Status of vaccine research and development of vaccines for *Staphylococcus aureus*\(^\ddagger\)

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**A B S T R A C T**

*Staphylococcus aureus* is a highly versatile gram positive bacterium that is resident as an asymptomatic colonizer on the skin and in the nasopharynx of approximately 30% of individuals. Nasopharyngeal colonization is a risk for acquiring *S. aureus* infections, which can cause a range of clinical symptoms that are commonly associated with skin and soft-tissue infections. The emergence of *S. aureus* strains that are highly resistant to antimicrobials has recently become a major public health concern. In low-income countries the incidence of *S. aureus* disease is highest in neonates and children up to one year of age and mortality rates are estimated to be up to 50%. In the United States, *S. aureus* infection accounts for approximately 300,000 hospitalizations per year. A vaccine against multi-drug resistant *S. aureus*, therefore, is urgently needed. Two vaccine candidates have previously been evaluated in late-stage clinical trials but have not demonstrated efficacy. At present, one vaccine candidate and two monoclonal antibody are undergoing clinical evaluation in target groups at high risk for *S. aureus* infection. This review provides an overview of current vaccine development efforts and presents the major technical and regulatory challenges to developing a licensed *S. aureus* vaccine.

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1. About the disease and pathogen

*Staphylococcus aureus* is a bacterium that is both an asymptomatic colonizer and frequent cause of disease in humans [1]. It is a highly versatile pathogen that causes a range of clinical manifestations of varying severity, and is the most commonly isolated pathogen in the setting of skin and soft-tissue infections, septic arthritis, pneumonia, endovascular infections, osteomyelitis, foreign-body associated infections, septicemia and toxic shock syndrome [2]. *S. aureus* infections strike people of all ages and backgrounds, but are most severe in young children, the elderly, the immunosuppressed and other individuals with major comorbidities [3,4]. The incidence of *S. aureus* infection in low income countries is highest in neonates and children up to one year of age with mortality rates of up to 50% [5], in contrast to high income countries where the disease appears to increase with age, or is most prevalent at the extremes of the age spectrum. However, there have been very few epidemiological studies in low and middle income countries, and it is likely that there is an under-reporting of *S. aureus*-associated disease generally, and particularly in the elderly in these settings [6,7].

*S. aureus* is known to be highly adaptable, and in recent history has shown a remarkable epidemiologic transition. Since 1959, when methicillin was first introduced, strains of methicillin-resistant *S. aureus* (MRSA) have been documented at a rapid and increasing rate. Hospital-associated MRSA (HA-MRSA) clones are now recognized to be the leading cause of nosocomial infections in low-, middle-, and high-income countries [5,8,9]. The emergence of community-associated MRSA (CA-MRSA) in the past several decades has also become a point of concern, as virulent strains of CA-MRSA are fast-spreading and can affect seemingly healthy individuals [10]. Vancomycin is currently the first-line treatment for severe CA-MRSA and HA-MRSA infections; however strains with reduced susceptibility to this antimicrobial (vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA)) have been reported with increasing frequency [11]. Since

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nasal carriage is a well-defined risk factor for infection, decoloni- 
sation protocols have had some moderate success in reducing the  
incidence of infection in specific hospitalized groups. However,  
clearance is not consistent or long-lived, and this strategy is not  
applicable to community settings. Efforts to prevent MRSA infec-
tion in high-income countries are well-resourced, but risk factors  
and infection are poorly monitored in low- and middle-income  
settings, where in some cases the counter MRSA antibiotics are  
available and frequently self-administered [12]. MRSA’s status as  
a global public health threat will only be exacerbated by inappro-
riate anti-microbial use and will further accelerate the spread of  
CA-MRSA and other resistant microorganisms.

S. aureus, which constitutes the normal flora in up to 30% of  
individuals (either persistently or intermittently), is typically  
spread via close contact from carriers to non-carriers. Transmis-
sion has been found to be most efficient within healthcare and  
athletic facilities [2,13]. In high income countries, target groups  
for potential S. aureus vaccination would be at-risk healthcare work-
ers, the elderly ≥65 years, and the immunocompromised, as well  
as patients with recurrent invasive staphylococcal infection [3,14].  
The global burden and spread of S. aureus infection is currently  
unknown and more data from low- and middle-income countries  
are needed, but in the US alone, S. aureus infection is reported as  
a discharge diagnosis for around 300,000 hospital stays per year.  
S. aureus infection is also associated with a five-fold increased risk  
of in-hospital death and three-fold higher cost of hospital stay  
compared to inpatients without infection [15]. A US study in 2003  
estimated that S. aureus infections accounted for $14.5 billion in all  
inpatient hospital stays and $12.3 billion for surgical stays [16].

