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FULL PAPER

# *Staphylococcus aureus* vaccine preclinical and clinical development: current state of the art

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SUMMARY

*Staphylococcus aureus* is a major pathogen in both community and hospital settings. It is a significant etiological agent to treat in healthcare-related infections due to both its ability to cause invasive infection as well as to form biofilm on biomaterials and the high prevalence of resistance to first line antibiotics. The most challenging preventive strategy is vaccine development to guarantee a full and durable protection from staphylococcal diseases in all different high-risk populations, even if the lack of a known correlate of protection from *S. aureus* is a major hindrance to this effort. We aimed to review the most recent advances in the field of vaccinology against *S. aureus*, highlighting the potential for future application of the different experimental vaccine types. Several vaccines have completed their preclinical phase of development and others have been tested in humans, however no successful phase III clinical trial has yet been completed.

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## INTRODUCTION

*Staphylococcus aureus* is a Gram-positive bacterium commonly colonizing humans. It can cause localized and serious invasive infections, as well as a severe septic shock syndrome (Krismer *et al.*, 2017; Que and Moreillon, 2015). Its clinical importance is also related to its ability to adhere and to form biofilms, mainly on biomaterials (e.g. orthopaedic joint prostheses, artificial heart valves, intravenous devices), causing difficult-to-treat infections (Figueiredo, 2017; Oliveira *et al.*, 2018). *S. aureus* is one of the most important etiologic agents of post-surgical complications and hospital-acquired or healthcare-associated infections and, moreover, it frequently develops resistance to beta-lactam agents. The prevalence of the methicillin-resistant *S. aureus* (MRSA) in Europe ranges from <1% to over 50% and multidrug-resistant isolates have been demonstrated both in the community and in the healthcare settings (Hassoun *et al*., 2017; Que and Moreillon, 2015; Reddy *et al*., 2017; March *et al.*, 2017). The high prevalence of antibiotic resistance makes it difficult to prescribe an effective empiric therapy. Moreover, in sub-chronic infections, bacterial culture may be difficult to obtain: in these cases, molecular diagnostic approaches may be required to improve sensitivity and to achieve a rapid diagnosis (Sambri *et al.*, 2017), failing the goal to switch to a specific therapy after an *in vitro* chemosusceptibility test. Glycopeptides can be considered the cornerstone of antibiotic therapy for MRSA infections and the first-choice in patients with beta-lactam allergy, although resistance to this class is emerging in several countries, and toxicity issues may represent a limitation. Alternative anti-MRSA antimicrobials are available, but resistance to these newer molecules has already been reported in clinical *S. aureus* isolates and it is increasing (Que and Moreillon, 2015; Foster, 2017; Musumeci *et al.*, 2016).

To overcome problems in the clinical management of staphylococcal infections, several newer approaches and their possible application using different preventive or therapeutic strategies are being evaluated (e.g. biocidal nano-molecules, passive immunotherapy) (Oliveira *et al.*, 2018; Siddiqi *et al.*, 2018; Sause *et al*., 2016). The most challenging preventive strategy is vaccine development whose objective is to obtain a full and durable protection from staphylococcal diseases in all different populations at risk. The lack of a known correlate of protection from *S. aureus* infection is a major hindrance to vaccine development (Proctor, 2012). For many years, efforts have been ongoing to gain a vaccine candidate, using recombinant or subunit antigens of *S. aureus* or an antigen delivering system, with promising results in preclinical development (Adhikari *et al.*, 2012; Wacker *et al.*, 2014; Becherelli *et al.*, 2013; Colonna *et al.*, 2013; Veloso *et al*., 2015, Bagnoli *et al*., 2015; Delfani *et al.*, 2015).

We aimed to review the most recent advances in the field of vaccinology against *S. aureus*, highlighting the potential for a future application of the different experimental vaccine types.

*Key words:*
*Staphylococcus aureus*, Vaccine, Prevention, Immunogenicity, Antigens.

