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Review

Updates on Staphylococcal Vaccines

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Abstract: *Staphylococcus aureus*, a prevalent human pathogen and a leading cause of hospital-acquired infections, is increasingly evolving antibiotic-resistant strains, increasing mortality and morbidity rates. Anti-staphylococcal vaccine research for prevention and treatment has become a priority. Antibodies against specific *S. aureus* components, toxins, and polysaccharides have demonstrated encouraging results in animal studies regarding protection against colonization or infection. However, human immunization trials have yielded less optimistic outcomes, with no anti-staphylococcal having passed clinical trials up to now. Although multiple formulation attempts triggered strong antibody responses, the vaccines could not effectively prevent *S. aureus* infections. This article delves into the results of immunotherapeutic strategies against *S. aureus* in both animal and human studies, discussing the feasibility of adequate immunization approaches against *S. aureus* in humans.

Keywords: staphylococcal vaccine; *Staphylococcus aureus*; autovaccine

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1. Introduction

The rise in *Staphylococcus aureus* antibiotic resistance poses a significant healthcare challenge of the twenty-first century [1]. Although new antibacterial medications are constantly being developed, there are still isolates identified that are resistant to even the most cutting-edge antibiotics, such as linezolid [1].

Gram-positive *S. aureus* is well known for the number and severity of infections it causes in hospitalized patients [2]. The illnesses include localized skin infections, bacteremia, and septic shock [2]. *S. aureus* frequently colonizes human skin and mucosa, especially the upper airways, although some strains appear to prefer the gastrointestinal tract [3].

In addition to being a significant cause of severe toxin-mediated diseases, such as toxic shock syndrome, epidermolysis syndromes, and gastroenteritis, *Staphylococcus aureus*—despite being a commensal of human skin and the nares—frequently causes bacteremia, skin and soft tissue infections, pneumonia, osteomyelitis, and septic arthritis [4,5].

S. aureus colonizes a wide variety of tissues, which can either result in less-severe manifestations such as folliculitis, or in potentially fatal infections like pneumonia, endocarditis, osteomyelitis, and sepsis [6,7]. The pathogen is known to cause recurring diseases, indicating that humans do not naturally develop a strong, long-lasting immune response against it [6]. In recent decades, the need for a vaccine to prevent the spread of *S. aureus*-related invasive disease has significantly grown [4]. It is vital to develop an effective vaccine to lower the frequency of fatal diseases [6]. Numerous antigens found

on the surfaces of *S. aureus* strains have been researched for their vaccination potential, either curative or preventive [6]. Understanding the immune response to a particular organism is frequently necessary for the development of a vaccine so that it may be improved upon through thoughtful vaccine design [5]. Natural anti-*S. aureus* immunity has been extensively studied, but further research is needed to bridge the gap to vaccines [2].

2. Approaches towards a *S. aureus* Vaccine

Preventing *S. aureus* disease would be the best strategy to reduce the morbidity and mortality that this organism causes [8]. Developing a widely effective vaccine is a challenging task, because of the various factors determining its virulence. Some of these factors are fibronectin-binding proteins, teichoic acid, clumping factors, hemolysin, phospholipase-C, metalloprotease, and capsular polysaccharides [8]. Based on these factors, tests were conducted on a range of *S. aureus* virulence factors to develop vaccines or other protective medications. Examples include the use of inactivated (whole-killed) staphylococci vaccines, bacterial interference tests, opsonophagocytosis assays, and genomic/bioinformatics approaches [8].

The numerous attempts to formulate vaccines against *S. aureus* can be categorized into two different methods: passive immunization (transfer of *S. aureus* antigen-specific antibodies, Table 1) or active immunization (vaccination with recombinant antigens to induce protective antibody responses, Table 2) [6].

Table 1. Passive immunization vaccines.

Name	Compound	Target	Company	Status	Reference
SEB-specific antibody	Chicken immunoglobulin IgY	Staphylococcal enterotoxin B	-	Animal model	[9]
Tefibazumab, Aurexis	humanized monoclonal antibody	Clumping Factor A	Bristol-Myers Squibb	Phase IIa	[10]
Altastaph	Polyclonal human immunoglobulin G	Capsular polysaccharide type 5 and type 8	Nabi Biopharmaceuticals	Phase II	[11]
Aurograb	Monoclonal antibody	ABC transporter	Novartis	Phase II	[12]
Pagimaximab	Humanized mouse chimeric monoclonal antibody	Lipoteichoic acid	Biosynexus	Phase IIb/III	[13]
DSTA4637S	Engineered human IgG1 monoclonal antibody	Wall teichoic acid at the surface of *S. aureus*	Genentech	Phase Ib	[14]
mAbtyrin	Human-derived anti-*S. aureus* monoclonal antibody (mAb)-centyrin fusion protein	Bacterial adhesins	Animal model	Animal model	[15]
ASN100	Monoclonal antibody combination of two fully human IgG1(κ) monoclonal antibodies, ASN-1, and ASN-2	Alpha-hemolysin (Hla) and five bicomponent leukocidins	Arsanis	Phase II	[16]
Tosatoxumab or AR-301	Fully human monoclonal IgG1 antibody	*S. aureus* alpha-toxin	Aridis Pharmaceuticals, Inc	Phase III	[17]