2. Overview of current efforts

A. Either vaccines currently available and their limitations or bio-
logical feasibility for vaccine development

Prior S. aureus infection does not provide protection against  
subsequent infection, but infections among carriers are less  
severe, suggesting that some form of immunity does develop  
during prolonged colonization. Although all adults have pre-
exisiting binding antibodies to S. aureus antigens, including  
capsule and clumping factor A (CfA), these do not typically  
include functional antibodies that have opsonophagocytic or  
neutralizing properties, and therefore do not provide protection  
against infection.

There is precedence for the development of safe and effective  
bacterial vaccines that target single antigens or toxins, partic-
cularly capsular polysaccharides. The most prominent examples  
are the tetanus toxoid and pneumococcal conjugate vaccines.  
Application of these technologies to S. aureus is complicated by  
the bacterium’s complex mechanisms of pathogenesis. S.  
aureus can comprise the normal human flora and, as such, has  
evolved a number of strategies to colonize and evade the host  
immunity, including polymorphic expression of surface anti-
gen and release of multiple redundant virulence factors [17,18].  
These include iron acquisition factors such as IsdB, manganese  
take receptors such as MntABC, fibronectin binding proteins  
(CfA, CfB), polysaccharide capsule molecules (CP5 and CP8)  
and toxic shock syndrome toxin (TSST). To date, vaccine can-
didates have targeted individual cell surface components, such  
as the polysaccharide capsule, extracellular polysaccharides or  
cell wall associated proteins that aid attachment, invasion or  
act as a receptor (e.g., hemoglobin for iron utilization). Although  
multiple vaccine candidates have shown promise through pre-
clinical development in a range of animal models, those that have  
reached late stage clinical testing have failed to demonstrate  
efficacy in human trials [19,29].

B. General approaches to vaccine development for this disease for  
low and middle income country markets

S. aureus has not been viewed as a high-priority pathogen in  
low-income countries. However, based on the limited data avail-
able, the incidence and mortality from multidrug-resistant S.  
aureus in these regions is likely significant. To date, only two  
vaccines have completed human efficacy, and neither have con-
templated target populations or indications that are prevalent  
in low- and middle-income countries [20]. StaphVAX is a hiva-
lent polysaccharide- and protein-conjugated vaccine, directed  
against S. aureus capsular polysaccharide types 5 and 8 (CP5  
and CP8), which are associated with approximately 80% of S.  
aureus clinical infections. The candidate was evaluated in two  
phase III studies to prevent bacteremia in end-stage renal dialysis  
patients in the 3–54 weeks following immunization. In the first  
40 weeks, bacteremia was reduced by 57% but efficacy dropped  
to 26% by week 54 [21]. A confirmatory Phase III study involving  
3600 hemodialysis patients who were evaluated for bacteremia  
showed no difference between vaccinated individuals and the  
placebo controls. The functional antibody titers induced by the  
vaccine in this second follow-up phase III study have not yet been  
made publicly available. Currently, then, the main reason for the  
second trial’s failure is being attributed to manufacturing incon-
sistencies between different vaccine lots used for the two studies  
[22]. Development of the candidate has been discontinued.

Another candidate, V710, elicits immunity against the cell-
wall anchored iron scavenger protein IsdB, and was evaluated in  
a Phase III randomized controlled trial in approximately  
8000 adults scheduled for cardiac surgery. This trial was termi-
nated when an interim analysis showed a statistically significant  
increase in mortality rate due to S. aureus infection and a signifi-
cantly higher rate of other adverse events [23].

Passive immunization strategies utilizing both polyclonal and  
monoclonal antibodies (mAbs) have been targeted for those who  
are immunocompromised and cannot mount an independent,  
robust immune response and for those at immediate risk of  
infection and do not have time to for an active immunization to  
take effect. Five antibody candidates have been developed and  
evaluated in late stage clinical studies; none have demonstrated  
efficacy [24].

The focus has been on development of a prophylactic product  
that will protect against life-threatening S. aureus infections, but  
it is hoped that such a vaccine would also protect against all  
S. aureus infections including more commonly encountered skin  
and soft tissue infections. To date, however, no product has been  
shown to protect against any tested outcome.