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## METHODS

We selected articles from Pubmed (https://www.ncbi.nlm.nih.gov/pubmed/) using the following key words: 'vac-

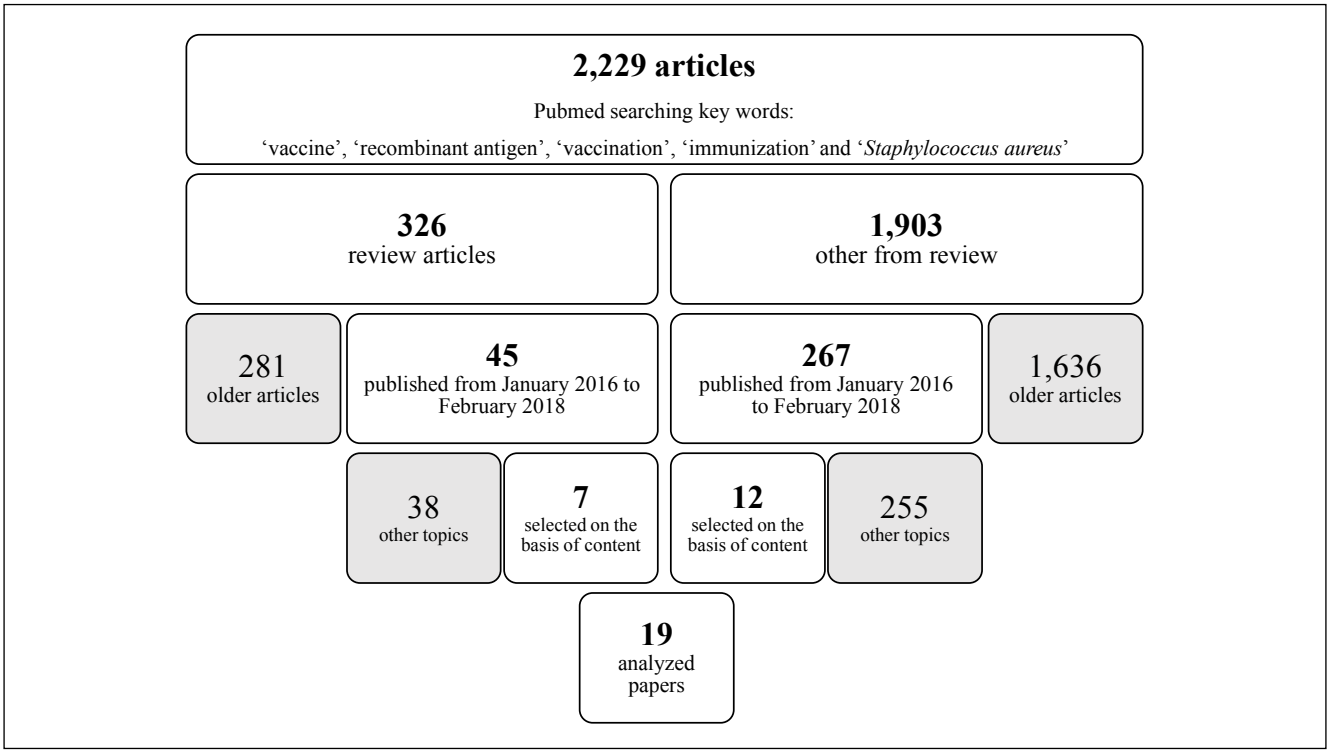


**Figure 1** - *Algorithm summarizing criteria of papers selection (numbers and reasons of exclusion in grey boxes).*

cine', 'recombinant antigen', 'vaccination', 'immunization'. Matching each term with '*Staphylococcus aureus*' we found 2,229 articles. We selected review articles (326 results) and further selected those starting from January 2016 up to February 2018, thus obtaining 45 articles. We made a further critical selection based on the content of the abstracts, finally finding 7 reviews focused on active immunization against *Staphyloccoccus aureus*. With the same key words and in the same time interval, original articles regarding new vaccine approaches and not included in the previous selected reviews, were also selected and analysed. A total of 17 papers were eventually included in our review. The criteria of article selection are summarized in *Figure 1*. Original studies reporting preclinical and clinical trials (where available) are listed in *Tables 1* and *2*.

