An Update on Clinical Burden, Diagnostic Tools, and Therapeutic Options of *Staphylococcus aureus*

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ABSTRACT: *Staphylococcus aureus* is an important pathogen responsible for a variety of diseases ranging from mild skin and soft tissue infections, food poisoning to highly serious diseases such as osteomyelitis, endocarditis, and toxic shock syndrome. Proper diagnosis of pathogen and virulence factors is important for providing timely intervention in the therapy. Owing to the invasive nature of infections and the limited treatment options due to rampant spread of antibiotic-resistant strains, the trend for development of vaccines and antibody therapy is increasing at rapid rate than development of new antibiotics. In this article, we have discussed elaborately about the host-pathogen interactions, clinical burden due to *S aureus* infections, status of diagnostic tools, and treatment options in terms of prophylaxis and therapy.

KEYWORDS: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, MRSA, detection, antibiotic resistance, vaccine, antibody therapy

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Introduction

Staphylococcus aureus is the leading cause of bacterial infections involving gastrointestinal, respiratory, skin and soft tissue, and blood stream infections. It is the leading cause of human disease not only in hospitalized individuals but also in individuals living in community and responsible for a variety of diseases ranging from mild skin and soft tissue suppurative (pus-forming) infections, food poisoning to highly serious diseases such as osteomyelitis, endocarditis, and toxic shock syndrome (TSS). The threat of antibiotic resistance in *S aureus* has risen enormously for several years and the health costs have increased dramatically. Different figures were provided by different nations regarding annual mortality due to antibiotic resistance with 22 000 extra deaths in the United States, 25 000 in Europe, and 12 500 in France.[1] Mortality due to methicillin-resistant *Staphylococcus aureus* (MRSA) is the leading cause of mortality due to bacterial infections, and the number of serious infections due to resistant strains has decreased in recent years. The pathogenesis of staphylococcal infections is multifactorial. However, there is some correlation to the presence of certain virulence factors with a particular disease. Therefore, timely detection of these virulence factors is crucial for undertaking appropriate therapeutic interventions. Molecular methods play an important role in detection and differentiation of pathogens. Numerous techniques have been reported for detection of *S aureus* virulence factors such as antibody,[2–5] polymerase chain reaction (PCR),[6,7] real-time PCRs (RT-PCRs),[8] and aptamer-based[9–11] methods. Many other sensitive methods such as immuno-PCRs,[12] mass spectrometric analysis,[13] and biosensor techniques[14,15] are also reported.

Despite its high incidence and frequency of causing life-threatening and drug-resistant infections, there is no successful vaccine to prevent *S aureus* infections. The initial efforts to develop a staphylococcal vaccine that targeted the capsular polysaccharides similar in line with other bacterial pathogens have not been met with success. However, vaccine therapies still hold great promise in broadening the available clinical tools against the global menace of antibiotic-resistant *S aureus* infections. Antibodies directed against the virulence determinants could neutralize these components and hence may help in reducing the severity of infection. Because toxins are prominent virulence determinants, targeting them and providing the antibodies as passive therapy might render the infections less invasive. The antigens which could induce both humoral and cell-mediated memory immune responses that might prevent the recurring infections elaborated. In this review, an updated information about *S aureus* virulence factors, pathogenesis, clinical burden, recent advances in *S aureus* diagnostics, therapy, and prophylaxis.

S aureus General Features, Growth, and Metabolism

Staphylococcus aureus is a gram-positive organism with aerobic to facultative anaerobic lifestyle and colonizes skin, nares, and axillae of humans. *Staphylococcus aureus* is a catalase-, urease-, and phosphatase-positive organism with most strains secreting coagulase and it also ferments mannitol sugar to lactic acid. Testing for catalase is an important criterion to distinguish *Staphylococci* from *Streptococci* and coagulase test for distinguishing *S aureus* from *S epidermidis*. It reduces nitrates to nitrites, liquefies gelatin, and is methyl red and Voges-Proskauer test positive. *Staphylococcus aureus* is lipolytic (lecithinase) when grown on media containing egg yolk. *Staphylococcus aureus* reduces tellurite in media containing potassium tellurite and produces shiny black color colonies. All strains of *S aureus* produce a heat-stable thermonuclease which has both endonuclease and exonuclease properties and can degrade

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both RNA and DNA. *Staphylococcus aureus* grows in irregular clusters because the cells divide successively in 3 perpendicular planes and the attachment of sister cells may not be in divisional plane but may adjust position while being attached.[16] It can remain viable even after many months of air-drying and resists the effect of chemicals and disinfectants.[17] Nutritional requirements of *S aureus* can be met by routine laboratory media, and most strains are metabolically versatile; that is, they can digest proteins, lipids and can ferment a variety of sugars. The average doubling time (mean generation time) of *S aureus* is as short as 20 minutes.

S aureus and Host Interactions

Staphylococcus aureus is part of normal microflora of humans and is found inhabiting in most human environments. The nares are the primary ecological niche for *S aureus*; however, multiple sites in the body such as skin, perineum, axillae, vagina, and gastrointestinal tract also were found to harbor this bacterium.[18] *Staphylococcus aureus* in general have a benign or commensal relationship with its host. However, they revert to pathogenic lifestyle once they gain entry into host tissues by injuries, inoculation by syringes, or by direct implantation with medical devices. A successful infection results when there is a shift of balance between host defenses and pathogen virulence mechanisms in favor of the pathogen.

Skin is a major physical and immunologic barrier; the keratinocytes in the epidermis express pattern recognition receptors (PRRs) such as toll-like receptors that recognize pathogen-associated molecular patterns of microbes.[19] After recognition, PRRs trigger early cutaneous immune responses such as recruitment of immune cells from circulation to site of recognition. Skin also harbors numerous resident immune cells such as Langerhans cells in the epidermis and dendritic cells, macrophages, mast cells, T and B cells, plasma cells, and natural killer (NK) cells in the dermis. Low temperature and pH of the skin surface resists growth of *S aureus*. Normal commensal organisms of skin such as *S epidermidis* and *Propionibacterium acnes* also prevent colonization and invasion by *S aureus* by secreting antimicrobial peptides such as phenol-soluble modulins (PSM-α and PSM-δ).[20] In addition, keratinocytes in the corneal layer of skin produces antimicrobial peptides that have bacteriostatic and bactericidal properties such as human β-defensins (hBD2, hBD3), cathelicidin (LL-37), and ribonuclease 7.[21–23]

