Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2910319 C 2022/06/14

(11)(21) **2 910 319**

(12) BREVET CANADIEN
CANADIAN PATENT

(13) **C**

(86) Date de dépôt PCT/PCT Filing Date: 2013/03/14

(87) Date publication PCT/PCT Publication Date: 2013/10/31

(45) Date de délivrance/Issue Date: 2022/06/14

(85) Entrée phase nationale/National Entry: 2015/10/23

(86) N° demande PCT/PCT Application No.: US 2013/031695

(87) N° publication PCT/PCT Publication No.: 2013/162746

(30) Priorités/Priorities: 2012/04/26 (US61/638.831);
2012/07/23 (US61/674.619)

(51) Cl.Int./Int.Cl. *A61K 38/43* (2006.01), *A61P 31/04* (2006.01), *C07K 19/00* (2006.01), *C12N 15/62* (2006.01), *C12N 15/63* (2006.01)

(72) Inventeurs/Inventors:
MCADOW, MOLLY, US;
DEDENT, ANDREA, US;
CHENG, ALICE, US;
EMOLO, CARLA, US;
MISSIAKAS, DOMINIQUE, US;
SCHNEEWIND, OLAF, US

(73) Propriétaire/Owner:
UNIVERSITY OF CHICAGO, US

(74) Agent: NORTON ROSE FULBRIGHT CANADA LLP/S.E.N.C.R.L., S.R.L.

(54) Titre : ANTIGENES DES STAPHYLOCOQUES COAGULASE ET LEURS METHODES D'UTILISATION
(54) Title: STAPHYLOCOCCAL COAGULASE ANTIGENS AND METHODS OF THEIR USE

(57) Abrégé/Abstract:
The present invention concerns methods and compositions for treating or preventing a bacterial infection, particularly infection by a Staphylococcus bacterium. The invention provides methods and compositions for stimulating an immune response against the bacteria. In certain embodiments, the methods and compositions involve coagulase Domains 1-2 and variants thereof.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 October 2013 (31.10.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/162746 A1

(51) International Patent Classification:
A61K 38/43 (2006.01) *C12N 15/63* (2006.01) *C07K 19/00* (2006.01) *A61P 31/04* (2006.01) *C12N 15/62* (2006.01)

(21) International Application Number: PCT/US2013/031695

(22) International Filing Date: 14 March 2013 (14.03.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/638.831 26 April 2012 (26.04.2012) US
61/674.619 23 July 2012 (23.07.2012) US

(71) Applicant: **UNIVERSITY OF CHICAGO** [US/US]; 5801 S.Ellis, Chicago, IL 60637 (US).

(72) Inventors: **MCADOW, Molly**; c/o UNIVERSITY OF CHICAGO, 5801 S. Ellis, Chicago, IL 60637 (US). **DEDENT, Andrea**; c/o UNIVERSITY OF CHICAGO, 5801 S. Ellis, Chicago, IL 60637 (US). **CHENG, Alice**; c/o UNIVERSITY OF CHICAGO, 5801 S. Ellis, Chicago, IL 60637 (US). **EMOLO, Carla**; c/o UNIVERSITY OF CHICAGO, 5801 S. Ellis, Chicago, IL 60637 (US). **MISSIAKAS, Dominique**; c/o UNIVERSITY OF CHICAGO, 5801 S. Ellis, Chicago, IL 60637 (US). **SCHNEEWIND, Olaf**; c/o UNIVERSITY OF CHICAGO, 5801 S. Ellis, Chicago, IL 60637 (US).

(74) Agent: **SHISHIMA, Gina N.**; Fulbright & Jaworski L.L.P., 98 San Jacinto Blvd., Suite 1100, Austin, TX 78701 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2013/162746 A1

(54) Title: STAPHYLOCOCCAL COAGULASE ANTIGENS AND METHODS OF THEIR USE

(57) Abstract: The present invention concerns methods and compositions for treating or preventing a bacterial infection, particularly infection by a *Staphylococcus bacterium*. The invention provides methods and compositions for stimulating an immune response against the bacteria. In certain embodiments, the methods and compositions involve coagulase Domains 1-2 and variants thereof.

DESCRIPTION

STAPHYLOCOCCAL COAGULASE ANTIGENS AND METHODS OF THEIR USE

BACKGROUND OF THE INVENTION

I. FIELD OF THE INVENTION

The present invention relates generally to the fields of immunology, microbiology, and pathology. More particularly, it concerns methods and compositions involving bacterial coagulase variants, which can be used to invoke an immune response against the bacteria.

II. BACKGROUND

The number of both community acquired and hospital acquired infections have increased over recent years with the increased use of intravascular devices. Hospital acquired (nosocomial) infections are a major cause of morbidity and mortality, more particularly in the United States, where it affects more than 2 million patients annually. The most frequent infections are urinary tract infections (33% of the infections), followed by pneumonia (15.5%), surgical site infections (14.8%) and primary bloodstream infections (13%) (Emorl and Gaynes, 1993).

The major nosocomial pathogens include *Staphylococcus aureus*, coagulase-negative Staphylococci (mostly *Staphylococcus epidermidis*), *enterococcus* spp., *Escherichia coli* and *Pseudomonas aeruginosa*. Although these pathogens cause approximately the same number of infections, the severity of the disorders they can produce combined with the frequency of antibiotic resistant isolates balance this ranking towards *S. aureus* and *S. epidermidis* as being the most significant nosocomial pathogens.

Date Reçue/Date Received 2021-01-25

Staphylococci can cause a wide variety of diseases in humans and other animals through either toxin production or invasion. Staphylococcal toxins are also a common cause of food poisoning, as the bacteria can grow in improperly-stored food.

Staphylococcus epidermidis is a normal skin commensal which is also an important opportunistic pathogen responsible for infections of impaired medical devices and infections at sites of surgery. Medical devices infected by *S. epidermidis* include cardiac pacemakers, cerebrospinal fluid shunts, continuous ambulatory peritoneal dialysis catheters, orthopedic devices and prosthetic heart valves.

Staphylococcus aureus is the most common cause of nosocomial infections with a significant morbidity and mortality. It is the cause of some cases of osteomyelitis, endocarditis, septic arthritis, pneumonia, abscesses, and toxic shock syndrome. *S. aureus* can survive on dry surfaces, increasing the chance of transmission. Any *S. aureus* infection can cause the staphylococcal scalded skin syndrome, a cutaneous reaction to exotoxin absorbed into the bloodstream. It can also cause a type of septicemia called pyaemia that can be life-threatening. Problematically, Methicillin-resistant Staphylococcus aureus (MRSA) has become a major cause of hospital-acquired infections.

S. aureus and *S. epidermidis* infections are typically treated with antibiotics, with penicillin being the drug of choice, whereas vancomycin is used for methicillin resistant isolates. The percentage of staphylococcal strains exhibiting wide-spectrum resistance to antibiotics has become increasingly prevalent, posing a threat for effective antimicrobial therapy. In addition, the recent emergence of vancomycin resistant *S. aureus* strain has aroused fear that MRSA strains are emerging and spreading for which no effective therapy is available.

An alternative to antibiotic treatment for staphylococcal infections is under investigation that uses antibodies directed against staphylococcal antigens. This therapy involves administration of polyclonal antisera (WO00/15238, WO00/12132) or treatment with monoclonal antibodies against lipoteichoic acid (WO98/57994).

An alternative approach would be the use of active vaccination to generate an immune response against staphylococci. The *S. aureus* genome has been sequenced and many of the coding sequences have been identified (WO02/094868, EP0786519), which can lead to the identification of potential antigens. The same is true for *S. epidermidis* (WO01/34809). As a refinement of this approach, others have identified proteins that are recognized by hyperimmune sera from patients who have suffered staphylococcal infection (WO01/98499, WO02/059148).

S. aureus secretes a plethora of virulence factors into the extracellular milieu (Archer, 1998; Dinges *et al.*, 2000; Foster, 2005; Shaw *et al.*, 2004; Sibbald *et al.*, 2006). Like most secreted proteins, these virulence factors are translocated by the Sec machinery across the plasma membrane. Proteins secreted by the Sec machinery bear an N-terminal leader peptide that is removed by leader peptidase once the pre-protein is engaged in the Sec translocon (Dalbey and Wickner, 1985; van Wely *et al.*, 2001). Recent genome analysis suggests that Actinobacteria and members of the Firmicutes encode an additional secretion system that recognizes a subset of proteins in a Sec-independent manner (Pallen, 2002). ESAT-6 (early secreted antigen target 6 kDa) and CFP-10 (culture filtrate antigen 10 kDa) of *Mycobacterium tuberculosis* represent the first substrates of this novel secretion system termed ESX-1 or Snm in *M. tuberculosis* (Andersen *et al.*, 1995; Hsu *et al.*, 2003; Pym *et al.*, 2003; Stanley *et al.*, 2003). In *S. aureus,* two ESAT-6 like factors designated EsxA and EsxB are secreted by the Ess pathway (<u>E</u>SAT-6 <u>s</u>ecretion <u>s</u>ystem) (Burts *et al.*, 2005).

The first generation of vaccines targeted against *S. aureus* or against the exoproteins it produces have met with limited success (Lee, 1996). There remains a need to develop effective vaccines against staphylococcal infections. Additional compositions for treating staphylococcal infections are also needed.

SUMMARY OF THE INVENTION

During infection, *Staphylococcus aureus* secrets two coagulases, Coa and vWbp, which upon association with host prothrombin and fibrinogen, convert soluble fibrinogen to insoluble fibrin, induce the formation of fibrin clots and enable the establishment of staphylococcal disease. Due to the fact that Coa and vWbp are important factors for staphylococcal coagulation and agglutination, which promote the pathogenesis of *S. aureus* abscess formation and lethal bacteremia in mice. Here the inventors demonstrate that antibodies directed against the variable prothrombin-binding portion of coagulases confer type-specific immunity through neutralization of *S. aureus* clotting activity and protect from staphylococcal disease. In particular, by combining variable portions of coagulases from North-American isolates into hybrid Coa and vWbp proteins, a subunit vaccine was derived that provides protection against challenge with different coagulase-type *S. aureus* strains.

Certain embodiments an immunogenic composition is provided comprising a staphylococcal coagulase Domains 1-2 (e.g., a Domains 1-2 from a staphylococcal Coa or vWbp protein). For example, the Domains 1-2 can comprise or consist of an amino acid sequence that is at least 80, 85, 90, 95, 98, 99 or 100% identical to an amino acid sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37) or SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41). In some aspects, a staphylococcal coagulase Domains 1-2 is comprised in a less than full-length coagulase protein. For example, the Domains 1-2 can be comprised in a less than full-length Coa protein (*e.g.,* that lacks all or part of a L or R Domain segment) or in a less than full-length vWbp protein (*e.g.,* that lacks all or part of a L or F Domain segment). In some aspects, a Domain 1-2 is a Domain 1-2 segment wherein the secretion signal sequence has been removed.

In certain embodiments, an immunogenic composition is provided comprising at least two different staphylococcal coagulase Domains 1-2. For example, a composition can comprise at least two different staphylococcal coagulase Domains 1-2 from a staphylococcal Coa or vWbp protein, wherein at least one Domain 1-2 is comprised in a less than full-length coagulase protein. In certain aspects, the sequence of the Domains 1-2 comprises or consists of an amino acid sequence that is at least 80% identical to an amino acid sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37) or SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41). In certain aspects, the sequence of the Domains 1-2 comprises or consists of an amino acid sequence that is at least 85, 90, 95, 98, 99 or 100% identical to an amino acid sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37) or SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41). In further aspects, at least one of the Domains 1-2 is comprised in a less than full-length coagulase protein sequence. In particular embodiments, the full length coagulase protein is a Coa protein comprising the sequence of SEQ ID NO: 42. In particular aspects, the full length coagulase protein is a vWbp protein comprising the sequence of SEQ ID NO: 75. In still further aspects, the a less than full-length Coa protein lacks all or part of a L or R Domain segment. In still further aspects, the truncated vWbp protein lacks all or part of a L or F Domain segment. The term "truncated" protein is used to refer to a protein or a polypeptide that does not achieve its full length, and thus is missing one or more of the amino acid residues that are present in a normal protein. The term "truncated relative to a full-length coagulase protein" is used to refer to a protein or a polypeptide that does not have the full length of a coagulase protein, and thus is missing at least one amino acid residues that are present in a coagulase protein.

In certain embodiments, one of the staphylococcal coagulase Domains 1-2 is from *S. aureus* Newman, 85/2082, MW2, MSSA476, N315, Mu50, MRSA252, CowanI, WIS or USA300 strain, or any other *S. aureus* strain. In some embodiments, one of the coagulase Domains 1-2 comprises a vWbp domains 1-2 from a *S. aureus* N315 or USA300.

In some aspects, one of the Domains 1-2 comprises a Coa Domains 1-2 at least 80% identical to an amino acid sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37). In further aspects, one of the Domains 1-2 comprises a Coa Domains 1-2 at least 85, 90, 95, 98, 99% identical to an amino acid sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37).

In another aspects, one of the Domains 1-2 comprises a vWbp Domains 1-2 at least 80% identical to a sequence of SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41). In further aspects, one of the Domains 1-2 comprises a vWbp Domains 1-2 at least 85, 90, 95, 98, 99% identical to a sequence of SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41).

In certain embodiments, one of the Domains 1-2 is a Coa Domains 1-2, further comprising an L or R domain from a staphylococcal Coa protein.

In certain embodiments, one of the Domains 1-2 is a vWbp Domains 1-2, further comprising an L or Fgb domain from a staphylococcal vWbp protein.

In some aspects, an immunogenic composition comprises at least three, four, or five different staphylococcal coagulase Domains 1-2. In further aspects, an immunogenic composition comprise at least four different staphylococcal coagulase Domains 1-2. In particular embodiments, the at least four different staphylococcal coagulase Domains 1-2 are staphylococcal Coa Domains 1-2 from strains MRSA252, MW2, N315 and USA300.

In some embodiments, it is contemplated that an immunogenic composition comprises at least two different staphylococcal coagulase Domains 1-2 that are comprised in a fusion protein.

In further embodiments, the immunogenic composition further comprises one or more additional staphylococcal antigen(s). In additional embodiments, the immunogenic composition may also include an adjuvant. In particular embodiments, the additional staphylococcal antigen(s) is Emp, EsxA, EsxB, EsaC, Eap, Ebh, EsaB, Coa, vWbp, vWh, Hla, SdrC, SdrD, SdrE, IsdA, IsdB, IsdC, ClfA, ClfB, SasF or a nontoxigenic SpA.

Embodiments include a recombinant polypeptide comprising at least two different staphylococcal coagulase Domains 1-2. The sequences of the Domains 1-2 are at least 80% identical to an amino acid sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37) or SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41). In some aspects, the sequence of the Domains 1-2 are at least 85, 90, 95, 98, 99% identical to an amino acid sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37) or SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41).

In further embodiments, a polynucleotide molecule comprising a nucleic acid sequence encoding a recombinant polypeptide comprising sequence encoding at least two different staphylococcal coagulase Domains 1-2 is contemplated. In further aspects, an expression vector comprises the nucleic acid sequence operably linked to an expression control sequence. In still further aspects, a host cell comprising the expression vector is also contemplated.

Embodiments include the use of the composition, the recombinant polypeptide, the polynucleotide molecule and the expression vector described herein to treat or prevent a staphylococcal infection in a subject. In some aspects, a composition comprising at least two different staphylococcal coagulase Domains 1-2 is used to treat or prevent a staphylococcal infection. The sequences of the Domains 1-2 are at least 80% identical to an amino acid sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37) or SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41) and at least one of the Domains 1-2 is a truncated coagulase protein sequence.

In some embodiments, a method to manufacture an immunogenic composition comprising mixing at least two different staphylococcal coagulase Domains 1-2 polypeptides is contemplated. The sequences of the Domains 1-2 are at least 80% identical to an amino acid sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37) or SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41) and at least one of the Domains 1-2 is a truncated coagulase protein sequence.

Embodiments include the use of at least two different staphylococcal coagulase Domains 1-2 described herein in methods and compositions for the treatment of bacterial and/or staphylococcal infection. Furthermore, certain embodiments provide methods and compositions that can be used to treat (*e.g.*, limiting staphylococcal abscess formation and/or persistence in a subject) or prevent bacterial infection. In some cases, methods for stimulating an immune response involve administering to the subject an effective amount of the immunogenic composition described herein and in certain aspects other bacterial proteins. Other bacterial proteins include, but are not limited to (i) a secreted virulence factor, and/or a cell surface protein or peptide, or (ii) a recombinant nucleic acid molecule encoding a secreted virulence factor, and/or a cell surface protein or peptide.

In other aspects, the subject can be administered with the immunogenic composition, the recombinant polypeptide, or the vector described herein. The recombinant polypeptide or the vector can be formulated in a pharmaceutically acceptable composition. The composition can further comprise one or more of at least or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 additional staphylococcal antigen or immunogenic fragment thereof (*e.g.*, Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla (*e.g.*, H35 mutants), IsdC, SasF, vWbp, or vWh). Additional staphylococcal antigens that can be used include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa (GenBank CAC80837), Aap (GenBank accession AJ249487), Ant (GenBank accession NP_372518), autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein (see PCT publications WO2007/113222, WO2007/113223, WO2006/032472, WO2006/032475, WO2006/032500.

The staphylococcal antigen or immunogenic fragment can be administered concurrently with the immunogenic composition comprising at least two different coagulase Domains 1-2, the recombinant polypeptide comprising at least two different Domains 1-2, and/or the vector

Date Reçue/Date Received 2021-01-25

comprising a nucleic acid sequence encoding at least two different Domains 1-2 described herein. The staphylococcal antigen or immunogenic fragment can be administered in the same composition with the immunogenic composition comprising at least two different Domains 1-2, the recombinant polypeptide comprising at least two different Domains 1-2, and/or the vector comprising a nucleic acid sequence encoding at least two different Domains 1-2 described herein. As used herein, the term "modulate" or "modulation" encompasses the meanings of the words "enhance," or "inhibit." "Modulation" of activity may be either an increase or a decrease in activity. As used herein, the term "modulator" refers to compounds that effect the function of a moiety, including up-regulation, induction, stimulation, potentiation, inhibition, down-regulation, or suppression of a protein, nucleic acid, gene, organism or the like.

A recombinant nucleic acid molecule can encode at least two different staphylococcal coagulase Domains 1-2 and at least one staphylococcal antigen or immunogenic fragment thereof. In particular aspects, one of the at least two different staphylococcal coagulase Domains 1-2 is a Coa Domains 1-2 at least 80% identical to an amino acid sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37). In still further aspects, one of the at least two different staphylococcal coagulase Domains 1-2 is a vWbp Domains 1-2 at least 80% identical to a sequence of SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41). In some aspects, the recombinant nucleic acid molecule comprises a sequence that encodes a truncated coagulase protein and the truncated coagulase protein includes either one of the at least two different staphylococcal coagulase Domains 1-2. In particular embodiments, the coagulase protein is a Coa protein comprising the sequence of SEQ ID NO: 42. In particular aspects, the coagulase protein is a vWbp protein comprising the sequence of SEQ ID NO: 75.