## PRECLINICAL STUDIES

About half of the analysed papers describe the preclinical phases of *S. aureus* vaccine candidates mainly using the murine model. This is a crucial stage in the development of immunization strategies, because a failure in this phase obviously threatens any further research. GlaxoSmith-Kline (GSK) approached active immunization in mice and rabbits using the capsular polysaccharide antigens serotype 5 and 8 (respectively CP5 and CP8), responsible for cellular adhesion, and detoxified α-hemolysin ($Hla_{H35L}$) that plays a crucial role in invasive infections (Giersing *et al.,* 2016, Reddy *et al.,* 2017). The vaccine was produced by recombinant technology in *Escherichia coli*, obtaining a bioconjugated and N-glycosylated protein (Wacker *et al.,* 2014). Even though elicited antibodies in immunized animals were protective against bacteraemia and pneumonia, there was no further development of this study (Reddy *et al.,* 2017). Nabi biopharmaceutical and Uniformed Services University of the Health Sciences (USUHS) evaluated the PentaStaph vaccine, still based on CP5, CP8 and Hla antigens, with the addition of the toxin Panton Valentine Leukocidin S (LukSPV) and wall teichoic acids (Reddy *et al.,* 2017). The efficacy was evaluated separately for each antigen component and studies seem ongoing regarding the pentavalent formulation: in 2009 PentaStaph was sold to GSK for further possible application (https://www.sec.gov/Archives/edgar/data/72444/000119312509167192/dex992.htm, last accessed February 28, 2018) but no final reporting paper is yet available.

$CRM_{197}$ (a nontoxic recombinant mutant of diphtheria toxin)-conjugated polysaccharide antigens CP5 and CP8 have been recently valuated as vaccine candidates by Cheng *et al*. in a murine model of bacteraemia, lethal sepsis, and skin infection. Even if a good antibody response was elicited and active immunization protected against staphylococcal bacteraemia, only the CP8-CRM component protected against dermonecrosis and neither CP5-CRM nor CP8-CRM protected against mortality in the sepsis model (Cheng *et al.*, 2017).

A multicomponent surface protein (SdrE, IsdA, SdrD, IsdB) target vaccine was developed by Novartis (now GSK) and revealed a protection from lethal doses of *S. aureus* strains in mice (Reddy *et al.,* 2017). The same company has recently created an alum adjuvated vaccine, named 4C-Staph. It was targeted on four different antigens: the previously described $Hla_{H35L}$ in combination with EsxAB, FhuD2, Csa1A. EsxAB is a fusion of two virulence secreted factors involved in abscess formation, FhuD2 is a lipoprotein involved in iron uptake, while the role of lipoprotein Csa1A is still not clearly understood (Mancini F, *et al.,* 2016; Dayan *et al.,* 2016). The beneficial effects of this quadrivalent vaccine have been shown in a murine model

**Table 1** - *Developing vaccines in preclinical phase.*

| *Vaccine* | *Developer* | *Target antigen (type)* | *Valued immune response* | *Status and results* | *Original article reference* |
|---|---|---|---|---|---|
| Glycovaxine | GSK | CP5/CP8/$Hla_{H35L}$ (recombinant) | Humoral | Completed, efficay, no further development | Wacker *et al.*, 2014 |
| PentaStaph | Nabi and USUHS | CP5/CP8/$Hla_{H35L}$ *plus* LukS-PV and wall teichoic acids (N.A.) | Humoral | Ongoing studies (sold to GSK), efficacy valuated separately for the each components | Schaffer and Lee, 2009 |
| N.A. | N.A. | CP5/CP8 (purified, $CRM_{197}$ conjugated) | Humoral | Completed, elicited protection in mice against bacteremia, but not lethal sepsis; in the skin infection model, only conjugated CP8 protected against dermonecrosis | Cheng *et al.*, 2017 |
| N.A. | Novartis (now GSK) | SdrE, IsdA, SdrD, IsdB surface proteins (recombinant, alum adjuvated) | Humoral | Ongoing, efficacy, protection from lethality in mouse infection model | Stranger-Jones *et al.*, 2006 |
| 4C-Staph | Novartis (now GSK) | FhuD2, EsxAB, $Hla_{H35L}$, Csa1A (purified, alum adjuvated) | Humoral and cellular | Completed, efficacy, reduction of murine lung infections and arthritis | Torre *et al.*, 2015 Mancini *et al.*, 2016 |
| N.A. | N.A. | ClfA (purified, or recombinant) | Humoral | Completed, efficacy, prevention of murine arthritis | Josefsson *et al.*, 2001 |
| N.A. | NIAID | AT62 (recombinant from Hla) | Humoral | Stopped, scarce control of murine skin infections. | Adhikari *et al.*, 2016 |
| N.A. | Pasture Institute of Iran and IAUPS | PBP2a (recombinant) | Humoral | Completed, efficacy, reduced mortaliy against bacteriemic MRSA infection | Haghighat *et al.*, 2017 |
| N.A. | N.A. | D-alanine auxotrophic mutant (live mutant bacterium) | Humoral and cellular | Completed, efficacy, reduction of abscesses formation in mice | Moscoso *et al.*, 2018 |
| SpA-$D_{KKAA}$-$FnBPA_{37-507}$ (SF) | NSFC | SpA/FnBPA (bivalent fusion vaccine, recombinant proteins) | Humoral and cellular | Completed, efficacy, reduction of pneumonia and skin abscesses in mice | Yang *et al.*, 2018 |