Colonization of *S aureus* is mediated by its adherence to surface components such as fibrinogen, fibronectin, and cytokeratins of nasal epithelium or cutaneous keratinocytes. It uses microbial surface components recognizing adhesive matrix molecules for binding such as fibronectin-binding proteins (FnbpA and FnbpB), fibrinogen-binding proteins (ClfA and ClfB), iron-regulated surface determinant (IsdA), and wall teichoic acid (WTA).[24,25] Superantigens such as staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB), and TSS toxin 1 (TSST-1) enhance fibronectin-mediated and fibrinogen-mediated *S aureus* colonization during atopic dermatitis by altering the levels of Th2 cytokine profiles (interleukin 4).[26] It has additional mechanisms to evade host antimicrobial peptides. For example, iron-regulated surface determinant (IsdA) renders *S aureus* resistant to β-defensins and cathelicidin and aureolysin, an extracellular metalloproteinase that inhibits cathelicidin activity.[27]

Staphylococcus aureus has several mechanisms to evade and kill host immune cells and inhibit neutrophil recruitment and antimicrobial activity. Toxins such as α-hemolysin, Panton-Valentine leukocidin (PVL), γ-hemolysin, leukocidin E/D, and PSM lyse the host cells, thus contributing to enhanced virulence. It inhibits the neutrophil recruitment by secretion of chemotaxis inhibitory protein of staphylococci (CHIPS) which reduces the endothelial expression of intercellular adhesion molecule 1 (ICAM-1).[28] Neutrophil killing of *S aureus* by reactive oxygen species is overcome by factors such as *S aureus* golden pigment[29] and superoxide dismutase.[30]

The success of *S aureus* strains is due to a unique combination of genetic factors that enable the bacteria to evade host immune system.[31] Recent findings suggest that cytolytic PSM-α, cytolysin α-toxin, and the global virulence regulator (*agr*) have demonstrated important roles in experimental skin infection models.[32] It was reported that high WTA amounts might permit *S aureus* to amplify early responses to abscess formation, thereby creating a microenvironment that protects bacteria from host responses.[31] Abscess formation and colony forming unit increase was observed when purified WTA along with bacterial inoculum of WTA^{low} producers was injected. Wall teichoic acid synthesis is one of the mechanisms that certain MRSA use to gain virulence and therefore could be an ideal target for development of novel anti-infective strategies. Therefore, understanding the host-pathogen interactions is important to identify targets for drug design, designing novel vaccines, and antibody-based therapies.

Clinical Significance of *S aureus*

Staphylococcus aureus has been a major human pathogen throughout the history and is also the leading cause of bacterial infections worldwide. It is responsible from mild to life-threatening diseases and can potentially infect any tissue in the human body. Among the various *S aureus* infections, they can be broadly classified into (1) superficial skin and soft tissue infections (SSTIs); (2) systemic and life-threatening infections such as endocarditis, osteomyelitis, pneumonia, meningitis, and bacteremia; and (3) toxinoses such as food poisoning, scalded skin syndrome, and TSS.[33] Severity of infection in general is dependent on virulence of the particular strain, inoculum size, and immune status of the individual. Staphylococcal infections are typically characterized by abscess filled with pus and damaged leukocytes surrounded by necrotic tissue. *Staphylococcus aureus* infections are caused either by autoinfection, infection with own carrier strain, or by cross infection, infection due to strain transmitted from another individual. *Staphylococcus aureus* has gained resistance to every antimicrobial therapy introduced so far. The massive consumption of antibiotics over the past 50 years has led to the rise in antibiotic resistance, and by far, the resistance against

the methicillin has gained utmost significance due to rising public health burden and mortality in comparison with methicillin-susceptible strains.[34] Although *S aureus* is an opportunistic pathogen, there are certain risk factors that increase the likelihood of an infection. Ideally, opportunistic pathogens attack when the body defenses are weakened.[35] Skin breakage and or immunosuppression along with nasal carriage are the major risk factors for *S aureus* infections.[36] Nasal carriage varies between individuals and is one of the major risk factors for subsequent *S aureus* infections.[37] In a general population, the average carrier rate is 37% (19%-55%) with some subpopulations showing a higher percentage such as patients with diabetes mellitus, human immunodeficiency virus, dialysis patients, and patients with atopic dermatitis.[37,38] Being a carrier is an important predisposition to subsequent infections. *Staphylococcus aureus* is the cause of large percentage of blood stream (22%) and SSTIs (39%).[39] Methicillin-resistant *S aureus* which was previously restricted to hospitals is increasingly seen in community. Worldwide, community-associated MRSA (CA-MRSA) is one of the major causes of SSTIs and sepsis cases. Two CA-MRSA clones USA300 and USA400 account for 60% to 75% of all *S aureus* infections in the community.[40]

S aureus Pathogenesis

Staphylococcus aureus is the most common cause of SSTIs, endocarditis, and second frequent cause of bacteremia. It is also a predominant cause of nosocomial-acquired infections such as intravenous catheter-associated infections, ventilator-associated pneumonia, postsurgical wound infections, invasive infections in neutropenic patients, and in patients undergoing solid organ or hematopoietic cell transplantations.[41] Methicillin-resistant *S aureus* kills ~19 000 hospitalized patients annually in United States, which is similar to combined deaths caused by AIDS, tuberculosis, and viral hepatitis.[42] *Staphylococcus aureus* can evade host defenses and antimicrobials by growing and persisting on biofilms formed on surfaces of the hosts and prosthetic devices.[43] *Staphylococcus aureus* bacteremia (SAB) may be complicated by endocarditis, metastatic infections, or sepsis.[44] Endothelial cell is central to all pathogenic process and its activation leads to endovascular infections. *Staphylococcus aureus* binds by adhesin-receptor interactions and is phagocytized inside the endothelial cells. Intracellular environment protects *S aureus* from host defense mechanisms and antibiotics. It was also reported that intracellular milieu of endothelial cell favors formation of small colony variants (SCVs).[45] These factors contribute to recurrent and persistent infections. *Staphylococcus aureus* escapes host defenses by invading and surviving inside the endothelial cells in patients with endocarditis.[46] *Staphylococcus aureus* also escapes from host defenses by forming SCVs which survive inside host cells without causing damage, and they are capable of reverting to virulent forms resulting in recurrent infection.[47,48]

Superantigens cause life-threatening TSS that is characterized by rapid onset of high fever, shock, and multiorgan failure. Superantigens are potent T-cell mitogens that bypass the normal antigen presentation and bind directly to invariant regions of major histocompatibility complex class II molecules of antigen-presenting cells. Major histocompatibility complex–bound superantigen attaches to the variable region of β chain receptor of T cells and causes massive expansion of clonal T cells (5%-10% in contrast to 0.01% for a normal processed antigen) leading to massive release of cytokines and chemokines by macrophages and T cells. The cytokines mediate the TSS leading to tissue damage.[49] Toxic shock syndrome toxin 1 contributes to 90% cases related to menstruation and is associated with the use of absorbent tampons. Other enterotoxins contribute to 50% of TSS cases that are not related to menstruation.[50]