Table 1. *Cont.*

Name	Compound	Target	Company	Status	Reference
Suvratoxumab or 'AR-320'	Human anti-alpha-toxin IgG1 monoclonal antibody	*S. aureus* alpha-toxin	Aridis Pharmaceuticals, Inc	Phase III, ongoing	[18]
INH-A21, Veronate	pooled human immunoglobulin purified from the serum of donors with high titers against ClfA and SdrG	Staphylococcal adhesins that bind fibrinogen and fibrin (*S. aureus* ClfA and *S. epidermidis* SdrG)	(Inhibitex)	Phase III, failed	[19]

Table 2. Active immunization vaccines.

Name	Target	Company	Status	Reference
-	Alpha-haemolysin (Hla)	-	Animal model	[20]
-	Clumping factor B (ClfB)	-	Animal model	[21]
Glycovaxine	CP5/CP8/HlaH35L (recombinant)	GSK	Animal model	[22]
4C-Staph	ferric hydroxamate uptakeuD2, EsxAB, HlaH35L, conserved staphylococcal antigens1A (purified, alum-adjuvanted)	Novartis	Animal model	[23]
SA75	Whole-cell vaccine	Vaccine Research International	Phase I	[23]
STEBvax	Enterotoxin B (rSEB)	Integrated BioTherapeutics	Phase I	[24]
SA4Ag (PF-06290510)	ClfA/MntC/CP5/CP8 (conjugated CP5/CP8 plus recombinant MntC/ClfA)	Pfizer	Phase IIb/III	[25]
StaphVax	capsule polysaccharides 5 and 8	Nabi Biopharmaceutical	Phase III	[26]
V710 (0657nl)	Iron surface determinant B (IsdB)	Merck	Phase III stopped	[2]
rFSAV	Hla, SpA, SEB, IsdB, MntC + Alum-adjuvanted	Olymvax	Phase II	[27]
SA5Ag		GSK	Phase II	[28]
NDV-3A	Als-3 (C. albicans cross reactive cell wall protein) + Alum	Novadigm Therapeutics	Phase II	[29,30]
IBT-V02	SEB, SEA, TSST-1, LukS, LukF, LukAB, Hla + alum	Integrated Biotherapeutics	Phase II	[31]
Lysigin	Whole-cell vaccine	Boehringer Ingelheim Vetmedica	Animal model	[32]
Startvac		Hipra	Animal model	[32]
Sa3Ag	*S. aureus* capsular polysaccharides	Pfizer	Phase II	[33]

Because *S. aureus* has more than 50 virulence factors, it can adapt to a wide range of host niches and cause a wide range of infections [34]. The pathogen must overcome host defenses, adhere to extracellular matrices and cells, and obtain vital nutrients—which are scarce in vivo—in order to establish and sustain an infection [34]. The innate immune system broadly recognizes invading pathogens through receptors that bind pathogen-

associated molecular patterns (PAMPs), such as single-stranded RNA, lipoteichoic acid, and peptidoglycan (PGN) [6].

Numerous studies have emphasized the role that humoral immunity plays in the prevention and management of *S. aureus* infections [35]. The host defends itself by deploying phagocytes, complement, and antimicrobial peptides (innate immune response) that fight the bacterium, followed by immunoglobulins that promote phagocytosis or trigger other antibody-mediated immunity mechanisms (adaptive immune response) [35]. A significant amount of research has been conducted to identify therapeutic approaches for strengthening and enhancing those host defense mechanisms [35]. Particularly in hospitalized acute patients with acquired immunodeficiencies, passively administered antibodies may prove beneficial in protecting against the quick and initial infection and, consequently, against its progression [35].

2.1. Microbial Factors

2.1.1. Clumping Factor A

Additional studies on *S. aureus* surface proteins, such as clumping factor A (ClfA), collagen binding protein (Cna), fibronectin-binding proteins, fibrinogen binding proteins, and secreted toxins, are currently being conducted [2]. A surface adhesin called ClfA attaches itself to the plasma fibrinogen C-terminus γ chain [36]. The multifunctional activities of this well-characterized virulence factor revolve around this interaction [36]. It facilitates the attachment of pathogens to platelets and encourages fibrin cross-linking, which leads to the formation of thrombi or blood clots [36]. Additionally, it has been demonstrated that ClfA is essential for the agglutination of *S. aureus* in the blood during infection, which results in sepsis and thromboembolic lesions in the heart tissue [36].