In certain embodiments, the composition or the polypeptide comprising at least two different staphylococcal coagulase Domains 1-2 may be used in combination with secreted factors or surface antigens including, but not limited to one or more of an isolated Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, or vWh polypeptide or immunogenic segment thereof. Additional staphylococcal antigens that can be used include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein. In certain embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more of Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh, 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein. can be specifically excluded from a formulation of the invention.

The following table lists the various combinations of staphylococcal coagulase Domains 1-2 and various other Staphylococcal antigens:

Table 1. Staphylococcal coagulase Domains 1-2 and staphylococcal antigen combinations.

Eap	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ebh		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Emp			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsaB				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsaC					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsxA						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

EsxB							+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrC								+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrD									+	+	+	+	+	+	+	+	+	+	+	+	+
SdrE										+	+	+	+	+	+	+	+	+	+	+	+
IsdA											+	+	+	+	+	+	+	+	+	+	+
IsdB												+	+	+	+	+	+	+	+	+	+
ClfA													+	+	+	+	+	+	+	+	+
ClfB														+	+	+	+	+	+	+	+
Coa															+	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

Ebh		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Emp			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsaB				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsaC					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsxA						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsxB							+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrC								+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrD									+	+	+	+	+	+	+	+	+	+	+	+	+

SdrE										+	+	+	+	+	+	+	+	+	+	+	+
IsdA											+	+	+	+	+	+	+	+	+	+	+
IsdB												+	+	+	+	+	+	+	+	+	+
ClfA													+	+	+	+	+	+	+	+	+
ClfB														+	+	+	+	+	+	+	+
Coa															+	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

Emp			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsaB				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsaC					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsxA						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsxB							+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrC								+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrD									+	+	+	+	+	+	+	+	+	+	+	+	+
SdrE										+	+	+	+	+	+	+	+	+	+	+	+
IsdA											+	+	+	+	+	+	+	+	+	+	+
IsdB												+	+	+	+	+	+	+	+	+	+
ClfA													+	+	+	+	+	+	+	+	+

ClfB														+	+	+	+	+	+	+	+
Coa															+	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

EsaB				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsaC					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsxA						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsxB							+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrC								+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrD									+	+	+	+	+	+	+	+	+	+	+	+	+
SdrE										+	+	+	+	+	+	+	+	+	+	+	+
IsdA											+	+	+	+	+	+	+	+	+	+	+
IsdB												+	+	+	+	+	+	+	+	+	+
ClfA													+	+	+	+	+	+	+	+	+
ClfB														+	+	+	+	+	+	+	+
Coa															+	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+

SasF																			+	+	+
vWbp																				+	+
vWh																					+

EsaC					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsxA						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsxB							+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrC								+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrD									+	+	+	+	+	+	+	+	+	+	+	+	+
SdrE										+	+	+	+	+	+	+	+	+	+	+	+
IsdA											+	+	+	+	+	+	+	+	+	+	+
IsdB												+	+	+	+	+	+	+	+	+	+
ClfA													+	+	+	+	+	+	+	+	+
ClfB														+	+	+	+	+	+	+	+
Coa															+	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

EsxA						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EsxB							+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

SdrC								+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrD									+	+	+	+	+	+	+	+	+	+	+	+	+
SdrE										+	+	+	+	+	+	+	+	+	+	+	+
IsdA											+	+	+	+	+	+	+	+	+	+	+
IsdB												+	+	+	+	+	+	+	+	+	+
ClfA													+	+	+	+	+	+	+	+	+
ClfB														+	+	+	+	+	+	+	+
Coa															+	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

EsxB							+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrC								+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrD									+	+	+	+	+	+	+	+	+	+	+	+	+
SdrE										+	+	+	+	+	+	+	+	+	+	+	+
IsdA											+	+	+	+	+	+	+	+	+	+	+
IsdB												+	+	+	+	+	+	+	+	+	+
ClfA													+	+	+	+	+	+	+	+	+
ClfB														+	+	+	+	+	+	+	+
Coa															+	+	+	+	+	+	+

Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

SdrC								+	+	+	+	+	+	+	+	+	+	+	+	+	+
SdrD									+	+	+	+	+	+	+	+	+	+	+	+	+
SdrE										+	+	+	+	+	+	+	+	+	+	+	+
IsdA											+	+	+	+	+	+	+	+	+	+	+
IsdB												+	+	+	+	+	+	+	+	+	+
ClfA													+	+	+	+	+	+	+	+	+
ClfB														+	+	+	+	+	+	+	+
Coa															+	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

SdrD									+	+	+	+	+	+	−	+	+	+	+	+	+
SdrE										+	+	+	+	+	−	+	+	+	+	+	+

IsdA											+	+	+	+	−	+	+	+	+	+	+
IsdB												+	+	+	−	+	+	+	+	+	+
ClfA													+	+	−	+	+	+	+	+	+
ClfB														+	−	+	+	+	+	+	+
Coa															−	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

SdrE										+	+	+	+	+	−	+	+	+	+	+	+
IsdA											+	+	+	+	−	+	+	+	+	+	+
IsdB												+	+	+	−	+	+	+	+	+	+
ClfA													+	+	−	+	+	+	+	+	+
ClfB														+	−	+	+	+	+	+	+
Coa															−	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

IsdA											+	+	+	+	−	+	+	+	+	+	+
IsdB												+	+	+	−	+	+	+	+	+	+
ClfA													+	+	−	+	+	+	+	+	+
ClfB														+	−	+	+	+	+	+	+
Coa															−	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

IsdB												+	+	+	+	+	+	+	+	+	+
ClfA													+	+	+	+	+	+	+	+	+
ClfB														+	+	+	+	+	+	+	+
Coa															+	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

ClfA													+	+	+	+	+	+	+	+	+

ClfB														+	+	+	+	+	+	+	+
Coa															+	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

ClfB														+	+	+	+	+	+	+	+
Coa															+	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

Coa															+	+	+	+	+	+	+
Hla																+	+	+	+	+	+
Hla_{H35A}																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+

vWh																					+

Hla																+	+	+	+	+	+
Hla$_{H35A}$																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

Hla$_{H35A}$																	+	+	+	+	+
IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

IsdC																		+	+	+	+
SasF																			+	+	+
vWbp																				+	+
vWh																					+

SasF																			+	+	+
vWbp																				+	+
vWh																					+

vWbp																			+	+
vWh																				+

vWh																				+

In still further aspects, the isolated recombinant polypeptide comprising at least two different staphylococcal coagulase Domains 1-2 described herein is multimerized, *e.g.*, dimerized or a linear fusion of two or more polypeptides or peptide segments. In certain aspects of the invention, a composition comprises multimers or concatamers of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more isolated cell surface proteins or segments thereof. Concatamers are linear polypeptides having one or more repeating peptide units. The at least two different staphylococcal coagulase Domains 1-2 can be consecutive or separated by a spacer or other peptide sequences, *e.g.*, one or more additional bacterial peptide. In a further aspect, the other polypeptides or peptides contained in the multimer or concatamer can include, but are not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 of Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh or immunogenic fragments thereof. Additional staphylococcal antigens that can be used in combination with at least two different staphylococcal coagulase Domains 1-2, include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein.

Certain embodiments include methods for eliciting an immune response against a staphylococcus bacterium or staphylococci in a subject comprising providing to the subject an effective amount of an immunogenic composition or a recombinant polypeptide comprising at least two different staphylococcal coagulase Domains 1-2 or a vector comprising a nucleic acid sequence encoding the same. In certain aspects, the methods for eliciting an immune response against a staphylococcus bacterium or staphylococci in a subject comprising providing to the subject an effective amount of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or more secreted proteins and/or cell surface proteins or segments/fragments thereof. A secreted protein or cell surface protein includes, but is not limited to Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, and/or vWh proteins and immunogenic fragments thereof. Additional staphylococcal antigens that can be used include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein.

Embodiments of the invention include compositions that include a polypeptide, peptide, or protein that comprises a sequence that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a staphylococcal coagulase Domains 1-2, in particular, a Coa Domains 1-2 (see, SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37)) or a vWbp Domains 1-2 (see, SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41)), or a second protein or peptide that is a secreted bacterial protein or a bacterial cell surface protein. Similarity or identity, with identity being preferred, is known in the art and a number of different programs can be used to identify whether a protein (or nucleic acid) has sequence identity or similarity to a known sequence. Sequence identity and/or similarity is determined using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith & Waterman (1981), by the sequence identity alignment algorithm of Needleman & Wunsch (1970), by the search for similarity method of Pearson & Lipman (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.), the Best Fit sequence program described by Devereux *et al.* (1984), preferably using the default settings, or by inspection. Preferably, percent identity is calculated by using alignment tools known to and readily ascertainable to those of skill in the art. Percent identity is essentially the number of identical amino acids divided by the total number of amino acids compared times one hundred.

Still further embodiments include methods for stimulating in a subject a protective or therapeutic immune response against a staphylococcus bacterium comprising administering to the subject an effective amount of a composition including (i) a immunogenic composition comprising at least two different staphylococcal coagulase Domains 1-2, *e.g.*, a Coa Domains 1-2 (see, SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37)) or a vWbp Domains 1-2 (see, SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41)) or a homologue thereof; or, (ii) a recombinant polypeptide comprising at least two different staphylococcal coagulase Domains 1-2 or homogues thereof; or, (iii) a nucleic acid molecule comprises a sequence encoding the at least two different staphylococcal Domains 1-2 or homologue thereof, or (iv) administering any of (i)-(iii) with any combination or permutation of bacterial proteins described herein. In a preferred embodiment the composition is not a staphylococcus bacterium. In certain aspects the subject is a human or a cow. In a further aspect the composition is formulated in a pharmaceutically acceptable formulation. The staphylococci may be *Staphylococcus aureus*.

Yet still further embodiments include vaccines comprising a pharmaceutically acceptable composition having at least two different staphylococcal coagulase Domains 1-2 described herein, or any other combination or permutation of protein(s) or peptide(s) described herein, wherein the composition is capable of stimulating an immune response against a staphylococcus bacterium. The vaccine may comprise at least two different staphylococcal coagulase Domains 1-2 described herein, or any other combination or permutation of protein(s) or peptide(s) described. In certain aspects, at least two different staphylococcal coagulase Domains 1-2 described herein, or any other combination or permutation of protein(s) or peptide(s) described are multimerized, *e.g.*, dimerized or concatamerized. In a further aspect, the vaccine composition is contaminated by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.5, 0.25, 0.05% (or any range derivable therein) of other Staphylococcal proteins. A composition may further comprise an isolated non-coagulase polypeptide. Typically the vaccine comprises an adjuvant. In certain aspects a protein or peptide of the invention is linked (covalently or non-covalently) to the adjuvant, preferably the adjuvant is chemically conjugated to the protein.

In still yet further embodiments, a vaccine composition is a pharmaceutically acceptable composition having a recombinant nucleic acid encoding a recombinant polypeptide containing at least two different staphylococcal coagulase Domains 1-2 described herein, or any other combination or permutation of protein(s) or peptide(s) described herein, wherein the composition is capable of stimulating an immune response against a staphylococcus bacteria. In certain embodiments the recombinant nucleic acid contains a heterologous promoter. Preferably the recombinant nucleic acid is a vector. More preferably the vector is a plasmid or a viral vector. In some aspects the vaccine includes a recombinant, non-staphylococcus bacterium containing the nucleic acid. The recombinant non-staphylococci may be Salmonella or another gram-positive bacteria. The vaccine may comprise a pharmaceutically acceptable excipient, more preferably an adjuvant.

Still further embodiments include methods for stimulating in a subject a protective or therapeutic immune response against a staphylococcus bacterium comprising administering to the subject an effective amount of a composition of at least two different staphylococcal coagulase Domains 1-2 described herein, or a recombinant polypeptide containing at least two different staphylococcal coagulase Domains 1-2, or a nucleic acid encoding the same, and further comprising one or more of a Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, or vWh protein or peptide thereof. In a preferred embodiment the composition comprises a non-staphylococcus bacterium. In a further aspect the composition is formulated in a pharmaceutically acceptable formulation. The staphylococci for which a subject is being treated may be *Staphylococcus aureus*. Methods of the invention may also additionaly include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or more secreted virulence factors and/or cell surface proteins, such as Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, or vWh in various combinations. In certain aspects a vaccine formulation includes Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, and vWh.

In certain aspects an antigen combination can include (1) at least two different staphylococcal coagulase Domains 1-2 and IsdA; (2) at least two different staphylococcal coagulase

Domains 1-2 and ClfB; (3) at least two different staphylococcal coagulase Domains 1-2 and SdrD; (4) at least two different staphylococcal coagulase Domains 1-2 and Hla or Hla variant; (5) at least two different staphylococcal coagulase Domains 1-2 and ClfB, SdrD, and Hla or Hla variant; (6) at least two different staphylococcal coagulase Domains 1-2, IsdA, SdrD, and Hla or Hla variant; (7) at least two different staphylococcal coagulase Domains 1-2, IsdA, ClfB, and Hla or Hla variant; (8) at least two different staphylococcal coagulase Domains 1-2, IsdA, ClfB, and SdrD; (9) at least two different staphylococcal coagulase Domains 1-2, IsdA, ClfB, SdrD and Hla or Hla variant; (10) at least two different staphylococcal coagulase Domains 1-2, IsdA, ClfB, and SdrD; (11) at least two different staphylococcal coagulase Domains 1-2, IsdA, SdrD, and Hla or Hla variant; (12) at least two different staphylococcal coagulase Domains 1-2, IsdA, and Hla or Hla variant; (13) at least two different staphylococcal coagulase Domains 1-2, IsdA, ClfB, and Hla or Hla variant; (14) at least two different staphylococcal coagulase Domains 1-2, ClfB, and SdrD; (15) at least two different staphylococcal coagulase Domains 1-2, ClfB, and Hla or Hla variant; or (16) at least two different staphylococcal coagulase Domains 1-2, SdrD, and Hla or Hla variant.

In certain aspects, a bacterium delivering a composition of the invention will be limited or attenuated with respect to prolonged or persistent growth or abscess formation. In yet a further aspect, at least two different staphylococcal coagulase Domains 1-2 can be overexpressed in an attenuated bacterium to further enhance or supplement an immune response or vaccine formulation.

The term "EsxA protein" refers to a protein that includes isolated wild-type EsxA polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria EsxA proteins.

The term "EsxB protein" refers to a protein that includes isolated wild-type EsxB polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria EsxB proteins.

The term "SdrD protein" refers to a protein that includes isolated wild-type SdrD polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria SdrD proteins.

The term “SdrE protein” refers to a protein that includes isolated wild-type SdrE polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria SdrE proteins.

The term “IsdA protein” refers to a protein that includes isolated wild-type IsdA polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria IsdA proteins.

The term “IsdB protein” refers to a protein that includes isolated wild-type IsdB polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria IsdB proteins.

The term “Eap protein” refers to a protein that includes isolated wild-type Eap polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria Eap proteins.

The term “Ebh protein” refers to a protein that includes isolated wild-type Ebh polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria Ebh proteins.

The term “Emp protein” refers to a protein that includes isolated wild-type Emp polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria Emp proteins.

The term “EsaB protein” refers to a protein that includes isolated wild-type EsaB polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria EsaB proteins.

The term “EsaC protein” refers to a protein that includes isolated wild-type EsaC polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria EsaC proteins.

The term “SdrC protein” refers to a protein that includes isolated wild-type SdrC polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria SdrC proteins.

The term "ClfA protein" refers to a protein that includes isolated wild-type ClfA polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria ClfA proteins.

The term "ClfB protein" refers to a protein that includes isolated wild-type ClfB polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria ClfB proteins.

The term "Coa protein" refers to a protein that includes isolated wild-type Coa polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria Coa proteins.

The term "Hla protein" refers to a protein that includes isolated wild-type Hla polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria Hla proteins.

The term "IsdC protein" refers to a protein that includes isolated wild-type IsdC polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria IsdC proteins.

The term "SasF protein" refers to a protein that includes isolated wild-type SasF polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria SasF proteins.

The term "vWbp protein" refers to a protein that includes isolated wild-type vWbp (von Willebrand factor binding protein) polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria vWbp proteins.

The term "vWh protein" refers to a protein that includes isolated wild-type vWh (von Willebrand factor binding protein homolog) polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria vWh proteins.

An immune response refers to a humoral response, a cellular response, or both a humoral and cellular response in an organism. An immune response can be measured by assays that include, but are not limited to, assays measuring the presence or amount of antibodies that specifically recognize a protein or cell surface protein, assays measuring T-cell activation or proliferation, and/or assays that measure modulation in terms of activity or expression of one or more cytokines.

In still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an EsxA protein.

In still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an EsxB protein.

In yet still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an SdrD protein.

In further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an SdrE protein.

In still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an IsdA protein.

In yet still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an IsdB protein.

Embodiments of the invention include compositions that include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a EsaB protein.

In a further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a ClfB protein.

In still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an IsdC protein.

In yet further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a SasF protein.

In yet still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a SdrC protein.

In yet still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a ClfA protein.

In yet still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an Eap protein.

In yet still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an Ebh protein.

In yet still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an Emp protein.

In yet still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an EsaC protein. Sequence of EsaC polypeptides can be found in the protein databases and include, but are not limited to accession numbers ZP_02760162 (GI:168727885), NP_645081.1 (GI:21281993), and NP_370813.1 (GI:15923279)

Date Reçue/Date Received 2021-01-25

In yet still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a Coa protein.

In yet still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a Hla protein.

In yet still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a vWa protein.

In yet still further embodiments of the invention a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a vWbp protein.

In certain aspects, a polypeptide or segment/fragment can have a sequence that is at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% or more identical to the amino acid sequence of the reference polypeptide. The term "similarity" refers to a polypeptide that has a sequence that has a certain percentage of amino acids that are either identical with the reference polypeptide or constitute conservative substitutions with the reference polypeptides.

The polypeptides described herein may include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more variant amino acids within at least, or at most 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 300, 400, 500, 550, 1000 or more contiguous amino acids, or any range derivable therein, of the sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37) or SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41).

A polypeptide segment as described herein may include 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 300, 400, 500, 550, 1000 or more contiguous amino acids, or any range derivable therein, of the sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37) or SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41).