Increase in percentage of SAB is due to increase in catheterization.[51] Patients with fever after 72 hours of catheter removal have increased risk of complications.[52] Incidence of endocarditis is more in case of intravenous drug users, elderly patients, patients with prosthetic values, and hospitalized patients.[44] *Staphylococcus aureus* has a tendency to spread to other sites in the body such as bones, joints, kidneys, and lungs leading to metastatic infections.[53,54] Pus collection at these sites serves as potential foci for persistent and recurrent infections.[53] Factors such as advance age, immunosuppression, chemotherapy, and invasive procedures may aid in progress of bacteremia and local infections to sepsis. Staphylococcal sepsis presents with fever, hypotension, tachycardia, and tachypnea. Severe cases progresses to multiorgan dysfunction and death.[55]

Antibiotic Resistance Mechanisms

Antibiotic resistance is the resistance of an organism, usually a pathogen to an antimicrobial drug that was effective originally for treating infections. Antibiotic resistance is a serious, ever-growing phenomenon and has emerged as a prominent global health concern in 21st century (http://en.wikipedia.org/wiki/Antibiotic_resistance). Evolution of antibiotic-resistant strains is a natural phenomenon that occurs due to erroneous replication or due to exchange of resistant traits between them. Use and misuse of antibiotics also lead to selection of antibiotic-resistant strains. Multidrug resistance is a common phenomenon among many pathogens such as pneumonia, micrococci, and staphylococci. *Staphylococcus aureus* is of major concern due to the intrinsic virulence, its ability to cause diverse life-threatening infections, and its ability to adapt to varied environmental conditions. *Staphylococcus aureus* isolates from blood cultures all over the world are increasingly resistant to multiple antibiotics.[56] Mechanisms leading to resistance to some of the broad antibiotic classes are in the following sections and in Table 2.

Penicillin resistance

Penicillin was discovered in 1928 by Alexander Fleming and is lethal to all sensitive cells by deactivation of cell wall–associated penicillin-binding protein (PBP) transpeptidases. Inactivated

transpeptidases are key to cross-linking of peptidoglycan stands which lead to weakened cell wall and death by osmotic lysis.[36] Penicillin treatment dramatically improved the prognosis of patients with *S aureus* infections. However, penicillin-resistant strains were discovered as early as 1942 in both hospitals and community,[57] and the incidence was ~80% by 1960 in hospitals and community. This pattern of resistance first appearing in hospitals and later spreading to community is now a common phenomenon observed with each new wave of antibiotic resistance. Resistance to penicillin is mediated by β-lactamase (*bla*Z), an extracellular enzyme which inactivates the β-lactam nucleus. *bla*Z gene is located on a transposable element on a plasmid with additional antibiotic-resistant genes (gentamicin and erythromycin). Spread of penicillin resistance occurs through spread of resistant strains.[56]

Methicillin resistance

To combat penicillin-resistant *S aureus*, a modified semisynthetic penicillin known as methicillin or meticillin was introduced which is immune to activity of β-lactamase. Soon after its introduction, reports of treatment failure with methicillin occurred by evolution of MRSA.[58] Methicillin resistance is mediated by chromosomally located *mecA* gene which codes for an altered PBP called PBP2a.[59] The *mecA* gene is part of a mobile genetic element (MGE) known as staphylococcal cassette chromosome *mec* (SSC*mec*).[60] PBP2a substitutes for other PBP in cross-linking of peptidoglycan chains because of its low affinity to β-lactams and therefore enables staphylococci survival even in high concentrations of these agents. The resistance to methicillin confers resistance to other β-lactams such as cephalosporins.[61] The therapeutic outcome of infection from an MRSA strain is more severe than from a methicillin-sensitive *S aureus* (MSSA) strain not only due to enhanced virulence but also due to the fact that MRSA occurs in older hospitalized patients and also due to limited antimicrobial drugs available to treat MRSA. Similar to penicillin resistance, MRSA strains carry multiple antibiotic-resistant genes. Methicillin-resistant *S aureus* has progressed into an important pathogen of humans and is endemic in hospitals worldwide. Recently, it has emerged in community with increased severity as CA-MRSA. The high mortality associated with some of the CA-MRSA infections is of particular concern. High morbidity of infections associated with CA-MRSA may be due to the presence of enterotoxins and PVL toxins. Treatment of MRSA-associated infections has become complicated owing to remarkable ability of this organism to develop antibiotic resistance.

Quinolone resistance

Fluoroquinolones were first introduced to treat gram-negative bacterial infections in 1980s. Due to broad antibacterial spectrum against gram positives, they have been used to treat infection caused by pneumococci and staphylococci.[56] Quinolone resistance quickly emerged in strains with methicillin resistance due to high antibiotic selection pressure in hospital setting resulting in selection and spread of resistant strains. Fluoroquinolone resistance is due to spontaneous chromosomal mutations in antibiotic targets, topoisomerase IV, or DNA gyrase or by the induction of multidrug efflux pump.[62] When quinolones are used to treat infections caused by other bacterial pathogens, the resident *S aureus* strains are likely to get exposed to suboptimal concentrations and are therefore at risk of colonization with resistant strains. Resistance to quinolones is achieved by stepwise acquisition of chromosomal mutations. ParC subunit mutations of topoisomerase IV are more critical for quinolone resistance in staphylococci as they are the primary drug targets.[63] Recently, there have been reports of plasmid-mediated resistance mechanisms, including the quinolone resistance proteins such Qnr, Aac(6′) Ib-cr, and QepA.[64]

Vancomycin resistance

Increased use of vancomycin to treat bacterial infections caused by MRSA, *Clostridium difficile*, and enterococci paid the way for emergence of vancomycin-resistant *S aureus* (VRSA). The first report of vancomycin-intermediate *S aureus* (VISA; minimum inhibitory concentration [MIC]: 8-16 μg/mL) came from Japan followed by more cases from many nations.[65,66] It was followed by reports of appearance of vancomycin-resistant strains with total resistance (MIC: >128 μg/mL) and a different mechanism of dissemination. In VISA strains, resistance is mediated by chromosomally located *vanA*; in contrast, VRSA acquire *vanA* operon by conjugal transfer from *Enterococcus faecalis* which is a more efficient means of disseminating resistance genes. Resistance is conferred by increased cell wall biosynthesis which leads to abnormally thick walls. The thick peptidoglycan wall ensnares the vancomycin within cell wall, denying the access to its cytoplasmic target *N*-acetyl-muramic acid precursor.[67,68]

Antibiotics have been considered as innovative therapy for many decades. In most cases, after widespread dissemination and prescription, they have been abandoned when it is not economically viable or it is not essential to pharmacopoeia. However, one interesting observation was that only 12.8% of invasive isolates were resistant to methicillin in some hospitals in 2015.[69] Some workers have even reported strains that are susceptible to penicillin. (Chabot et al, 2015).[70,71] It is essential that we maintain the full repertoire of all antibiotics as part "revival of old antibiotics" to face a particular therapeutic situation. Interestingly, *C difficile* infections have become more common hospital-associated infections than MRSA infections which have decreased dramatically.[72] Some argue that the exaggeration which presently exists regarding antimicrobial resistance is likely an evolutionary trend of our societies to panic to when faced with new phenomenon (Duborg et al, 2015).[73]

Clinical burden due to S aureus infections

Health care systems of many areas in the world including North America, Europe, Australia, and Asia have witnessed increasing levels of MRSA due to epidemics of highly transmissible clones.