*Legend:* GSK: GlaxoSmithKline; CP: capsular polysaccharide antigens; Hla/AT: α-toxin; CRM: cross-reacting mutant, a nontoxic recombinant mutant of diphtheria toxin; USUHS: Uniformed Services University of the Health Sciences; LukS-PV: Panton–Valentine leukocidin component S; N.A.: not available; Sdr: serine-aspartate repeat proteins; Isd: iron-regulated surface determinant; Fhu: ferric hydroxamate uptake; Esx: secretion system protein; Csa: conserved staphylococcal antigens; Clf: clumping factor; NIAID: National Institute of Allergy and Infectious Diseases, USA; IAUPS: Islamic Azad University, Pharmaceutical Sciences Branch; PBP: penicillin binding protein; NSFC: National Natural Science Foundation of China; Sp: staphylococcal protein; FnBP: fibronectin-binding protein.

of joint and lung infections, with robust antibody response and CD4+ T lymphocyte activation (Corrado *et al.,* 2016). To date, there is no information on further development (Reddy *et al.*, 2017; Giersing *et al.,* 2016).

Another potential vaccine *S. aureus* antigen is the surface protein Clamping factor A (ClfA) that allows adhesion to several human tissues by fibrinogen binding. The successful preclinical study on ClfA opened the way to its application in multiple antigen vaccines, which are in advanced stages of development (Lacey *et al.,* 2016; Dayan *et al.,* 2016). An equally successful preclinical performance was not achieved by a recombinant vaccine (AT62, by the National Institute of Allergy and Infectious Diseases, USA) based on the α-hemolysin (Hla) subunit, that showed a weak activity in preventing murine surgical wound infections, despite a robust antibody response. The Hla subunit seems nevertheless to be suitable for the development of multivalent vaccines (Adhikari *et al.,* 2016). An interesting immunization target under evaluation, by the Pasture Institute of Iran and Pharmaceutical Sciences Branch of Islamic Azad University, is the Penicillin Binding Protein 2A (PBP2a) involved in beta-lactam resistance due to target mutation. Vaccine based on PBP2a reduced the mortality rate and protected mice against lethal MRSA challenge (Haghighat *et al.,* 2017). Other possible vaccine candidates are a mutant live *S. aureus*, unable to synthetize cell wall D-alanine (Moscoso *et al.,* 2018) and a bivalent fusion vaccine based on the D domain of staphylococcal protein A (SpA) and the A domain of fibronectin-binding protein A (FnBPA), by the National Natural Science Foundation of China (Yang *et al.,* 2018). Vaccination with the mutant live *S. aureus* resulted in a protective effect against *S. aureus* bacteremia in mice (Moscoso *et al.,* 2018). The bivalent fusion vaccine showed a protective efficacy in murine pneumonia and a skin abscess model (Yang *et al.,* 2018).

## CLINICAL STUDIES

### *Phase I*

Despite the efficacy obtained in the preclinical studies, some of the evaluated vaccine candidates did not undergo further development. A composed target vaccine (conjugated to tetanus toxin CP5/CP8 polysaccharides *plus* re-

