in Staphylococcus aureus and Staphylococcus epidermidis biofilms. Appl Environ Microbiol. 2008;74(2):470–476.

[184] Vinogradov E, Sadovskaya I, Li J, et al. Structural elucidation of the extracellular and cell-wall teichoic acids of Staphylococcus aureus MN8m, a biofilm forming strain. Carbohydr Res. 2006;341(6):738–743.

[185] Corrigan RM, Rigby D, Handley P, et al. The role of Staphylococcus aureus surface protein SasG in adherence and biofilm formation. Microbiology. 2007;153(Pt 8):2435–2446.

[186] Periasamy S, Joo HS, Duong AC, et al. Staphylococcus aureus biofilms develop their characteristic structure. Proc Natl Acad Sci U S A. 2012;109(4):1281–1286.

[187] Mootz JM, Malone CL, Shaw LN, et al. Staphopains modulate Staphylococcus aureus biofilm integrity. Infect Immun. 2013;81(9):3227–3238.

[188] Otto M. Staphylococcal infections: mechanisms of biofilm maturation and detachment as critical determinants of pathogenicity. Annu Rev Med. 2013;64:175–188.

[189] Thurlow LR, Hanke ML, Fritz T, et al. Staphylococcus aureus biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. J Immunol. 2011;186(11):6585–6596.

[190] Beenken KE, Dunman PM, McAleese F, et al. Global gene expression in Staphylococcus aureus biofilms. J Bacteriol. 2004;186(14):4665–4684.

[191] Hanke ML, Kielian T. Deciphering mechanisms of staphylococcal biofilm evasion of host immunity. Front Cell Infect Microbiol. 2012;2:62.

[192] Rogers DE. Studies on bacteriemia. I Mechanisms Relating to the Persistence of Bacteremia in Rabbits following the Intravenous Injection of Staphylococci J Exp Med. 1956;103(6):713–742.

[193] Gresham HD, Lowrance JH, Caver TE, et al. Survival of Staphylococcus aureus inside neutrophils contributes to infection. J Immunol. 2000;164(7):3713–3722.

[194] Kubica M, Guzik K, Koziel J, et al. A potential new pathway for Staphylococcus aureus dissemination: the silent survival of S. Aureus Phagocytosed by Human...
Monocyte-derived Macrophages PLoS One. 2008;3(1): e1409.

[195] Garzoni C, Kelley WL. Staphylococcus aureus: new evidence for intracellular persistence. Trends Microbiol. 2009;17(2):59–65.

[196] Que YA, Haeligger JA, Piroth L, et al. Fibrinogen and fibronectin binding cooperate for valve infection and invasion in Staphylococcus aureus experimental endocarditis. J Exp Med. 2005;201(10):1627–1635.

[197] Bur S, Preer KT, Herrmann M, et al. Staphylococcus aureus extracellular adherence protein promotes bacterial internalization by keratinocytes independent of fibronectin-binding proteins. J Invest Dermatol. 2013;133(8):2004–2012.

[198] Schwarz-Linek U, Werner JM, Pickford AR, et al. Pathogenic bacteria attach to human fibronectin through a tandem beta-zipper. Nature. 2003;423 (6936):177–181.

[199] Moldovan A, Fraunholz MJ. In or out: phagosomal escape of Staphylococcus aureus. Cell Microbiol. 2019;21(3):e12997.

[200] Proctor RA, von Eiff C, Kahl BC, et al. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol. 2006;4(4):295–305.

[201] Vaudaux P, Francois P, Bisognano C, et al. Increased expression of clumping factor and fibronectin-binding proteins by hemB mutants of Staphylococcus aureus expressing small colony variant phenotypes. Infect Immun. 2002;70(10):5428–5437.

[202] Peetemans M, Vanasse T, Liesenborghs L, et al. Plasminogen activation by staphylokinase enhances local spreading of S. Aureus in Skin Infections. BMC Microbiol. 2014;14:310.

[203] Cho JS, Pietras EM, Garcia NC, et al. 17 is essential for host defense against cutaneous Staphylococcus aureus infection in mice. J Clin Invest. 2010;120(5):1762–1773.