Comparable to the murine sepsis models employed in this investigation, several such antigens have been tested. ClfA has been tested in passive immunization studies using a ClfA-specific monoclonal antibody [2].

Clinical Phase I and II studies have demonstrated early promise for Veronate®, a human polyclonal immunoglobulin preparation derived from plasma donors with naturally occurring levels of IgG antibodies against ClfA [19]. However, the Phase III confirmatory study did not meet its intended endpoints for protection in infants with extremely low birth weight [19].

In prophylactic and therapeutic rabbit models, AurexisTM, a humanized IgG1 antibody with high affinity for ClfA, demonstrated efficacy [35]. In a Phase II trial, 60 adults with *S. aureus* bacteremia treated with vancomycin received a single dose (20 mg/kg) of AurexisTM; the results showed no worsening of sepsis and no statistically significant reduction in deaths when compared to the control group [35].

S. aureus can attach to human fibrinogen through the adhesion of ClfA, and antibodies against this protein can prevent this binding [37]. A *Staphylococcus aureus* four-antigen vaccine (SA4Ag) was developed to protect surgical patients against invasive disease [37]. The vaccine contains mutant clumping factor A (Y338A-ClfA) and manganese transporter subunit C (MntC), as well as conjugates of capsular polysaccharide types 5 and 8 (CRM197) [37].

2.1.2. Capsular Polysaccharides

Over 80% of *S. aureus strains* produce capsular polysaccharide serotypes 5 or 8, most strains being encapsulated [35]. It has been demonstrated that capsules increase virulence and induce the production of protective antibodies [35].

In a 10-month study period, infection was reduced by 56% in hemodialysis patients who received a vaccine against capsular polysaccharides conjugated with nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A [26]. Two *S. aureus* capsule serotypes (5 and 8), which account for around 70% of *S. aureus* clinical isolates, are the target of this vaccine [26].

Despite the fact that capsular polysaccharides grant the bacteria effective means of immune evasion, anti-polysaccharide antibodies effectively attach to the surface of bacterial cells and promote phagocytosis [36]. Developing a single, all-encompassing anti-

S. aureus vaccine proved challenging due to the numerous staphylococcal antigens and diverse pathogenic pathways [38]. However, there has been substantial progress in the development of vaccines against *S. aureus* [38]. Approaches such as StaphVAX and V710, although unsuccessful, are a step forward for further research and offer important lessons on what should be improved down the line [38]. In an effort to address the heterogeneity of virulence factor expression throughout the infection process and the diversity of clinical pathology associated with *S. aureus* infections, current development efforts employ multi-antigen approaches [38]. StaphVax vaccine, which consists of *Pseudomonas* exotoxin A-conjugated type 5 and type 8 capsular polysaccharides, has not met its Phase III clinical endpoint [39]. Instead of a breakdown in the mechanism of action, it has been proposed that this may have been caused by variations in the quality of the conjugates used in the trial [39].

2.1.3. Manganese Transporter C (MntC)

Important immunodominant antigens, or *S. aureus* surface proteins, are frequently the first molecules to interact with host cells and tissues [38]. Because these proteins are crucial for adhesion and nutrient acquisition and because their occurrence and structure are highly conserved across strains, they are the primary targets for the development of *S. aureus* vaccines [38]. Several studies have indicated that the formulation of an effective *S. aureus* vaccine through subunit vaccination is a promising trend [38]. These studies have examined proteins such as Clumping Factor A (ClfA) and Manganese Transporter C (MntC) and have demonstrated their capacity to elicit a protective immune response [38]. MntC is a highly conserved (>98% sequence identity) lipoprotein [23], part of the manganese transporter MntABC, which is expressed during the formation of biofilms in animal models and is rapidly upregulated in vivo [37]. Manganese uptake by *S. aureus* via oxygen-radical detoxification is crucial for both growing and for avoiding neutrophil killing [37]. Manganese Transporter C is the surface-exposed-metal-binding subunit of MntABC, a heterotrimeric membrane transporter that is involved in the uptake of manganese [36]. Manganese is an important metal ion for many pathogens [40]. MntC functions essentially as a metal-binding protein and has been demonstrated to provide protective immunity against *S. aureus* infections in animal model systems [40]. The sequestration of metal ions necessary for bacterial survival is a major host defense mechanism against bacterial invasion [36]. *S. aureus* and other bacteria have evolved strategies to quickly scavenge divalent cations, such as manganese and iron [36].