In yet still further embodiments, a composition may include a polynucleotide that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a nucleic acid sequence encoding a Coa protein. In certain aspects, the nucleic acid sequence encoding a Coa protein of strain USA300 will have all or part of the nucleic acid sequence provided herein. In certain aspects, the nucleic acid sequence encoding a Coa protein of strain N315 will have all or part of the nucleic acid sequence provided herein In certain aspects, the nucleic acid sequence encoding a Coa protein of strain MW2 will have all or part of the nucleic acid sequence of provided herein. In certain aspects, the nucleic acid sequence encoding a Coa protein of strain MRSA252 will have all or part of the nucleic acid sequence of provided herein. In certain aspects, the nucleic acid sequence encoding a Coa protein of strain WIS will have all or part of the nucleic acid sequence of provided herein. In certain aspects, the nucleic acid sequence encoding a Coa protein of strain MU50 will have all or part of the nucleic acid sequence of provided herein. In certain aspects, the nucleic acid sequence encoding a Coa protein of strain 85/2082 will have all or part of the nucleic acid sequence of provided herein. In certain aspects, the nucleic acid sequence encoding a Coa protein of strain Newman will have all or part of the nucleic acid sequence of provided herein.

In yet still further embodiments, a composition may include a polynucleotide that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a nucleic acid sequence encoding a vWbp fusion protein. In certain aspects, the nucleic acid sequence encoding a vWpb protein of strain USA300 will have all or part of the nucleic acid sequence provided herein. In certain aspects, the nucleic acid sequence encoding a vWbp protein of strain N315 will have all or part of the nucleic acid sequence provided herein. In certain aspects, the nucleic acid sequence encoding a vWbp protein of strain Newman will have all or part of the nucleic acid sequence provided herein. In certain aspects, the nucleic acid sequence encoding a vWbp protein of strain MRSA252 will have all or part of the nucleic acid sequence provided herein. In certain aspects, the nucleic acid sequence encoding a vWbp protein of strain MW2 will have all or part of the nucleic acid sequence provided herein.

In yet still further embodiments, a composition may include a polynucleotide that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a nucleic acid sequence encoding a Coa Domains 1-2. In certain aspects, the nucleic acid sequence encoding a Coa Domains 1-2 of strain N315 will have all or part of the nucleic acid sequence provided herein. In certain aspects, the nucleic acid sequence encoding a Coa Domains 1-2 of strain MW2 will have all or part of the nucleic acid sequence provided herein. In certain aspects, the nucleic acid sequence encoding a Coa Domains 1-2 of strain MRSA252 will have all or part of the nucleic acid sequence provided herein. In certain aspects, the nucleic acid sequence encoding a Coa Domains 1-2 of strain WIS will have all or part of the nucleic acid sequence provided herein.

In particular aspects, a composition may comprise a polynucleotide that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a nucleic acid sequence encoding five different Coa Domains 1-2 from strains WIS, MRSA252, N315, MW2, and USA300, respectively. In still further aspects, the nucleic acid sequence encoding five different Coa Domains 1-2 will have all or part of the nucleic acid sequence provided herein.

In yet still further embodiments, a composition may include a polynucleotide that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a nucleic acid sequence encoding a vWbp Domains 1-2. In certain aspects, the nucleic acid sequence encoding a vWbp Domains 1-2 of strain N315 will have all or part of the nucleic acid sequence provided herein. In certain aspects, the nucleic acid sequence encoding a vWbp Domains 1-2 of strain MW2 will have all or part of the nucleic acid sequence provided herein. In certain aspects, the nucleic acid sequence encoding a vWbp Domain 1-2 of strain MRSA252 will have all or part of the nucleic acid sequence provided herein.

The compositions may be formulated in a pharmaceutically acceptable composition. In certain aspects of the invention the staphylococcus bacterium is an *S. aureus* bacterium.

In further aspects, a composition may be administered more than one time to the subject, and may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more times. The administration of the compositions include, but is not limited to oral, parenteral, subcutaneous, intramuscular, intravenous, or various combinations thereof, including inhalation or aspiration.

In still further embodiments, a composition comprises a recombinant nucleic acid molecule encoding a polypeptide described herein or segments/fragments thereof. Typically a recombinant nucleic acid molecule encoding a polypeptide described herein contains a heterologous promoter. In certain aspects, a recombinant nucleic acid molecule of the invention is a vector, in still other aspects the vector is a plasmid. In certain embodiments the vector is a viral vector. In certain aspects a composition includes a recombinant, non-staphylococcus bacterium containing or expressing a polypeptide described herein. In particular aspects the recombinant non-staphylococcus bacteria is *Salmonella* or another gram-positive bacteria. A composition is typically administered to mammals, such as human subjects, but administration to other animals that are capable of eliciting an immune response is contemplated. In further aspects the staphylococcus bacterium containing or expressing the polypeptide is *Staphylococcus aureus*. In further embodiments the immune response is a protective immune response.

In further embodiments a composition comprises a recombinant nucleic acid molecule encoding all or part of one or more of a Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, SpA, vWbp, or vWh protein or peptide or variant thereof. Additional staphylococcal antigens that can be used in combination with the polypeptides described herein include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein. In particular aspects, a bacteria is a recombinant non-staphylococcus bacteria, such as a *Salmonella* or other gram-positive bacteria.

Compositions discussed herein are typically administered to human subjects, but administration to other animals that are capable of eliciting an immune response to a staphylococcus bacterium is contemplated, particularly cattle, horses, goats, sheep and other domestic animals, *i.e.*, mammals.

In certain aspects the staphylococcus bacterium is a *Staphylococcus aureus*. In further embodiments the immune response is a protective immune response. In still further aspects, the methods and compositions of the invention can be used to prevent, ameliorate, reduce, or treat infection of tissues or glands, *e.g.*, mammary glands, particularly mastitis and other infections. Other methods include, but are not limited to prophylactically reducing bacterial burden in a subject not exhibiting signs of infection, particularly those subjects suspected of or at risk of being colonized by a target bacteria, *e.g.*, patients that are or will be at risk or susceptible to infection during a hospital stay, treatment, and/or recovery.

Any embodiment discussed with respect to one aspect of the invention applies to other aspects of the invention as well. In particular, any embodiment discussed in the context of a composition comprising at least two different staphylococcal coagulse Domains 1-2 or a recombinant polypeotide comprising the same or a nucleic acid encoding the same may be implemented with respect to other antigens, such as Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh, 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein (or nucleic acids), and vice versa. It is also understood that any one or more of Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh, 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein can be specifically excluded from a claimed composition.

Embodiments include compositions that contain or do not contain a bacterium. A composition may or may not include an attenuated or viable or intact staphylococcal bacterium. In certain aspects, the composition comprises a bacterium that is not a staphylococcal bacterium or does not contain staphylococcal bacteria. In certain embodiments a bacterial composition comprises an isolated or recombinantly expressed at least two different staphylococcal coagulase Domains 1-2 described herein or a nucleotide encoding the same. The composition may be or include a recombinantly engineered staphylococcus bacterium that has been altered in a way that comprises specifically altering the bacterium with respect to a secreted virulence factor or cell surface protein. For example, the bacteria may be recombinantly modified to express more of the virulence factor or cell surface protein than it would express if unmodified.

The term "isolated" can refer to a nucleic acid or polypeptide that is substantially free of cellular material, bacterial material, viral material, or culture medium (when produced by recombinant DNA techniques) of their source of origin, or chemical precursors or other chemicals (when chemically synthesized). Moreover, an isolated compound refers to one that can be administered to a subject as an isolated compound; in other words, the compound may not simply be considered "isolated" if it is adhered to a column or embedded in an agarose gel. Moreover, an "isolated nucleic acid fragment" or "isolated peptide" is a nucleic acid or protein fragment that is not naturally occurring as a fragment and/or is not typically in the functional state.

Moieties, such as polypeptides, peptides, antigens, or immunogens, may be conjugated or linked covalently or noncovalently to other moieties such as adjuvants, proteins, peptides, supports, fluorescence moieties, or labels. The term "conjugate" or "immunoconjugate" is broadly used to define the operative association of one moiety with another agent and is not intended to refer solely to any type of operative association, and is particularly not limited to chemical "conjugation." Recombinant fusion proteins are particularly contemplated. Compositions of the invention may further comprise an adjuvant or a pharmaceutically acceptable excipient. An adjuvant may be covalently or non-covalently coupled to a polypeptide or peptide of the invention. In certain aspects, the adjuvant is chemically conjugated to a protein, polypeptide, or peptide.

The term "providing" is used according to its ordinary meaning to indicate "to supply or furnish for use." In some embodiments, the protein is provided directly by administering the protein, while in other embodiments, the protein is effectively provided by administering a nucleic acid that encodes the protein. In certain aspects the invention contemplates compositions comprising various combinations of nucleic acid, antigens, peptides, and/or epitopes.

The subject will have (*e.g.*, are diagnosed with a staphylococcal infection), will be suspected of having, or will be at risk of developing a staphylococcal infection. Compositions of the present invention include immunogenic compositions wherein the antigen(s) or epitope(s) are contained in an amount effective to achieve the intended purpose. More specifically, an effective amount means an amount of active ingredients necessary to stimulate or elicit an immune response, or provide resistance to, amelioration of, or mitigation of infection. In more specific aspects, an effective amount prevents, alleviates or ameliorates symptoms of disease or infection, or prolongs the survival of the subject being treated. Determination of the effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. For any preparation used in the methods of the invention, an effective amount or dose can be estimated initially from *in vitro* studies, cell culture, and/or animal model assays. For example, a dose can be formulated in animal models to achieve a desired immune response or circulating antibody concentration or titer. Such information can be used to more accurately determine useful doses in humans.

The embodiments in the Example section are understood to be embodiments of the invention that are applicable to all aspects of the invention.

The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." It is also contemplated that anything listed using the term "or" may also be specifically excluded.

Throughout this application, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

Following long-standing patent law, the words "a" and "an," when used in conjunction with the word "comprising" in the claims or specification, denotes one or more, unless specifically noted.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DESCRIPTION OF THE DRAWINGS

So that the matter in which the above-recited features, advantages and objects of the invention as well as others which will become clear are attained and can be understood in detail, more particular descriptions and certain embodiments of the invention briefly summarized above are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate certain embodiments of the invention and therefore are not to be considered limiting in their scope.

FIGs. 1A-1D. Immune responses to coagulase. (A) Drawing to illustrate the primary structure of coagulase from *S. aureus* Newman (Coa_{NM}), which was purified via an N-terminal His_6 tag from *E. coli*. Coa_{NM} encompasses the D1 and D2 domains involved in prothrombin binding, the linker (L) domain and the Repeat (R) domain, which is comprised of tandem repeats of a 27 residue peptide sequence that binds to fibrinogen. In addition to Coa_{NM}, the $D1_{Coa}$, $D2_{Coa}$, $D12_{Coa}$, L_{Coa}, and R_{Coa} domains were purified. (B) Rabbits were immunized with purified Coa_{NM} and immune sera examined by ELISA for serum IgG reactive with Coa_{NM}, $D1_{Coa}$, $D2_{Coa}$, $D12_{Coa}$, L_{Coa} or CT_{Coa}. (C) The association of $D12_{Coa}$ with human prothrombin or the binding of CT_{Coa} to fibrinogen were measured by ELISA and perturbed with increasing concentrations rabbit IgG directed against Coa_{NM} or the plague vaccine antigen V10 as a control. (D) Affinity purified rabbit IgG specific for Coa_{NM} (α-Coa_{NM}), $D12_{Coa}$ (α-$D12_{Coa}$) or CT_{Coa} (α-CT_{Coa}) were added to citrate-treated mouse blood and inoculated with *S. aureus* Newman to monitor the inhibition of staphylococcal coagulation.

FIGs. 2A-2C. Coagulase domains as vaccine antigens. (A) Recombinant purified Coa_{NM}, $D12_{Coa}$ and CT_{Coa} were used to immunize BALB/c mice (n=5) with a prime-booster regimen and immune sera were analyzed by ELISA for reactivity of mouse serum IgG towards purified Coa_{NM}, $D12_{Coa}$ or CT_{Coa}. (B) Cohorts of BALB/c mice (n=10) with a prime-booster regimen of purified Coa_{NM}, $D12_{Coa}$ and CT_{Coa} and challenged by intravenous injection with *S. aureus* Newman (1×10^8 CFU). Survival of animals was monitored over 10 days. (C) Affinity purified rabbit IgG specific for Coa_{NM} (α-Coa_{NM}), $D12_{Coa}$ (α-$D12_{Coa}$), CT_{Coa} (α-CT_{Coa}) or V10 (α-V10) was injected at a concentration of 5 mg/kg body weight into the peritoneal cavity of naïve BALB/c mice. Passively immunized mice were challenged by intravenous injection with *S. aureus* Newman (1×10^8 CFU) and survival of animals was monitored over 10 days.

FIGs. 3A-3D. Immune responses to von Willebrand Factor binding protein (vWbp). (A) Drawing to illustrate the primary structure of vWbp from *S. aureus* Newman ($vWbp_{NM}$), which was purified via an N-terminal His_6 tag from *E. coli*. $vWbp_{NM}$ encompasses the D1 and D2 domains involved in prothrombin binding, the linker (L) domain and the fibrinogen binding (Fgb) domain. In addition to $vWbp_{NM}$, the $D1_{vWbp}$, $D2_{vWbp}$, $D12_{vWbp}$, L_{vWbp}, Fgb_{vWbp} and the CT_{vWbp} domains were purified. (B) Rabbits were immunized with purified $vWbp_{NM}$ and immune sera examined by ELISA for serum IgG reactive with $vWbp_{NM}$, the $D1_{vWbp}$,

$D2_{vWbp}$, $D12_{vWbp}$, L_{vWbp}, Fgb_{vWbp} and the CT_{vWbp}. (C) The association of $D12_{vWbp}$ with human prothrombin or the binding of CT_{vWbp} to fibrinogen were measured by ELISA and perturbed with increasing concentrations rabbit IgG directed against $vWbp_{NM}$ or the plague vaccine antigen V10 as a control. (D) Affinity purified rabbit IgG specific for $vWbp_{NM}$ (α-$vWBp_{NM}$), $D12_{vWbp}$ (α-$D12_{vWbp}$) or CT_{vWbp} (α-CT_{vWbp}) were added to citrate-treated mouse blood and inoculated with *S. aureus* Newman to monitor the inhibition of staphylococcal coagulation.

FIGs. 4A-4C. von Willebrand Factor binding protein (vWbp) domains as vaccine antigens. (A) Recomb28nant purified $vWbp_{NM}$, $D12_{vWbp}$ and CT_{vWbp} were used to immunize BALB/c mice (n=5) with a prime-booster regimen and immune sera were analyzed by ELISA for reactivity of mouse serum IgG towards purified $vWbp_{NM}$, $D12_{vWbp}$ and CT_{vWbp}. (B) Cohorts of BALB/c mice (n=10) with a prime-booster regimen of purified $vWbp_{NM}$, $D12_{vWbp}$ and CT_{vWbp} and challenged by intravenous injection with *S. aureus* Newman (1×10^8 CFU). Survival of animals was monitored over 10 days. (C) Affinity purified rabbit IgG specific for $vWbpp_{NM}$ (α-$vWbp_{NM}$), $D12_{vWbp}$ (α-$D12_{vWbp}$), CT_{vWbp} (α-CT_{vWbp}) or V10 (α-V10) was injected at a concentration of 5 mg/kg body weight into the peritoneal cavity of naïve BALB/c mice. Passively immunized mice were challenged by intravenous injection with *S. aureus* Newman (1×10^8 CFU) and survival of animals was monitored over 10 days.

FIGs. 5A-5F. Immunization of mice with Coa_{NM}/$vWbp_{NM}$ vaccine and the spectrum of disease protection against different *S. aureus* isolates. (A) Recombinant Coa_{NM}/$vWbp_{NM}$ or mock (PBS) vaccine were used to immunize BALB/c mice (n=5) with a prime-booster regimen. Immune sera were analyzed by ELISA for reactivity of mouse serum IgG towards purified Coa_{NM} and $vWbp_{NM}$. Cohorts of BALB/c mice (n=10) were immunized with a prime-booster regimen of purified Coa_{NM}/$vWbp_{NM}$ or mock vaccine and challenged by intravenous injection with *S. aureus* USA300 (B), N315 (C) , MW2 (D) , CowanI (E) or WIS (F). Survival of animals was monitored over 10 days.

FIGs. 6A-6C Immunogenicity of the Coa_4/$vWbp_2$ vaccine. (A) Drawing to illustrate the design of the Coa_4 and $vWbp_2$ vaccine components. Coa_4 is comprised of an N-terminal His6 tag, the Coa D12 domains of *S. aureus* strains MRSA252, MW2, N315 and the full length mature sequence of Coa from strain USA300 in addition to a C-terminal STREP tag. $vWbp_2$ is comprised of an N-terminal His6 tag, the vWBp D12 domains of *S. aureus* N315 and the full length mature sequence of vWbp from strain USA300 in addition to a C-terminal STREP tag. (B) Coa_4 and $vWbp_2$ were purified from *E. coli* via Ni-NTA and Streptavidin affinity chromatography and analyzed by Coomassie stained SDS-PAGE.

FIGs. 7A-7F Immunization of mice with the $Coa_4/vWbp_2$ vaccine and the spectrum of disease protection against different *S. aureus* isolates. (A) $Coa_4/vWbp_2$ or mock (PBS) vaccine were used to immunize BALB/c mice (n=5) with a prime-booster regimen. Immune sera were analyzed by ELISA for reactivity of mouse serum IgG towards purified Coa_4 and $vWbp_2$. (B) Cohorts of BALB/c mice (n=10) were immunized with a prime-booster regimen of purified $Coa_4/vWbp_2$ or mock vaccine and challenged by intravenous injection with *S. aureus* USA300 (B), N315 (C), MW2 (D), CowanI (E) or WIS (F). Survival of animals was monitored over 10 days.

FIG. 8A-B: Coa sequence alignments. (A) Alignment of Coa nucleic acid sequences from five *S. aureus* strains. (B) Alignment of amino acid sequences of Coa Domains 1-2 from selected *S. aureus* strains.

FIG. 9A-C: vWbp sequence alignments. (A) Alignment of vWbp nucleic acid sequences from five *S. aureus* strains. (B) Alignment of amino acid sequences of vWbp (Domain 1 sequence is shaded) from selected *S. aureus* strains. (C) Alignment of amino acid sequences of vWbp from selected *S. aureus* strains without the two truncated alleles.

DETAILED DESCRIPTION

Staphylococcus aureus, a Gram-positive microbe that colonizes the human skin and nares, causes invasive diseases such as skin and soft tissue infections, bacteremia, sepsis and endocarditis (Lowy 1998). The emergence of antibiotic-resistant strains, designated community-acquired (CA-MRSA) or hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA), presents a formidable therapeutic challenge (Klevens 2008). Although several vaccine development efforts have been launched, an FDA-licensed *S. aureus* vaccine is not yet available (DeDent 2012).

