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New insights into the prevention of staphylococcal infections and toxic shock syndrome

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Abstract

Staphylococcus aureus is a major human pathogen capable of causing various diseases, from skin infections to life-threatening pneumonia and toxic shock syndrome. *S. aureus* exoproteins, including superantigens, contribute significantly to the pathogenesis of this organism. Antibiotics inhibit growth, but often provide no protection from *S. aureus* exoproteins. With the emergence of antibiotic-resistant *S. aureus*, new therapeutic options to treat or prevent *S. aureus*-associated diseases are critical. Most *S. aureus* infections begin on the skin or mucosal surfaces from direct inflammatory or cytotoxic effects of exotoxins. Therefore, antitoxin therapies that prevent toxin production and prevent their effects on host cells are being researched. Current treatments for staphylococcal diseases and recent developments in antitoxin therapeutic agents and vaccines are reviewed.

Keywords

α -toxin; antibiotic; antimicrobial resistance; exotoxin; necrotizing pneumonia; skin and soft-tissue infection; *Staphylococcus aureus*; toxic shock syndrome

Staphylococcus aureus

Burden of disease: epidemiology

Staphylococcus aureus is a Gram-positive bacterium capable of infecting virtually every tissue of the body and causing infections ranging from minor skin infections to life-threatening infections, such as bacteremia, endocarditis, necrotizing pneumonia and toxic shock syndrome (TSS). *S. aureus* is a major cause of healthcare-associated infections with the incidence of *S. aureus*-related infections estimated to be 13.8 per 1000 hospitalizations in US hospitals in 2005 [1]. The increasing prevalence of antibiotic-resistant *S. aureus*, most well-known as methicillin-resistant *S. aureus* (MRSA), has emerged as a serious public health issue both in the hospital and community settings. MRSA isolates account for more than 60% of nosocomial *S. aureus* infections in intensive care units (ICUs) [2] and more than 70% of culturable *S. aureus* skin and soft-tissue infections (SSTIs) in the community

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[3]. Generally speaking, MRSA strains are not considered to be more virulent than their methicillin-susceptible *S. aureus* (MSSA) counterparts. However, patients infected with MRSA are likely to have longer durations of hospital stays, higher hospital charges and higher mortality rates than patients infected with MSSA [4,5]. Invasive (severe) MRSA infections alone are estimated to cause approximately 19,000 deaths in the USA annually, which is similar to the total number of deaths due to AIDS, tuberculosis and viral hepatitis combined [6,7].

Types of human diseases

Owing to frequent exposures to antibiotics in the healthcare setting, the proportion of MRSA in hospitals steadily increased from approximately 36% in 1992 to more than 60% in 2003 in ICU patients [2]. Healthcare-associated MRSA (HA-MRSA) primarily affects people who have a compromised immune system and people with prior surgery or implanted medical devices, and typically cause septicemia, pneumonia and device-associated infections. HA-MRSA isolates are most frequently identified as pulsed-field gel electrophoresis (PFGE) USA100 and USA200, and most carry staphylococcal chromosomal cassettes (*SCCmec*) type I to III, which contains *mecA*, the methicillin-resistant gene that encodes penicillin-binding protein 2a (PBP2a), and multiple other antibiotic resistance genes [8]. These HA-MRSA isolates are typically resistant to multiple antibiotics, including penicillins, β -lactams, erythromycin, clindamycin, fluoroquinolones and sometimes aminoglycosides.

S. aureus is also a common cause of SSTIs in the community. MRSA has been traditionally associated with hospitals; however, since the late 1990s there have been increasing reports of MRSA infections in people with no contact with the healthcare system or predefined risk factors for HA-MRSA. These MRSA isolates were therefore referred to as community-associated MRSA (CA-MRSA). The prevalence of MRSA in the community was only approximately 1% according to a national nasal colonization survey conducted by the CDC from 2001 to 2004 [9]. However, CA-MRSA strains are virulent and emerging as a major cause of severe SSTIs and, less frequently, necrotizing pneumonia in the community [8]. In 2004, a study of adult patients with purulent SSTIs from 11 emergency departments throughout the USA indicated that *S. aureus* was responsible for 76% of the SSTIs and 78% of the *S. aureus* isolated were MRSA (~60% of all infections) [10]. Epidemiological evidence indicates that CA-MRSA isolates are distinct from HA-MRSA isolates in many ways [8]. CA-MRSA isolates are most frequently identified as PFGE USA300 and USA400. Most of them carry *SCCmec* type IV or V and are susceptible to most antibiotics except β -lactams and erythromycin [8]. These CA-MRSA isolates have started to spread into hospital settings; therefore, USA300 and USA400 are emerging as major causes of HA-MRSA infections [1,6,11,12]. It is no surprise that the *SCCmec* pattern differences in HA-MRSA and CA-MRSA are also becoming less distinct owing to the clonal blending [12,13].

In addition to the difference in demographic distribution and antibiotic susceptibilities, HA-MRSA and CA-MRSA isolates have different exotoxin profiles, which have been associated with their genetic backgrounds. For example, Pantón–Valentine leukocidin (PVL) is epidemiologically associated with CA-MRSA isolates [13]. Certain superantigens, that is, staphylococcal enterotoxin B (SEB) and SEC, have also been associated with CA-MRSA; however, there are large variations in the prevalence of these superantigens in the epidemiological studies [8,13,14].

Virulence factors & pathogenesis

S. aureus produces an array of virulence factors (Table 1) to facilitate its pathogenesis. Initially researchers focused on the role of cell surface virulence factors, such as capsule; however, recently researchers have started to recognize the overall importance of

staphylococcal exoproteins, such as cytolysins and superantigens, in the initiation and progression of infections via direct tissue damage to mucosal membranes and skin [15,16]. The antibiotics of choice for treating *S. aureus*-related illnesses, β -lactams for MSSA and vancomycin for MRSA, kill the organism by lysis of the cell wall or inhibition of cell wall biosynthesis, but are not able to inhibit *S. aureus* exoprotein production or neutralize the existing toxins' effects on host cells. In fact, β -lactams may even induce production of cytolysins and other virulence-related exoproteins when inadequately used for treating MRSA, which potentially worsens clinical outcomes [17].

Given the emergence of MRSA and the high mortality associated with *S. aureus* infections, there is a demand for novel antistaphylococcal approaches to manage the increasing burden of *S. aureus* on human health. Owing to the important roles of *S. aureus* exotoxins in pathogenesis, strategies targeting *S. aureus* virulence factors (exotoxins) have started to gain interest as targets to prevent and/or decrease the morbidity and mortality associated with *S. aureus* diseases. Historically, cell surface virulence factors have been targeted for their role in pathogenesis and as potential vaccination candidates.