After immunizing healthy subjects, vaccines containing SEB (STEBVax, IBT/NIAID) or MntC (SA4Ag, Pfizer) successfully completed Phase I clinical trials that revealed no safety concerns and a favorable immunogenicity profile [41]. In a sepsis and pneumonia model, the average survival rates were only 53% and 36%, respectively, despite the fact that 2C-Staph (containing recombinant proteins staphylococcal enterotoxin B and manganese transport protein C) without adjuvant induced higher survival rates than single-antigen groups [41]. Most of the recent vaccines contain combinations of antigens.

In a murine study, the phagocytosis of *S. aureus* by macrophages was effectively facilitated by MntC-specific antiserum [42]. Furthermore, the death rate by challenge and the bacterial load in the organs were both clearly reduced by MntC-specific antiserum [42]. These findings suggest that polyclonal antibodies specific to MntC can effectively mediate immune protection against *S. aureus* infection [42]. MntC may be a therapeutic target for the development of antibiotics; anti-MntC monoclonal antibodies have been shown to bind to *S. aureus* cells, and MntC may define possible antigen combinations for multi-component vaccines [40].

2.1.4. IsdA or IsdB

Iron-regulated surface determinant (Isd) proteins extract heme bound iron from host hemoproteins, helping the bacteria to obtain the necessary iron for bacterial growth [43]. In a murine study, when combined with amorphous aluminum hydroxyphosphate sulfate

adjuvant IsdB proved very immunogenic [2]. In a mouse model of infection, the activation of IsdB-specific antibody responses was associated with considerable and repeatable protection against a variety of clinical isolates of *S. aureus*, including methicillin-resistant strains [2]. The HarA protein (also known as IsdH) is a receptor for hemoglobin, haptoglobin, and their complex [43]. Mice immunized against IsdB were not shielded from deadly infections when challenged with a strain with IsdB HarA deletions, suggesting the specificity of an immune response directed against surface-expressed IsdB [2].

Compared to the mice that perished from the illness, the IsdB-immunized animals that survived the fatal challenge showed higher antibody responses [2]. This emphasizes the function of IsdB antibody responses in sepsis protection [2].

In iron-restricted environments, the *S. aureus* cell surface expresses the 72 kDa antigen known as IsdB to remove heme iron from hemoglobin and import it [44]. IsdB is upregulated during pathogenesis in vivo because mammalian blood and tissues are a low-iron environment [44]. IsdB is highly conserved in a variety of *S. aureus* clinical isolates, including both methicillin-resistant and methicillin-sensitive strains. It is also present in humans and in all mammals tested to date [44].

It is interesting to note that TSA (Trypticase Soy Agar), a medium that suppresses the expression of IsdB, is frequently used to prepare the bacteria to challenge animals [2]. Under these growth conditions, there is no detectable amount of IsdB on the surface of *S. aureus* [2]. Nonetheless, studies on immunity and the capacity to detect protein IsdB surface expression in vivo-grown bacteria imply that the protein is quickly expressed during infection [2]. In a randomized Phase 2b/3 trial, V710, which contained the iron uptake component IsdB, failed to achieve its primary efficacy endpoint (preventing life-threatening postoperative *S. aureus* infections after cardiothoracic surgery) [45]. Furthermore, V710 was linked to a higher mortality rate in patients who acquired *S. aureus* infections [45].

Preoperative vaccination with V710 did not significantly lower the composite incidence of *S. aureus* bacteremia and deep sternal wound infection in a study of adult patients undergoing cardiothoracic surgery [45]. The lack of efficacy was not solely attributable to a failure to increase IgG levels against homologous IsdB because vaccination with V710 elicited consistent humoral responses [45].

Recombinant IsdB, or the V710 vaccine, induced protection through Th17 pathways that have previously been linked to unfavorable results in clinical trials [46]. The V710 vaccine trial was discontinued by the data monitoring committee due to hyperimmune reactions in participants who had acquired invasive *S. aureus* infections [46]. Consistently low IL-2 levels were present prior to vaccination in patients who died after receiving the V710 vaccine, according to a follow-up study conducted to potentially explain these results [46]. This immunological dysregulation correlate was revealed by the vaccine, validating the general theory that vaccine safety and efficacy depend on a protective but restrained immune response [46]. Given that IL-2 is required for the development of regulatory T cells, it is possible that patients with low IL-2 levels may be deficient in the production of Treg cells, which regulate the inflammatory response in the system [46].

2.2. Host Factors

2.2.1. T Cells

T cells are essential for vaccine-mediated protection against infections, according to an expanding body of data [44]. The control and activation of neutrophils and macrophages are closely associated with Th1 or Th17-polarized immune responses [44]. IL-17 primarily mediates chemotaxis and activation of neutrophils during *S. aureus* infection [44]. IFN-gamma is a crucial cytokine that renders macrophages more effective at killing microbes [44]. Previous research showed that the IsdB or ClfA40-559 vaccines caused the production of IL-17, which was essential for the vaccines' protective efficacy against *S. aureus* infection [44].