A hallmark of *S. aureus* isolates is their ability to form clots when inoculated into human citrate-plasma or blood (Much 1908). This phenotype has been linked to the secretion of coagulase (Coa) (Cheng 2010), which binds prothrombin and alters the enzyme's active site through insertion of their N-terminal residues at exosite 1, thereby converting fibrinogen to fibrin (Friedrich 2003). The mature form of Coa is comprised of the N-terminal D1 and D2 domains, which provide for association with and activation of prothrombin (Panizzi 2004) (Fig. 1A). A linker domain (L) connects D12 and the R region with tandem repeats of a 27 residue peptide that bind fibrinogen (Panizzi 2006) (Fig. 1A). Prothrombin Coa complex (staphylocoagulase) converts soluble fibrinogen to insoluble fibrin, forming the mesh network of a clot (Friedrich 2003; Kroh 2009).

When injected into animals, purified Coa clots blood *in vivo* and this is thought to promote staphylococcal escape from phagocytic killing (Hale 1945; Smith 1956). More recently, coagulase typing, i.e. the neutralization of *S. aureus* coagulation of citrate-plasma with specific antiserum was used to distinguish ten different serological Coa types (Kanemitsu 2001). Coagulase (Coa) types were also analyzed by DNA sequencing, which revealed significant variation within *coa* sequences for the D1-2 domain and little variation for the linker and repeat regions, respectively (Watanabe 2005). To address the question whether sequence variation within *S. aureus coa* genes is the result of negative selection, as might occur when infected individuals develop antibody responses against secreted Coa, Watanabe and colleagues sequenced the *coa* genes from 126 *S. aureus* isolates, which were simultaneously analyzed for coagulase-serotype and clonal cluster (CC) type. The latter is accomplished via multi-locus sequence typing (MLST), which examines sequences from seven different genes (*arc*, *aro*, *glp*, *gmk*, *pta*, *tpi*, and *yqi*) (Enright 2000). With the exception of CC1 and CC8 strains, most of the isolates that were defined by MLST were of the same *coa* sequence-type (Watanabe 2009). Variation of *coa* sequences is likely generated via horizontal gene transfer (phage transduction or DNA transformation), as *coa* genes of the same sequence-type are found scattered across the MLST tree (Watanabe 2009). Together with the observation that pooled human immunoglobulin neutralizes most, but not all, coagulase-types (Streitfeld 1959), these results suggest that *coa* gene diversification may enable *S. aureus* to circumvent the humoral immune responses of hosts with prior exposure to the pathogen (Watanabe 2009). Thus, Coa may represent a protective antigen of *S. aureus* and should be carefully analyzed for its possible use as a vaccine antigen.

Nearly a century after the first description of staphylococcal coagulase, Bjerketorp and colleagues discovered vWbp (Bjerketorp 2002). vWbp is a secreted protein that, in addition to binding von Willebrand Factor, also associates with prothrombin to convert fibrinogen to fibrin (Friedrich 2003; Kroh 2009; Bjerketorp 2004). vWbp displays sequence homology to the Coa D12 domains (Watanabe 2005; Bjerketorp 2004), however its C-terminal domain lacks the L and R domains of Coa, which are replaced by unique vWF and fibrinogen binding sites (Cheng 2010; Bjerketorp 2002). Genome sequencing discovered two distinct *vwb* alleles with variation in the predicted D1-2 domains (Watanabe 2005). Immunization of mice with purified recombinant Coa or vWbp alone were not sufficient to elicit protective immune responses against challenge with the same coagulase-type *S. aureus* strain, however antibodies against both, Coa and vWbp, protected animals against *S. aureus* abscess formation and lethal bacteremia (Cheng 2010). Similarly, *S. aureus* Newman mutants lacking *coa* and *vwb*, but not variants with single gene deletions, displayed significant defects in mouse models of abscess formation or lethal bacteremia (Cheng 2010). Coa and vWbp secretion enables *S. aureus to* agglutinate in the presence of plasma, resulting in thrombo-embolic lesions as well as endocarditis and promoting the lethal outcome of staphylococcal bacteremia (McAdow 2011; Panizzi 2011). Blocking coagulases with univalent direct thrombin inhibitors delays the time-to-death associated with lethal *S. aureus* challenge, further highlighting the importance of coagulases for staphylococcal disease (McAdow 2011).

Early work on coagulase demonstrated that, following *S. aureus* infection, humans as well as animals generate Coa-specific antibodies (Tager 1948; Lominski 1946). When transferred to naïve rabbits, these antibodies may neutralize *S. aureus* coagulation and, at least in some cases, may confer immunity to challenge with *S. aureus* (Lominski 1949; Lominski 1962). Active immunization of rabbits with preparations containing coagulase could prolong the life of rabbits that had been challenged by intravenous inoculation with lethal doses of *S. aureus* (Boake 1956). Comparison of different (phage-typed) *S. aureus* isolates for inhibition of plasma clotting by coagulase-antiserum revealed both phage type-specific and non-specific neutralization (Lominski 1946; Lominski 1962; Rammelkamp 1950; Duthie 1952; Harrison 1964). These data supported a general concept for the existence of serological types of Coa, which are not strictly linked to *S. aureus* phage-types (Rammelkamp 1956).

Purified coagulase toxoid, encompassing purified Coa from *S. aureus* strains M1 and Newman adsorbed to aluminum phosphate, was examined for therapeutic immunization of 71 patients with chronic furunculosis (Harrison 1963). As compared to placebo, coagulase immunization generated a rise in coagulase-specific antibody titers but failed to improve the clinical outcome of chronic furunculosis (Harrison 1963). Of note, the development of neutralizing antibodies or the possibility of type-specific immunity were not examined

(Harrison 1963). Thus, although early work revealed preclinical efficacy of coagulase subunit vaccines, clinical studies failed to demonstrate efficacy in a human trial. As most of these studies were conducted from 1945-1965, one must consider the limited tools for the isolation of highly purified coagulases as well as the inability to type *S. aureus* strains or coagulase vaccine preparations on the basis of their nucleotide sequence. Further, earlier studies were conducted without knowledge of vWbp or of the molecular mechanisms of Coa- and vWbp-mediated prothrombin activation and fibrinogen cleavage (Friedrich 2003; Kroh 2009).

The inventors recently observed that both coagulases secreted by *S. aureus* Newman, Coa_{NM} and $vWbp_{NM}$, are sufficient for the ability of this strain to cause abscess formation and rapidly lethal bacteremia in mice (Cheng 2010). In active and passive immunization experiments, antibodies against both Coa_{NM} and $vWbp_{NM}$ were required to confer protection against abscess formation or lethal bacteremia (Cheng 2010). On the basis of these observations, the inventors hypothesize that coagulases may function as protective antigens that elicit antibody responses against Coa and vWbp, which protect animals and humans against *S. aureus* disease (Cheng 2010). In agreement with this model, expression of *coa* and *vwb* is a universal trait of *S. aureus* strains (Cheng 2011). Of note, the *coa* gene of *S. aureus* isolates is variable (McCarthy 2010), with greater variation in amino acid sequence than even the tandem repeats of the protein A (*spa*) gene; the variation in *spa* is used for epidemiological typing experiments (Watanabe 2009; Koreen 2004). *S. aureus* mutants that are unable to express *coa* have not yet been isolated from humans with manifest staphylococcal disease. The *vwb* gene is less variable (McCarthy 2010). Analyzing currently available *S. aureus* genome sequences for *vwb* homology, the inventors identified three alleles. Two of the *vwb* alleles varied in their coding sequence for the D12 domain (*S. aureus* N315 and USA300 are representatives for these alleles), whereas the third allele harbored a nucleotide deletion in codon 102, creating a frameshift that results in a nonsense mutation in codon 107 (*S. aureus* MRSA252).

Enabled by these observations, the inventors examined immune responses to coagulases and demonstrated that antibodies against the D1-2 domain neutralize staphylococcal coagulation in a type-specific manner. By injecting mice with a $Coa_4/vWbp_2$ vaccine that harbors antigenic determinants from the major North American isolates [CC1, CC5 (USA100), CC8 (USA300), CC30, CC45] (Klevens 2007; Patel 2011), mice could be protected against challenge with several different *S. aureus* strains.

Coa and vWbp immunization of rabbits or mice generated predominantly antibodies against the D1-2 domain of Coa_{NM} or $vWbp_{NM}$. D1-2-specific antibodies neutralized the coagulase activities of *S. aureus* Newman and, when transferred to naïve animals, conferred protection against lethal bacteremia. Neutralization and disease protection of Coa_{NM}- and $vWbp_{NM}$-specific antibodies occurred in a type-specific manner, not unlike the type-specific immunity reported for *Streptococcus pyogenes* M proteins (Lancefield 1928; Lancefield 1962) or the pilus (T) antigens of *S. pyogenes* and *Streptococcus agalactiae* (Mora 2005; Niccitelli 2011). Informed by the structural vaccinology approach for pilus antigens (Nuccitelli 2011; Schneewind 2011), the inventors engineered two polypeptides that encompasses the D1-2 domains of the major Coa and vWbp types from the North American *S. aureus* isolates: CC1, CC5, CC8, CC30 and CC45 strains (Tenover 2012). The purified products, Coa_4 and $vWbp_2$, were used as antigens and elicited antibody responses against the D12 domains of every Coa and vWbp type examined. Immunization of mice with Coa_4/$vWbp_2$ provided protection against lethal bacteremia challenge with representative *S. aureus* CC1, CC5, CC8, CC30 and CC45 strains. Thus, the design criteria of the Coa_4/$vWbp_2$ vaccine, to generate universal immune responses against Coa and vWbp against clinically relevant *S. aureus*, have been met. In addition to type-specific neutralization of Coa and vWbp via antibodies directed against the D12 domain, antibodies against the R (Coa) and CT domains (vWbp) also provided protection against *S. aureus* disease.

I. STAPHYLOCOCCAL ANTIGENS

A. Staphylococcal Coagulases

Coagulases are enzymes produced by *Staphylococcus* bacteria that convert fibrinogen to fibrin. Coa and vW_h activate prothrombin without proteolysis (Friedrich *et al.*, 2003). The coagulase·prothrombin complex recognizes fibrinogen as a specific substrate, converting it directly into fibrin. The crystal structure of the active complex revealed binding of the D1 and D2 domains to prothrombin and insertion of its Ile1-Val^2 N-terminus into the Ile^{16} pocket, inducing a functional active site in the zymogen through conformational change (Friedrich *et al.*, 2003). Exosite I of α-thrombin, the fibrinogen recognition site, and proexosite I on prothrombin are blocked by the D2 of Coa (Friedrich *et al.*, 2003). Nevertheless, association of the tetrameric $(Coa{\cdot}prothrombin)_2$ complex binds fibrinogen at a new site with high affinity (Panizzi *et al.*, 2006). This model explains the coagulant properties and efficient fibrinogen conversion by coagulase (Panizzi *et al.*, 2006).

Fibrinogen is a large glycoprotein (*Mr* ~340,000), formed by three pairs of Aα-, Bβ-, and γ-chains covalently linked to form a "dimer of trimers," where A and B designate the fibrinopeptides released by thrombin cleavage (Panizzi *et al.*, 2006). The elongated molecule folds into three separate domains, a central fragment E that contains the N-termini of all six chains and two flanking fragments D formed mainly by the C-termini of the Bβ- and γ-chains. These globular domains are connected by long triple-helical structures. Coagulase-prothrombin complexes, which convert human fibrinogen to the self-polymerizing fibrin, are not targeted by circulating thrombin inhibitors (Panizzi *et al.*, 2006). Thus, staphylococcal coagulases bypass the physiological blood coagulation pathway.

All *S. aureus* strains secrete coagulase and vWbp (Bjerketorp *et al.*, 2004; Field and Smith, 1945). Although early work reported important contributions of coagulase to the pathogenesis of staphylococcal infections (Ekstedt and Yotis, 1960; Smith et al., 1947), more recent investigations with molecular genetics tools challenged this view by observing no virulence phenotypes with endocarditis, skin abscess and mastitis models in mice (Moreillon *et al.*, 1995; Phonimdaeng *et al.*, 1990). Generating isogenic variants of *S. aureus* Newman, a fully virulent clinical isolate (Duthie *et al.*, 1952), it is described herein that *coa* mutants indeed display virulence defects in a lethal bacteremia and renal abscess model in mice. In the inventors experience, *S. aureus* 8325-4 is not fully virulent and it is presumed that mutational lesions in this strain may not be able to reveal virulence defects *in vivo*. Moreover, antibodies raised against Coa or vWbp perturb the pathogenesis of *S. aureus* Newman infections to a degree mirroring the impact of gene deletions. Coa and vWbp contribute to staphylococcal abscess formation and lethal bacteremia and may also function as protective antigens in subunit vaccines.

Biochemical studies document the biological value of antibodies against Coa and vWbp. By binding to antigen and blocking its association with clotting factors, the antibodies prevent the formation of Coa·prothrombin and vWbp·prothrombin complexes. Passive transfer studies revealed protection of experimental animals against staphylococcal abscess formation and lethal challenge by Coa and vWbp antibodies. Thus, Coa and vWbp neutralizing antibodies generate immune protection against staphylococcal disease.

Earlier studies revealed a requirement of coagulase for resisting phagocytosis in blood (Smith *et al.*, 1947) and the inventors observed a similar phenotype for Δ*coa* mutants in lepirudin-treated mouse blood (see Example 3 below). As vWbp displays higher affinity for human prothrombin than the mouse counterpart, it is suspected the same may be true for Δ*vWbp* variants in human blood. Further, expression of Coa and vWbp in abscess lesions as well as their striking distribution in the eosinophilic pseudocapsule surrounding (staphylococcal abscess communities (SACs) or the peripheral fibrin wall, suggest that secreted coagulases contribute to the establishment of these lesions. This hypothesis was tested and, indeed, Δ*coa* mutants were defective in the establishment of abscesses. A corresponding test, blocking Coa function with specific antibodies, produced the same effect. Consequently, it is proposed that the clotting of fibrin is a critical event in the establishment of staphylococcal abscesses that can be targeted for the development of protective vaccines. Due to their overlapping function on human prothrombin, both Coa and vWbp are considered excellent candidates for vaccine development.

A. Staphylcoccal Protein A (SpA)

All *Staphylococcus aureus* strains express the structural gene for Protein A (spa) (Jensen, 1958 ; Said-Salim *et al.*, 2003), a well characterized virulence factor whose cell wall anchored surface protein product (SpA) encompasses five highly homologous immunoglobulin binding domains designated E, D, A, B, and C (Sjodahl, 1977). These domains display ~ 80% identity at the amino acid level, are 56 to 61 residues in length, and are organized as tandem repeats (Uhlen *et al.*, 1984). SpA is synthesized as a precursor protein with an N-terminal YSIRK/GS signal peptide and a C-terminal LPXTG motif sorting signal (DeDent *et al.*, 2008; Schneewind *et al.*, 1992). Cell wall anchored Protein A is displayed in great abundance on the staphylococcal surface (DeDent *et al.*, 2007; Sjoquist *et al.*, 1972). Each of its immunoglobulin binding domains is composed of anti-parallel α-helices that assemble into a three helix bundle and bind the Fc domain of immunoglobulin G (IgG) (Deisenhofer, 1981; Deisenhofer *et al.*, 1978), the VH3 heavy chain (Fab) of IgM (*i.e.*, the B cell receptor) (Graille *et al.*, 2000), the von Willebrand factor at its A1 domain [vWF AI is a ligand for platelets] (O'Seaghdha *et al.*, 2006) and the tumor necrosis factor α (TNF-α) receptor I (TNFRI) (Gomez *et al.*, 2006), which is displayed on surfaces of airway epithelia (Gomez *et al.*, 2004; Gomez *et al.*, 2007).

SpA impedes neutrophil phagocytosis of staphylococci through its attribute of binding the Fc component of IgG (Jensen, 1958; Uhlen *et al.*, 1984). Moreover, SpA is able to activate intravascular clotting via its binding to von Willebrand factor AI domains (Hartleib *et al.*, 2000). Plasma proteins such as fibrinogen and fibronectin act as bridges between staphylococci (ClfA and ClfB) and the platelet integrin GPIIb/IIIa (O'Brien *et al.*, 2002), an activity that is supplemented through Protein A association with vWF AI, which allows staphylococci to capture platelets via the GPIb-α platelet receptor (Foster, 2005; O'Seaghdha *et al.*, 2006). SpA also binds TNFRI and this interaction contributes to the pathogenesis of staphylococcal pneumonia (Gomez *et al.*, 2004). SpA activates proinflammatory signaling through TNFR1 mediated activation of TRAF2, the p38/c-Jun kinase, mitogen activate protein kinase (MAPK) and the Rel-transcription factor NF-KB. SpA binding further induces TNFR1 shedding, an activity that appears to require the TNF-converting enzyme (TACE)(Gomez *et al.*, 2007). All of the aforementioned SpA activities are mediated through its five IgG binding domains and can be perturbed by the same amino acid substitutions, initially defined by their requirement for the interaction between Protein A and human IgG1 (Cedergren *et al.*, 1993.

SpA also functions as a B cell superantigen by capturing the Fab region of VH3 bearing IgM, the B cell receptor (Gomez *et al.*, 2007; Goodyear *et al.*, 2003; Goodyear and Silverman, 2004; Roben *et al.*, 1995). Following intravenous challenge, staphylococcal Protein A (SpA) mutations show a reduction in staphylococcal load in organ tissues and dramatically diminished ability to form abscesses (described herein). During infection with wildtype *S. aureus*, abscesses are formed within forty-eight hours and are detectable by light microscopy of hematoxylin-eosin stained, thin-sectioned kidney tissue, initially marked by an influx of polymorphonuclear leukocytes (PMNs). On day 5 of infection, abscesses increase in size and enclosed a central population of staphylococci, surrounded by a layer of eosinophilic, amorphous material and a large cuff of PMNs. Histopathology revealed massive necrosis of PMNs in proximity to the staphylococcal nidus at the center of abscess lesions as well as a mantle of healthy phagocytes. The inventors also observed a rim of necrotic PMNs at the periphery of abscess lesions, bordering the eosinophilic pseudocapsule that separated healthy renal tissue from the infectious lesion. Staphylococcal variants lacking Protein A are unable to establish the histopathology features of abscesses and are cleared during infection.

In previous studies, Cedergren *et al.* (1993) engineered five individual substitutions in the Fc frgament binding sub-domain of the B domain of SpA, L17D, N28A, I31A and K35A. These authors created these proteins to test data gathered from a three dimensional structure of a complex between one domain of SpA and Fc_1. Cedergren *et al.* determined the effects of these mutations on stability and binding, but did not contemplate use of such substitutions for the production of a vaccine antigen.