Cell surface factors: capsule & fibronectin-binding proteins

S. aureus cell wall-associated virulence factors include capsular polysaccharides (CPs), staphyloxanthin (carotenoid pigment), and a group of proteins known as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs).

Capsular polysaccharides are produced by approximately 90% of clinical isolates, and two serotypes, CP5 and CP8, account for approximately 75% of isolates recovered from humans [18]. The main function of capsules in staphylococcal virulence is to impede phagocytosis by neutrophils, but it has also been shown to enhance bacterial colonization and persistence on mucosal surfaces [18]. *S. aureus* golden pigment, staphyloxanthin, also functions to resist killing by neutrophils (reactive oxidant-based phagocytosis) [19]. Inhibition of staphyloxanthin biosynthesis allows *S. aureus* to become more vulnerable to innate immune clearance, and resulting in a significant reduction of *S. aureus* bacterial load in the kidney in a mouse model with intraperitoneal bacteria challenge [19]. MSCRAMMs, such as clumping factors (Clf), fibronectin-binding proteins (FnBP), collagen adhesion and protein A, play important roles in microbial adhesion to host proteins (i.e., fibronectin, fibrinogen and collagen) and establish the first step of an infection. These proteins also prevent the organism from recognition by the host immune system [20]. For example, Clf and FnBP are able to cause platelet activation, which results in clotting. Protein A binds to the Fc portion of immunoglobulin to prevent opsonization.

Secreted factors: lipases, cytolysins, superantigens & proteases

In contrast to the protective and passive role of cell wall-associated virulence factors, secreted *S. aureus* virulence factors play active roles in disarming host immunity by disrupting host cells and tissues and interfering with the host immune system to release nutrients and facilitate bacteria dissemination. The secreted virulence factors are comprised of four main categories: superantigens, pore-forming toxins, various exoenzymes and miscellaneous proteins (Table 1).

Superantigens: staphylococcal enterotoxins & TSS toxin-1

Superantigens are a group of *S. aureus* exotoxins capable of inducing a variety of human diseases, including TSS. More than 20 different superantigens have been identified from *S. aureus* isolates, including staphylococcal enterotoxins (SEs), enterotoxin-like proteins (SE-Is), and TSS toxin-1 (TSST-1). More than 60% of clinical *S. aureus* isolates carry at least one superantigen [21,22]. These superantigens are able to activate T lymphocytes and

antigen-presenting cells (APCs), such as macrophages or dendritic cells, nonspecifically by cross-linking V β regions of the T-cell receptor (TCR) and MHC class II molecules of the APCs in a nonantigen-specific manner (Figure 1) [23]. As a result, superantigens are able to activate 5–30% of T cells (compared with 0.001% by a normal antigen), which induces a massive release of cytokines and chemokines from both T cells and APCs and causes the symptoms observed in staphylococcal TSS [23]. Clinical features of TSS include fever ($\geq 38.9^{\circ}\text{C}$), rash, late desquamation of the palms of the hands and feet (1–2 weeks after disease onset), hypotension, and multiorgan dysfunction, which is acute and potentially life threatening (Table 2).

Based on the site of infection, TSS can be divided into two categories, menstrual and non-menstrual TSS. Menstrual TSS usually occurs within 2 days after the initiation of menstruation or within 2 days after completion of menstruation, and it is associated with tampon usage in women colonized vaginally by superantigen-producing *S. aureus*. TSST-1 is responsible for more than 90% of menstrual TSS [24]. Non-menstrual TSS occurs as a complication of *S. aureus* infections after surgical procedures, burns or post-influenza pneumonia. TSST-1 is responsible for approximately half of non-menstrual TSS cases, and SEC and SEB are responsible for the majority of the remaining cases [16,24]. The prevalence of the TSST-1 gene (*tst*) is approximately 25% in all clinical isolates, but is carried by more than 80% of USA200 isolates, whereas the prevalence of SEB and SEC is only approximately 10% in clinical isolates, with USA400 isolates producing either SEB or SEC [20].

Absence or an insufficient amount of neutralizing antibodies is a risk factor for TSS; therefore, children and young women are especially at risk of developing TSS. Recurrent TSS, both menstrual and non-menstrual TSS, has been reported in patients who failed to develop neutralizing antibodies [24,25]. The incidence of menstrual TSS is estimated to be approximately one per 100,000 women of menstrual ages (15–44 years old) in the USA [26]. A surveillance study conducted in the Minneapolis–St Paul area during 2000–2003 suggested a local increase of TSS (including both menstrual and non-menstrual TSS), which rose from 0.9 (in 2000) to 3.4 (in 2003) cases per 100,000 women of menstrual age per year [27]. Based on a study comparing superantigen profiles of *S. aureus* vaginal colonizing isolates from 1980 and 1981 to 2003–2005 in the Minneapolis–St Paul area, the increased incidence of TSS in the above study was most likely due to the increase in the prevalence of vaginal *S. aureus* (from 12 to 23%) or non-menstrual TSS associated cases, instead of an increasing proportion of TSST-1⁺ isolates in vaginal colonization strains [22]. A French surveillance study collecting 55 TSS cases over 30 months during 2003 to 2006 suggested that non-menstrual staphylococcal TSS is associated with more severe neurological disorders and higher mortality rates and non-menstrual staphylococcal TSS may be more prevalent than menstrual TSS [28]. Since the diagnosis of TSS relies on clinical symptoms, which may vary significantly due to patient sensitivity and/or other underlying conditions, the prevalence of TSS may be under-reported.

Superantigens can also serve as allergens to stimulate IgE production (via T-cell dependent B-cell activation), mast cell activation and local inflammation [29]. Additionally, superantigens have also been shown to induce steroid resistance via activating MAPK cascades and inducing expression of glucocorticoid receptor GR β [30,31]. Therefore, superantigens have also been associated with autoimmune diseases, such as Kawasaki syndrome [32,33], rheumatoid arthritis [29], psoriasis [34] and atopic dermatitis [35,36], nasal polyps [37], and asthma [38]. For most of these diseases, the mechanism of superantigen involvement has not been characterized. However, there is no doubt that these toxins function (in many ways) to disturb host immune response to ensure the survival and persistence of *S. aureus* in host niches.

Superantigens may also have direct effects on other cells to facilitate *S. aureus* pathogenesis on mucosal surfaces or skin. TSST-1 was determined to have dose-dependent proinflammatory and cytotoxic effects on endothelial cells, which may facilitate capillary leakage and cause TSS [39]. TSST-1 is also proinflammatory to epithelial cells, which stimulates recruitment of neutrophils that may lead to tissue destruction and increased permeability of the mucosal tissue [40].

Superantigens are pyrogenic and capable of enhancing sensitivity of rabbits to endotoxin (lipopolysaccharides). Therefore, they have been suggested to play a role in sepsis where a person may become infected with Gram-negative and *S. aureus* organisms [41]. Some superantigens, namely SEs, can also induce emesis and diarrhea when ingested and SEs are a common cause of food poisoning, where SE-induced inflammation in abdominal viscera has been suggested to induce the symptoms [41]. However, no intestinal epithelial receptor for SEs has been identified.