[204] Spellberg B, Ibrahim AS, Yeaman MR, et al. The antifungal vaccine derived from the recombinant N terminus of Als3p protects mice against the bacterium Staphylococcus aureus. Infect Immun. 2008;76 (10):4574–4580.

[205] Hewitt CR, Lamb JR, Hayball J, et al. Major histo-compatibility complex independent clonal T cell anergy by direct interaction of Staphylococcus aureus enterotoxin B with the T cell antigen receptor. J Exp Med. 1992;175(6):1493–1499.

[206] Llewelyn M, Cohen J. Superantigens: microbial agents that corrupt immunity. Lancet Infect Dis. 2002;2 (3):156–162.

[207] Ziegler C, Goldmann O, Hobelka E, et al. The dynamics of T cells during persistent Staphylococcus aureus infection: from antigen-reactivity to in vivo energy. EMBO Mol Med. 2011;3(11):652–666.

[208] Tebartz C, Horst SA, Sparwasser T, et al. A major role for myeloid-derived suppressor cells and a minor role for regulatory T cells in immunosuppression during Staphylococcus aureus infection. J Immunol. 2015;194 (3):1100–1111.

[209] Lindsay JA. Staphylococci: evolving Genomes. Microbiol Spectr. 2019;7:6.

[210] Malachowa N, DeLeo FR. Mobile genetic elements of Staphylococcus aureus. Cell Mol Life Sci. 2010;67 (18):3057–3071.

[211] Novick RP, Islands P. Their Role in Staphylococcal Microbiol. Spectr. 2019;7:3.

[212] Klaui AJ, Boss R, Graber HU. Characterization and Comparative Analysis of the Staphylococcus aureus Genomic Island vSabeta: an In Silico Approach. J Bacteriol. 2019;201:22.

[213] Huseby M, Shi K, Brown CK, et al. Structure and biological activities of beta toxin from Staphylococcus aureus. J Bacteriol. 2007;189(23):8719–8726.

[214] Carroll JD, Cafferkey MT, Coleman DC. Serotype F double- and triple-converting phage incrementally inactivate the Staphylococcus aureus beta-toxin determinant by a common molecular mechanism. FEMS Microbiol Lett. 1993;106(2):147–155.

[215] Katayama Y, Baba T, Sekine M, et al. Beta-hemolysin promotes skin colonization by Staphylococcus aureus. J Bacteriol. 2013;195(6):1194–1203.

[216] Langley R, Patel D, Jackson N, et al. Staphylococcal superantigen super-domains in immune evasion. Crit Rev Immunol. 2010;30(2):149–165.

[217] Nguyen MT, Kraft B, Yu W, et al. The nuSaalpha Specific Lipoprotein Like Cluster (lpl) of S. Aureus USA300 Contributes to Immune Stimulation and Invasion in Human Cells PLoS Pathog. 2015;11(6): e1004984.

[218] Joo HS, Cheung GY, Otto M. Antimicrobial activity of community-associated methicillin-resistant Staphylococcus aureus is caused by phenol-soluble modulin derivatives. J Biol Chem. 2011;286(11):8933–8940.

[219] Jefferson KK, Cramong SE, Gotz F, et al. Identification of a 5-nucleotide sequence that controls expression of the ica locus in Staphylococcus aureus and characterization of the DNA-binding properties of IcaR. Mol Microbiol. 2003;48(4):889–899.

[220] Cerca N, Brooks JL, Jefferson KK. Regulation of the intercellular adhesin locus regulator (icaR) by SarA, sigmaB, and IcaR in Staphylococcus aureus. J Bacteriol. 2008;190(19):6530–6533.

[221] Joo HS, Otto M. Toxin-mediated gene regulatory mechanism in Staphylococcus aureus. Microb Cell. 2016;4(1):29–31.

[222] Le KY, Otto M. Quorum-sensing regulation in staphylococci-an overview. Front Microbiol. 2015;6:1174.

[223] Recsei P, Kreiswirth B, O’Reilly M, et al. Regulation of exoprotein gene expression in Staphylococcus aureus by agr. Mol Gen Genet. 1986;202(1):58–61.