However, the true extent of MRSA is not known correctly. In many countries, surveillance is mandatory only in severe forms of disease such as bacteremia. It is highly possible that the percentage of population presenting with actual disease is only the "tip of the iceberg" and that the actual clinical spectrum includes all possible individuals colonized with MRSA but may never develop any clinical disease but can be dangerous to others (Gould, 2005).[74] One important factor often forgotten is the additional economic burden incurred on the patients and health care systems. With the increasing incidences of MSSA and MRSA infections, there is a gradual increase in rates of bacteremia and huge additional costs toward treatment. Added to this failure of treatment due to inappropriate antimicrobials or lack of efficacy of anti-MRSA drugs, excess toxicity of new antimicrobials over routine ones is likely to increase the morbidity and mortality. Financial burden of MRSA is very high given the wide spectrum of clinical infections. Direct costs include providing care to MRSA-infected patients, antibiotic treatment costs, indirect costs such as morbidity and diminished quality of life, and infrastructure costs of surveillance and control (Gould, 2005). In one study, after reviewing subjects, extra costs for treatment was estimated in the range of US $3000 to US $30 000 depending on clinical infection and severity.[75]

Different classes of S aureus virulence factors

Staphylococcus aureus produces many potential virulence factors belonging to various classes categorized based on their functionality such as adherence, invasion and penetration, host evasion, enzymes, toxins, and surface proteins. Some of the major classes of virulence factors that contribute to *S aureus* infection capabilities include (1) *surface proteins* (adhesins, clumping factors, IsdA, fibrinogen-binding, and fibronectin-binding proteins) that are involved in the adherence and colonization of host tissues; (2) *invasins* that promote bacterial spread in host tissues (leukocidins, kinases, and hyaluronidase); (3) *surface factors* which inhibit phagocytic engulfment (capsule); (4) *biochemical properties* that enhance their survival abilities inside the host (carotenoids and catalase); (5) *immunological disguises* (coagulase and protein A); (6) *membrane-damaging toxins* that lyse host cells (hemolysins, leukotoxin, and leukocidin); (7) *secretory toxins* that damage host tissues and promote symptoms of disease (enterotoxins A-G, TSST-1, exfoliative toxin); (8) inherent and acquired *resistance to antimicrobial agents*.[16] α- and γ-hemolysins are encoded in the core genome and thus are produced by most strains. Toxins such as enterotoxin A, exfoliative toxins, TSST-1, and PVL are encoded on MGEs of bacteriophages and hence are present in only certain strains.[76] *Staphylococcus aureus* is capable of sensing the surrounding environment and adjust the production of virulence factors suitable for colonization, dissemination, and for causing infection.[77] Successful infection of a strain into specific host is multifactorial and depends on the virulence factors secreted by the strain. However, there are certain correlations to the expression of particular virulence determinants which suggest their involvement in certain diseases. Expression of secretory toxins occurs primarily during postexponential growth phase and is controlled by at least 3 global regulatory systems, namely, the accessory gene regulator (*agr*), the staphylococcal accessory regulator (*sar*), and extracellular protein regulator (*xpr*).[78] Evidence for staphylococcal matrix-binding proteins as virulence factors came from adherence assay studies involving defective mutants. Defective fibrinogen-binding and fibronectin-binding *S aureus* mutants have reduced virulence in rat endocarditis model.[79] Mutants deficient in collagen-binding protein has reduced virulence in mouse septic arthritis model.[80] The role of some of the important classes of virulence factors such as hemolysins, leukocidins, and superantigens needs more discussion.

S aureus enzymes

The primary role of staphylococcal enzymes is to provide the nutrients for cell growth and division, and only certain enzymes play key role in the pathogenesis. Proteolytic enzymes of *S aureus* are involved in the inactivation of antimicrobial peptides and also for modulating and activating other virulence factors (zymogens) such as clumping factors, staphylococcal protein A (SpA), and fibrinogen-binding proteins. The major proteolytic enzymes consist of a metalloproteinase (aureolysin, Aur), a serine glutamyl endopeptidase (serine protease, SspA), and 2 related cysteine proteinases referred to as staphopain (ScpA) and the cysteine protease (SspB).[81] Hyaluronidase produced by most *S aureus* strains helps in degrading hyaluronic acid from connective tissue and promotes bacterial spread inside host tissues. Coagulase protects bacteria from host defenses by forming fibrin clot around the foci of infection.[82]

Hemolysins

Among the membrane-damaging toxins, α-hemolysin is the most potent pore-forming toxin, expressed as monomer by almost all the clinical isolates of *S aureus*. α-Hemolysin monomers oligomerize to form a functional heptameric toxin with a central pore through which cell contents are leaked. There is a direct correlation between the levels of α-hemolysin expression and the virulence of a particular strain suggesting its prominent role in pathogenesis.[83] Platelets and monocytes are the most susceptible cells to the action of α-hemolysin, and the method of cells lysis is likely by osmotic lysis.[16] β-toxin is a sphingomyelinase which damages membranes rich in lipids, and most of the human isolates do not express this toxin. It is encoded by a lysogenic bacteriophage.[84]

Leukocidins

The PVL and γ-hemolysins are the staphylococcal bicomponent toxins with leukocytotoxic activity requiring the action of 2 components, the S and the F subunits. Leukocidins are associated with a total of 5 genes: γ-hemolysins are encoded by 3 ORFs, *hlgA*, *hlgB*, and *hlgC*, and PVL is encoded by 2

cotranscribed ORFs, the *lukS-PV* and *lukF-PV*. Among these, *hlgA, hlgC*, and *lukS-PV* function as S component, whereas *hlgB* and *lukF-PV* function as F component. The γ-hemolysin is present in almost 99% of *S aureus* strains, and hence, its involvement in pathogenesis is difficult to ascertain. In contrast, PVL toxin has increasingly been associated not only with community-acquired primary SSTIs but also with severe necrotizing pneumonia in young and healthy individuals.[85]