Cytotoxins

S. aureus secrete a large number of pore-forming toxins, including cytolysins (α -, β -, γ -, and δ -toxins), leukocidin family (leukocidin, LukD/E, LukM and PVL), and phenol-soluble modulins (PSMs). Although these toxins are structurally diverse and have various target specificity (i.e., erythrocytes, leukocytes and epithelial cells), their function on host cells is similar. They form pores in the membranes of target cells and cause leakage (osmotic swelling) and inflammation of the cells at low doses, and cell lysis at high doses.

α -toxin is the most well-characterized cytolysin. The gene encoding α -toxin, *hla*, is carried by virtually all clinical isolates; however, some strains, mostly vaginal TSS isolates, do not produce grossly detectable α -toxin due to a silencing point mutation in the gene structure (pseudogene) [41–43]. α -toxin is secreted as a water-soluble monomer by *S. aureus* and engages surface receptors of sensitive host cells (such as erythrocytes, lymphocytes, macrophages and epithelial cells), where they assemble into cylindrical heptamers (1–2 nm wide) in cell membranes [41]. α -toxin has a wide-spectrum of activity against various cells, including erythrocytes, leukocytes (i.e., monocytes, macrophages and polymorphonuclear [PMN] cells), platelets, epithelial cells and fibroblasts [44]. The toxin has been shown to be hemolytic, dermonecrotic, neurotoxic and lethal when injected intravenously. However, the significance of α -toxin in human disease has not been established conclusively. α -toxin is highly dermonecrotic in humans and is thus linked to furuncles [41]. The role of α -toxin in necrotizing pneumonia was determined recently using a murine model [15]. The mortality rate of mice challenged with high dose ($\sim 10^8$ colony forming units [cfu]) of CA-MRSA isolates were shown to be correlated with the concentration of α -toxin secreted by the strains. In addition, mice with α -toxin-specific antibodies were protected from lung tissue damage and death from pneumonia despite no significant change (~ 1 -log decrease) in bacterial load in the lung tissues. In addition, the proinflammatory activity of low concentrations of α -toxin (5 $\mu\text{g}/\text{ml}$) to epithelial cells was observed to increase mucosal permeability and significantly facilitate TSST-1 penetration through multi-layered porcine vaginal mucosa, a tissue model that physiologically resembles human vaginal mucosa [16].

β -toxin (sphingomyelinase C) is the only cytolysin with a known enzymatic mode of action, which is to specifically hydrolyze its target, sphingomyelin [45,46]. Therefore, the sensitivity of human cells to the toxicity of β -toxin depends upon the distribution of sphingomyelin on the membrane. β -toxin is cytotoxic to monocytes, erythrocytes, neutrophils and lymphocytes (especially proliferating T-cells) [45]. The toxin may also contribute to *S. aureus* immune invasion by inhibiting the production of endothelial IL-8, which activates neutrophil transmigration [47]. Additionally, a recent study identified a

unique role for β -toxin as a catalyst in the formation of nucleoprotein matrix in staphylococcal biofilms [48].

The cytolysin, γ -toxin and the leukocidin family, including PVL, leukocidin E/D and leukocidin M/F-PV, are bi-component toxins. Each of these toxins is composed of two separately secreted proteins, referred to as fast-eluting (F) and slow-eluting (S) components. Amino acid sequences of the S-components (HlgA, HlgC, LukM, LukE, LukS-PV) and F-components (HlgB, LukD, LukF-PV) of these bi-component toxins share approximately 70% (ranging 55–81%) sequence identity within groups (S or F) and 30% between groups (S vs F) [44,49]. These subcomponents (S or F proteins) alone do not have biological activity, but they can assemble with one another (S + F) to form heptamers (with stoichiometry of 3:4 or 4:3) or hexamers (3:3). γ -toxin (composed of HlgA or HlgC, and HlgB) lyses both leukocytes and erythrocytes, while PVL (composed of LukS-PV and LukF-PV) is more toxic to leukocytes than red blood cells [49]. These toxins were shown to induce different levels of inflammatory reactions when injected into the rabbit eye vitreous humor [50] and rabbit skin [51]. γ -toxin is produced by more than 90% of clinical isolates, whereas PVL (<5%) and other leukocidins, such as LukE/D (~30%), are much less prevalent [20]. LukM/F-PV is seldom isolated from *S. aureus* in humans [44].

Although only detected in less than 5% of all clinical isolates, PVL is epidemiologically associated with CA-MRSA isolates [52]. However, whether or not PVL is responsible for the virulence of CA-MRSA isolates is debatable because of conflicting results in animal models. Labandeira-Rey *et al.* indicated that PVL is sufficient to cause necrotizing pneumonia by comparing clinical pneumonia strains, isogenic PVL-positive and PVL-negative *S. aureus* laboratory strains, and purified PVL in a mouse pneumonia model [53]. Voyich and colleagues, however, concluded that PVL is not an important virulence factor based on their observation comparing PVL-positive strains to genetically close PVL-negative isolates and their own isogenic PVL-knockout strains using mouse sepsis and abscess models; the PVL-positive and PVL-negative strains were comparable at the levels of mortality and skin damage [54]. Presently, whether or not PVL, as well as other cytolysins, play clinically relevant roles in *S. aureus* etiology in humans remains to be determined.

δ -toxin is a single 26-amino-acid α -helix peptide, which is capable of lysing human erythrocytes, neutrophils, as well as various mammalian cells via its amphipathic (surfactant) activity [41]. δ -toxin also serves as the effector protein of the accessory gene regulator (Agr), which is a *S. aureus* global virulence regulatory system. The toxin is produced by 97% of *S. aureus* isolates. The role of δ -toxin in *S. aureus* pathogenesis remains unclear; however, a recent study by Wang *et al.* suggested that δ -toxin and structurally similar peptides, PSMs, may be responsible for the virulence of CA-MRSA isolates [55]. The authors determined that CA-MRSA isolates produce high amounts of PSMs (including δ -toxin) and isogenic PSM deletion strains, especially PSM α -deletion isolates, were less virulent than their parental strains in mouse bacteremia and skin infection models [55].

S. aureus secrete multiple extracellular enzymes, including a glycerol ester hydrolase (lipase), a nuclease, a hyaluronidase, a staphylokinase, and multiple proteases, including serine proteases, cysteine proteases, aureolysin (metalloenzymes) and staphopains, which presumably function to disrupt host tissues and/or inactivate host antimicrobial mechanisms such as lipids, defensins, antibodies and complement mediators, and thereby facilitate bacterial dissemination. The clinical significance of these enzymes has not been well characterized.