3. Technical and regulatory assessment

Active and passive immunization approaches have been based on  
increasing the concentration of opsonic antibodies to single  
surface antigens, and all have failed to demonstrate protection.  
Antigenic variation and the multiple invasion and colonization  
mechanisms of S. aureus, absence of representative preclinical  
models and lack of immune correlates or surrogates of protec-
tion all present significant obstacles to the rational design,  
development and evaluation of potentially successful vaccine  
candidates.

Leaders in the field have been speculating about the future  
directions for S. aureus vaccine development, particularly in the  
aftermath of the failed efficacy trials [25–28]. First and foremost,  
a multi-antigen approach that targets both cell surface compo-
ents and secreted virulence factors, and potentially responses  
against the disease causing toxins, have been hypothesized as  
essential to the success of a vaccine. Because S. aureus causes
a broad range of diseases, preclinical testing should involve the use of multiple models that approximate the different diseases that the pathogen causes in humans. Many candidates have been evaluated in murine models, due to convenience and low cost, and have demonstrated proof-of-concept in terms of preclinical protection through the elicitation of opsonic antibodies. However, these data have not translated into human efficacy [19,29]. In addition to developing a number of representative preclinical models, it is also important to evaluate protection against diverse S. aureus clinical isolates, and to assess development of in vitro assays that measure functional responses through the induction of both humoral and T-cell mediated responses, particularly toxin neutralization, opsonophagocytic killing by neutrophils, and IL-17 secretion.

The optimal population for clinical proof-of-concept or efficacy studies is challenging to identify. Generally speaking, the more immune compromised the patient, the greater the severity of S. aureus infection. However, these individuals are less likely to mount an effective immune response after vaccination. Patient groups who are generally healthy but elect to undergo surgery or some other invasive procedure are typical target populations for efficacy studies, as vaccination can be administered prior to a known period of increased risk (approximately 90 days) and be compared to a placebo. Regardless of the population in which vaccine proof-of-concept is demonstrated, an efficacious vaccine will ultimately be targeted to patients at who are already at high risk of infection and are not in good health at baseline (immunocompromised or with co-morbidities). Prospective studies across a number of elective surgery populations that define the risk, characteristics of surgical procedures, co-morbidities and infections rates are needed to accurately identify the optimal population in which to test S. aureus vaccines, as well as to plan how such a vaccine may be most impactful.

Recent analysis of the data from V710 vaccine recipients indicates that the combination of low pre-vaccination IL-2 levels, vaccination with V710, and post-operative S. aureus infection were associated with increased mortality following cardiothoracic surgery [30]. Nine of ten V710 recipients with undetectable preoperative IL17a levels and postoperative S. aureus infections died. Investigation into the pathophysiologic mechanism of this outcome is ongoing.

A vaccine that offers sterilizing protection from S. aureus may be an overly ambitious target, but would be ideal. The alternative to a vaccine that protects against all clinical S. aureus syndromes is to develop vaccines against each or a subset of specific clinical manifestations, however this would be very costly, and certainly challenging for use in low income countries. A more achievable end point may be reduction in the severity of infection, as this may still have a very important impact on mortality.

S. aureus vaccines could also be considered a high priority within the antimicrobial resistance (AMR) agenda together with, for example, Group A Streptococcal, P. falciparum malaria and tuberculosis vaccines. However, the AMR research agenda has not yet fully incorporated vaccine R&D perspectives. A discussion about incentivizing vaccine development to address AMR concerns is needed at national and international policy levels.