[224] Redfield RJ. Is quorum sensing a side effect of diffusion sensing? Trends Microbiol. 2002;10(8):365–370.

[225] Cheung GY, Wang R, Khan BA, et al. Role of the accessory gene regulator agr in community-associated methicillin-resistant Staphylococcus aureus pathogenesis. Infect Immun. 2011;79(5):1927–1935.

[226] Novick RP, Ross HH, Prijon SJ, et al. Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. Embo J. 1993;12(10):3967–3975.

[227] 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. 2008;32(1):150–158.

[228] Geisinger E, Adhikari RP, Jin R, et al. Inhibition of rot translation by RNAIII, a key feature of agr function. Mol Microbiol. 2006;64(4):1038–1048.

[229] Ji G, Beavis RC, Novick RP. Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. Proc Natl Acad Sci U S A. 1995;92(26):12055–12059.

[230] Ji G, Beavis R, Novick RP. Bacterial interference caused by autoinducing peptide variants. Science. 1997;276(5321):2027–2030.

[231] Otto M. Staphylococcus aureus and Staphylococcus epidermidis peptide pheromones produced by the accessory gene regulator agr system. Peptides. 2001;22(10):1603–1608.

[232] Williams MR, Costa SK, Zaramela LS, et al. Quorum sensing between bacterial species on the skin protects against epidermal injury in atopic dermatitis. Sci Transl Med. 2019;11:1490.

[233] Cheung AL, Eberhardt KJ, Chung E, et al. Diminished virulence of a sar-/agr- mutant of Staphylococcus aureus in the rabbit model of endocarditis. J Clin Invest. 1994;94(5):1815–1822.

[234] Wright JS 3rd, Jin R, Novick RP. Transient interference with staphylococcal quorum sensing blocks abscess formation. Proc Natl Acad Sci U S A. 2005;102(5):1691–1696.

[235] Bubeck Wardenburg J, Patel RJ, Schneewind O. Surface proteins and exotoxins are required for the pathogenesis of Staphylococcus aureus pneumonia. Infect Immun. 2007;75(2):1040–1044.

[236] Montgomery CP, Boyle-Vavra S, Daum RS. Importance of the global regulators Agr and SarS in the pathogenesis of CA-MRSA USA300 infection. PLoS One. 2010;5(12):e15177.

[237] Abdelnour A, Arvidson S, Bremell T, et al. The accessory gene regulator (agr) controls Staphylococcus aureus virulence in a murine arthritis model. Infect Immun. 1993;61(9):3879–3885.

[238] Gillaspy AF, Hickmon SG, Skinner RA, et al. Role of the accessory gene regulator (agr) in pathogenesis of staphylococcal osteomyelitis. Infect Immun. 1995;63(9):3373–3380.

[239] Nakamura Y, Takahashi H, Takaya A, et al. Staphylococcus Agr virulence is critical for epidermal colonization and associates with atopic dermatitis development. Sci Transl Med. 2020;12:551.

[240] Pollitt EJ, West SA, Crusz SA, et al. Cooperation, quorum sensing, and evolution of virulence in Staphylococcus aureus. Infect Immun. 2014;82(3):1045–1051.

[241] Traber KE, Lee E, Benson S, et al. agr function in clinical Staphylococcus aureus isolates. Microbiology. 2008;154(PT 8):2265–2274.

[242] He L, Le KY, Khan BA, et al. Resistance to leukocytes ties benefits of quorum sensing dysfunctionality to biofilm infection. Nat Microbiol. 2019;4(7):1114–1119.

[243] 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. 2004;190(6):1140–1149.

[244] Chen J, Novick RP. svrA, a multi-drug exporter, does not control agr. Microbiology. 2007;153(PT 5):1604–1608.

[245] Villaruz AE, Bubeck Wardenburg J, Khan BA, et al. A point mutation in the agr locus rather than expression of the Panton-Valentine leukocidin caused previously reported phenotypes in Staphylococcus aureus pneumonia and gene regulation. J Infect Dis. 2009;200(5):724–734.