T cells derived from the thymus express distinct T cell receptors that can identify antigen-derived peptides bound to major histocompatibility complex molecules on antigen-presenting cells [47]. The presence of detectable T cell responses in humans, as well as

the pathogen's ability to modulate T cells by expression of numerous T cell superantigens, support the involvement of T cells during *S. aureus* infection, much like B cells and antibodies [47]. T cells, however, have not been shown to be required for the protection of mice against *S. aureus* [47]. Moreover, *S. aureus* is infrequently identified as the source of infection in individuals with T cell deficiencies; however, the high susceptibility of these patients to other pathogens complicates our ability to accurately evaluate the role of T cells in *S. aureus* immunity in this setting [47].

Data from recent studies indicate that the secret to developing a vaccine may lie in finding antigens that can both stimulate humoral immunity and cell-mediated immunity [48]. One of the membrane-anchored proteins involved in the stress response, alkaline shock protein 23, has been identified as an *S. aureus* CD4+ T cell antigen [48]. On post-immunization days 21, 35, and 42, serum titers of IgG, IgG1, and IgG2a produced in response to the protein were assessed using indirect ELISA and compared to control mice injected with PBS [48]. As compared to the control group, the results indicated that the protein generated much greater antibody responses in vaccinated mice [48].

In both local and disseminated infection models, T cells were found to be crucial in preventing *S. aureus* infection [44]. These studies demonstrated a relationship between T cells and neutrophils, proving the significance of IL-17A in neutrophil recruitment, chemotaxis enhancement, and neutrophil priming for bactericidal activity [44].

Different immune responses mediate protection depending on the site and type of the *S. aureus* infection or colonization [49]. Antigen-specific cellular (Th1 and Th17) responses are crucial for defense against skin and soft-tissue infections (SSTI) (dermonecrosis and abscesses) and gastrointestinal infections, but they do not supply significant protection against i.v. infection [49]. Some antibodies, for example, protect mice against *S. aureus* i.v. infection and, to a lesser extent, dermonecrosis, but are ineffective in preventing skin abscesses or gastrointestinal colonization [49]. When vaccination activates all three immune pathways, the best support against *S. aureus* is elicited [49]. In order to fully protect against *S. aureus*, vaccination strategies that aim to elicit multipronged B and T cell responses to the pathogen's antigens may be essential [49].

2.2.2. Antibody Therapy

A promising human monoclonal antibody therapy, 514G3, is currently being designed to treat *S. aureus* bacteremia [50]. The immune profile of a healthy human donor was used to isolate 514G3, which is specific to the *Staphylococcus* protein A (SpA) [50]. Early in 2017, 514G3 finished a double-blind, placebo-controlled Phase 1/2 clinical trial with over 50 patients in a hospital setting [50]. Arsanis, Inc. has developed ASN100, a monoclonal antibody treatment against *S. aureus* that targets leukocidins ED, GH, α-hemolysin, Panton-Valentine leukocidin, γ-hemolyins AB, and γ-hemolyins CB. ASN100 was specifically targeted at patients on respiratory support who were at risk for pneumonia caused by *S. aureus* [50]. After a Phase 1 trial's endpoints were reached, a Phase 2 clinical trial has been announced [50].

AltaStaph is a hyperimmune polyclonal antibody compound obtained from healthy individuals who have received a *S. aureus* capsular polysaccharide vaccine conjugated with *Pseudomonas aeruginosa* recombinant exoprotein A [51]. AltaStaph elicits significant levels of antibodies against type 5 and type 8 capsular polysaccharides [52]. It provides passive protection in a variety of animal staphylococcal sepsis models and demonstrates opsonizing action in opsonophagocytosis tests in vitro [52]. AltaStaph has been investigated in humans, particularly in low-birth-weight and very-low-birth-weight newborns [52]. In a trial including patients with *S. aureus* bacteremia, passive immunization with AltaStaph produced higher levels of anti-type 5 and anti-type 8 capsular antibodies for up to six weeks when compared to placebo [51].

Gram-positive bacteria like *S. aureus* cannot be eliminated by complement lysis alone; immune cells must engage in opsonophagocytic killing in order to defend the host [4].

According to earlier research, IsdA- or IsdB-specific antibodies encourage isolated human PMNs to kill staphylococci by opsonophagocytic activity [4].

Antibody-mediated protection after active and passive immunization in pre-clinical models, the widespread presence of anti-staphylococcal serum antibodies, the modulation of antibody responses by *S. aureus* virulence factors, and human data relating antibody protective titers all imply that they may very well contribute to the protective response [47]. The possibility of ineffective or harmful antibody responses is further supported by published data, highlighting the need to better understand protective antibody response characteristics in order to clarify vaccine-design contributions to natural susceptibility [47].