Exfoliative toxins (ETA and ETB) are also a type of serine proteases (although not as prevalent as other extracellular proteases). Different from other proteases, ETs have been indicated as major toxins responsible for bullous impetigo and staphylococcal scalded skin syndrome [56].

S. aureus also secrete chemotaxis inhibitory protein of *S. aureus* (CHIPS) [20], staphylococcal inhibitor of complement (SCIN) [57], extracellular adherence protein (Eap) [58], and staphylococcal superantigen-like (SSL) proteins. More than ten SSLs have been identified, based on their structure similarity to superantigens. SSLs do not have superantigenicity but rather display a wide array of activities targeting key elements in the innate immune system [59].

Treatment & prevention

Antimicrobials: resistance & effects on exotoxins

Anti-staphylococcal antibiotics target multiple pathways that are essential for bacterial survival including bacterial cell-wall synthesis (i.e. β -lactams, glycopeptides), folic acid metabolism (sulfonamides), and bacterial protein synthesis (i.e., macrolides, lincosamides and aminoglycosides) (Table 3). As an extension of its ability to adapt to ecological niches, *S. aureus* is excellent in developing resistance mechanisms to antibiotics, either by acquiring genes encoding enzymes that inactivate the antibiotics (penicillinases, β -lactamases and aminoglycoside-modification enzymes), altering target molecules (PBP2a of MRSA and D-ala-D-Lac of peptidoglycan precursors of vancomycin-resistant *S. aureus*), limiting access of the antibiotics to their targets via thickening of the cell wall structures (vancomycin-intermediate resistant and possibly daptomycin-resistant strains), and/or increasing active efflux of the antibiotic from inside the cell (fluoroquinolones, macrolides and tetracycline), to ensure its survival [60].

β -lactams are first-line therapy for MSSA infections. β -lactam antibiotics bind to penicillin-binding-proteins (PBP) and inhibit cell wall biosynthesis. When β -lactam antibiotic concentrations are above the MIC of the organisms they are bactericidal with a rapid rate of killing. However, subgrowth inhibitory concentrations of β -lactams have been shown to induce significantly the expression of α -toxin, PVL and TSST-1 by *S. aureus in vitro* [17,61]. This observation implies that inadequate use of β -lactams, for example, in the case of MRSA infections, may enhance tissue damage associated with the infection by increased production of exotoxins.

By contrast, vancomycin, the current drug of choice for MRSA infections, has minimal effect on the production of virulence factors [17]. Vancomycin inhibits bacterial cell wall biosynthesis by forming complexes with the D-ala-D-ala portion of peptide precursor unit to prevent the cross-linking of the cell wall peptidoglycan. Given vancomycin binds directly to the cell wall compared with the inhibition of cross-linking enzymes, such as β -lactams, there is no cross-resistance between the two antibiotics [62]. However, vancomycin has its limitations owing to an overall decrease in MRSA susceptibility. Therefore, experts recently published practice guidelines for the appropriate use of vancomycin [63]. They advised that the area under the curve:minimum inhibitory concentration (AUC:MIC) ratio was the most useful pharmacodynamic parameter to predict vancomycin effectiveness and suggested a target AUC:MIC ratio of 400 or greater to treat *S. aureus*. In addition, trough serum concentration monitoring was suggested as the most accurate method to monitor vancomycin serum levels. The guidelines suggest increasing vancomycin trough concentrations to 15–20 mg/l (from 5 to 15 mg/l) to attain the targeted AUC:MIC ratio of 400 with increased monitoring for nephrotoxicity. In the event of vancomycin failure or resistance (i.e., MIC > 2 mg/l) other antibiotics, such as aminoglycosides, macrolides,

clindamycin, Synercid[®] (quinupristin and dalfopristin), daptomycin, tetracyclines, linezolid, doxycycline, tigecycline and/or trimethoprim–sulfamethoxazole (TMP–SMX), are recommended [63,64]. The ability of the antibiotic to inhibit protein synthesis should also be considered in the selection of antimicrobial agents to treat infections caused by toxin-producing Gram-positive pathogens [17].

Antibiotics that inhibit protein synthesis are known to have some degree of antivirulence effects. Macrolides bind reversibly to 23S ribosomal RNA (rRNA) of the 50S subunit of bacterial ribosome inhibiting RNA-dependent protein synthesis. Clindamycin (a lincosamide) at subgrowth inhibitory concentrations was able to inhibit almost completely the translation of α -toxin, PVL and/or TSST-1 [17,65], and subinhibitory concentrations of erythromycin (a macrolide) or aminoglycosides partially reduced α -toxin expression [61,66]. Linezolid (an oxazolidinone), a synthetic protein inhibitor that binds to the 50S ribosome to prevent the formation of the 70S initiation complex, is also capable of repressing toxin production similar to clindamycin, and has been reported to treat a staphylococcal TSS case with good therapeutic outcome [17,67]. In addition, tetracyclines and macrolides have also been characterized for their anti-inflammatory effects *in vitro* and *in vivo*, independent of their antimicrobial effect [68,69]. Doxycycline (a tetracycline) was shown to inhibit superantigen-induced T-cell proliferation and cytokine and chemokine production by PMN cells *in vitro* [70]. Therefore, these antibiotics, especially tetracyclines, may be beneficial in the treatment of TSS and SSTIs caused by CA-MRSA in adults.

The development of anti-staphylococcal agents has predominately focused on the elimination of bacteria cells (antimicrobials). This strategy has neglected the contribution of secreted proteins to disease for decades. With the emergence of antibiotic-resistant *S. aureus* possessing multiple secreted virulence factors and decreased rate of antimicrobial development, alternative approaches to ameliorate *S. aureus* diseases and TSS are needed.

Intravenous immunoglobulin for TSS

In addition to antibiotics and supportive treatments, intravenous immunoglobulin (IVIG) is also used to treat TSS. IVIG contains a cocktail of pooled human antibodies derived from hundreds of donors, which are able to neutralize various bacterial toxins as well as modulate immune responses. IVIG was shown to significantly improve clinical manifestations within hours of administration in patients suffering from TSS (although most evidence was from group A streptococcal TSS), which was presumably due to the combination of its toxin neutralization and immunomodulation effects [71–74]. However, no strong evidence supports the benefit of IVIG as adjunctive therapy for severe sepsis and septic shock [75]. Meta-analysis studies indicate that IVIG decreases the mortality associated with sepsis and septic shock, but the clinical benefit may be minor due to lack of significant clinical improvement, high cost and limited supply [76,77]. The lack of treatment benefit with IVIG has been hypothesized to be due to low and inconsistent anti-toxin antibodies in different lots.