### Table 1

<table>
<thead>
<tr>
<th>Candidate name/Identifier</th>
<th>Developer</th>
<th>Vaccine approach</th>
<th>Pre-clinical</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Status</th>
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<td><strong>Active prophylactic vaccines</strong></td>
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<td></td>
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<tr>
<td>PF-06290510/S4Ag</td>
<td>Pfizer</td>
<td>CIA/MntC/CP5/CP8 conjugated to CRM197</td>
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<td></td>
<td>Safety and Efficacy of SAAAg Vaccine in Adults Having Elective Posterior Instrumented Lumbar Spinal Fusion Procedure (STRIVE): NCT01827358</td>
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<tr>
<td>GSK2392103A</td>
<td>GSK</td>
<td>CP5/CP8/TT/AT/ClfA plus AS03B</td>
<td></td>
<td>X</td>
<td></td>
<td>SAAAg Safety, Tolerability, and Immunogenicity Study in Japanese Adults: NCT02492958</td>
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<td><strong>Glycosylated CP5, CP8, and HLA1351</strong></td>
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<tr>
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<td>Vaccine Research International</td>
<td>Whole cell vaccine</td>
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<td>X</td>
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<td>No longer under active development <a href="http://www.vri.org.uk/PhaseITrial.pdf">http://www.vri.org.uk/PhaseITrial.pdf</a></td>
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<td>Novartis</td>
<td>FliC2, EsxA, Hla, Sur-2</td>
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<td>X</td>
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<td>Pan Chai University</td>
<td>S. aureus ghosts</td>
<td>X</td>
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<td>[36]</td>
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<td>MEDI4893</td>
<td>Medimmune</td>
<td>mAb binding to S. aureus toxin</td>
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<td>Dose-ranging efficacy and safety in mechanically Ventilated Adults: NCT02296320</td>
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<td>AR-301</td>
<td>Aridis</td>
<td>mAb</td>
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<td>X</td>
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<td>Phase II Safety, Pharmacokinetics and Efficacy of KRSA301 in Severe Pneumonia (S. aureus) as an adjunctive therapy to standard of care antibiotics in hospital-acquired pneumonia (HAP) and ventilator associated pneumonia (VAP) patients: NCT01589185</td>
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</tbody>
</table>
4. Status of vaccine R&D activities

Following the failure of single antigen vaccine approaches, most development efforts are now focused on multiple antigen approaches. Pfizer’s SA4Ag candidate is currently the most advanced and is comprised of four antigens: the adhesion molecule ClfA, the manganese transporter MntC and anti-phagocytic capsular polysaccharides 5 and 8 [31]. This combination is designed to elicit broad humoral and cellular immune responses against multiple virulence mechanisms involved in the establishment and maintenance of infection. Results from a phase I study in which healthy adults of ages 50–85 (n = 312) or 18–24 (n = 96) years randomly received a one of three dosages of SA4Ag as a single intramuscular injection or placebo showed that the 50- to 85-year age stratum elicited robust immune responses to all component antigens, as well as functional responses as measured by an opsonophagocytosis assay. The rise in functional antibody titers against S. aureus was maintained through at least 12 months. A phase IIb placebo-controlled safety and efficacy study in adults undergoing elective spinal fusion surgery is underway (referred to as STRIVE: STaphylococcus aureus SuRgical Inpaint Vaccine Efficacy). SA4Ag was granted Fast Track designation by the U.S. Food and Drug Administration (FDA) in February 2014.

Other multi-antigen preclinical candidates targeting conserved capsular polysaccharide conjugates and/or protein antigens, some including toxins such as alpha toxin, TSST, and SEB are in development (Table 1). In the face of past failures, current efforts are also focused on further characterizing the immunopathology and immunity of S. aureus infections to identify new antigenic targets, and developing more representative preclinical [32] models in which opsonising and/or neutralizing immune responses are measured.

Passive immunization with mAbs is an alternative strategy to manage S. aureus infection in the context of increasing antimicrobial resistance. Questions about affordability and access of mAb interventions for low- and middle-income countries need to be clarified. As with previous vaccine candidates, clinical trials examining the efficacy of human antibodies have failed to meet their study end points and have been discontinued [19,29]. A new generation of mAbs is now undergoing clinical evaluation. Aridis Pharmaceuticals’ candidate AR-301 (Salvecin™) is a fully human monoclonal IgG1 antibody targeting S. aureus alpha-toxin that is undergoing evaluation as an adjunctive therapy to standard-of-care antibiotic treatment for hospital-acquired pneumonia (HAP) and ventilator associated pneumonia (VAP) [38]. Medimmune’s MEDI4893 long half-life mAb is also targeting the alpha toxin, and is currently in a Phase 2 dose-ranging study to evaluate safety and efficacy in mechanically ventilated adult subjects [39].

5. Likelihood for financing

The relatively well-characterized incidence of S. aureus in high-income countries and the market potential for a vaccine has meant that development, particularly through late-stage clinical testing, has to date been advanced by pharmaceutical companies. Basic research into S. aureus pathogenesis, development of improved preclinical models and functional assays is being supported by government funding agencies such as the European Commission and the National Institutes of Health. However, there are no efforts to develop a vaccine against S. aureus infections in low- and middle-income countries as no market has yet been established there.

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References


