# *Staphylococcus aureus* vaccine. Use of ISD system components in the development of a *S. aureus* vaccine.

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## Background

*Staphylococcus aureus* is a gram positive ubiquitous pathogen that [1,2] may cause a variety of diseases ranging from moderate to severe skin and soft tissue infections to serious infections. [3,4]. Over the past decade different clonal types resistant to a wide range of antibiotics have emerged, as well as methicillin resistant *Staphylococcus aureus* (MRSA) [4,5], and becoming the predominant strain in *S. aureus* infections [2]. This increased underlines the need to develop new strategies to prevent infection by *S. aureus* [2].
Despite the need for a *S. aureus* vaccine and the continuing efforts of the scientific community, clinical trials have failed [1]. This emphasizes the need to develop new vaccine strategies that provide cellular memory, particularly Th17 [2,5].

## Objectives

- To develop a vaccine strategy against *Staphylococcus aureus* that involves multiple antigens that would trigger various immune mechanisms, especially Th17-mediated response and IL17, is essential for eliminating the infection.
- Mass vaccination strategy for the general public, including sectors of the population with a high risk of infection.

## *S. aureus* immunology

Even though *S. aureus* stimulates the humoral response, this may not play an important role in the removal of the pathogen [6].
The main immune mechanisms required for protection against staphylococcal infections include neutrophils and T cells, which regulate the phagocytic activity [3]. Various studies establish the connection between T cells and neutrophils through the IL-17 cytokine, which is important for the recruitment and activation of neutrophils at the site of infection, increases chemotaxis and their synergistic actions with the TLR2 *(Figure 1)* [2,6].

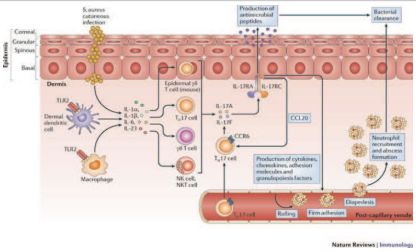

*Figure 1:* IL-17 activates production of antimicrobial peptides, chemokines and adhesion molecules that promote the recruitment of neutrophils from the circulation to the site of infection. Neutrophils help control infection spread and are required for bacterial clearance. IL-17-mediated signalling also promotes $T_H17$ cells recruitment [14].

## Isd system

Iron is essential in the pathogenesiS [2,7]. *S. aureus* has developed a collection system called iron-regulated surface determinants (Isd) for heme acquisition [7]. Isd system is comprised of cell wall-anchored surface proteins (IsdA, IsdB, IsdC and IsdH), a membrane transporter (IsdD, IsdE and IsdF), a transpeptidase (SrtB) and two cytoplasmatic heme-degrading monooxygenases (IsdG and IsdH) (*Figure 2*) [7,8].
The Joshi *et al.* study using a murine sepsis model demonstrated that the protein IsdB provides cellular immunity, specifically Th17. They also found pre-existing titers of antibodies against this protein in serum of various mammals [6].
This project will make a subunit vaccine consisting of Isd system surface proteins, IsdA, IsdB, IsdC and IsdH, formulated with AAHSA (amorphous aluminum hydroxyphosphate sulfate adjuvant).

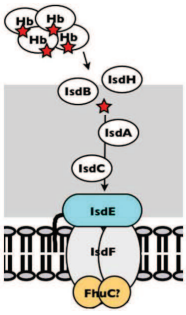


*Figure 2:* The different Isd system components involved in heme uptake (red star) of/from hemoglobin (Hb) [13].