Experimental approaches to inhibit toxins/superantigens

Since most *S. aureus* infections begin on the skin or mucosal surfaces with direct inflammatory or cytotoxic effects of exotoxins, anti-staphylococcal therapies targeting toxins (neutralization), toxin production or the effects of toxins on mammalian cells could prevent or ameliorate *S. aureus* infections and TSS (Table 4).

Glycerol monolaurate

Glycerol monolaurate (GML) is a naturally derived lauric acid glycerol monoester commonly used in the food and cosmetic industries as an emulsifier and preservative. GML

was originally recognized for its ability to inhibit the growth of *S. aureus*, block the induction of β -lactamases, and delay the production of *S. aureus* exoproteins, such as TSST-1 and α -toxin via interfering with bacterial cell membrane signal transduction [78–80]. More recently, studies have shown that GML has immunomodulation effects on mammalian cells via membrane stabilization and thereby protects these cells and experimental animals from toxic effects (i.e., osmosis change and cell lysis) due to various bacterial toxins [81]. GML prevents human and rabbit erythrocytes from lysis by bacterial hemolysins, including purified α - and β -toxins, inhibits superantigen-stimulated proliferation of lymphocytes, and reduces the production of proinflammatory cytokines and chemokines by epithelial cells in response to *S. aureus* and purified TSST-1 [81,82]. GML, as vaginal gel, prevents lethality in rabbits challenged vaginally with purified TSST-1 [81]. The compound, however, is not stable in the presence of *S. aureus* and can be hydrolyzed by *S. aureus* esterase (lipase) into glycerol and lauric acid [78,80]. In an effort to overcome the limitation of inactivation, compounds with ether linkage have been suggested as potential alternatives to GML. A structurally similar ether compound, 1-*O*-dodecyl-*rac*-glycerol (DDG) was observed to have anti-staphylococcal effects, and to be more stable than GML to chemical and enzymatic hydrolysis; however, it was less effective than GML in preventing TSS *in vivo* in a rabbit Wiffleball model of TSS [83–85]. The ability to inhibit toxin production independent of growth inhibition was indicated to be a main reason why GML was more efficacious than DDG in preventing TSS in that model [85]. Given compounds like GML are inexpensive and easily accessible; they may provide clinical benefit as topical applications to prevent *S. aureus* infections and TSS.

Hemoglobin subunits

A pilot study to measure TSST-1 and α -toxin on used tampons from four healthy women with *S. aureus* on tampons and from two women with tampon-associated mTSS determined TSST-1 and α -toxin were present only in the sections containing little or no menstrual blood (low hemoglobin density) [86]. This finding led to the characterization of α - and β -globins of hemoglobin as inhibitors of *S. aureus* exotoxin production, including superantigens, cytolysins, and lipase, despite not affecting bacterial growth [87]. The hemoglobin subunits were suggested to target the two-component system, SrrA–SrrB, an oxygen sensing system that regulates exotoxin production of *S. aureus*. The structure that is responsible for the toxin inhibitory effect remains to be determined; however, this serves as a potential approach to prevent staphylococcal TSS.

High-affinity V β antagonists

Based on the concern of superantigens, especially SEB, as bioterrorism agents, high affinity (picomolar-affinity) V β antagonists that block the binding of SEB to T-cell receptors have been investigated as treatment for superantigen intoxication [88]. The engineered protein binds to the particular TCR V β 8 binding site on SEB with 3 million greater affinity than the wild-type V β 8.2:SEB interaction, and thereby prevents SEB-mediated T-cell activation. The peptide antagonist was determined to prevent lethality in rabbits administered SEB intravenously with the protective capacity of the V β agent being 2000-times greater than that of IVIG. A single-chain construct with both TSST-1 and SEB antagonists was shown to neutralize these superantigens *in vitro*, which shows the potentials of such a reagent to neutralize multiple clinically important superantigens at one time [89,90]. The efficacy of superantigen TCR antagonists in humans remains to be determined; however, this approach appears to be a promising treatment alternative to IVIG.

Vaccination

Currently, there is no US FDA-approved vaccine or immunotherapy to prevent or treat *S. aureus* diseases. Based on previous successes with other bacterial pathogens, most

companies have designed their vaccines by targeting a single cell wall-associated virulence factor (Table 5). The failure of these monovalent designs has been in part attributed to the functional redundancy of *S. aureus* virulence factors. *S. aureus* can easily down-regulate the targeted virulence factor and compensate its function using other virulence factors so that there is no significant change in pathogenicity. Multivalent strategies are therefore proposed. However, due to the limited understanding of the overall roles these proteins play in *S. aureus* pathogenesis, there is still a debate as to which protein(s) would provide clinically significant protection against *S. aureus* infections.

Vaccination against cell wall-associated staphylococcal components

Cell wall-associated staphylococcal components, such as CP, ClfA and ATP-binding transporters, were the focus of antistaphylococcal vaccines and immunotherapies (Table 5). In theory, these vaccines would elicit functional antibodies that would bind to the target antigen(s) on the bacterial surface and trigger complement activation, which in turn augments the process of uptake and killing of bacteria by phagocytic cells, such as neutrophils and macrophages. Despite promising experimental results in animal models (mostly murine models), this approach has not successfully provided effective clinical outcomes in Phase II and III human clinical trials [91]. Later studies using murine bacteremia and wound infection models indicated the expression of CP and ClfA are variable among strains, which may account for their failures as an antistaphylococcal vaccine [92]. A study testing passive immunization with antibodies to CP and ClfA in a mouse model of mastitis indicated that administration of either antibody alone significantly reduced the bacterial load, but induced the presence of stable unencapsulated *S. aureus* mutants and small colony variants, which are less susceptible to antibiotics. The emergence of the mutants was abrogated when both antibodies to CP and ClfA were administered together [93]. These studies suggested that a multi-antigen (multivalent) approach is probably necessary to ensure broad coverage against the versatile pathogen.

Despite previous studies suggesting cell wall-associated proteins are not ideal targets, the potential of the *S. aureus* surface protein, iron surface determinant B (IsdB; Merck *S. aureus* vaccine: V710), is being assessed currently as a prophylactic vaccine against *S. aureus* infection in patients scheduled for cardiothoracic surgery (NCT00518687). IsdB is an iron-sequestering protein that is conserved in diverse *S. aureus* clinical isolates, both methicillin-resistant and methicillin-sensitive, and it is expressed on the surface of all isolates tested. Vaccination against IsdB was highly immunogenic in mice and had significant protection in animal models of *S. aureus* infection [94]. A study to evaluate the safety and immunogenicity of V710 in adult patients with end-stage renal disease on chronic hemodialysis was completed recently; however, results have yet to be reported (NCT00572910). Other cell wall-associated vaccine targets with human clinical trials ongoing include MSCRAMMs (SA3Ag) by Pfizer and lipoteichoic acid, a humanized monoclonal antibody (pagibaximab by Biosynexus), which is being evaluated currently for safety and efficacy compared to placebo in preventing staphylococcal sepsis in very low birth weight infants (NCT00646399) [95].