High IgG levels were produced in sera after MntC vaccination, and IgG1 was the most common IgG subtype [42]. Antibodies have been demonstrated to offer some protection against *S. aureus* infection in a number of scenarios [42]. It is possible that these antibodies mediate antibody-dependent cell killing activities by interacting with the pathogen soon after infection [42].

It was previously believed that the uptake and destruction of *S. aureus* by phagocytes is mediated through complement and antibodies, with neutrophils being crucial to the healing process [44]. However, *S. aureus* was actually shown to survive inside neutrophils, aggravating the disease [44]. In some models, the humoral immune response induced by *S. aureus* did not contribute significantly to bacterial clearance [44]. Although antibodies undoubtedly play a part in defense, the effectiveness of vaccine protection may not be determined by antibodies because both animals and humans have enough opsonins at rest to allow for neutrophilic phagocytic uptake [44].

2.3. Other Approaches

In the sepsis and pneumonia models, intramuscular or intranasal vaccination with the 2C-Staph augmented with a nano-emulsion vaccine led to greater survival rates ($p < 0.05$) when compared to the 2C-Staph and 2C-Staph/MF59 (MF59—an oil-in-water emulsion adjuvant) vaccines [37]. Overall, the nano-emulsion (NE) adjuvant-formulated 2C-Staph improved the immune responses [37].

In another study, mice were immunized with lethally irradiated MRSA (strain USA300 LAC) to investigate the immune response generated against the different epitopes presented by this whole-cell vaccination [44]. When mice were given a lethal dose of live MRSA intravenously, vaccination had no protective effect [44]. However, when mice were given a sublethal dose of of live MRSA, vaccination resulted in higher mortality rates when compared to mock-vaccinated mice. Vaccination increased the bacterial burden in the lungs, spleen, and blood on days two and five after infection, failing to control bacterial growth in blood and multiple organs [44].

Toxins, either alone or in combination with vaccines, may constitute a further vaccination-field strategy as they share similar domains that may be targeted; Pfizer's SA4Ag vaccine candidate, for instance, showed encouraging outcomes up to Phase IIb [45]. It was constructed of recombinant MntC, ClfA, and both CP5 and CP8 conjugated to a detoxified version of diphtheria toxin [45]. IBT-V02 contained seven *S. aureus* toxoids: Hla; Panton-Valentine Leukocidin F and S subunits; Leukocidin A/B; SEA; SEB; and Toxic shock syndrome toxinand showed successful results in Phase II as well as in assays mimicking pre-infection with *S. aureus* [44].

The inclusion of toxin components to multi-component prophylactic vaccine formulations is dubious because of the heterogeneous nature of *S. aureus* toxins, and the fact that they are typically released after an infection has already been established [31].

The *spa* gene encodes protein A, a highly conserved *S. aureus* cell wall protein that binds with high affinity to the Fc region of human IgG1 and IgG2 and of mouse IgG2a and IgG2b [31]. This prevents functional antibodies from obstructing essential functions like adhesion and opsonophagocytosis [31].

A culture of pathogenic microorganisms isolated from an infection site has been used to make autovaccines [51]. Nonetheless, the suggested methods for administering and

preparing autovaccines vary somewhat based on the manufacturer [51]. Generally, the cultured microorganisms are inactivated by heat or chemicals (0.4% formalin solution) and suspended in a sterile 0.9% NaCl solution [51]. Based on McFarland quantification, the inactivated whole-cell suspension is bottled in the laboratory in a series of vials with densities of 5×10^8, 1×10^9, and 2.5×10^9 bacteria/mL. Either 0.5% phenol or 0.1 mg/mL thiomersal are added for preservation [51]. Finally, cultivation in a liquid enrichment medium is used to test the sterility of these suspensions [51].

The first infection cannot be prevented since creating an autogenous vaccination requires it [53,54]. The number of bacteria used, the inactivation process, and the adjuvants all affect vaccination efficacy [53]. Research on animals has shown that opsonization and phagocytosis, in conjunction with the involvement of particular antibodies against the capsular polysaccharides, are the primary factors influencing protective immunity against extracellular microorganisms, such as Gram-positive bacteria [55]. Following an infection challenge, this mechanism provides effective protection. IgG2a class antibodies are essential for defense against *S. aureus* [55]. Several authors have addressed the role of IgG2a in protection against this disease [55]. Antibodies generated by vaccination could have had a role in the C57BL/6 mice's protection and decreased bacterial burdens in the inflammatory environment [55]. The C57BL/6 mice that were not immunized had greater bacterial loads in their air pouches [55].