Brown *et al.* (1998) describe studies designed to engineer new proteins based on SpA that allow the use of more favorable elution conditions when used as affinity ligands. The mutations studied included single mutations of Q13A, Q14H, N15A, N15H, F17H, Y18F, L21H, N32H, or K39H. Brown *et al.* report that Q13A, N15A, N15H, and N32H substitutions made little difference to the dissociation constant values and that the Y18F substitution resulted in a 2 fold decrease in binding affinity as compared to wild type SpA. Brown *et al.* also report that L21H and F17H substitutions decrease the binding affinity by five-fold and a hundred-fold respectively. The authors also studied analogous substitutions in two tandem domains. Thus, the Brown *et al.* studies were directed to generating a SpA with a more favorable elution profile, hence the use of His substitutions to provide a pH sensitive alteration in the binding affinity. Brown *et al.* ia silent on the use of SpA as a vaccine antigen.

Graille *et al.* (2000) describe a crystal structure of domain D of SpA and the Fab fragment of a human IgM antibody. Graille *et al.* define by analysis of a crystal structure the D domain amino acid residues that interact with the Fab fragment as residues Q26, G29, F30, Q32, S33, D36, D37, Q40, N43, E47, or L51, as well as the amino acid residues that form the interface between the domain D sub-domains. Graille *et al.* define the molecular interactions of these two proteins, but is silent in regard to any use of substitutions in the interacting residues in producing a vaccine antigen.

O'Seaghdha *et al.* (2006) describe studies directed at elucidating which sub-domain of domain D binds vWF. The authors generated single mutations in either the Fc or VH3 binding sub-domains, i.e., amino acid residues F5A, Q9A, Q10A, F13A, Y14A, L17A, N28A, I31A, K35A, G29A, F30A, S33A, D36A, D37A, Q40A, E47A, or Q32A.. The authors discovered that vWF binds the same sub-domain that binds Fc. O'Seaghda *et al.* define the sub-domain of domain D responsible for binding vWF, but is silent in regard to any use of substitutions in the interacting residues in producing a vaccine antigen.

Gomez *et al.* (2006) describe the identifcation of residues responsible for activation of the TNFR1 by using single mutations of F5A, F13A, Y14A, L17A, N21A, I31A, Q32A, and

K35A. Gomez *et al.* is silent in regard to any use of substitutions in the interacting residues in producing a vaccine antigen.

Recombinant affinity tagged Protein A, a polypeptide encompassing the five IgG domains (EDCAB) (Sjodahl, 1977) but lacking the C-terminal Region X (Guss *et al.*, 1984), was purified from recombinant *E. coli* and used as a vaccine antigen (Stranger-Jones *et al.*, 2006). Because of the attributes of SpA in binding the Fc portion of IgG, a specific humoral immune response to Protein A could not be measured (Stranger-Jones *et al.*, 2006). The inventors have overcome this obstacle through the generation of SpA-DQ9,10K;D36,37A. BALB/c mice immunized with recombinant Protein A (SpA) displayed significant protection against intravenous challenge with *S. aureus* strains: a 2.951 log reduction in staphylococcal load as compared to the wild-type ($P > 0.005$; Student's t-test) (Stranger-Jones *et al.*, 2006). SpA specific antibodies may cause phagocytic clearance prior to abscess formation and/or impact the formation of the aforementioned eosinophilic barrier in abscesses that separate staphylococcal communities from immune cells since these do not form during infection with Protein A mutant strains. Each of the five SpA domains (*i.e.*, domains formed from three helix bundles designated E, D, A, B, and C) exerts similar binding properties (Jansson *et al.*, 1998). The solution and crystal structure of the domain D has been solved both with and without the Fc and VH3 (Fab) ligands, which bind Protein A in a non-competitive manner at distinct sites (Graille *et al.*, 2000). Mutations in residues known to be involved in IgG binding (FS, Q9, Q10, S11, F13, Y14, L17, N28, I31 and K35) are also required for vWF AI and TNFR1 binding (Cedergren *et al.*, 1993; Gomez *et al.*, 2006; O'Seaghdha *et al.*, 2006), whereas residues important for the VH3 interaction (Q26, G29, F30, S33, D36, D37, Q40, N43, E47) appear to have no impact on the other binding activities (Graille *et al.*, 2000; Jansson *et al.*, 1998). SpA specifically targets a subset of B cells that express VH3 family related IgM on their surface, *i.e.*, VH3 type B cell receptors (Roben *et al.*, 1995). Upon interaction with SpA, these B cells proliferate and commit to apoptosis, leading to preferential and prolonged deletion of innate-like B lymphocytes (*i.e.*, marginal zone B cells and follicular B2 cells)(Goodyear *et al.*, 2003; Goodyear *et al.*, 2004).

Molecular basis of Protein A surface display and function. Protein A is synthesized as a precursor in the bacterial cytoplasm and secreted via its YSIRK signal peptide at the cross wall, *i.e.* the cell division septum of staphylococci (DeDent *et al.*, 2007; DeDent *et al.*, 2008). Following cleavage of the C-terminal LPXTG sorting signal, Protein A is anchored to bacterial peptidoglycan crossbridges by sortase A (Mazmanian *et al.*, 1999; Schneewind *et al.*, 1995; Mazmanian *et al.*, 2000). Protein A is the most abundant surface protein of staphylococci; the molecule is expressed by virtually all *S. aureus* strains (Cespedes *et al.*, 2005; Kennedy *et al.*, 2008; Said-Salim *et al.*, 2003). Staphylococci turn over 15-20% of their cell wall per division cycle (Navarre and Schneewind, 1999). Murine hydrolases cleave the glycan strands and wall peptides of peptidoglycan, thereby releasing Protein A with its attached C-terminal cell wall disaccharide tetrapeptide into the extracellular medium (Ton-That *et al.*, 1999). Thus, by physiological design, Protein A is both anchored to the cell wall and displayed on the bacterial surface but also released into surrounding tissues during host infection (Marraffini *et al.*, 2006).

Protein A captures immunoglobulins on the bacterial surface and this biochemical activity enables staphylococcal escape from host innate and acquired immune responses (Jensen, 1958; Goodyear *et al.*, 2004). Interestingly, region X of Protein A (Guss *et al.*, 1984), a repeat domain that tethers the IgG binding domains to the LPXTG sorting signal / cell wall anchor, is perhaps the most variable portion of the staphylococcal genome (Said-Salim, 2003; Schneewind *et al.*, 1992). Each of the five immunoglobulin binding domains of Protein A (SpA), formed from three helix bundles and designated E, D, A, B, and C, exerts similar structural and functional properties (Sjodahl, 1977; Jansson *et al.*, 1998). The solution and crystal structure of the domain D has been solved both with and without the Fc and V_H3 (Fab) ligands, which bind Protein A in a non-competitive manner at distinct sites (Graille 2000).

In the crystal structure complex, the Fab interacts with helix II and helix III of domain D via a surface composed of four VH region β-strands (Graille 2000). The major axis of helix II of domain D is approximately 50° to the orientation of the strands, and the interhelical portion of domain D is most proximal to the C0 strand. The site of interaction on Fab is remote from the Ig light chain and the heavy chain constant region. The interaction involves the following domain D residues: Asp-36 of helix II, Asp-37 and Gln-40 in the loop between helix II and helix III and several other residues (Graille 2000). Both interacting surfaces are composed predominantly of polar side chains, with three negatively charged residues on domain D and two positively charged residues on the 2A2 Fab buried by the interaction, providing an overall electrostatic attraction between the two molecules. Of the five polar interactions identified between Fab and domain D, three are between side chains. A salt bridge is formed between Arg-H19 and Asp-36 and two hydrogen bonds are made between Tyr-H59 and Asp-

37 and between Asn-H82a and Ser-33. Because of the conservation of Asp-36 and Asp-37 in all five IgG binding domains of Protein A, the inventors mutated these residues.

The SpA-D sites responsible for Fab binding are structurally separate from the domain surface that mediates Fcγ binding. The interaction of Fcγ with domain D primarily involves residues in helix I with lesser involvement of helix II (Gouda *et al.*, 1992; Deisenhofer, 1981). With the exception of the Gln-32, a minor contact in both complexes, none of the residues that mediate the Fcγ interaction are involved in Fab binding. To examine the spatial relationship between these different Ig-binding sites, the SpA domains in these complexes have been superimposed to construct a model of a complex between Fab, the SpA-domain D, and the Fcγ molecule. In this ternary model, Fab and Fcγ form a sandwich about opposite faces of the helix II without evidence of steric hindrance of either interaction. These findings illustrate how, despite its small size (*i.e.*, 56–61 aa), an SpA domain can simultaneously display both activities, explaining experimental evidence that the interactions of Fab with an individual domain are noncompetitive. Residues for the interaction between SpA-D and Fcγ are Gln-9 and Gln-10.

In contrast, occupancy of the Fc portion of IgG on the domain D blocks its interaction with vWF A1 and probably also TNFR1 (O'Seaghdha *et al.*, 2006). Mutations in residues essential for IgG Fc binding (F5, Q9, Q10, S11, F13, Y14, L17, N28, I31 and K35) are also required for vWF A1 and TNFR1 binding (O'Seaghdha *et al.*, 2006; Cedergren *et al.*, 1993; Gomez *et al.*, 2006), whereas residues critical for the VH3 interaction (Q26, G29, F30, S33, D36, D37, Q40, N43, E47) have no impact on the binding activities of IgG Fc, vWF A1 or TNFR1 (Jansson *et al.*, 1998; Graille *et al.*, 2000). The Protein A immunoglobulin Fab binding activity targets a subset of B cells that express V_H3 family related IgM on their surface, *i.e.*, these molecules function as VH3type B cell receptors (Roben *et al.*, 1995). Upon interaction with SpA, these B cells rapidly proliferate and then commit to apoptosis, leading to preferential and prolonged deletion of innate-like B lymphocytes (*i.e.*, marginal zone B cells and follicular B2 cells) (Goodyear and Silverman, 2004; Goodyear and Silverman, 2003). More than 40% of circulating B cells are targeted by the Protein A interaction and the V_H3 family represents the largest family of human B cell receptors to impart protective humoral responses against pathogens (Goodyear and Silverman, 2004; Goodyear and Silverman, 2003). Thus, Protein A functions analogously to staphylococcal superantigens (Roben *et al.*, 1995), albeit that the latter class of molecules, for example SEB, TSST-1, TSST-2, form complexes with the T cell receptor to inappropriately stimulate host immune responses and thereby precipitating characteristic disease features of staphylococcal infections (Roben et al., 1995; Tiedemann et al., 1995). Together these findings document the contributions of Protein A in establishing staphylococcal infections and in modulating host immune responses.

C. Other Staphylococcal Antigens

Research over the past several decades identified *S. aureus* exotoxins, surface proteins and regulatory molecules as important virulence factors (Foster, 2005; Mazmanian *et al.*, 2001; Novick, 2003). Much progress has been achieved regarding the regulation of these genes. For example, staphylococci perform a bacterial census via the secretion of auto-inducing peptides that bind to a cognate receptor at threshold concentration, thereby activating phospho-relay reactions and transcriptional activation of many of the exotoxin genes (Novick, 2003). The pathogenesis of staphylococcal infections relies on these virulence factors (secreted exotoxins, exopolysaccharides, and surface adhesins). The development of staphylococcal vaccines is hindered by the multifaceted nature of staphylococcal invasion mechanisms. It is well established that live attenuated micro-organisms are highly effective vaccines; immune responses elicited by such vaccines are often of greater magnitude and of longer duration than those produced by non-replicating immunogens. One explanation for this may be that live attenuated strains establish limited infections in the host and mimic the early stages of natural infection. Embodiments of the invention are directed to compositions and methods including variant coagulase polypeptides and peptides, in particular, one or more coagulase Domains 1-2, as well as other immunogenic extracellular proteins, polypeptides, and peptides (including both secreted and cell surface proteins or peptides) of gram positive bacteria for the use in mitigating or immunizing against infection. In particular embodiments the bacteria is a staphylococcus bacteria. Extracellular proteins, polypeptides, or peptides include, but are not limited to secreted and cell surface proteins of the targeted bacteria.

The human pathogen *S. aureus* secretes EsxA and EsxB, two ESAT-6 like proteins, across the bacterial envelope (Burts *et al.*, 2005). Staphylococcal *esxA* and *esxB* are clustered with six other genes in the order of transcription: *esxA esaA essA esaB essB essC esaC esxB*. The acronyms esa, ess, and esx stand for ESAT-6 secretion accessory, system, and extracellular, respectively, depending whether the encoded

Date Reçue/Date Received 2021-01-25

proteins play an accessory (esa) or direct (ess) role for secretion, or are secreted (esx) in the extracellular milieu. The entire cluster of eight genes is herein referred to as the Ess cluster. EsxA, esxB, essA, essB, and essC are all required for synthesis or secretion of EsxA and EsxB. Mutants that fail to produce EsxA, EsxB, and EssC display defects in the pathogenesis of *S. aureus* murine abscesses, suggesting that this specialized secretion system may be a general strategy of human bacterial pathogenesis. Secretion of non-WXG100 substrates by the ESX-1 pathway has been reported for several antigens including EspA, EspB, Rv3483c, and Rv3615c (Fortune *et al.*, 2005; MacGurn *et al.*, 2005; McLaughlin *et al.*, 2007; Xu *et al.*, 2007). The alternate ESX-5 pathway has also been shown to secrete both WXG100 and non-WXG100 proteins in pathogenic mycobacteria (Abdallah *et al.*, 2007; Abdallah *et al.*, 2006).

The *Staphylococcus aureus* Ess pathway can be viewed as a secretion module equipped with specialized transport components (Ess), accessory factors (Esa) and cognate secretion substrates (Esx). EssA, EssB and EssC are required for EsxA and EsxB secretion. Because EssA, EssB and EssC are predicted to be transmembrane proteins, it is contemplated that these proteins form a secretion apparatus. Some of the proteins in the *ess* gene cluster may actively transport secreted substrates (acting as motor) while others may regulate transport (regulator). Regulation may be achieved, but need not be limited to, transcriptional or post-translational mechanisms for secreted polypeptides, sorting of specific substrates to defined locations (*e.g.,* extracellular medium or host cells), or timing of secretion events during infection. At this point, it is unclear whether all secreted Esx proteins function as toxins or contribute indirectly to pathogenesis.

Staphylococci rely on surface protein mediated-adhesion to host cells or invasion of tissues as a strategy for escape from immune defenses. Furthermore, *S. aureus* utilize surface proteins to sequester iron from the host during infection. The majority of surface proteins involved in staphylococcal pathogenesis carry C-terminal sorting signals, *i.e.*, they are covalently linked to the cell wall envelope by sortase. Further, staphylococcal strains lacking the genes required for surface protein anchoring, *i.e.,* sortase A and B, display a dramatic defect in the virulence in several different mouse models of disease. Thus, surface protein antigens represent a validated vaccine target as the corresponding genes are essential for the development of staphylococcal disease and can be exploited in various embodiments of the invention. The sortase enzyme superfamily are Gram-positive transpeptidases responsible for anchoring surface protein virulence factors to the peptidoglycan cell wall layer. Two sortase isoforms have been identified in *Staphylococcus aureus*, SrtA and SrtB. These enzymes have been shown to recognize a LPXTG motif in substrate proteins. The SrtB isoform appears to be important in heme iron acquisition and iron homeostasis, whereas the SrtA isoform plays a critical role in the pathogenesis of Gram-positive bacteria by modulating the ability of the bacterium to adhere to host tissue via the covalent anchoring of adhesins and other proteins to the cell wall peptidoglycan. In certain embodiments the coagulase variants, in particular, one or more coagulase Domains 1-2 described herein can be used in combination with other staphylococcal proteins such as Coa, Eap, Ebh, Emp, EsaC, EsaB, EsxA, EsxB, Hla, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, IsdC, SasF, vWbp, and/or vWh proteins.

Certain aspects of the invention include methods and compositions concerning proteinaceous compositions including polypeptides, peptides, or nucleic acid encoding coagulase variants, in particular, one or more coagulase Domains 1-2 described herein and other staphylococcal antigens such as other proteins transported by the Ess pathway, or sortase substrates. These proteins may be modified by deletion, insertion, and/or substitution.

The Esx polypeptides include the amino acid sequence of Esx proteins from bacteria in the Staphylococcus genus. The Esx sequence may be from a particular staphylococcus species, such as *Staphylococcus aureus*, and may be from a particular strain, such as Newman. In certain embodiments, the EsxA sequence is SAV0282 from strain Mu50 (which is the same amino acid sequence for Newman) and can be accessed using Genbank Accession Number Q99WU4 (gi|68565539). In other embodiments, the EsxB sequence is SAV0290 from strain Mu50 (which is the same amino acid sequence for Newman) and can be accessed using Genbank Accession Number Q99WT7 (gi|68565532). In further embodiments, other polypeptides transported by the Ess pathway may be used, the sequences of which may be identified by one of skill in the art using databases and internet accessible resources.

The sortase substrate polypeptides include, but are not limited to the amino acid sequence of SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, IsdC or SasF proteins from bacteria in the Staphylococcus genus. The sortase substrate polypeptide sequence may be from a particular staphylococcus species, such as *Staphylococcus aureus*, and may be from a particular strain, such as Newman. In certain embodiments, the SdrD sequence is from strain N315 and can be accessed using Genbank Accession Number NP_373773.1 (gi|15926240). In other embodiments, the SdrE sequence is from strain N315 and can be accessed using Genbank

Date Reçue/Date Received 2021-01-25

Accession Number NP_373774.1 (gi|15926241). In other embodiments, the IsdA sequence is SAV1130 from strain Mu50 (which is the same amino acid sequence for Newman) and can be accessed using Genbank Accession Number NP_371654.1 (gi|15924120). In other embodiments, the IsdB sequence is SAV1129 from strain Mu50 (which is the same amino acid sequence for Newman) and can be accessed using Genbank Accession Number NP_371653.1 (gi|15924119). In further embodiments, other polypeptides transported by the Ess pathway or processed by sortase may be used, the sequences of which may be identified by one of skill in the art using databases and internet accessible resources.

Examples of various proteins that can be used in the context of the present invention can be identified by analysis of database submissions of bacterial genomes, including but not limited to accession numbers NC_002951 (GI:57650036 and GenBank CP000046), NC_002758 (GI:57634611 and GenBank BA000017), NC_002745 (GI:29165615 and GenBank BA000018), NC_003923 (GI:21281729 and GenBank BA000033), NC_002952 (GI:49482253 and GenBank BX571856), NC_002953 (GI:49484912 and GenBank BX571857), NC_007793 (GI:87125858 and GenBank CP000255), NC_007795 (GI:87201381 and GenBank CP000253).

As used herein, a "protein" or "polypeptide" refers to a molecule comprising at least ten amino acid residues. In some embodiments, a wild-type version of a protein or polypeptide are employed, however, in many embodiments of the invention, a modified protein or polypeptide is employed to generate an immune response. The terms described above may be used interchangeably. A "modified protein" or "modified polypeptide" or a "variant" refers to a protein or polypeptide whose chemical structure, particularly its amino acid sequence, is altered with respect to the wild-type protein or polypeptide. In some embodiments, a modified/variant protein or polypeptide has at least one modified activity or function (recognizing that proteins or polypeptides may have multiple activities or functions). It is specifically contemplated that a modified/variant protein or polypeptide may be altered with respect to one activity or function yet retain a wild-type activity or function in other respects, such as immunogenicity.