Phenol-soluble modulins

Phenol-soluble modulins are recently discovered amphipathic, α-helical peptides secreted by members of staphylococci. Phenol-soluble modulins are key virulence determinants in highly virulent *S aureus* strains. Phenol-soluble modulin α peptides of *S aureus* lyse neutrophils after they are phagocytized. Phenol-soluble modulins are also key factors for biofilm formation and their dissemination in biofilm-associated infections. The surfactant properties of PSMs facilitate their growth on epithelial surfaces. Phenol-soluble modulin can be grouped in to smaller (~20-25 amino acids [aa]) α-type PSMs and longer (~44 aa) β-type PSMs.[86]

Superantigens

Toxic shock syndrome is a rare condition associated with menstruating women using tampons and is characterized by rapid onset of fever and multiorgan failure. This condition is caused by TSST-1 belonging to a class of staphylococcal superantigens which causes massive activation of T lymphocytes.[87] Gene encoding TSST-1 is located on a less transmissible pathogenic island designated as SapI 1, and hence, *tst-1* is present in only few restricted clones.[88] Staphylococcal enterotoxins (SEs) belong to a group of structurally related superantigen family of toxins whose presence is correlated with increased virulence in nosocomial infections. Staphylococcal enterotoxin A has been associated with more severe infections such as staphylococcal food poisoning (SFP) and septic shock in comparison with other enterotoxins. Most of the enterotoxins are carried by plasmids, phages, pathogenicity islands, or MGEs.[89] Exfoliative toxins (ETA, ETB, ETC, and ETD) cause exfoliation of skin epidermis followed by secondary infections. ETA and ETB are the important isoforms in humans predominantly affecting neonates and are associated with staphylococcal bullous impetigo and staphylococcal scalded skin syndrome. Prevalence of exfoliative toxins is not so frequent in *S aureus*.[87]

Staphylococcal food poisoning

Staphylococcus aureus is one of the most frequent pathogens responsible for food-borne outbreaks worldwide. It causes SFP after ingestion of foods containing preformed heat-stable enterotoxins. Contamination in SFP cases occurs commonly due to improper or extensive manual handling of foods rich in proteins combined with inadequate heating and improper storage.[91] Foods commonly contaminated with SEs are meat and meat products, poultry and egg products, milk and milk products, and confectionary products such cream-filled pastries and cakes.[92] Staphylococcal food poisoning was the fourth most frequent cause of food-borne illness in European Union in 2008 (EFSA, 2010).[89] Although *S aureus* cells can be killed by heating, the enterotoxins are very stable even after rigorous heating. Staphylococcal enterotoxins are resistant to proteases such as pepsin, trypsin, papain, and rennin and thus they are active even after ingestion in the intestine. At present, there are 23 enterotoxins or enterotoxin-like genes.[93] Staphylococcal enterotoxins are globular, single-polypeptide proteins which are related structurally with molecular weights ranging from 22 to 29 kDa. Staphylococcal food poisoning is associated with rapid onset of symptoms within 2 to 8 hours from the time of ingestion of contaminated food. Symptoms typically include nausea, vomiting, abdominal cramping, and occasionally with diarrhea and fever.[94] Severity of SFP is dependent on amount of SE ingested and the health status of the individual. In most cases, the symptoms subside within 24 to 48 hours; however, in case of infants and elderly people, it requires hospitalization.[95] In cases of severe dehydration, it requires supplementation with intravenous fluid administration. Staphylococcal enterotoxin A is the most frequently encountered SE among SFP cases.[96] Enterotoxin A (*sea*) is very different from all other SE genes such as enterotoxin B (*seb)*, enterotoxin C (*sec*), and enterotoxin D (*sed*) because it is carried by polymorphic family of lysogenic or temperate phages.[97]

Detection methods for S aureus and its toxins

Pathogenesis of *S aureus* diseases is a multifactorial phenomenon. However, there is some relationship with the presence of certain virulence factors to a particular disease. Although *S aureus* produces various toxins and enzymes, there is direct correlation between virulence of a particular strain with the amount of α-hemolysin secreted. The presence of this bacterium or its enterotoxins in processed foods is a general indication of poor sanitation. Mere isolation of *S aureus*–viable cells may not be sufficient to cause food poisoning. It should have the capacity to secrete enterotoxins (SEs). Staphylococcal enterotoxins also play an important role in food poisoning and TSS cases. Staphylococcal enterotoxins are globular, single polypeptides which constitute a family of related proteins with similarities at structural and aa levels. Although heat treatment used commonly in food processing industries destroys *S aureus* vegetative cells, the heat-stable enterotoxins secreted by this organism are resistant to high temperatures for extended periods. Food intoxication caused by SEs is referred to as SFP and is one of the common forms of food-borne illnesses reported worldwide. Staphylococcal food poisoning is characterized by nausea, vomiting, and abdominal cramps. Staphylococcal enterotoxins are also responsible for autoimmune responses due to their superantigenic nature resulting in TSS. Staphylococcal enterotoxin A is one of the most commonly encountered enterotoxins among SFP cases.[96] Staphylococcal

enterotoxin B is another enterotoxin responsible for food poisoning and is also a potent T-cell mitogen and hence is listed as a category B bioweapon agent.[98] Concentrations of 0.5 to 1 ng/mL of SEs are sufficient to induce food poisoning. Therefore, detection and quantification of SEs from food is a more appropriate approach than detection of *S aureus* viable cells from foods. Laboratory methods for identification of *S aureus* from food sample, wound, or blood culture require isolation and biochemical test procedures which require considerable time and resources. Several methods have been reported for rapid identification so that most appropriate therapeutic interventions can be undertaken. Commercially, many kits are available for the enumeration of *S aureus* from food and environmental samples and also for detection of SEs from isolates as well as food samples. Polymerase chain reaction has revolutionized several areas of molecular biology particularly in the field of molecular diagnostics of infectious diseases. Polymerase chain reaction methods for *S aureus* identification includes PCRs for species-specific genes such as 16S RNA,[99] thermonuclease (*nuc*),[100] and acriflavine resistance[101] gene. Molecular methods play an important role in detection and differentiation of pathogens. Numerous techniques have been reported for detection of SEs such as antibody,[4,5] PCR,[6,7] RT-PCRs,[8] and aptamer-based[9–11] methods. Many other sensitive methods such as immuno-PCRs,[12] mass spectrometric analysis,[13] and biosensor techniques[14,15] are also reported. Although these methods are sensitive, they are relatively expensive and thus cannot be used in routine testing of multiple samples.