Vaccination against exotoxins: cytolytins & superantigens

Vaccination with cytolytins and superantigens may provide several levels of protection. First, antibodies (i.e., secretory IgA) in the mucosa neutralize *S. aureus* toxic exoproteins before they damage host epithelial cells, which protects the integrity of mucosal barrier. Secondly, antibodies (i.e., IgG) in the serum neutralize toxins to prevent their ability to bind T cells and APCs, and the IgG facilitates bacterial clearance by host immune mechanisms. Third, antibodies against superantigens can prevent the toxin's function to alter proper immune response. Superantigens impede proper humoral response (antibody generation) to

the bacteria and also deplete certain subtypes of T-cells (via T-cell anergy); therefore, neutralizing superantigens allows the host to derive anti-staphylococcal immunity. Additionally, antibodies against superantigens directly prevent TSS, a relatively rare but serious staphylococcal disease. This anti-toxin approach may be especially important as passive immunization in high risk populations of *S. aureus* infections, that is, premature neonates, elderly and immunocompromised patients, who already lack suitable ability to develop proper immune response.

Active and passive immunization with the cytolysin, α -toxin, was shown to prevent mice from lethal staphylococcal pneumonia, despite minimal effect on the overall bacterial load in the lung tissue [15]. PVL was also investigated as a vaccine target against emerging staphylococcal infections caused by USA300 isolates. Vaccination with PVL subunits, especially LukS-PV, was shown to be protective in a murine pneumonia and skin model [96]. However, the role of PVL in USA300 pathogenesis is still in debate due to inconsistent results in various murine models [53,54,96,97]. Despite the controversy, it is possible that immunization against PVL would provide cross-protection against other staphylococcal cytolysins, such as γ -toxin and other leukocidins, due to their sequence homology. The safety and efficacy of recombinant α -toxin and LukS-PV as vaccine antigens is currently in progress (NCT01011335). In addition, vaccine studies with mutant SEB toxoid, which has lost the ability to bind MHC II yet has retained its immunogenicity (STEBVax) is being examined in Phase I trials for its ability to induce high SEB-neutralizing hypersera, to compensate for potentially low titers in pooled IVIG preparations, that can be used to treat TSS caused by SEB (NCT00974935).

Impact of *S. aureus* vaccination

As MRSA is a major cause of infections in hospitals and SSTIs in the community, an efficacious vaccine would have a substantial impact on mortality, morbidity and overall healthcare-associated costs. A study evaluated recently the overall optimal vaccination strategies, which would have the greatest impact on morbidity and mortality. Since an estimated 108,000 cases of invasive MRSA occur in the USA every year, if all persons 65 years of age or older were vaccinated and hospitalized persons 15–64 years of age were vaccinated at hospital discharge this would result in 19,030 cases being prevented (17.6% reduction) and prevent 4260 deaths (20.9% reduction) [98]. These estimates were calculated using moderate assumptions of efficacy (40%) and coverage (64%) in people 65 years of age or older and 60% efficacy and 25–45% coverage in people 15–64 years of age. Therefore, a *S. aureus* vaccine with 40–60% efficacy in a population at risk of an MRSA infection could still provide substantial benefit to society and a country's resources. The next 5–10 years will be important as *S. aureus* vaccine targets that provide optimal efficacy are researched and evaluated.

Expert commentary

As MRSA isolates currently account for more than 60% of nosocomial *S. aureus* infections in the ICUs and more than 70% of *S. aureus* SSTIs in the community, healthcare professionals are challenged with managing antimicrobial resistant strains. Complicating management is the spread of CA-MRSA isolates into the hospital setting. CA-MRSA isolates have different antimicrobial susceptibility profiles and differences in the overall exotoxin production, including superantigens, and the cytolysins, PVL and α -toxin.

Vancomycin is the most widely used antimicrobial agent for the treatment of MRSA infections; however, the antibiotic has its limitations due to use only as an intravenous formulation, moderate penetration into the sites of infection, nephrotoxicity associated with

higher doses and an overall increase in average MIC. Therefore, experts published practice guidelines for the appropriate use of vancomycin, suggesting increased trough concentrations to 15–20 mg/l to attain the targeted AUC:MIC ratio of 400 with increased monitoring for nephrotoxicity. Alternative therapies were recommended in patients with *S. aureus* infections that demonstrate a vancomycin MIC of 2 mg/l or greater because the target AUC: MIC ratio (400) is unlikely to be achieved at the site of infection. Alternative and newer therapies include clindamycin, linezolid, daptomycin, tigecycline, telavancin, tetracyclines and TMP–SMX with or without rifampin. The selection of the appropriate antimicrobial agent is highly dependent on the antimicrobial susceptibility profiles as well as site of infections and cost constrains. As mentioned throughout this article, the importance of exotoxins (especially superantigens and cytolysins) in the pathogenesis of *S. aureus* diseases has received increased research attention over the past several years and antimicrobials or therapeutics that inhibit protein synthesis (such as clindamycin or linezolid) or neutralize exotoxins (such as IVIG) are important additions to consider in the management of *S. aureus* infections.

Five-year view

We speculate that the field of *S. aureus* and TSS research will evolve considerably over the next 5–10 years. With several (active and passive) vaccine candidates in clinical trials currently, it is possible a *S. aureus* vaccine may be available for individuals at risk by the end of the next decade. The prevalence of MRSA in the hospital and community setting will most likely continue to rise, which will increase the need for real-time diagnostics to inform the clinician of antimicrobial resistance and the presence of specific superantigens or cytolysins so optimal therapy can be utilized. Owing to continued increase in resistance and availability of newer antibiotics with less toxicity, vancomycin will most likely no longer be the antimicrobial of choice for MRSA infections in the hospital setting.

Therapeutics targeted at the effects of exotoxins on mucosal & skin surfaces

Despite these advances, the study of the pathogenesis of *S. aureus* will continue to evolve with an overall focus on the role of exotoxins and their effects on mucosal and skin surfaces, that is, the site of infection. Recently, a study described the role of metalloprotease 10 (ADAM10) on host cells, which interacts with α -toxin and is required to initiate the cytolytic pore and inflammatory effects of α -toxin on mammalian cells [99]. This is the first study identifying a host receptor for α -toxin, despite decades of research describing the effects of this toxin on mammalian cells, and this finding will most likely lead to new therapeutic anti-staphylococcal agents. Likewise, superantigens have been reported to induce changes in epithelial cellular morphology and secretion of proinflammatory cytokines/chemokines from vaginal, bronchial, nasal and intestinal cells [100–103]. Although the host receptor(s) for superantigens has yet to be determined, the ability of a topical agent, GML, to reduce exotoxin production and overall proinflammatory cytokines at the site of infection and increase survival in a rabbit model of TSS [85], suggests anti-inflammatory effects on mucosal surfaces are an important consideration in the development of therapeutics.