**Table 2** - *Developing vaccines in clinical phases.*

| *Vaccine* | *Developer* | *Target antigen (type)* | *Valued immune response* | *Phase* | *Status and results* | *Key review or original article reference* |
|---|---|---|---|---|---|---|
| GSK2392103A | GSK | CP5/CP8/Hla/ClfA (conjugated CP5/CP8 plus recombinant Hla/ClfA) | N.A. | Phase I | Completed, no further development | Levy *et al.*, 2015 |
| NDV3 | NovaDigm Therapeutics | rAls3p-N (C. albicans surface protein cross reacting with S. aureus; alum adjuvated) | Humoral and cellular | Phase I | Completed, safety and immunogenicity, stopped phase II due to enrolment problems | Schmidt *et al.*, 2012 |
| SA75 | Vaccine Research International | Whole cell vaccine | Humora and cellualr | Phase I | Completed, safety and tolerability, no further development | Giersing *et al.*, 2016 |
| N.A. | Integrated BioTherapeutics | Enterotoxins A and C1, TSST (recombinant) | Humoral | Phase I | Completed, safety, evaluating possible phase II trial | Roetzer *et al.*, 2016 |
| STEBvax | Integrated BioTherapeutics and NIAID | Enterotoxin B (rSEB) (recombinant, alum adjuvated) | Humoral | Phase I | Completed, safety, demostrated production of toxin neutralizing antibodies. | Chen *et al.*, 2016 |
| SA4Ag (PF-06290510) | Pfizer | ClfA/MntC/CP5/CP8 (conjugated CP5/CP8 plus recombinant MntC/ClfA) | Humoral and cellurar | Phase I | Completed, safety, robust immune response, ongoing phase IIb in adults receiving spinal surgery. | Creech *et al.*, 2017 Begier *et al.*, 2017 Frenck *et al.*, 2017 |
| N.A. | Nabi | rAT(α-toxin)/rLukS-PV (recombinant) | Humoral | Phase I | Completed, safety, robust immune response. | Landrum *et al.*, 2017 |
| StaphVAX | Nabi | CP5/CP8 (purified and conjugated capsular polysaccharides) | Humoral | Phase III | Stopped, no differences between vaccine and placebo in end-stage renal patients | Fattom *et al.*, 2004 Fattom *et al.*, 2015 |
| V710 | Merck | IsdB (purified surface protein) | Humoral | Phase III | Stopped, increased mortality in vaccinated subjects post-cardiothoracic surgery | Fowler *et al.*, 2013 McNeely *et al.*, 2014 |

Legend: GSK: GlaxoSmithKline; CP: capsular polysaccharide antigens; Hla/AT: α-toxin; Clf: clumping factor; Als3p: agglutinin like sequence 3 protein; TSST: toxic shock syndrome toxin; NIAID: National Institute of Allergy and Infectious Diseases, USA; Mnt: manganese transporter protein; LukS-PV: Panton–Valentine leukocidin component S; Isd: iron surface determinant.

combinant Hla/ClfA proteins) was developed by GSK, and it completed the phase I clinical trial (Dayan *et al.,* 2016; Mohamed *et al.,* 2017). This vaccine elicited an increase in functional humoral antibody responses that could kill CP5-expressing strains in opsonophagocytic assays after a single dose, but an inefficient T-cell activation. No safety concerns arose during this study but this vaccine was not further developed (Levy *et al.,* 2015; Giersing *et al.,* 2016; Reddy *et al.,* 2017). A hypothetically promising immunization strategy was proposed by NovaDigm Therapeutics with the so called NDV3 vaccine. This vaccine consists of an alum adjuvated, recombinant antigen rAls3p-N (agglutinin like sequence 3 protein), a C. *albicans* surface protein that cross reacts with *S. aureus* (Lacey *et al.*, 2016). NDV3 previously demonstrated a preclinical efficacy in reducing murine skin abscesses, so it was carried on phase I, showing safety and immunogenicity (Dayan *et al.,* 2016). NDV3 is currently under study for the prevention of *Candida* vaginitis (Giersing *et al.*, 2016). A cell wall vaccine, SA75 by Vaccine Research International, has shown good tolerability and safety during phase I, but it was not further developed (Giersing *et al.*, 2016). Indeed, preclinical studies on similar types of cell wall vaccines showed controversial results, with sufficient immunogenicity only after intravenous injection, even if an efficient cellular and humoral response was observed in the murine model of skin and soft tissue infections (Selle *et al.*, 2016, Zhang *et al.*, 2017). Secreted virulence factors have also been evaluated in phase I trials. Recombinant staphylococcal enterotoxins A and C1 by Integrated BioTherapeutics showed a safe profile (Roetzer *et al.*, 2017). Moreover, Integrated BioTherapeutics, in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID), demonstrated a production of functional toxin-neutralizing antibodies in adults after immunization with STEBVax, an alum adjuvated recombinant enterotoxin B (rSEB) (Chen *et al.*, 2016).