[246] Adhikari RP, Arvidson S, Novick RP. A nonsense mutation in agrA accounts for the defect in agr expression and the avirulence of Staphylococcus aureus 8325-4 traP:kam. Infect Immun. 2007;75(9):4534–4540.

[247] Xiong A, Singh VK, Cabrera G, et al. Molecular characterization of the ferric-uptake regulator, fur, from Staphylococcus aureus. Microbiology. 2000;146(Pt 3):659–668.

[248] 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. 2001;183(4):1113–1123.

[249] Johnson M, Sengupta M, Purves J, et al. Fur is required for the activation of virulence gene expression through the induction of the sae regulatory system in Staphylococcus aureus. Int J Med Microbiol. 2011;301(1):44–52.

[250] Torres VJ, Attia AS, Mason WJ, et al. Staphylococcus aureus fur regulates the expression of virulence factors that contribute to the pathogenesis of pneumonia. Infect Immun. 2010;78(4):1618–1628.

[251] Horsburgh MJ, Ingham E, Foster SJ. In Staphylococcus aureus, fur is an interactive regulator with PerR, contributes to virulence, and Is necessary for oxidative stress resistance through positive regulation of catalase and iron homeostasis. J Bacteriol. 2001;183(2):468–475.

[252] Tiwari N, Lopez-Redondo M, Miguel-Romero L, et al. The SrrAB two-component system regulates Staphylococcus aureus pathogenicity through redox sensitive cytoines. Proc Natl Acad Sci U S A. 2020;117(20):10989–10999.

[253] Kinkel TL, Roux CM, Dunman PM, et al. The Staphylococcus aureus SrrAB two-component system promotes resistance to nitrosative stress and hypoxia. mBio. 2013;4(6):e00696–13.

[254] 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. 2007;65(5):1276–1287.

[255] Cheung AL, Nishina KA, Trotonda MP, et al. The SarA protein family of Staphylococcus aureus. Int J Biochem Cell Biol. 2008;40(3):355–361.

[256] Liu Y, Manna AC, Pan CH, et al. Structural and function analyses of the global regulatory protein SarA from Staphylococcus aureus. Proc Natl Acad Sci U S A. 2006;103(7):2392–2397.
[257] Beenken KE, Mrak LN, Griffin LM, et al. Epistatic relationships between sarA and agr in Staphylococcus aureus biofilm formation. PLoS One. 2010;5(5):e10790.

[258] Liu Q, Yeo WS, Bae T. The SaeRS Two-Component System of Staphylococcus aureus. Genes (Basel). 2016;7:10.

[259] Liang X, Zheng L, Landwehr C, et al. Global regulation of gene expression by ArlRS, a two-component signal transduction regulatory system of Staphylococcus aureus. J Bacteriol. 2005;187(15):5486–5492.

[260] Ingavale S, van Wamel W, Luong TT, et al. Rat/MgrA, a regulator of autolysis, is a regulator of virulence genes in Staphylococcus aureus. Infect Immun. 2005;73(3):1423–1431.

[261] Jonsson IM, Lindholm C, Luong TT, et al. mgrA regulates staphylococcal virulence important for induction and progression of septic arthritis and sepsis. Microbes Infect. 2008;10(12–13):1229–1235.

[262] Li L, Wang G, Cheung A, et al. MgrA Governs Adherence, Host Cell Interaction, and Virulence in a Murine Model of Bacteremia Due to Staphylococcus aureus. J Infect Dis. 2019;220(6):1019–1028.

[263] Luong TT, Dunman PM, Murphy E, et al. Transcription Profiling of the mgrA Regulon in Staphylococcus aureus. J Bacteriol. 2006;188(5):1899–1910.

[264] Chen PR, Bae T, Williams WA, et al. An oxidation-sensing mechanism is used by the global regulator MgrA in Staphylococcus aureus. Nat Chem Biol. 2006;2(11):591–595.

[265] Chen PR, Nishida S, Poor CB, et al. A new oxidative sensing and regulation pathway mediated by the MgrA homologue SarZ in Staphylococcus aureus. Mol Microbiol. 2009;71(1):198–211.