An overall understanding of the exoproteins most important in the ability of *S. aureus* to cause infection and the corresponding host immunological responses (i.e., mucosal response) is critical and will open new areas of research. New therapeutic agents are being developed currently to support this area of research, including anti-toxin production and neutralizing agents, and/or receptor antagonists, mucosal anti-inflammatory agents and vaccines derived from toxoids.

Key issues

- Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates account for more than 60% of nosocomial *S. aureus* infections in the intensive care units and more than 70% of *S. aureus* skin and soft-tissue infections in the community.
- Hospital acquired (HA)-MRSA and community acquired (CA)-MRSA isolates have distinct exotoxin profiles. Panton–Valentine leukocidin (PVL) is epidemiologically associated with CA-MRSA isolates. Staphylococcal enterotoxin B (SEB) and SEC, have also been associated with CA-MRSA.
- *S. aureus* produces an array of virulence factors to facilitate its pathogenesis. Initially researchers focused on the role of cell surface virulence factors, such as capsule; however, recently researchers have started to recognize the overall importance of exoproteins, such as cytolysins and superantigens, in the initiation and progression of infections via direct tissue damage to mucosal membranes and skin.
- Antibiotics of choice for treating *S. aureus*-related illnesses, β -lactams for methicillin-susceptible *S. aureus* (MSSA) and vancomycin for MRSA, kill the organism by lysis of the cell wall or inhibit cell wall biosynthesis, but are not able to inhibit *S. aureus* exoprotein production or neutralize the existing toxins' effects on β -lactams may host cells. even induce production of cytolysins and other virulence-related exoproteins when inadequately used for treating MRSA, which potentially worsens clinical outcomes.
- Protein-synthesis inhibition is important in the selection of antimicrobial agents to treat infections caused by toxin-producing Gram-positive pathogens. Linezolid and clindamycin have proven efficacious in reducing exotoxin production even at inhibitory concentrations.
- Intravenous immunoglobulin (IVIG) has been used to treat toxic shock syndrome due to its ability to neutralize clinical benefits of such treatment in staphylococcal toxic shock syndrome (TSS) may be unproven. New anti-toxin therapeutic approaches such as hypersera, V β antagonists, hemoglobin subunit inhibitors and glycerol monolaurate are in development for TSS prevention and as treatments.
- Vaccination targets to date have been cell-surface targets, including iron-regulated surface determinant B (IsdB), but exotoxins (PVL and α -toxin) and the superantigen (SEB) are being evaluated in clinical trials as vaccine targets.
- Future areas of research include a focus on the exotoxins such as cytolysins and superantigens and their effects on mucosal surfaces; mucosal receptor antagonists, anti-inflammatory agents and a multivalent approach to vaccination that includes the cytolysins and superantigens.

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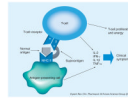


Figure 1. Superantigen mechanism of action

Superantigens bind to MHC class II molecules of antigen presenting cells (i.e., macrophages) and V β region of T-cell receptor in a non-antigen-specific manner, which leads to massive release of cytokines and chemokines, as well as the clonal expansion of certain clonal types of T cells.

Table 1Secreted *Staphylococcus aureus* virulence factors.

Secreted virulence factor	Putative function
Toxic shock syndrome toxin 1; staphylococcal enterotoxins; staphylococcal enterotoxin-like toxins	Activate T cells and macrophages
Cytolysins (α -, β -, γ -, δ -toxins); phenol- soluble modulins-like peptides; leukocidins (PVL, LukD/E)	Induce apoptosis (at low concentration) and lysis of various cell types, including erythrocytes, lymphocytes, monocytes, epithelial cells; target specificity varies
Lipase	Inactivate fatty acids
Hyaluronidase	Degradation of hyaluronic acid
Serine proteases; cysteine proteases (including staphopains); aureolysin	Inactivate neutrophil proteolytic activity; inactivate antimicrobial peptides
Staphylokinase	Plasminogen activation; inactivate antimicrobial peptides
Exfoliative toxins	Act as serine proteases; activate T cells
Chemotaxis inhibitory protein of <i>Staphylococcus aureus</i> ; Staphylococcal inhibitor of complement	Inhibit complement
Staphylococcal superantigen-like proteins; extracellular adherence protein	Inhibit complement C5 and IgA; inhibit neutrophil migration

PVL: Pantón–Valentine leukocidin

Table 2

CDC case definition of staphylococcal toxic shock syndrome.

Criteria	Definition
<i>Clinical</i>	
Fever	Temperature greater than or equal to 102.0°F (≥38.9°C)
Rash	Diffuse macular erythroderma
Desquamation	1–2 weeks after onset of illness, particularly on the palms and soles
Hypotension	Systolic blood pressure ≤90 mmHg for adults or <5th percentile by age for children aged <16 years; orthostatic drop in diastolic blood pressure ≥15 mmHg from lying to sitting, orthostatic syncope or orthostatic dizziness
<i>Multisystem involvement (≥3 organ systems)</i>	
Gastrointestinal	Vomiting or diarrhea at onset of illness
Muscular	Severe myalgia or creatine phosphokinase level at least twice the upper limit of normal
Mucosal	Mucous membrane: vaginal, oropharyngeal or conjunctival hyperemia
Renal	Blood urea nitrogen or creatinine at least twice the upper limit of normal for laboratory or urinary sediment with pyuria (≥5 leukocytes per high-power field) in the absence of urinary tract infection
Hepatic	Total bilirubin, alanine aminotransferase enzyme or spirate aminotransferase enzyme levels at least twice the upper limit of normal for laboratory
Hematologic	Platelets <100,000/mm ³
CNS	Disorientation or alterations in consciousness without focal neurologic signs when fever and hypotension are absent
<i>Laboratory</i>	
Culture	If obtained, negative results on blood, throat, or cerebrospinal fluid cultures (blood culture may be positive for <i>Staphylococcus aureus</i>)
Titer	If obtained, no rise in titer to Rocky Mountain spotted fever, leptospirosis or measles
<i>Case classification</i>	
Probable	A case which meets the laboratory criteria and in which four of the five clinical findings described above are present
Confirmed	A case which meets the laboratory criteria and in which all five of the clinical findings described above are present, including desquamation, unless the patient dies before desquamation occurs

Data from [201].

Table 3

Antistaphylococcal therapies and effects on exotoxins.