The SA4Ag vaccine by Pfizer is composed of four *S. aureus* virulence factors: CP5 and CP8 conjugated with diphteric toxoid *plus* recombinant-mutated ClfA and recombinant-mutated MntC (manganese transporter protein C). A previous use of an SA3Ag vaccine (lacking of MntC) and of SA4Ag showed an acceptable safety for both, but SA4Ag showed a more robust humoral immune response (Xu *et*

*al.*, 2018, Esposito *et al.*, 2016; Begier *et al.*, 2017; Creech *et al.*, 2017, Mohamed *et al.*, 2017).
One of the most recent phase I trials was conducted on the bivalent recombinant α-toxin and Panton Valentine Leukocidin vaccine (rAT/r rLukS-PV) produced by Nabi. It was investigated on healthy militarie personnel obtaining positive results in terms of safety and long-term immunogenicity (Landrum *et al.*, 2017).

### *Phase II*

There are no ongoing phase II studies.
Phase II of the previously described NDV3 by NovaDigm Therapeutics was stopped due to enrolment difficulties (Lacey *et al.*, 2016). The use of the previously described recombinant staphylococcal enterotoxins A and C1 by Integrated BioTherapeutics is under evaluation for a phase II trial (Roetzer *et al.,* 2017).
SA4Ag (PF-06290510) is the only candidate tested in an ongoing phase IIb trial: the STRIVE (*STaphylococcus aureus* suRgical InpatientVaccine Efficacy) study aims to confirm the phase I results in a wider target population of adults receiving spinal surgery (Begier *et al.,* 2017, *et al.*, 2016, Mohamed *et al.,* 2017).

### *Phase III*

Two phase III trials testing a purified CP5/CP8 conjugated with recombinant pseudomonal exotoxin A, StaphVax, by Nabi as well as a purified surface protein IsdB, V710 by Merck, were interrupted due to the absence of difference in the primary endpoint between vaccine and placebo for StaphVax and an increased mortality in exposed subjects for V710 (Giersing *et al.*, 2016; Dayan *et al.,* 2016; Reddy *et al.,* 2017; Missiakas and Schneewind, 2016; Mohamed *et al.*, 2017; Pozzi *et al.*, 2017; Lacey *et al.*, 2016). No other clinical phase III trial is ongoing or under evaluation. Possible manufacturing matters causing failure of StaphVax were hypothesized (Fattom *et al.,* 2015; Dayan *et al.* 2016), but its capsular polysaccharide antigens are further being evaluated within the PentaStaph vaccine, as previously described.

## CONCLUSIONS

Development of an effective vaccination against *S. aureus* seems to be a major priority in terms of prevention at the individual patient level and as a public health measure, with the additional aim of reducing the economic impact of these infectious complications.
Despite the plethora of preclinical studies in recent years, clinical trials are still far from approaching a potential application in clinical practice.
The multiple staphylococcal antigens and different pathogenic pathways make it difficult to imagine a single and universal anti-*S. aureus* vaccine. Some authors referred to the bacterial complexity in the failure of tested vaccine candidates (Lacey *et al.,* 2016; Dayan *et al.* 2016). Vaccines targeting each different type of staphylococcal infection have been proposed as a possible future approach (Lacey *et al.,* 2016).
Differences in staphylococcal pathogenic mechanisms in humans, compared to those in animal models, could represent another major problem to translate results from the preclinical development into the clinical phases. Animals, in particular mice, may be a suboptimal model to study staphylococcal infections (Proctor, 2012): "humanized" mice, rabbits and guinea pigs have been proposed as more reliable animal models (Parker, 2017; Malachowa, 2016; Kim, 2015). Other intriguing and advanced experimental studies explore the potential of reverse vaccinology or immunoproteomics (Holtfreter *et al.*, 2016; Stentzel *et al.*, 2016).
More studies and clinical trials are needed to reach the objective of an effective and widely employable anti-staphylococcal vaccine.

### Conflict of interest

DR has received non financial support from ViiV Healthcare, Abbvie, Astellas and Gilead, all outside the submitted work.
CSR: nothing to declare.
BR received consultant fees from Janssen, ViiV Healthcare, Abbvie, Merck-Sharp and Dohme, Bristol-Myers Squibb and Gilead Sciences, all outside the submitted work.
ADL has received research grants from ViiV, Gilead and Merck-Sharp and Dohme and has been a paid consultant for ViiV, Gilead, Janssen-Cilag and Merck-Sharp and Dohme.
FM has received non financial support from Angelini and Astellas, outside the submitted work. She has done contract research for Novartis Vaccine and Diagnostic S.r.l. (now GSK Vaccine S.r.l.) on behalf of the University Hospital of Siena; she is Infectious Diseases Consultant for GSK (consultancy fee on behalf of University of Siena).

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