[266] Kullik II, Giachino P. The alternative sigma factor sigmaB in Staphylococcus aureus: regulation of the sigB operon in response to growth phase and heat shock. Arch Microbiol. 1997;167(2–3):151–159.

[267] Bischoff M, Entenza JM, Giachino P. Influence of a functional sigB operon on the global regulators sar and agr in Staphylococcus aureus. J Bacteriol. 2001;183(17):5171–5179.

[268] Nicholas RO, Li T, McDevitt D, et al. Isolation and characterization of a sigB deletion mutant of Staphylococcus aureus. Infect Immun. 1999;67(7):3667–3669.

[269] Tuchscherr L, Bischoff M, Lattar SM, et al. Sigma Factor SigB Is Crucial to Mediate Staphylococcus aureus Adaptation during Chronic Infections. PLoS Pathog. 2015;11(4):e1004870.

[270] Dickey SW, Cheung GYC, Otto M. Different drugs for bad bugs: antivirulence strategies in the age of antibiotic resistance. Nat Rev Drug Discov. 2017;16(7):457–471.

[271] Diep BA, Hilliard JJ, Le VT, et al. Targeting Alpha Toxin To Mitigate Its Lethal Toxicity in Ferret and Rabbit Models of Staphylococcus aureus Necrotizing Pneumonia. Antimicrob Agents Chemother. 2017;61(4).

[272] Yu XQ, Robbie GJ, Wu Y, et al. Pharmacokinetics of MED4893, an Investigational, Extended-Half-Life, Anti-Staphylococcus aureus Alpha-Toxin Human Monoclonal Antibody, in Healthy Adults. Antimicrob Agents Chemother. 2017;61(1).

[273] Rouha H, Badarau A, Visram ZC, et al. Five birds, one stone: neutralization of alpha-hemolysin and 4 bi-component leukocidins of Staphylococcus aureus with a single human monoclonal antibody. MAbs. 2015;7(1):243–254.

[274] Stulik L, Rouha H, Labrousse D, et al. Preventing lung pathology and mortality in rabbit Staphylococcus aureus pneumonia models with cytoxin-neutralizing monoclonal IgGsPenetrating the epithelial lining fluid. Sci Rep. 2019;9(1):5339.

[275] Khan BA, Yeh AJ, Cheung GY, et al. Investigational therapies targeting quorum-sensing for the treatment of Staphylococcus aureus infections. Expert Opin Investig Drugs. 2015;24(5):689–704.

[276] Lyon GJ, Wright JS, Christopoulos A, et al. Reversible and specific extracellular antagonism of receptor-histidine kinase signaling. J Biol Chem. 2002;277(8):6247–6253.

[277] Piewngam P, Zheng Y, Nguyen TH, et al. Pathogen elimination by probiotic Bacillus via signalling interference. Nature. 2018;562(7728):532–537.

[278] Parlet CP, Kavanaugh JS, Crosby HA, et al. Apicidin Attenuates MRSA Virulence through Quorum-Sensing Inhibition and Enhanced Host Defense. Cell Rep. 2019;27(1):187–98 e6.

[279] Sully EK, Malachowa N, Elmore BO, et al. Selective chemical inhibition of agr quorum sensing in Staphylococcus aureus promotes host defense with minimal impact on resistance. PLoS Pathog. 2014;10(6):e1004174.

[280] Li M, Cheung GY, Hu J, et al. Comparative analysis of virulence and toxin expression of global community-associated methicillin-resistant Staphylococcus aureus strains. J Infect Dis. 2010;202(12):1866–1876.

[281] Diep BA, Le VT, Badiou C, et al. IVIG-mediated Protection against Necrotizing Pneumonia Caused by MRSA. Sci Transl Med. 2016;8(357):357ra124.

[282] Bae T, Schneewind O. Allelic replacement in Staphylococcus aureus with inducible counter-selection. Plasmid. 2006;55(1):58–63.

[283] Monk IR, Shah IM, Xu M, et al. Transforming the untransformable: application of direct transformation to manipulate genetically Staphylococcus aureus and Staphylococcus epidermidis. mBio. 2012;3:2.