Predicting bacterial immunogens is an essential step in the reverse-vaccinology process [56]. Reverse vaccinology is a vaccine-prediction process that uses a genome sequence and the resultant complete proteome repertoire to identify candidates [48]. The entire spectrum of potential antigens is found in microbial genomes, which serve as the data foundation for the creation of vaccines [38].

The initial aim of reverse vaccinology was to screen a pathogen's whole genome in silico for genes encoding proteins with properties that make them attractive targets for vaccinations, such as proteins that are likely to be surface exposed and that are highly conserved between strains [57]. These selected proteins were then placed in *Escherichia coli* and used to immunize mice in order to evaluate immunogenicity and protection based on the analysis of antisera by flow cytometry, serum bactericidal activity, opsonophagocytosis tests, or animal models of infection [57]. This has progressed to screening several genomes within a species [57].

Proteins can be employed as vaccine candidates in one or more cases [48]. Generally speaking, peptide-derived epitopes can be employed in lieu of the entire protein in order to trigger an immune response [48]. In order to be considered potential vaccine candidates, the proteins (and their derived epitopes) must be highly antigenic, surface exposed, and accountable for the generated pathogenicity [48]. Through in silico techniques, the time and expense required to find viable vaccine candidates among bacterial species' proteins can be greatly decreased [56].

Artificial intelligence systems search for the microbial components that are least likely to change or mutate in order to ensure that a vaccine is effective for an extended period of time [49].

3. Application of *S. aureus* Vaccines

Staphylococcus aureus has the ability to permanently colonize humans; as a result, the bacteria and the host immune system constantly interact [36]. The finding that all people already have antibodies against *S. aureus* antigens supports this [36]. *S. aureus* colonization increases the risk of infection following surgery or trauma, despite the fact that it generally does not trigger infection in healthy persons [42]. Vaccines are promising substitutes for harmful antibiotics [42].

In a trial aimed at reducing *S. aureus* colonization, antibody concentrations dramatically increased following conjugate-vaccine immunization, regardless of age, occupation, or colonization status [53]. Despite this increase in humoral antibody concentrations, five addi-

tional patients became colonized, and 74% of those with persistent colonization maintained their status [53].

Active immunization can considerably lower lung bacterial loads one day after *S. aureus* pneumonia infection [58]. Although the bacterial loads in the control group decreased with time, they remained significantly higher than those in the $IsdB_{151-277}ClfA_{33-213}$ group [58]. While pneumonia severity was controlled in the $IsdB_{151-277}ClfA_{33-213}$ group, it grew worse over time in the control group [58]. Another respiratory disease that would benefit from a *S. aureus* vaccine is bronchiectasis [54,55]. Genetic and environmental factors contribute to the development of bronchiectasis, though its exact etiopathology is still unknown [54,55]. It is an irreversible lung disease characterized by chronic inflammation of the of the proximal and medium-sized bronchi (>2 mm in diameter), and recurrent bacterial infections [54]. Although this pathology is common, it represents a significant cause of respiratory morbidity [54]. This compromises mucociliary clearance, rendering airways more susceptible to pathogen colonization [54].

When challenged with *S. aureus*, mice immunized with recombinant ClfA showed fewer instances of severe arthritis than mice immunized with a control antigen [59]. The protection provided by active immunization is antibody mediated, as evidenced by the fact that mice passively immunized with rat and rabbit anti-ClfA antibodies were protected against *S. aureus* arthritis and sepsis-induced death [59]. When considered collectively, these data strongly imply that ClfA is a key virulence factor for septic arthritis and a fantastic target for the development of immune therapies against *S. aureus* [59].

The direct binding of platelets by bacteria is a critical factor in the development of infective endocarditis (IE) [60]. For pathogenetic events that take place after the initial colonization of the valve surface—vegetation development and septic embolization—staphylococcus–platelet binding appears to be essential [60].

Inducing coagulation may promote the formation of new platelet and fibrin deposits on top of the infection nidus, protecting vegetation-adherent bacteria from further mechanical detachment and/or cellular host defense systems [61].

The likelihood of valvular infection depends on both the level of post-challenge bacteremia and the ligand-receptor interactions between the surface components of damaged valves and bacteria [61].

Although the proportion of staphylococci and streptococci has varied over time and by region, these two organisms have together accounted for about 80% of IE cases [62]. While the percentage of IE caused by viridans-group streptococci has decreased, the prevalence of *S. aureus* and coagulase-negative staphylococci has increased in tandem with the rise in healthcare-associated IE [62]. The third most common cause of IE is enterococci, which are becoming increasingly associated with medical interactions [62]. When infections with Gram-negative and fungal pathogens do arise in IE, they are mainly linked to health care [62].