In certain embodiments the size of a protein or polypeptide (wild-type or modified) may comprise, but is not limited to, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22,

Date Reçue/Date Received 2021-01-25

23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825, 850, 875, 900, 925, 950, 975, 1000, 1100, 1200, 1300, 1400, 1500, 1750, 2000, 2250, 2500 amino molecules or greater, and any range derivable therein, or derivative of a corresponding amino sequence described or referenced herein. It is contemplated that polypeptides may be mutated by truncation, rendering them shorter than their corresponding wild-type form, but also they might be altered by fusing or conjugating a heterologous protein sequence with a particular function (*e.g.*, for targeting or localization, for enhanced immunogenicity, for purification purposes, *etc.*).

As used herein, an "amino molecule" refers to any amino acid, amino acid derivative, or amino acid mimic known in the art. In certain embodiments, the residues of the proteinaceous molecule are sequential, without any non-amino molecule interrupting the sequence of amino molecule residues. In other embodiments, the sequence may comprise one or more non-amino molecule moieties. In particular embodiments, the sequence of residues of the proteinaceous molecule may be interrupted by one or more non-amino molecule moieties.

Accordingly, the term "proteinaceous composition" encompasses amino molecule sequences comprising at least one of the 20 common amino acids in naturally synthesized proteins, or at least one modified or unusual amino acid.

Proteinaceous compositions may be made by any technique known to those of skill in the art, including (i) the expression of proteins, polypeptides, or peptides through standard molecular biological techniques, (ii) the isolation of proteinaceous compounds from natural sources, or (iii) the chemical synthesis of proteinaceous materials. The nucleotide as well as the protein, polypeptide, and peptide sequences for various genes have been previously disclosed, and may be found in the recognized computerized databases. One such database is the National Center for Biotechnology Information's Genbank and GenPept databases (on the World Wide Web at ncbi.nlm.nih.gov/). The coding regions for these genes may be amplified and/or expressed using the techniques disclosed herein or as would be known to those of ordinary skill in the art.

Amino acid sequence variants of coagulases, in particular, of coagulase Domains 1-2, SpA and other polypeptides of the invention can be substitutional, insertional, or deletion variants. A variation in a polypeptide of the invention may affect 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more non-contiguous or contiguous amino acids of the polypeptide, as compared to wild-type. A variant can comprise an amino acid sequence that is at least 50%, 60%, 70%, 80%, or 90%, including all values and ranges there between, identical to any sequence provided or referenced herein, e.g., a sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37) or SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41). A variant can include 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more substitute amino acids. A polypeptide processed or secreted by the Ess pathway or other surface proteins (see Table 1) or sortase substrates from any staphylococcus species and strain are contemplated for use in compositions and methods described herein.

Deletion variants typically lack one or more residues of the native or wild-type protein. Individual residues can be deleted or a number of contiguous amino acids can be deleted. A stop codon may be introduced (by substitution or insertion) into an encoding nucleic acid sequence to generate a truncated protein. Insertional mutants typically involve the addition of material at a non-terminal point in the polypeptide. This may include the insertion of one or more residues. Terminal additions, called fusion proteins, may also be generated. These fusion proteins include multimers or concatamers of one or more peptide or polypeptide described or referenced herein.

Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide, with or without the loss of other functions or properties. Substitutions may be conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine.

Alternatively, substitutions may be non-conservative such that a function or activity of the polypeptide is affected. Non-conservative changes typically involve substituting a residue with one that is chemically dissimilar, such as a polar or charged amino acid for a nonpolar or uncharged amino acid, and vice versa.

Table 2. Exemplary surface proteins of *S. aureus* strains.

SAV #	SA#	Surface	MW2	Mu50	N315	Newman	MRSA252*	MSSA476*
SAV0111	SA0107	Spa	492	450	450	520	516	492
SAV2503	SA2291	FnBPA	1015	1038	1038	741	-	1015
SAV2502	SA2290	FnBPB	943	961	961	677	965	957
SAV0811	SA0742	ClfA	946	935	989	933	1029	928
SAV2630	SA2423	ClfB	907	877	877	913	873	905
Np	Np	Cna	1183	-	-	-	1183	1183
SAV0561	SA0519	SdrC	955	953	953	947	906	957
SAV0562	SA0520	SdrD	1347	1385	1385	1315	-	1365
SAV0563	SA0521	SdrE	1141	1141	1141	1166	1137	1141
Np	Np	Pls	-	-	-	-	-	-
SAV2654	SA2447	SasA	2275	2271	2271	2271	1351	2275
SAV2160	SA1964	SasB	686	2481	2481	2481	2222	685
	SA1577	SasC	2186	213	2186	2186	2189	2186
SAV0134	SA0129	SasD	241	241	241	241	221	241
SAV1130	SA0977	SasE/IsdA	350	350	350	350	354	350
SAV2646	SA2439	SasF	635	635	635	635	627	635
SAV2496		SasG	1371	525	927	-	-	1371
SAV0023	SA0022	SasH	772	-	772	772	786	786
SAV1731	SA1552	SasI	895	891	891	891	534	895
SAV1129	SA0976	SasJ/IsdB	645	645	645	645	652	645
	SA2381	SasK	198	211	211	-	-	197
	Np	SasL	-	232	-	-	-	-
SAV1131	SA0978	IsdC	227	227	227	227	227	227

Proteins of the invention may be recombinant, or synthesized *in vitro*. Alternatively, a non-recombinant or recombinant protein may be isolated from bacteria. It is also contemplated that a bacteria containing such a variant may be implemented in compositions and methods of the invention. Consequently, a protein need not be isolated.

The term "functionally equivalent codon" is used herein to refer to codons that encode the same amino acid, such as the six codons for arginine or serine, and also refers to codons that encode biologically equivalent amino acids (see Table 3, below).

Table 3 Codon Table

Amino Acids			Codons
Alanine	Ala	A	GCA GCC GCG GCU
Cysteine	Cys	C	UGC UGU
Aspartic acid	Asp	D	GAC GAU
Glutamic acid	Glu	E	GAA GAG
Phenylalanine	Phe	F	UUC UUU
Glycine	Gly	G	GGA GGC GGG GGU
Histidine	His	H	CAC CAU
Isoleucine	Ile	I	AUA AUC AUU
Lysine	Lys	K	AAA AAG
Leucine	Leu	L	UUA UUG CUA CUC CUG CUU
Methionine	Met	M	AUG
Asparagine	Asn	N	AAC AAU
Proline	Pro	P	CCA CCC CCG CCU
Glutamine	Gln	Q	CAA CAG
Arginine	Arg	R	AGA AGG CGA CGC CGG CGU
Serine	Ser	S	AGC AGU UCA UCC UCG UCU
Threonine	Thr	T	ACA ACC ACG ACU
Valine	Val	V	GUA GUC GUG GUU
Tryptophan	Trp	W	UGG
Tyrosine	Tyr	Y	UAC UAU

It also will be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids, or 5' or 3' sequences, respectively, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein activity (*e.g.*, immunogenicity) where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region.

The following is a discussion based upon changing of the amino acids of a protein to create a variant polypeptide or peptide. For example, certain amino acids may be substituted for other amino acids in a protein structure with or without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's functional activity, certain amino acid substitutions can be made in a protein sequence, and in its underlying DNA coding sequence, and nevertheless produce a protein with a desirable property. It is thus contemplated by the inventors that various changes may be made in the DNA sequences of genes.

It is contemplated that in compositions of the invention, there is between about 0.001 mg and about 10 mg of total polypeptide, peptide, and/or protein per ml. The concentration of protein in a composition can be about, at least about or at most about 0.001, 0.010, 0.050, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 mg/ml or more (or any range derivable therein). Of this, about, at least about, or at most about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% may be a coagulase Domains 1-2 or a coagulase or its variant and may be used in combination with other peptides or polypeptides, such as other bacterial peptides and/or antigens.

The present invention contemplates the administration of staphylococcal coagulase Domains 1-2 or variants thereof to effect a preventative therapy or therapeutic effect against the development of a disease or condition associated with infection by a staphylococcus pathogen.

In certain aspects, combinations of staphylococcal antigens are used in the production of an immunogenic composition that is effective at treating or preventing staphylococcal infection. Staphylococcal infections progress through several different stages. For example, the staphylococcal life cycle involves commensal colonization, initiation of infection by accessing adjoining tissues or the bloodstream, and/or anaerobic multiplication in the blood. The interplay between *S. aureus* virulence determinants and the host defense mechanisms can induce complications such as endocarditis, metastatic abscess formation, and sepsis syndrome. Different molecules on the surface of the bacterium are involved in different steps of the infection cycle. Combinations of certain antigens can elicit an immune response which protects against multiple stages of staphylococcal infection. The effectiveness of the immune response can be measured either in animal model assays and/or using an opsonophagocytic assay.

B. Polypeptides and Polypeptide Production

The present invention describes polypeptides, peptides, and proteins and immunogenic fragments thereof for use in various embodiments of the present invention. For example, specific polypeptides are assayed for or used to elicit an immune response. In specific embodiments, all or part of the proteins of the invention can also be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, (1984); Tam *et al.*, (1983); Merrifield, (1986); and Barany and Merrifield (1979).

Alternatively, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a peptide of the invention is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression.

One embodiment of the invention includes the use of gene transfer to cells, including microorganisms, for the production and/or presentation of polypeptides or peptides. The gene for the polypeptide or peptide of interest may be transferred into appropriate host cells followed by culture of cells under the appropriate conditions. The generation of recombinant expression vectors, and the elements included therein, are well known in the art and briefly discussed herein. Alternatively, the protein to be produced may be an endogenous protein normally synthesized by the cell that is isolated and purified.

Another embodiment of the present invention uses autologous B lymphocyte cell lines, which are transfected with a viral vector that expresses an immunogen product, and more specifically, a protein having immunogenic activity. Other examples of mammalian host cell lines include, but are not limited to Vero and HeLa cells, other B- and T- cell lines, such as CEM, 721.221, H9, Jurkat, Raji, as well as cell lines of Chinese hamster ovary, W138, BHK, COS-7, 293, HepG2, 3T3, RIN and MDCK cells. In addition, a host cell strain may be chosen that modulates the expression of the inserted sequences, or that modifies and processes the gene product in the manner desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed.

A number of selection systems may be used including, but not limited to HSV thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase, and adenine

Date Reçue/Date Received 2021-01-25

phosphoribosyltransferase genes, in tk-, hgprt- or aprt- cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection: for dhfr, which confers resistance to trimethoprim and methotrexate; gpt, which confers resistance to mycophenolic acid; neo, which confers resistance to the aminoglycoside G418; and hygro, which confers resistance to hygromycin.

Animal cells can be propagated *in vitro* in two modes: as non-anchorage-dependent cells growing in suspension throughout the bulk of the culture or as anchorage-dependent cells requiring attachment to a solid substrate for their propagation (*i.e.*, a monolayer type of cell growth).

Non-anchorage dependent or suspension cultures from continuous established cell lines are the most widely used means of large scale production of cells and cell products. However, suspension cultured cells have limitations, such as tumorigenic potential and lower protein production than adherent cells.

Where a protein is specifically mentioned herein, it is preferably a reference to a native or recombinant protein or optionally a protein in which any signal sequence has been removed. The protein may be isolated directly from the staphylococcal strain or produced by recombinant DNA techniques. Immunogenic fragments of the protein may be incorporated into the immunogenic composition of the invention. These are fragments comprising at least 10 amino acids, 20 amino acids, 30 amino acids, 40 amino acids, 50 amino acids, or 100 amino acids, including all values and ranges there between, taken contiguously from the amino acid sequence of the protein. In addition, such immunogenic fragments are immunologically reactive with antibodies generated against the Staphylococcal proteins or with antibodies generated by infection of a mammalian host with Staphylococci. Immunogenic fragments also include fragments that when administered at an effective dose, (either alone or as a hapten bound to a carrier), elicit a protective or therapeutic immune response against Staphylococcal infection, in certain aspects it is protective against *S. aureus* and/or *S. epidermidis* infection. Such an immunogenic fragment may include, for example, the protein lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment according to the invention comprises substantially all of the extracellular domain of a protein which has at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, or at least 97-99% identity, including all values and ranges there between, to a sequence selected segment of a polypeptide described or referenced herein.

Also included in immunogenic compositions of the invention are fusion proteins composed of one or more Staphylococcal proteins, or immunogenic fragments of staphylococcal proteins. Such fusion proteins may be made recombinantly and may comprise one portion of at least 1, 2, 3, 4, 5, or 6 staphylococcal proteins or segments. Alternatively, a fusion protein may comprise multiple portions of at least 1, 2, 3, 4 or 5 staphylococcal proteins. These may combine different Staphylococcal proteins and/or multiples of the same protein or proten fragment, or immunogenic fragments in the same protein (forming a multimer or a concatamer). Alternatively, the invention also includes individual fusion proteins of Staphylococcal proteins or immunogenic fragments thereof, as a fusion protein with heterologous sequences such as a provider of T-cell epitopes or purification tags, for example: β-galactosidase, glutathione-S-transferase, green fluorescent proteins (GFP), epitope tags such as FLAG, myc tag, poly histidine, or viral surface proteins such as influenza virus haemagglutinin, or bacterial proteins such as tetanus toxoid, diphtheria toxoid, or CRM197.

II. NUCLEIC ACIDS

In certain embodiments, the present invention concerns recombinant polynucleotides encoding the proteins, polypeptides, peptides of the invention. The nucleic acid sequences for coagulases, coagulases Domains 1-2, SpA, and other bacterial proteins are included, and can be used to prepare peptides or polypeptides.

As used in this application, the term "polynucleotide" refers to a nucleic acid molecule that either is recombinant or has been isolated free of total genomic nucleic acid. Included within the term "polynucleotide" are oligonucleotides (nucleic acids of 100 residues or less in length), recombinant vectors, including, for example, plasmids, cosmids, phage, viruses, and the like. Polynucleotides include, in certain aspects, regulatory sequences, isolated substantially away from their naturally occurring genes or protein encoding sequences. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be RNA, DNA (genomic, cDNA or synthetic), analogs thereof, or a combination thereof. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide.

Date Reçue/Date Received 2021-01-25

In this respect, the term "gene," "polynucleotide," or "nucleic acid" is used to refer to a nucleic acid that encodes a protein, polypeptide, or peptide (including any sequences required for proper transcription, post-translational modification, or localization). As will be understood by those in the art, this term encompasses genomic sequences, expression cassettes, cDNA sequences, and smaller engineered nucleic acid segments that express, or may be adapted to express, proteins, polypeptides, domains, peptides, fusion proteins, and mutants. A nucleic acid encoding all or part of a polypeptide may contain a contiguous nucleic acid sequence of: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1095, 1100, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 9000, 10000, or more nucleotides, nucleosides, or base pairs, including all values and ranges therebetween, of a polynucleotide encoding one or more amino acid sequence described or referenced herein. It also is contemplated that a particular polypeptide may be encoded by nucleic acids containing variations having slightly different nucleic acid sequences but, nonetheless, encode the same or substantially similar protein (see Table 3 above).

In particular embodiments, the invention concerns isolated nucleic acid segments and recombinant vectors incorporating nucleic acid sequences that encode one or more coagulase Domains 1-2, or variants thereof . The term "recombinant" may be used in conjunction with a polynucleotide or polypeptide and generally refers to a polypeptide or polynucleotide produced and/or manipulated *in vitro* or that is a replication product of such a molecule.

In other embodiments, the invention concerns isolated nucleic acid segments and recombinant vectors incorporating nucleic acid sequences that encode a coagulase polypeptide or peptide or a variant thereof to generate an immune response in a subject. In various embodiments the nucleic acids of the invention may be used in genetic vaccines.

The nucleic acid segments used in the present invention can be combined with other nucleic acid sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant nucleic acid protocol. In some cases, a nucleic acid sequence may encode a polypeptide sequence with additional heterologous coding sequences, for example to allow for purification of the polypeptide, transport, secretion, post-translational modification, or for therapeutic benefits such as targeting or efficacy. As discussed above, a tag or other heterologous polypeptide may be added to the modified polypeptide-encoding sequence, wherein "heterologous" refers to a polypeptide that is not the same as the modified polypeptide.

In certain other embodiments, the invention concerns isolated nucleic acid segments and recombinant vectors that include within their sequence a contiguous nucleic acid sequence encoding one of the sequence of SEQUENCE TABLE NO. 1 (SEQ ID NOs: 33-37) or SEQUENCE TABLE NO. 2 (SEQ ID NOs: 38-41) or any other nucleic acid sequences encoding coagulases or other secreted virulence factors and/or surface proteins including proteins transported by the Ess pathway, processed by sortase, or proteins.

In certain embodiments, the present invention provides polynucleotide variants having substantial identity to the sequences disclosed herein; those comprising at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher sequence identity, including all values and ranges there between, compared to a polynucleotide sequence of this invention using the methods described herein (e.g., BLAST analysis using standard parameters).

The invention also contemplates the use of polynucleotides which are complementary to all the above described polynucleotides.

A. Vectors

Polypeptides of the invention may be encoded by a nucleic acid molecule comprised in a vector. The term "vector" is used to refer to a carrier nucleic acid molecule into which a heterologous nucleic acid sequence can be inserted for introduction into a cell where it can be replicated and expressed. A nucleic acid sequence can be "heterologous," which means that it is in a context foreign to the cell in which the vector is being introduced or to the nucleic acid in which is incorporated, which includes a sequence homologous to a sequence in the cell or nucleic acid but in a position within the host cell or nucleic acid where it is ordinarily not found. Vectors

Date Reçue/Date Received 2021-01-25

include DNAs, RNAs, plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (*e.g.*, YACs). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques (for example Sambrook *et al.*, 2001; Ausubel *et al.*, 1996). In addition to encoding one or more coagulase Domains 1-2 or variant thereof, the vector can encode other polypeptide sequences such as a one or more other bacterial peptide, a tag, or an immunogenicity enhancing peptide. Useful vectors encoding such fusion proteins include pIN vectors (Inouye *et al.*, 1985), vectors encoding a stretch of histidines, and pGEX vectors, for use in generating glutathione S-transferase (GST) soluble fusion proteins for later purification and separation or cleavage.

The term "expression vector" refers to a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. Expression vectors can contain a variety of "control sequences," which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well and are described herein.