Among the immunological assays, Western blots, radio immunoassay, enzyme-linked immunosorbent assays (ELISAs), and reversed passive latex agglutination assay have been described for detection and quantification of exotoxins such as α-hemolysin, enterotoxins, and PVL toxins. Immunoassays could be used to detect SEs directly from culture or from contaminated food material. There are many commercially available kits such as VIDAS, TRANSIA, TECRA, and RIDASCREEN for the detection of SEs available commonly in sandwich ELISA formats (Table 1). Many in-house assays[4,5,96] have also been reported for detection of SEs. Among the various antibody-based formats reported so far, immuno-PCR is a sensitive diagnostic technique which combines the specificity of ELISA with the sensitivity of PCR and it offers the advantages of high sensitivity and easy automation for detection multiple analytes by differential capture of antigens. Immuno-PCR has established itself as a potential diagnostic tool and has been applied for the detection of various bacterial[102] and viral pathogens,[103] bacterial toxins,[104] and mycotoxins.[105] Most of the immunoassays employ antibodies from mammalian sources such as rabbit, mice, sheep, and goat. The major hindrance with the specificity of these immunoassays is the presence of a 42-kDa SpA secreted by all *S aureus* strains. Staphylococcal protein A is an immunoglobulin-binding protein present on cell wall and is also secreted into the medium during exponential growth phase. Staphylococcal protein A causes false positives in antibody-based tests involving *S aureus* antigens due to its ability to bind various classes and subclasses of immunoglobulins. Staphylococcal protein A mediates this activity by binding to Fc region of most immunoglobulin classes and to F(ab)2 region of certain immunoglobulin classes. A variety of methods have been proposed to overcome SpA interference in immunoassays. However, there are limitations with these assays and are not completely free from the effect of protein A.

In recent times, there is an increasing use of antibodies from avian (immunoglobulin Y [IgY]) sources, especially from chickens, because raising antibodies from chickens are more convenient, hygienic, inexpensive, and isolation does not require invasive methods unlike from mammalian sources. Egg yolks are abundant sources of IgY, and single yolk can yield IgY in the range of 10 to 20 mg/mL. There are several advantages with IgY and, most importantly, IgY does not have any affinity to immunoglobulin-binding proteins such as protein A, protein G, and protein L. Chicken antibodies were used in many assays where there is a marked effect of SpA on immunoassays due to its binding ability to mammalian immunoglobulins.[2,3,129]

Next-generation sequencing (NGS) offers potential solution to challenges in detection of infectious diseases. Next-generation sequencing offers huge potential in sequencing all the nucleic acids present in a sample allowing limitless multiplex interrogations, thereby providing higher levels of diagnostic interpretation through complete characterization of genomic content.[130] However, an unbiased NGS requires a substantial amount of sequence depth to separate low-prevalence pathogens from overwhelming host nucleic acids. Targeted NGS such as using pathogen-specific signatures for amplification could be a possible mitigation strategy. Next-generation sequencing would offer immense aid in diagnosing serious *S aureus* infections such as bacteremia and pneumonia and especially in low-income countries.

Treatment, therapies, and prevention

Most of the *S aureus* isolated from hospitals and community are resistant to multiple antibiotics which therefore makes the treatment of *S aureus* infections complicated. Treatment of infections by multidrug-resistant *S aureus* is possible only with last line of antibiotics such as vancomycin and linezolid. Additional nonspecific mechanisms such as biofilm formation on medical devices also aid in the resistance to antimicrobial agents. At present, little interest is being shown for the development of novel antibiotics due to the high cost, limited success rate, and possible emergence of antibiotic resistance. Therefore, researchers have intensified their interest toward the development of vaccines and therapeutic antibodies because they can be raised easily and inexpensively in comparison with development of novel antibiotics. Moreover, vaccination might be beneficial to people at high risk such as dialysis patients, patients at risk of endocarditis, patients undergoing surgery, sports persons, prison inmates, and health care workers who are the potential sources of dissemination of hospital-associated MRSA in hospitals and to patients. However, in contrast to

Table 1. Commercial test kits available for detection of *Staphylococcus aureus* and its enterotoxins.

S. NO.	TEST KIT	MANUFACTURER	FOODS COVERED	FEATURES
Conventional and rapid methods for the enumeration of *S aureus*				
1.	BBL CHROMagar *Staph aureus* agar medium[106]	BD Diagnostics	Cooked roast beef, smoked salmon, shell eggs, and certain uncooked foods such as dairy products, salads, and sandwiches	Results available within 24 h, does not require supplements, easy to read and interpret, and more sensitive
2.	RAPID'Staph medium[107]	Bio-Rad laboratories	Food products intended for human and animal consumption; environmental samples	Results in 24 h after 24 h enrichment, easy to read, and highly selective
3.	TECRA *Staphylococcus aureus* VIA ELISA[108]	3M Microbiology	Not stated	Food supplements such as fish oil, green tea, alfalfa, brewer's yeast, mustard seeds, and lecithin concentrate
4.	3M Petrifilm Staph Express Count Plates (thin-film medium)[109]	3M Microbiology	Selected dairy foods	Easy inoculation and interpretation; fast, accurate results in as little as 22 h; saves incubator space; and easy to enumerate with built-in grid
5.	Baird-Parker agar[110]	Numerous vendors	Food, environmental samples, and clinical specimens	Requires 46 to 48 h for result interpretation, can differentiate coagulase-positive and coagulase-negative staphylococci, staphylococci
6.	Rabbit plasma fibrinogen agar[111]	Oxoid	N/A	Inclusion of rabbit plasma for better coagulase activity and lower concentration of potassium tellurite favor the growth of all *S aureus*
7.	BBL Staphyloslide Latex Test Kit[112]	BD Diagnostics	N/A	Differentiates staphylococci with coagulase and protein A from other staphylococci, results in as few as 20 s
8.	Staphytect Plus[113]	Oxoid	N/A	Latex slide agglutination test for differentiation of *S aureus* by detection of clumping factor, protein A, and certain polysaccharides from other staphylococci
9.	Microgen Staph latex test[115]	Microgen	Food, clinical, and environmental samples after plating on selective agar	Sensitive and specific latex agglutination for the identification of *S aureus* offering rapid and accurate identification of *S aureus* in 2 min
10.	Phadebact Staph Aureus test[116]	Bactus AB, Sweden	N/A	Intended for the detection of coagulase (clumping factor) and/or protein A associated with *S aureus* obtained from primary cultures. Test colonies should be fresh preferably grown on blood agar plates
11.	Staphylase Test[117]	Oxoid	N/A	Detects the presence of clumping factor through clumping of fibrinogen-sensitized sheep red blood cells
12.	Pastorex Staph Plus[118]	Bio-Rad	N/A	Rapid agglutination test for the simultaneous detection of the fibrinogen affinity antigen (clumping factor), protein A, and the capsular polysaccharides of *S aureus*
13.	Bacto Staph (Berke and Tilton, 1986)[114]	Difco Laboratories		A suspension of yellow latex particles sensitized with specific plasma proteins
14.	Staphaurex and Staphaurex Plus[119,120]	Remel	N/A	Staphaurex Rapid latex test for the detection of clumping factor and protein A associated with *S aureus* Staphaurex Plus—yellow latex particles coated with human fibrinogen for detection of clumping factor coated with specific IgG for detection of protein A and surface antigens