Because of the severity of *S. aureus* infection, individuals in high-risk groups who are exposed to situations that enhance their chances of contracting *S. aureus* bacteremia should consider receiving suitable prophylaxis [63]. Patients are frequently prescribed prophylactic antibiotics; however, a lot of *S. aureus* strains, particularly those with nosocomial origins, are multiresistant. Additionally, certain antibiotic classes may cause allergies in certain patients [63]. Up to now, a number of studies have been carried out with the goal of creating a preventive vaccine, but they have been unsuccessful in creating a safe and reliable *S. aureus* IE vaccine [63].

For patients in critical care units or undergoing planned surgery, a vaccine that triggers an immediate protective response against *S. aureus* infection would be ideal [15]. The 4C-Staph vaccine, when appropriately adjuvanted, may help to accomplish this [15]. In a study, a novel small molecule targeting TLR7 adsorbed to alum was added to 4C-Staph to create 4C-Staph/T7–alum [15]. This vaccine formulation was tested as a single dose in mice models of *S. aureus* kidney abscess and peritonitis [15].

The 4C-Staph/T7–alum vaccine outperformed the 4C-Staph–alum formulation by more than 100 times, lowering the bacterial load in the kidneys of mice infected intraperitoneally and protecting around 80% of vaccinated animals from the catastrophic results associated with an intraperitoneal infection (i.p.) challenge [15]. Surprisingly, compared to 54% of 4C-Staph–alum vaccinated mice, 91% of mice that survived the i.p. had no detectable staphylococci in their kidneys [15]. These observations demonstrated that 4C-Staph/T7–alum facilitated more-effective control of bacteria than 4C-Staph–alum. Nonetheless, they revealed that using survival rates alone to estimate *S. aureus* vaccination efficiency might lead to overestimation of outcomes [15].

Several factors complicate the clinical evaluation of an investigational *S. aureus* vaccine at each stage of its development, one of which is the high likelihood that the final target population for any vaccine will be larger than can be investigated in efficacy trials [34]. When one takes into account the diversity of human populations at risk for *S. aureus* infection as well as the various comorbidities within those populations, one can appreciate the complexity of any potential clinical development plan [34].

4. Failure to Obtain an *S. aureus* Vaccine

Active- or passive-immunization-based *S. aureus* vaccine studies have been tried before, but none have been successful. There have been several proposed explanations for these unsuccessful trials: one antigen might not be enough to protect people against *S. aureus* infections; functional antibody levels were not assessed or defined; or humoral immunity is not enough to shield people from *S. aureus* infections. Formulating a vaccine against *S. aureus* infections continues to pose several challenges.

The failure of vaccination protectivity, as seen in preclinical infection models, is one of the most significant factors impeding vaccine development—a successful translation for protective efficacy in human subjects requires a clever solution to this impediment. Nonetheless, it is possible that the reaction of repeatedly immunized naive animals does not accurately mirror the maturation of a human immune response influenced by environmental exposure [37]. One example might be the response to ClfA [37]. Even after several vaccinations with adjuvant, naive mice respond poorly to ClfA, whereas humans and non-human primates respond favorably after just one dose [37].

The vaccine recipient's immune history could contribute to the inability to successfully adapt experimental vaccines to clinical practice; vaccines are tested in naive mice before being applied to humans, who always show inherent immune responses against *S. aureus.* Natural exposure to *S. aureus* "imprints" the immune system resulting in vaccine resistance, a pathogen-developed trait that helps the microbe survive during colonization or infection.

The absence of an adjuvant could be one reason responsible for the failure in obtaining a vaccine. The majority of the evidence supporting this theory comes from pre-clinical research using murine models that show improved immunogenicity in *S. aureus* vaccine formulations after the addition of an adjuvant. Others show distinct immune profiles in mice vaccinated with identical antigens but different adjuvant systems.

5. Conclusions

Developing a vaccine against *S. aureus* is challenging due to its complex pathogenesis involving numerous virulence factors. Past studies on vaccines aimed to tackle major mechanisms of *S. aureus* disease: immune evasion (capsule), host cell binding and immune evasion (ClfA), and nutrient acquisition (MntC). By targeting early-expressed *S. aureus* antigens and incorporating multiple targets, such as CP5, CP8, ClfA, and MntC, the vaccine seeks to induce functional antibodies capable of combating the pathogen's defense mechanisms. Past attempts at both active and passive immunization focused on increasing opsonizing antibodies against surface antigens like SdrG, IsdB, SEB, ClfA, and various capsules. However, trials relying on single-antigen-based vaccines have shown limited success, highlighting the need for multi-antigen approaches. Moreover, the use of innovative technologies or adjuvants in vaccine formulation may enhance clinical efficacy and speed

the design process. Considering *S. aureus*'s wide impact across different populations and diseases, successful prophylactic vaccines should target diverse patient groups.

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