## Material and Methods

### Antigens production

PCR amplify genes *isdA, isdB, isdC* and *isdH* adding restriction enzyme targets BamHI and NdeI *(Figure 3) (Table 2)*

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Separete PCR product by agarose eletrophoresis

↓

Cut the plasmid pET-15b and PCR products with BamHI and NdeI

↓

Ligation *(Figure 3)*

↓

Transform in *E. coli* BL21 (DE3) by electroporation

↓

Selection the plasmids with the insert in LB + Amp

↓

Seed the colonies in LB liquid medium until an $Abs_{600nm}$ = 0.8

↓

Induce expresion of T7 RNA polymerase with IPTG for 3 hours

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Lyse cells by sonication

↓

Recovering chimeric proteins *(Figure 3)*

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Purify proteins that are labelled with a histidine tag in an affinity chromatography

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Remove histidine tail with thrombin

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Purify proteins by gel filtration

### Murine sepsis model and lethal challenge

Formulate puryfy protein with adjuvant AAHSA

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Inject 50 µl/doses intramuscularly to BALB/c mice on days 0, 7 and 21 *(Figure 4)*

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Day 28: perform and ELISA to determine antibody titer and a flow citometry to determine Th17 levels (*Figure 4*)

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Day 35: infect mice with lethal dose of *S. aureus*. Perform a survival study of 10 days *(Figure 4)*

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Perform a statistical analysis

Vaccine: 20mg/dose antigen + 450mg/dose AAHSA

Control: 450mg/dose AAHSA

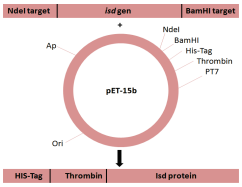


*Figure 3:* Sketch of genic construction used in gene amplification for the production of antigens and chimeric protein obtained.

**Table 2: Primers**

| | |
|---|---|
| IsdAF | 5'- CATCATG GCAGAAAATACAAATA -3' |
| IsdAR | 5'- GGATCCTTACATTTTAGATTGACT-3' |
| IsdBF | 5'- CATCATGTTATTTAGATTCTTTTCT -3' |
| IsdBR | 5'- GGATCCATGACAAAACATTAT -3' |
| IsdCF | 5'- CATCATGTTAGTTTTTACGTTTTCT -3' |
| IsdCR | 5'- GGATCCATGAACAAACAGC -3' |
| IsdHF | 5'- CATCATGTTGAAAAATATTTTAAAAG -3' |
| IsdHR | 5'- GGATCCTTATTCCACATTGC -3' |
| pET-15bF | 5'- GATCTCGATCCCGCGAA-3' |
| pET-15bR | 5'- CCTCAAATGGGCTCTTAAAACC-3' |

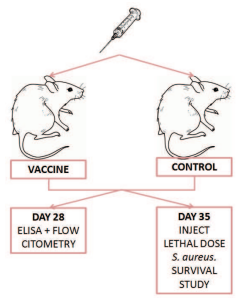


*Figure 4:* Murine sepsis model and lethal challenge diagram to test the *S. aureus* vaccine developed in this project.

## Expected results

The vaccine formed by the Isd system surface proteins should provide protective memory against *S. aureus*. The immune response must be mediated by Th17 cells, so that it is expected to increase production of the cytokine IL-17 and th17 cell in the vaccinated individuals compared to the control ones. Likewise an increase of survival in mice immunized in the lethal challenge is expected. It should also increase the titer of antibodies against proteins of the vaccine as a consequence of enhancing the T response [6,9,10].

## Project dissemination

After the concession of the project, undertake the necessary research and experiments to obtain viable results and to perform a patent. Submit an application to the *Oficina Española de Patentes y Marcas* (OEPM http://www.oepm.es/es/index.html) with international scope by applying the Patents Cooperation Treaty (PCT). Once the patent is granted, publish one or more scientific papers with the obtained results so far in one of the following scientific journals:
- Vaccine (http://www.journals.elsevier.com/vaccine/)
- Journal of Bacteriology (http://jb.asm.org/).

## Project Benefits

The project provides a solution to a major problem of public health, which is the increasing infections by *S.aureus*, particularly multi-resistant strains.
The scientific community has attempted to solve this problem from different angles, but so far has failed.
This project offers an easily constructed vaccine that steers the immune response to a cellular one, more particularly to Th17 which is essential to bacterial clearance. Moreover, the vaccine is composed of various antigens, resulting in a multivalent approach.

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