Classification	Antimicrobial agents	Mechanism of action	Main resistance mechanism(s)	Effect on exotoxin at subgrowth inhibitory concentrations
β -lactam	Methicillin, oxacillin and cephalosporins	Inhibits cell wall biosynthesis	β -lactamase (penicillinase); modified penicillin binding protein PBP2a (<i>mecA</i>)	\uparrow
Glycoprotein	Vancomycin	Inhibits cell wall synthesis by inhibiting the incorporation of newly synthesized precursors in the cell wall peptidoglycan	Thickened cell wall that traps vancomycin (increased and structurally varied pentapeptides); change of binding site (D-ala-D-ala to D-ala-D-Lac)	\leftrightarrow
Lipopeptide	Daptomycin	Depolarization of the membrane	Decreased outer membrane permeability	NA
Macrolide	Erythromycin, clarithromycin and azithromycin	Reversibly binds to 23S ribosomal RNA (rRNA) of the 50S subunit of bacterial ribosome inhibiting RNA-dependent protein synthesis	A methylase that dimethylates an adenine within the 23S ribosomal binding site of the macrolides (<i>erm</i> ; inducible); an efflux pump (<i>mef</i> [E]) that expels the macrolides	\downarrow
Lincosamide	Clindamycin	Binds to the 50S ribosome and inhibits protein synthesis	<i>erm</i> ; cross-resistance to macrolides	$\downarrow\downarrow$
Oxazolidinone	Linezolid	Binds to 23S rRNA of the 50S ribosomal subunits and prevents formation of the 70S initiation complex	Mutation(s) in the 23S rRNA	$\downarrow\downarrow$
Streptogramin	Quinupristin/dalfopristin	Inhibits protein synthesis	<i>erm</i> ; cross-resistance to macrolides and clindamycin	NA
Aminoglycoside	Gentamicin, streptomycin, kanamycin and amikacin	Binds to the 30S ribosome and inhibits protein synthesis	Aminoglycoside-modifying enzymes	\downarrow
Tetracycline	Tetracycline, minocycline, doxycycline and tigecycline	Binds to the 30S ribosome and inhibits protein synthesis	Efflux transporters	\downarrow
Sulfonamide	Trimethoprim sulfamethoxazole	Inhibits folic acids biosynthesis	Single amino acid substitution in dihydrofolate reductase	NA

NA: Not available.

Table 4

Experimental agents for prevention of staphylococcal TSS/exoprotein toxicity.

Reagent	Mechanisms of action	Supporting evidence
GML	Inhibit the growth of <i>Staphylococcus aureus</i> Delay the production of <i>S. aureus</i> exoproteins Immunomodulation effects on mammalian cells via membrane stabilization	<i>In vitro</i> : GML reduces the production of proinflammatory cytokines and chemokines by epithelial cells in response to <i>S. aureus</i> and purified TSST-1 [81,82] <i>In vivo</i> : GML, as 5% vaginal gel, prevents lethality in rabbits challenged vaginally with purified TSST-1 [81] Tampon coated with GML reduces <i>S. aureus</i> growth, exotoxin production and vaginal IL-8 secretion [104]
Hemoglobin subunit inhibitors	Target two-component and quorum sensing systems to inhibit exoprotein production [87]	<i>In vitro</i> : mixtures of α and β hemoglobin ($\geq 1 \mu\text{g/ml}$) inhibit <i>S. aureus</i> exoproteins [87] <i>In vivo</i> : TSST-1 and α -toxin were only detected in tampon sections containing little or no menstrual blood, despite the high bacterial counts [86]
V β peptides (SEB antagonists)	A synthesized immunoglobulin-like peptide competes with the particular TCR binding site to prevent SEB-mediated T-cell activation and lethality in rabbits intravenously administered with SEB [88]	<i>In vivo</i> : rabbits injected with V β protein (dose $\geq 32.5 \mu\text{g/kg}$) survived through endotoxin enhancement model of TSS [88] The protective capacity of the V β agent was 2000-times greater than that of IVIG

GML: Glycerol monolaurate.

Table 5

Experimental staphylococcal vaccines and immunotherapies in clinical trials.

Bacterial target	Putative role in virulence	Proposed strategy (product/company)	Trial conclusion
Capsule polysaccharide (CP5 and CP8)	Avoidance of phagocytosis	Active immunization (StaphVAX [®] /Nabi)	Vaccine efficacy (57%) only last up to 40 weeks after immunization in end-stage renal disease (ESRD) hemodialysis patients (n = 1804). However, no significant protection was detected against bacteria in a confirmatory follow-up trial (n = 3600)
		Human polyclonal antiserum (Altastaph [®] /Nabi)	No reduction in preventing <i>Staphylococcus aureus</i> bacteremia in very low-birth weight (<1500 g) neonates (n = 206) No treatment effect in children ≥7 years with <i>S. aureus</i> bacteremia and persistent fever (n = 40)
Clumping factor A	Attachment to fibrinogen and biomaterial surfaces	Selected IVIG (INH-A21; Veronate [®] /Inhibitex)	No prevention in mortality or the rates of late-onset sepsis in infants (n = 1983)
		Humanized monoclonal antibody (tefibazumab; Aurexis [®] /Inhibitex)	No difference in composite clinical end points (a relapse, a complication, or death of bacteria) in hospitalized adult patients with bacteremia as an add-on therapy to standard therapy (n = 63)
ATP-binding cassette transporter	Nutrient uptake and cell attachment	Human-derived single-chain variable antibody fragment (Aurograb [®] /NeuTec)	Lack of efficacy as add-on therapy to vancomycin to treat deep-seated MRSA infections in Phase II trial (development terminated)
Lipoteichoic acid [†]	Bacteria cell wall component	Humanized monoclonal antibody (pagibaximab/Biosynexus)	Pilot study showed reduced staphylococcal sepsis rate in very low-birth weight neonates (n = 88) Safety and efficacy for prevention of staphylococcal sepsis in very low birth weight

Bacterial target	Putative role in virulence	Proposed strategy (product/company)	Trial conclusion
			neonates (Phase II/III) is in progress (NCT00646399)
Iron-regulated surface determinant B [†]	Iron uptake	Active immunization (V710/Merck)	Safety and immunogenicity study in 198 patients with ESRD on chronic hemodialysis was completed February 2010 Safety and efficacy in prevention of serious <i>S. aureus</i> infections in adult patients within 90 days after selective cardiothoracic surgery is in process (NCT00518687)
SA3Ag [†]	MSCRAMMs	Active immunization (Pfizer)	Phase I trial is in progress (NCT01018641)
<i>Staphylococcus aureus</i> toxoids (α -toxin and LukS-PV) [†]	Staphylococcal cytotoxic exoproteins	Active immunization (Nabi)	Phase I/II trial is in progress (NCT01011335)
Staphylococcal enterotoxin B [†]	Toxic shock syndrome	Active immunization (STEBVax/NIAID)	Phase I is in progress (NCT00974935)

[†]There are active clinical trials investigating the product.

MSCRAMM: Microbial surface components recognizing adhesive matrix molecules; NIAID: The National Institute of Allergy and Infectious

Data from [106,202].