1. Promoters and Enhancers

A "promoter" is a control sequence. The promoter is typically a region of a nucleic acid sequence at which initiation and rate of transcription are controlled. It may contain genetic elements at which regulatory proteins and molecules may bind such as RNA polymerase and other transcription factors. The phrases "operatively positioned," "operatively linked," "under control," and "under transcriptional control" mean that a promoter is in a correct functional location and/or orientation in relation to a nucleic acid sequence to control transcriptional initiation and expression of that sequence. A promoter may or may not be used in conjunction with an "enhancer," which refers to a cis-acting regulatory sequence involved in the transcriptional activation of a nucleic acid sequence.

Naturally, it may be important to employ a promoter and/or enhancer that effectively directs the expression of the DNA segment in the cell type or organism chosen for expression. Those of skill in the art of molecular biology generally know the use of promoters, enhancers, and cell type combinations for protein expression (see Sambrook *et al.*, 2001). The promoters

Date Reçue/Date Received 2021-01-25

employed may be constitutive, tissue-specific, or inducible and in certain embodiments may direct high level expression of the introduced DNA segment under specified conditions, such as large-scale production of recombinant proteins or peptides.

Various elements/promoters may be employed in the context of the present invention to regulate the expression of a gene. Examples of such inducible elements, which are regions of a nucleic acid sequence that can be activated in response to a specific stimulus, include but are not limited to Immunoglobulin Heavy Chain (Banerji *et al.*, 1983; Gilles *et al.*, 1983; Grosschedl *et al.*, 1985; Atchinson *et al.*, 1986, 1987; Imler *et al.*, 1987; Weinberger *et al.*, 1984; Kiledjian *et al.*, 1988; Porton *et al.*; 1990), Immunoglobulin Light Chain (Queen *et al.*, 1983; Picard *et al.*, 1984), T Cell Receptor (Luria *et al.*, 1987; Winoto *et al.*, 1989; Redondo *et al.*; 1990), HLA DQ α and/or DQ β (Sullivan *et al.*, 1987), β Interferon (Goodbourn *et al.*, 1986; Fujita *et al.*, 1987; Goodbourn *et al.*, 1988), Interleukin-2 (Greene *et al.*, 1989), Interleukin-2 Receptor (Greene *et al.*, 1989; Lin *et al.*, 1990), MHC Class II 5 (Koch *et al.*, 1989), MHC Class II HLA-DRα (Sherman *et al.*, 1989), β-Actin (Kawamoto *et al.*, 1988; Ng *et al.*; 1989), Muscle Creatine Kinase (MCK) (Jaynes *et al.*, 1988; Horlick *et al.*, 1989; Johnson *et al.*, 1989), Prealbumin (Transthyretin) (Costa *et al.*, 1988), Elastase I (Ornitz *et al.*, 1987), Metallothionein (MTII) (Karin *et al.*, 1987; Culotta *et al.*, 1989), Collagenase (Pinkert *et al.*, 1987; Angel *et al.*, 1987), Albumin (Pinkert *et al.*, 1987; Tronche *et al.*, 1989, 1990), α-Fetoprotein (Godbout *et al.*, 1988; Campere *et al.*, 1989), γ-Globin (Bodine *et al.*, 1987; Perez-Stable *et al.*, 1990), β-Globin (Trudel *et al.*, 1987), c-fos (Cohen *et al.*, 1987), c-Ha-Ras (Triesman, 1986; Deschamps *et al.*, 1985), Insulin (Edlund *et al.*, 1985), Neural Cell Adhesion Molecule (NCAM) (Hirsh *et al.*, 1990), α1-Antitrypain (Latimer *et al.*, 1990), H2B (TH2B) Histone (Hwang *et al.*, 1990), Mouse and/or Type I Collagen (Ripe *et al.*, 1989), Glucose-Regulated Proteins (GRP94 and GRP78) (Chang *et al.*, 1989), Rat Growth Hormone (Larsen *et al.*, 1986), Human Serum Amyloid A (SAA) (Edbrooke *et al.*, 1989), Troponin I (TN I) (Yutzey *et al.*, 1989), Platelet-Derived Growth Factor (PDGF) (Pech *et al.*, 1989), Duchenne Muscular Dystrophy (Klamut *et al.*, 1990), SV40 (Banerji *et al.*, 1981; Moreau *et al.*, 1981; Sleigh *et al.*, 1985; Firak *et al.*, 1986; Herr *et al.*, 1986; Imbra *et al.*, 1986; Kadesch *et al.*, 1986; Wang *et al.*, 1986; Ondek *et al.*, 1987; Kuhl *et al.*, 1987; Schaffner *et al.*, 1988), Polyoma (Swartzendruber *et al.*, 1975; Vasseur *et al.*, 1980; Katinka *et al.*, 1980, 1981; Tyndell *et al.*, 1981; Dandolo *et al.*, 1983; de Villiers *et al.*, 1984; Hen *et al.*, 1986; Satake *et al.*, 1988; Campbell *et al.*, 1988), Retroviruses (Kriegler *et al.*, 1982, 1983; Levinson *et al.*,

Date Reçue/Date Received 2021-01-25

1982; Kriegler *et al.*, 1983, 1984a, b, 1988; Bosze *et al.*, 1986; Miksicek *et al.*, 1986; Celander *et al.*, 1987; Thiesen *et al.*, 1988; Celander *et al.*, 1988; Choi *et al.*, 1988; Reisman *et al.*, 1989), Papilloma Virus (Campo *et al.*, 1983; Lusky *et al.*, 1983; Spandidos and Wilkie, 1983; Spalholz *et al.*, 1985; Lusky *et al.*, 1986; Cripe *et al.*, 1987; Gloss *et al.*, 1987; Hirochika *et al.*, 1987; Stephens *et al.*, 1987), Hepatitis B Virus (Bulla *et al.*, 1986; Jameel *et al.*, 1986; Shaul *et al.*, 1987; Spandau *et al.*, 1988; Vannice *et al.*, 1988), Human Immunodeficiency Virus (Muesing *et al.*, 1987; Hauber *et al.*, 1988; Jakobovits *et al.*, 1988; Feng *et al.*, 1988; Takebe *et al.*, 1988; Rosen *et al.*, 1988; Berkhout *et al.*, 1989; Laspia *et al.*, 1989; Sharp *et al.*, 1989; Braddock *et al.*, 1989), Cytomegalovirus (CMV) IE (Weber *et al.*, 1984; Boshart *et al.*, 1985; Foecking *et al.*, 1986), Gibbon Ape Leukemia Virus (Holbrook *et al.*, 1987; Quinn *et al.*, 1989).

Inducible elements include, but are not limited to MT II - Phorbol Ester (TFA)/Heavy metals (Palmiter *et al.*, 1982; Haslinger *et al.*, 1985; Searle *et al.*, 1985; Stuart *et al.*, 1985; Imagawa *et al.*, 1987, Karin *et al.*, 1987; Angel *et al.*, 1987b; McNeall *et al.*, 1989); MMTV (mouse mammary tumor virus) – Glucocorticoids (Huang *et al.*, 1981; Lee *et al.*, 1981; Majors *et al.*, 1983; Chandler *et al.*, 1983; Lee *et al.*, 1984; Ponta *et al.*, 1985; Sakai *et al.*, 1988); β-Interferon - poly(rI)x/poly(rc) (Tavernier *et al.*, 1983); Adenovirus 5 E2 – ElA (Imperiale *et al.*, 1984); Collagenase - Phorbol Ester (TPA) (Angel *et al.*, 1987a); Stromelysin - Phorbol Ester (TPA) (Angel *et al.*, 1987b); SV40 - Phorbol Ester (TPA) (Angel *et al.*, 1987b); Murine MX Gene - Interferon, Newcastle Disease Virus (Hug *et al.*, 1988); GRP78 Gene - A23187 (Resendez *et al.*, 1988); α-2-Macroglobulin - IL-6 (Kunz *et al.*, 1989); Vimentin – Serum (Rittling *et al.*, 1989); MHC Class I Gene H-2κb – Interferon (Blanar *et al.*, 1989); HSP70 – ElA/SV40 Large T Antigen (Taylor *et al.*, 1989, 1990a, 1990b); Proliferin - Phorbol Ester/TPA (Mordacq *et al.*, 1989); Tumor Necrosis Factor – PMA (Hensel *et al.*, 1989); and Thyroid Stimulating Hormone α Gene - Thyroid Hormone (Chatterjee *et al.*, 1989).

The particular promoter that is employed to control the expression of peptide or protein encoding polynucleotide of the invention is not believed to be critical, so long as it is capable of expressing the polynucleotide in a targeted cell, preferably a bacterial cell. Where a human cell is targeted, it is preferable to position the polynucleotide coding region adjacent to and under the control of a promoter that is capable of being expressed in a human cell. Generally speaking, such a promoter might include either a bacterial, human or viral promoter.

In embodiments in which a vector is administered to a subject for expression of the protein, it is contemplated that a desirable promoter for use with the vector is one that is not down-regulated by cytokines or one that is strong enough that even if down-regulated, it produces an effective amount of at least two different staphylococcal conagulase Domains 1-2 for eliciting an immune response. Non-limiting examples of these are CMV IE and RSV LTR. Tissue specific promoters can be used, particularly if expression is in cells in which expression of an antigen is desirable, such as dendritic cells or macrophages. The mammalian MHC I and MHC II promoters are examples of such tissue-specific promoters.

2. Initiation Signals and Internal Ribosome Binding Sites (IRES)

A specific initiation signal also may be required for efficient translation of coding sequences. These signals include the ATG initiation codon or adjacent sequences. Exogenous translational control signals, including the ATG initiation codon, may need to be provided. One of ordinary skill in the art would readily be capable of determining this and providing the necessary signals.

In certain embodiments of the invention, the use of internal ribosome entry sites (IRES) elements are used to create multigene, or polycistronic, messages. IRES elements are able to bypass the ribosome scanning model of 5'□ methylated Cap dependent translation and begin translation at internal sites (Pelletier and Sonenberg, 1988; Macejak and Sarnow, 1991). IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, creating polycistronic messages. Multiple genes can be efficiently expressed using a single promoter/enhancer to transcribe a single message (see U.S. Patents 5,925,565 and 5,935,819).

3. Selectable and Screenable Markers

In certain embodiments of the invention, cells containing a nucleic acid construct of the present invention may be identified *in vitro* or *in vivo* by encoding a screenable or selectable marker in the expression vector. When transcribed and translated, a marker confers an identifiable change to the cell permitting easy identification of cells containing the expression vector. Generally, a selectable marker is one that confers a property that allows for selection.

Date Reçue/Date Received 2021-01-25

A positive selectable marker is one in which the presence of the marker allows for its selection, while a negative selectable marker is one in which its presence prevents its selection. An example of a positive selectable marker is a drug resistance marker.

B. Host Cells

As used herein, the terms "cell," "cell line," and "cell culture" may be used interchangeably. All of these terms also include their progeny, which is any and all subsequent generations. It is understood that all progeny may not be identical due to deliberate or inadvertent mutations. In the context of expressing a heterologous nucleic acid sequence, "host cell" refers to a prokaryotic or eukaryotic cell, and it includes any transformable organism that is capable of replicating a vector or expressing a heterologous gene encoded by a vector. A host cell can, and has been, used as a recipient for vectors or viruses. A host cell may be "transfected" or "transformed," which refers to a process by which exogenous nucleic acid, such as a recombinant protein-encoding sequence, is transferred or introduced into the host cell. A transformed cell includes the primary subject cell and its progeny.

Host cells may be derived from prokaryotes or eukaryotes, including bacteria, yeast cells, insect cells, and mammalian cells for replication of the vector or expression of part or all of the nucleic acid sequence(s). Numerous cell lines and cultures are available for use as a host cell, and they can be obtained through the American Type Culture Collection (ATCC), which is an organization that serves as an archive for living cultures and genetic materials (www.atcc.org).

C. Expression Systems

Numerous expression systems exist that comprise at least a part or all of the compositions discussed above. Prokaryote- and/or eukaryote-based systems can be employed for use with the present invention to produce nucleic acid sequences, or their cognate polypeptides, proteins and peptides. Many such systems are commercially and widely available.

The insect cell/baculovirus system can produce a high level of protein expression of a heterologous nucleic acid segment, such as described in U.S. Patents 5,871,986, 4,879,236, and which can be bought, for example, under the name MAXBAC® 2.0 from INVITROGEN® and BACPACK™ BACULOVIRUS EXPRESSION SYSTEM FROM CLONTECH®.

In addition to the disclosed expression systems of the invention, other examples of expression systems include STRATAGENE®'s COMPLETE CONTROL™ Inducible Mammalian Expression System, which involves a synthetic ecdysone-inducible receptor, or its pET Expression System, an *E. coli* expression system. Another example of an inducible expression system is available from INVITROGEN®, which carries the T-REX™ (tetracycline-regulated expression) System, an inducible mammalian expression system that uses the full-length CMV promoter. INVITROGEN® also provides a yeast expression system called the *Pichia methanolica* Expression System, which is designed for high-level production of recombinant proteins in the methylotrophic yeast Pichia methanolica. One of skill in the art would know how to express a vector, such as an expression construct, to produce a nucleic acid sequence or its cognate polypeptide, protein, or peptide.

III. POLYSACCHARIDES

The immunogenic compositions of the invention may further comprise capsular polysaccharides including one or more of PIA (also known as PNAG) and/or *S. aureus* Type V and/or type VIII capsular polysaccharide and/or *S. epidermidis* Type I, and/or Type II and/or Type III capsular polysaccharide.

A. PIA (PNAG)

It is now clear that the various forms of staphylococcal surface polysaccharides identified as PS/A, PIA and SAA are the same chemical entity - PNAG (Maira-Litran *et al.*, 2004). Therefore the term PIA or PNAG encompasses all these polysaccharides or oligosaccharides derived from them.

PIA is a polysaccharide intercellular adhesin and is composed of a polymer of β-(1→6)-linked glucosamine substituted with N-acetyl and O-succinyl constituents. This polysaccharide is present in both *S. aureus* and *S. epidermidis* and can be isolated from either source (Joyce *et al.*, 2003; Maira-Litran *et al.*, 2002). For example, PNAG may be isolated from *S. aureus* strain MN8m (WO04/43407). PIA isolated from *S. epidermidis* is a integral constituent of biofilm. It is responsible for mediating cell-cell adhesion and probably also functions to shield the growing colony from the host's immune response. The polysaccharide previously known as poly-N-succinyl-β-(1→6)-glucosamine (PNSG) was recently shown not to have the expected structure since the identification of N- succinylation was incorrect

(Maira-Litran *et al.*, 2002). Therefore the polysaccharide formally known as PNSG and now found to be PNAG is also encompassed by the term PIA.

PIA (or PNAG) may be of different sizes varying from over 400kDa to between 75 and 400kDa to between 10 and 75kDa to oligosaccharides composed of up to 30 repeat units (of β-(1→6)-linked glucosamine substituted with N-acetyl and O-succinyl constituents). Any size of PIA polysaccharide or oligosaccharide may be use in an immunogenic composition of the invention, in one aspect the polysaccharide is over 40kDa. Sizing may be achieved by any method known in the art, for instance by microfluidization, ultrasonic irradiation or by chemical cleavage (WO 03/53462, EP497524, EP497525). In certain aspects PIA (PNAG) is at least or at most 40-400kDa, 40-300kDa, 50-350kDa, 60-300kDa, 50-250kDa and 60-200kDa.

PIA (PNAG) can have different degree of acetylation due to substitution on the amino groups by acetate. PIA produced *in vitro* is almost fully substituted on amino groups (95- 100%). Alternatively, a deacetylated PIA (PNAG) can be used having less than 60%, 50%, 40%, 30%, 20%, 10% acetylation. Use of a deacetylated PIA (PNAG) is preferred since non-acetylated epitopes of PNAG are efficient at mediating opsonic killing of Gram positive bacteria, preferably *S. aureus* and/or *S. epidermidis*. In certain aspects, the PIA (PNAG) has a size between 40kDa and 300kDa and is deacetylated so that less than 60%, 50%, 40%, 30% or 20% of amino groups are acetylated.

The term deacetylated PNAG (dPNAG) refers to a PNAG polysaccharide or oligosaccharide in which less than 60%, 50%, 40%, 30%, 20% or 10% of the amino agroups are acetylated. In certain aspects, PNAG is deaceylated to form dPNAG by chemically treating the native polysaccharide. For example, the native PNAG is treated with a basic solution such that the pH rises to above 10. For instance the PNAG is treated with 0.1-5 M, 0.2-4 M, 0.3-3 M, 0.5-2 M, 0.75-1.5 M or 1 M NaOH , KOH or NH_4OH. Treatment is for at least 10 to 30 minutes, or 1, 2, 3, 4, 5, 10, 15 or 20 hours at a temperature of 20-100, 25-80, 30-60 or 30-50 or 35-45 °C. dPNAG may be prepared as described in WO 04/43405.

The polysaccharide(s) can be conjugated or unconjugated to a carrier protein.

B. Type 5 and Type 8 polysaccharides from *S. aureus*

Most strains of *S. aureus* that cause infection in man contain either Type 5 or Type 8 polysaccharides. Approximately 60% of human strains are Type 8 and approximately 30% are Type 5. The structures of Type 5 and Type 8 capsular polysaccharide antigens are described in Moreau *et al.*, (1990) and Fournier *et al.*, (1984). Both have FucNAcp in their repeat unit as well as ManNAcA which can be used to introduce a sulfhydryl group. The structures are:

Type 5

→4)-β-D-ManNAcA(3OAc)-(1→4)-α-L-FucNAc(1→3)-β-D-FucNAc-(1→

Type 8

→3)-β-D-ManNAcA(4OAc)-(1→3)-α-L-FucNAc(1→3)-β-D-FucNAc-(1→

Recently (Jones, 2005) NMR spectroscopy revised the structures to:

Type 5

→4)-β-D-ManNAcA-(1→4)-α-L-FucNAc(3OAc)-(1→3)-β-D-FucNAc-(1→

Type 8

→3)-β-D-ManNAcA(4OAc)-(1→3)-α-L-FucNAc(1→3)-α-D-FucNAc(1→

Polysaccharides may be extracted from the appropriate strain of *S. aureus* using method well known to of skill in the art, *See* U.S. Patent 6,294,177. For example, ATCC 12902 is a Type 5 *S. aureus* strain and ATCC 12605 is a Type 8 *S. aureus* strain.

Polysaccharides are of native size or alternatively may be sized, for instance by microfluidisation, ultrasonic irradiation, or by chemical treatment. The invention also covers oligosaccharides derived from the type 5 and 8 polysaccharides from *S. aureus*. The type 5 and 8 polysaccharides included in the immunogenic composition of the invention are preferably conjugated to a carrier protein as described below or are alternatively unconjugated. The immunogenic compositions of the invention alternatively contains either type 5 or type 8 polysaccharide.