(Continued)

Table 1. (Continued)

S. NO.	TEST KIT	MANUFACTURER	FOODS COVERED	FEATURES
Genetic methods for the detection of *S aureus*				
1.	BAX System (Real-Time PCR Assay for *Staphylococcus aureus*)[121]	DuPont Qualicon, Inc.	Ground beef, soy protein isolate, and soy-based and milk-based powdered infant formula	Real-time PCR assay for reliable genetic detection of *S aureus* the day after sampling. Requires less than 90 min of real-time processing, detects 1 cfu/g in powdered infant formula, and detects threshold of 10 cfu in line with meat industry standards
2.	GENE-TRAK (based on DNA hybridization)[122]	Neogen	Isolates	Employs *S aureus*–specific DNA probes and a colorimetric detection system for the detection of *S aureus* in food samples following broth culture enrichment
Rapid test kits for detection of staphylococcal enterotoxins				
1.	VIDAS Staph enterotoxin (SET) Immunoassay[123]	BioMérieux	Dairy products, meat, and seafood, etc	N/A
2.	VIDAS Staph enterotoxin II (SET2) Immunoassay[124]	BioMérieux	Milk and milk products, canned foods, dehydrated foods, meat, seafood, and shellfish, etc	Detects SEA-SEE, total run time of 80 min and the results are obtained on the same day
3.	TECRA Staphylococcal Enterotoxin VIA[125]	3M Microbiology	Canned mushrooms, nonfat dry milk, canned lobster bisque, beef and pasta, cooked chicken, and cheese	Fast, reliably detects SEA, SEB, SEC1, SEC2, SEC3, SED, and SEE from food and food-related products and enrichment cultures with a sensitivity of 1 ng/mL
4.	RIDASCREEN Immunoassay[125,151]	R-Biopharm, Darmstadt, Germany	Various including cheese	Sandwich enzyme immunoassay for the identification of SETs A, B, C, D, E in fluid and solid foods as well as in bacterial cultures
5.	Transia (Transiatube and TransiaPlate) Immunoaffinity, ELISA[126]	Diffchamb, Lyon, France	Milk and dairy products	
6.	SET-RPLA[127]	Oxoid	A wide variety of food and food products such as dairy, meat, and meat products	Used to detect staphylococcal enterotoxins in a wide variety of foods and to give a semiquantitative result. Sensitivity of the test is 1 ng/mL of extract
7.	SET-RPLA “SEIKEN” RPLA[128]	Denka Seiken		RPLA test employing separately sensitized with highly specific antibodies for SEs A, B, C, and D. Results are semiquantitative

Abbreviations: IgG, immunoglobulin G; N/A, not applicable; PCR, polymerase chain reaction; RPLA, reversed passive latex agglutination assay; SEs, staphylococcal enterotoxins; SEA, staphylococcal enterotoxin A; SEB, staphylococcal enterotoxin B; SEE, staphylococcal enterotoxin E; SETs, staphylococcal enterotoxins.
Table adapted and modified from Hudson.[126]

other bacterial pathogens, there is no vaccine available yet that stimulates active immunity against staphylococcal infections in humans. This may be due to the fact that *S aureus* is a permanent or transient colonizer in part of the population and it has developed mechanisms to thwart human immune mechanisms such as immunologic disguises, toxins that lyse white blood cells, avoiding complement deposition, dysregulated immune hyperactivation, and evasion of phagocytic killing.[41,131,132] In addition, hyperimmune serum or monoclonal antibodies could be given to patients undergoing surgery as a form of passive immunization. There is evidence that preexisting antibodies against TSST-1 protects people from TSST-1–induced disease.[133] Therefore, this research is aimed at increasing the preexisting antibody titers to some of the key virulence determinants to reduce the severity of infection. Several active and passive immunization strategies are being undertaken and are mainly targeted at molecules involved in pathogenesis.

The selection of antigen for Merck V710 vaccine is based on study involving screening *S aureus* peptide libraries with human serum. The surface protein IsdB which plays role in heme acquisition and iron uptake was selected as antigen. This vaccine was found highly immunogenic and was protective against diverse strains in animal infection models.[134,135] Due to promising results with capsular polysaccharides as vaccine targets with *Haemophilus influenza* and *Streptococcus pneumoniae*, Nabi has developed a StaphVax vaccine based on type 5 and 8–based capsular polysaccharides bound to pseudomonal exotoxoid A as carrier. Passive immunization studies were promising with mouse and rat infection models of bacteremia. However, in phase 3 clinical trials involving hemodialysis, patient’s

Table 2. List of active immunization approaches against *Staphylococcus aureus.*

S. NO.	TARGET ANTIGEN	NAME	COMPANY	STATUS
Single targets				
1.	IsdB[134]	V710	Merck	Phase 2
2.	Capsular polysaccharides types 5 and 8[141]	StaphVax	Nabi	Phase III failed
3.	α-toxin (H35L)[139]			Preclinical (reduced lethality in mouse lung infection model)
4.	Panton-Valentine leukocidin (PVL)[142]			Preclinical (controversial results on efficacy in mouse lung infection)
5.	PNAG (PIA)[143]			Preclinical (protection in murine bacteremia)
6.	Enterotoxin B (SEB)[144]		Integrated BioTherapeutics	Phase 1 (protects monkeys from infection by SEB-positive strain). As antibiological biowarfare
7.	Enterotoxins A and C1, TSST		Integrated BioTherapeutics	Preclinical
Composite targets				
8.	Capsular polysaccharide types 5 and 8, nontoxic derivatives of α-toxin and PVL, wall teichoic acids[137]	PentaStaph	Nabi	Preclinical
9.	ClfA/MntC/CP5/CP8 conjugated to CRM197[145]	SA4Ag	Pfizer	Phase 3 failed
10.	CP5/CP8/TT/AT/ClfA plus AS03B[146]	GSK2392103A	GSK	Phase 1 failed
11.	Capsular polysaccharide types 5 and 8, ClfA		Wyeth/Pfizer	Phase 1
12.	Multicomponent surface proteins (SdrE, IsdA, SdrD, IsdB)		Novartis	Preclinical (protects from lethality in mouse infection model)
13.	Iron-regulated proteins[147]		Syntiron	Preclinical
14.	Candidal adhesion protein fragment Als3p[152]	NDV3	NovaDigm	Phase 1 failed
15.	Glycosylated CP5, CP8, and HlaH35L[132]	Glycovaxin	GSK	Preclinical failed

Table adapted and modified from Otto.[132]

protection was seen only until 40 weeks. Decrease in protection was correlated with decrease in *S aureus* antibodies. Therefore, the company has stopped further development of StaphVax vaccine. However, StaphVax could be administered to patients who need protection for shorter duration or people visiting hospitals for short duration such as surgery. Despite the presence of impressive opsonophagocytic anticapsular antibodies, they failed to protect patients for longer durations. Failure of capsular polysaccharides as vaccine candidates in *S aureus* in contrast to success in *H influenza* is due to the fact that role of capsular polysaccharide in *S aureus* pathogenesis is very limited.[136,137]

α-Hemolysin is a potent cytolytic toxin encoded on core genome and is present in most *S aureus* strains which makes it an ideal vaccine target. Earlier studies involving α-toxin and whole killed *S aureus* did not show efficacy in preventing infection in dialysis patients.[138] However, a nontoxic, nonhemolytic variant of α-hemolysin H35L has proven to be valuable for vaccine development.[139] Role of PVL as a vaccine candidate is highly controversial as its role in contribution to pathogenesis. Panton-Valentine leukocidin had no protective effect against CA-MRSA strain USA300 clone in mouse lung infection model; however, α-hemolysin showed strong protective effect.[140] After failure of StaphVax, Nabi has further added 3 antigens in its vaccine, namely, WTA, nontoxic α-hemolysin variant, and PVL. This vaccine is now called PentaStaph owing to the 5 antigen components in the formulation. Furthermore, a variety of targets such as surface proteins and adhesins have been evaluated as vaccine candidates in different studies. These active immunization strategies have been summarized in Table 2.

Due to the limited success with vaccines strategies against *S aureus*, there has been shift toward passive immunotherapy approaches. Most of these strategies are aimed at neutralizing the virulence determinants in particular toxins and surface components (Table 3). Because *S aureus* has a diverse array of virulence factors, passive immunotherapy approaches should be aimed at several different virulence determinants. A multivalent antigen offers more promise than distinct individual antigens in that they might induce complementary and nonoverlapping

Table 3. List of passive immunotherapy approaches against *Staphyococcus aureus.*

S. NO.	TARGET	NAME	COMPANY	STATUS	REMARKS
Single target					
1.	Capsular polysaccharides types 5 and 8	Altastaph	Nabi	Phase 2 failed	Polyclonal serum from individuals treated with StaphVax
2.	ClfA (surface protein)	Aurexis	Inhibitex	Phase 2 failed	mAb
3.	ABC transporter	Aurograb	NeuTec/Novartis	Development stopped	Ab fragment
4.	Lipoteichoic acid	Pagibaximab	Biosynexus	Phase 2 finished	Humanized mouse chimeric Ab
5.	α-toxin (nontoxic derivative H35L)			Preclinical (protective in mouse lung infection)	Polyclonal Ab, mAb
6.	PVL			Preclinical (no protection in mouse lung infection)	Polyclonal
7.	Enterotoxin B (SEB)[152]			Preclinical (protects monkeys from infection by SEB-positive strain)	Possible antibiological warfare drug
8.	Agr AIP 4			Preclinical (protects mice from abscess formation, death)	Specific for *S aureus* Agr subgroup 4
9.	Protein A		Elusys/Pfizer		Heteropolymeric Ab against protein A and human CR1
10.	α-toxin	AR-301 (Salvecin)	Aridis	Phase 2 failed	Monoclonal Ab adjunctive therapy to standard of care antibiotics in hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia
11.	α-toxin	MEDI4893	MedImmune	Phase 2 failed	Dose-ranging efficacy and safety in mechanically ventilated adults
Composite targets					
10.	ClfA, SdrG	Veronate	Inhibitex	Phase 3 failed	Serum from donors with high titers against ClfA and SdrG
11	Anti-WTA THIOMAB covalently linked to rifalogue by cathepsin cleavable linker	AAC		Preclinical	Tested in mice model with better protection than vancomycin

Abbreviations: PVL, Panton-Valentine leukocidin; WTA, wall teichoic acid.
Table adapted and modified from Otto.[132]

immune mechanisms of protection across diverse human populations.

It is now understood that the primary immune mechanisms required for protection against *S aureus* infections include phagocytes and T lymphocytes (Th17 cells).[41] In addition, antigen selection should be such that they induce strong humoral as well as T-cell immune responses that react to broadest possible *S aureus* strains. In this regard, multivalent antigens will be more likely to induce both humoral and T-cell immune responses and might give protection to broad array of *S aureus* strains. Among T cells, Th17 cells are important in vaccine-mediated protection against *S aureus* in mouse model and they act by recruitment of neutrophils to the site infection and promoting their killing.[148] Invasive infections of *S aureus* result in generation of memory immune response as seen by high antibody titers postinfection. However, whether this memory immune response will protect against recurrent infection is not well established.[149,150] In various studies involving disparate populations, it was observed that 10% to 30% of cutaneous abscesses resulted in recurrence.[151,152] Therefore, natural infection with *S aureus* does not result in a protective immune memory response which leads to further recurrence. Possible reasons for failure with traditional vaccines may be due to immune evasion mechanisms of *S aureus*, for example, the killing of phagocytes by leukolytic toxins. Lessons

from clinical and preclinical research reports suggest to the use of surface proteins and toxins with proven role in pathogenesis as promising targets for vaccine development. The use of therapeutic antibodies represents a novel, adjunctive, or alternative strategy to specifically target toxins with a demonstrated role in *S aureus* virulence.

Conclusions

Despite numerous efforts in developing a vaccine for combatting *S aureus*, no vaccine was successful in providing a memory immune response to previous infection. Lessons from clinical and preclinical research reports suggest to the use of surface proteins and toxins with proven role in pathogenesis as promising targets for vaccine development. The use of therapeutic antibodies represents a novel, adjunctive, or alternative strategy to specifically target toxins with a demonstrated role in *S aureus* virulence. The development of novel antibody-based therapies might offer hope in treatment of severe and invasive infections as an adjunctive to antibiotic treatment. The antibodies should target and neutralize virulence factors, immune evasion molecules, and surface factors to target them for destruction.

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Author Contributions

PNR is involved in literature collection, writing, editing and revising manuscript. KS involved in proof reading and providing critical correction in language and material. VRD is involved in preparing manuscript outline, proof reading and undertaking manuscript revision.

Criteria for Literature Search and Selection

Data for this review were identified from searches in PubMed, Google Scholar, and ScienceDirect and from references of popular articles. Some of the data presented were also identified from the extensive literature collections of the authors. Some of the key words for searching and selection of literature were *S aureus*, virulence factors, antibiotic resistance, host-pathogen interactions, superantigens, toxins, detection, immunodiagnostics, ELISA, subunit vaccine, prophylaxis, therapy, etc. Only articles written in English language were chosen for review. No data restriction was set during literature search.

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