C. *S. aureus* 336 antigen

In an embodiment, the immunogenic composition of the invention comprises the *S. aureus* 336 antigen described in U.S. Patent 6,294,177. The 336 antigen comprises β-linked hexosamine, contains no O-acetyl groups, and specifically binds to antibodies to *S. aureus* Type 336 deposited under ATCC 55804. In an embodiment, the 336 antigen is a polysaccharide which is of native size or alternatively may be sized, for instance by microfluidisation, ultrasonic irradiation, or by chemical treatment. The invention also covers oligosaccharides derived from the 336 antigen. The 336 antigen can be unconjugated or conjugated to a carrier protein.

D. Type I, II and III polysaccharides from *S. epidermidis*

Amongst the problems associated with the use of polysaccharides in vaccination, is the fact that polysaccharides *per se* are poor immunogens. It is preferred that the polysaccharides utilized in the invention are linked to a protein carrier which provide bystander T-cell help to improve immunogenicity. Examples of such carriers which may be conjugated to polysaccharide immunogens include the Diphtheria and Tetanus toxoids (DT, DT CRM197 and TT respectively), Keyhole Limpet Haemocyanin (KLH), and the purified protein derivative of Tuberculin (PPD), *Pseudomonas aeruginosa* exoprotein A (rEPA), protein D from *Haemophilus influenzae*, pneumolysin or fragments of any of the above. Fragments suitable for use include fragments encompassing T-helper epitopes. In particular the protein D fragment from *H. influenza* will preferably contain the N-terminal 1/3 of the protein. Protein D is an IgD-binding protein from *Haemophilus influenzae* (EP 0 594 610 B1) and is a potential immunogen. In addition, staphylococcal proteins may be used as a carrier protein in the polysaccharide conjugates of the invention.

A carrier protein that would be particularly advantageous to use in the context of a staphylococcal vaccine is staphylococcal alpha toxoid. The native form may be conjugated to a polysaccharide since the process of conjugation reduces toxicity. Preferably genetically detoxified alpha toxins such as the His35Leu or His35Arg variants are used as carriers since residual toxicity is lower. Alternatively the alpha toxin is chemically detoxified by treatment with a cross-linking reagent, formaldehyde or glutaraldehyde. A genetically detoxified alpha toxin is optionally chemically detoxified, preferably by treatment with a cross-linking reagent, formaldehyde or glutaraldehyde to further reduce toxicity.

The polysaccharides may be linked to the carrier protein(s) by any known method (for example those methods described in U.S. Patents 4,372,945, 4,474,757, and 4,356,170). Preferably, CDAP conjugation chemistry is carried out (see WO95/08348). In CDAP, the cyanylating reagent 1-cyano-dimethylaminopyridinium tetrafluoroborate (CDAP) is preferably used for the synthesis of polysaccharide-protein conjugates. The cyanilation reaction can be performed under relatively mild conditions, which avoids hydrolysis of the alkaline sensitive polysaccharides. This synthesis allows direct coupling to a carrier protein.

Conjugation preferably involves producing a direct linkage between the carrier protein and polysaccharide. Optionally a spacer (such as adipic dihydride (ADH)) may be introduced between the carrier protein and the polysaccharide.

IV. IMMUNE RESPONSE AND ASSAYS

As discussed above, the invention concerns evoking or inducing an immune response in a subject against a coagulase or one or more coagulase Domains 1-2 or variants thereof. In one embodiment, the immune response can protect against or treat a subject having, suspected of having, or at risk of developing an infection or related disease, particularly those related to staphylococci. One use of the immunogenic compositions of the invention is to prevent nosocomial infections by inoculating a subject prior to undergoing procedures in a hospital or other environment having an increased risk of infection.

A. Immunoassays

The present invention includes the implementation of serological assays to evaluate whether and to what extent an immune response is induced or evoked by compositions of the invention. There are many types of immunoassays that can be implemented. Immunoassays encompassed by the present invention include, but are not limited to, those described in U.S. Patent 4,367,110 (double monoclonal antibody sandwich assay) and U.S. Patent 4,452,901 (western blot). Other assays include immunoprecipitation of labeled ligands and immunocytochemistry, both *in vitro* and *in vivo*.

Immunoassays generally are binding assays. Certain preferred immunoassays are the various types of enzyme linked immunosorbent assays (ELISAs) and radioimmunoassays (RIA) known in the art. Immunohistochemical detection using tissue sections is also particularly useful. In one example, antibodies or antigens are immobilized on a selected surface, such as a well in a polystyrene microtiter plate, dipstick, or column support. Then, a test composition suspected of containing the desired antigen or antibody, such as a clinical sample, is added to the wells. After binding and washing to remove non specifically bound immune complexes, the bound antigen or antibody may be detected. Detection is generally achieved by the addition of another antibody, specific for the desired antigen or antibody, that is linked to a detectable label. This type of ELISA is known as a "sandwich ELISA." Detection also may be achieved by the addition of a second antibody specific for the desired antigen, followed by the addition of a third antibody that has binding affinity for the second antibody, with the third antibody being linked to a detectable label.

Competition ELISAs are also possible implementations in which test samples compete for binding with known amounts of labeled antigens or antibodies. The amount of reactive species in the unknown sample is determined by mixing the sample with the known labeled species before or during incubation with coated wells. The presence of reactive species in the sample acts to reduce the amount of labeled species available for binding to the well and thus reduces the ultimate signal. Irrespective of the format employed, ELISAs have certain features in common, such as coating, incubating or binding, washing to remove non specifically bound species, and detecting the bound immune complexes.

Antigen or antibodies may also be linked to a solid support, such as in the form of plate, beads, dipstick, membrane, or column matrix, and the sample to be analyzed is applied to the immobilized antigen or antibody. In coating a plate with either antigen or antibody, one will generally incubate the wells of the plate with a solution of the antigen or antibody, either overnight or for a specified period. The wells of the plate will then be washed to remove incompletely-adsorbed material. Any remaining available surfaces of the wells are then "coated" with a nonspecific protein that is antigenically neutral with regard to the test antisera. These include bovine serum albumin (BSA), casein, and solutions of milk powder. The coating allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific binding of antisera onto the surface.

B. Diagnosis of Bacterial Infection

In addition to the use of proteins, polypeptides, and/or peptides, as well as antibodies binding these polypeptides, proteins, and/or peptides, to treat or prevent infection as described above, the present invention contemplates the use of these polypeptides, proteins, peptides, and/or antibodies in a variety of ways, including the detection of the presence of Staphylococci to diagnose an infection, whether in a patient or on medical equipment which may also become infected. In accordance with the invention, a preferred method of detecting the presence of infections involves the steps of obtaining a sample suspected of being infected by one or more staphylococcal bacteria species or strains, such as a sample taken from an individual, for example, from one's blood, saliva, tissues, bone, muscle, cartilage, or skin. Following isolation of the sample, diagnostic assays utilizing the polypeptides, proteins, peptides, and/or antibodies of the present invention may be carried out to detect the presence of staphylococci, and such assay techniques for determining such presence in a sample are well known to those skilled in the art and include methods such as radioimmunoassay, western blot analysis and ELISA assays. In general, in accordance with the invention, a method of diagnosing an infection is contemplated wherein a sample suspected of being infected with staphylococci has added to it the polypeptide, protein, peptide, antibody, or monoclonal antibody in accordance with the present invention, and staphylococci are indicated by antibody binding to the polypeptides, proteins, and/or peptides, or polypeptides, proteins, and/or peptides binding to the antibodies in the sample.

Accordingly, antibodies in accordance with the invention may be used for the prevention of infection from staphylococcal bacteria (*i.e.*, passive immunization), for the treatment of an ongoing infection, or for use as research tools. The term "antibodies" as used herein includes monoclonal, polyclonal, chimeric, single chain, bispecific, simianized, and humanized or primatized antibodies as well as Fab fragments, such as those fragments which maintain the binding specificity of the antibodies, including the products of an Fab immunoglobulin expression library. Accordingly, the invention contemplates the use of single chains such as the variable heavy and light chains of the antibodies. Generation of any of these types of antibodies or antibody fragments is well known to those skilled in the art. Specific examples of the generation of an antibody to a bacterial protein can be found in U.S. Patent Application Pub. No. 20030153022.

Any of the above described polypeptides, proteins, peptides, and/or antibodies may be labeled directly with a detectable label for identification and quantification of staphylococcal bacteria. Labels for use in immunoassays are generally known to those skilled in the art and include enzymes, radioisotopes, and fluorescent, luminescent and chromogenic substances, including colored particles such as colloidal gold or latex beads. Suitable immunoassays include enzyme-linked immunosorbent assays (ELISA).

Date Reçue/Date Received 2021-01-25

C. Protective Immunity

In some embodiments of the invention, proteinaceous compositions confer protective immunity to a subject. Protective immunity refers to a body's ability to mount a specific immune response that protects the subject from developing a particular disease or condition that involves the agent against which there is an immune response. An immunogenically effective amount is capable of conferring protective immunity to the subject.

As used herein in the specification and in the claims section that follows, the term polypeptide or peptide refer to a stretch of amino acids covalently linked there amongst via peptide bonds. Different polypeptides have different functionalities according to the present invention. While according to one aspect, a polypeptide is derived from an immunogen designed to induce an active immune response in a recipient, according to another aspect of the invention, a polypeptide is derived from an antibody which results following the elicitation of an active immune response in, for example, an animal, and which can serve to induce a passive immune response in the recipient. In both cases, however, the polypeptide is encoded by a polynucleotide according to any possible codon usage.

As used herein the phrase "immune response" or its equivalent "immunological response" refers to the development of a humoral (antibody mediated), cellular (mediated by antigen-specific T cells or their secretion products) or both humoral and cellular response directed against a protein, peptide, carbohydrate, or polypeptide of the invention in a recipient patient. Such a response can be an active response induced by administration of immunogen or a passive response induced by administration of antibody, antibody containing material, or primed T-cells. A cellular immune response is elicited by the presentation of polypeptide epitopes in association with Class I or Class II MHC molecules, to activate antigen-specific CD4 (+) T helper cells and/or CD8 (+) cytotoxic T cells. The response may also involve activation of monocytes, macrophages, NK cells, basophils, dendritic cells, astrocytes, microglia cells, eosinophils or other components of innate immunity. As used herein "active immunity" refers to any immunity conferred upon a subject by administration of an antigen.

As used herein "passive immunity" refers to any immunity conferred upon a subject without administration of an antigen to the subject. "Passive immunity" therefore includes, but is not limited to, administration of activated immune effectors including cellular mediators or protein mediators (*e.g.*, monoclonal and/or polyclonal antibodies) of an immune response. A

Date Reçue/Date Received 2021-01-25

monoclonal or polyclonal antibody composition may be used in passive immunization for the prevention or treatment of infection by organisms that carry the antigen recognized by the antibody. An antibody composition may include antibodies that bind to a variety of antigens that may in turn be associated with various organisms. The antibody component can be a polyclonal antiserum. In certain aspects the antibody or antibodies are affinity purified from an animal or second subject that has been challenged with an antigen(s). Alternatively, an antibody mixture may be used, which is a mixture of monoclonal and/or polyclonal antibodies to antigens present in the same, related, or different microbes or organisms, such as gram-positive bacteria, gram-negative bacteria, including but not limited to staphylococcus bacteria.

Passive immunity may be imparted to a patient or subject by administering to the patient immunoglobulins (Ig) and/or other immune factors obtained from a donor or other non-patient source having a known immunoreactivity. In other aspects, an antigenic composition of the present invention can be administered to a subject who then acts as a source or donor for globulin, produced in response to challenge with the antigenic composition ("hyperimmune globulin"), that contains antibodies directed against Staphylococcus or other organism. A subject thus treated would donate plasma from which hyperimmune globulin would then be obtained, *via* conventional plasma-fractionation methodology, and administered to another subject in order to impart resistance against or to treat staphylococcus infection. Hyperimmune globulins according to the invention are particularly useful for immune-compromised individuals, for individuals undergoing invasive procedures or where time does not permit the individual to produce their own antibodies in response to vaccination. See U.S. Patents 6,936,258, 6,770,278, 6,756,361, 5,548,066, 5,512,282, 4,338,298, and 4,748,018, for exemplary methods and compositions related to passive immunity.

For purposes of this specification and the accompanying claims the terms "epitope" and "antigenic determinant" are used interchangeably to refer to a site on an antigen to which B and/or T cells respond or recognize. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of

Date Reçue/Date Received 2021-01-25

determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, *e.g.*, Epitope Mapping Protocols (1996). Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen. T-cells recognize continuous epitopes of about nine amino acids for CD8 cells or about 13-15 amino acids for CD4 cells. T cells that recognize the epitope can be identified by *in vitro* assays that measure antigen-dependent proliferation, as determined by ^{3}H-thymidine incorporation by primed T cells in response to an epitope (Burke *et al.*, 1994), by antigen-dependent killing (cytotoxic T lymphocyte assay, Tigges *et al.*, 1996) or by cytokine secretion.

The presence of a cell-mediated immunological response can be determined by proliferation assays (CD4 (+) T cells) or CTL (cytotoxic T lymphocyte) assays. The relative contributions of humoral and cellular responses to the protective or therapeutic effect of an immunogen can be distinguished by separately isolating IgG and T-cells from an immunized syngeneic animal and measuring protective or therapeutic effect in a second subject.

As used herein and in the claims, the terms "antibody" or "immunoglobulin" are used interchangeably and refer to any of several classes of structurally related proteins that function as part of the immune response of an animal or recipient, which proteins include IgG, IgD, IgE, IgA, IgM and related proteins.

Under normal physiological conditions antibodies are found in plasma and other body fluids and in the membrane of certain cells and are produced by lymphocytes of the type denoted B cells or their functional equivalent. Antibodies of the IgG class are made up of four polypeptide chains linked together by disulfide bonds. The four chains of intact IgG molecules are two identical heavy chains referred to as H-chains and two identical light chains referred to as L-chains.

In order to produce polyclonal antibodies, a host, such as a rabbit or goat, is immunized with the antigen or antigen fragment, generally with an adjuvant and, if necessary, coupled to a carrier. Antibodies to the antigen are subsequently collected from the sera of the host. The polyclonal antibody can be affinity purified against the antigen rendering it monospecific.

Monoclonal antibodies can be produced by hyperimmunization of an appropriate donor with the antigen or *ex-vivo* by use of primary cultures of splenic cells or cell lines derived from spleen (Anavi, 1998; Huston *et al.*, 1991; Johnson *et al.*, 1991; Mernaugh *et al.*, 1995).

As used herein and in the claims, the phrase "an immunological portion of an antibody" includes a Fab fragment of an antibody, a Fv fragment of an antibody, a heavy chain of an antibody, a light chain of an antibody, a heterodimer consisting of a heavy chain and a light chain of an antibody, a variable fragment of a light chain of an antibody, a variable fragment of a heavy chain of an antibody, and a single chain variant of an antibody, which is also known as scFv. In addition, the term includes chimeric immunoglobulins which are the expression products of fused genes derived from different species, one of the species can be a human, in which case a chimeric immunoglobulin is said to be humanized. Typically, an immunological portion of an antibody competes with the intact antibody from which it was derived for specific binding to an antigen.

Optionally, an antibody or preferably an immunological portion of an antibody, can be chemically conjugated to, or expressed as, a fusion protein with other proteins. For purposes of this specification and the accompanying claims, all such fused proteins are included in the definition of antibodies or an immunological portion of an antibody.

As used herein the terms "immunogenic agent" or "immunogen" or "antigen" are used interchangeably to describe a molecule capable of inducing an immunological response against itself on administration to a recipient, either alone, in conjunction with an adjuvant, or presented on a display vehicle.

D. Treatment Methods

A method of the present invention includes treatment for a disease or condition caused by a staphylococcus pathogen. An immunogenic polypeptide of the invention can be given to induce an immune response in a person infected with staphylococcus or suspected of having been exposed to staphylococcus. Methods may be employed with respect to individuals who have tested positive for exposure to staphylococcus or who are deemed to be at risk for infection based on possible exposure.

In particular, the invention encompasses a method of treatment for staphylococcal infection, particularly hospital acquired nosocomial infections. The immunogenic compositions and vaccines of the invention are particularly advantageous to use in cases of elective surgery. Such patients will know the date of surgery in advance and could be inoculated in advance. The immunogenic compositions and vaccines of the invention are also advantageous to use to inoculate health care workers.

In some embodiments, the treatment is administered in the presence of adjuvants or carriers or other staphylococcal antigens. Furthermore, in some examples, treatment comprises administration of other agents commonly used against bacterial infection, such as one or more antibiotics.

The use of peptides for vaccination can require, but not necessarily, conjugation of the peptide to an immunogenic carrier protein, such as hepatitis B surface antigen, keyhole limpet hemocyanin, or bovine serum albumin. Methods for performing this conjugation are well known in the art.

V. VACCINE AND OTHER PHARMACEUTICAL COMPOSITIONS AND ADMINISTRATION

A. Vaccines

The present invention includes methods for preventing or ameliorating staphylococcal infections, particularly hospital acquired nosocomial infections. As such, the invention contemplates vaccines for use in both active and passive immunization embodiments. Immunogenic compositions, proposed to be suitable for use as a vaccine, may be prepared from immunogenic coagulases or a fragment thereof or a variant thereof, e.g., one or more coagulase Domains 1-2 . In other embodiments, coagulases, a fragment thereof or a variant thereof, can be used in combination with other secreted virulence proteins, surface proteins or immunogenic fragments thereof. In certain aspects, antigenic material is extensively dialyzed to remove undesired small molecular weight molecules and/or lyophilized for more ready formulation into a desired vehicle.

Other options for a protein/peptide-based vaccine involve introducing nucleic acids encoding the antigen(s) as DNA vaccines. In this regard, recent reports described construction of recombinant vaccinia viruses expressing either 10 contiguous minimal CTL epitopes (Thomson, 1996) or a combination of B cell, cytotoxic T-lymphocyte (CTL), and T-helper (Th) epitopes from several microbes (An, 1997), and successful use of such constructs to immunize mice for priming protective immune responses. Thus, there is ample evidence in the literature for successful utilization of peptides, peptide-pulsed antigen presenting cells (APCs), and peptide-encoding constructs for efficient *in vivo* priming of protective immune responses. The use of nucleic acid sequences as vaccines is exemplified in U.S. Patents 5,958,895 and 5,620,896.

The preparation of vaccines that contain polypeptide or peptide sequence(s) as active ingredients is generally well understood in the art, as exemplified by U.S. Patents 4,608,251; 4,601,903; 4,599,231; 4,599,230; 4,596,792; and 4,578,770. Typically, such vaccines are prepared as injectables either as liquid solutions or suspensions: solid forms suitable for solution in or suspension in liquid prior to injection may also be prepared. The preparation may also be emulsified. The active immunogenic ingredient is often mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants that enhance the effectiveness of the vaccines. In specific embodiments, vaccines are formulated with a combination of substances, as described in U.S. Patents 6,793,923 and 6,733,754.

Vaccines may be conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkalene glycols or triglycerides: such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10%, preferably about 1% to about 2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 10% to about 95% of active ingredient, preferably about 25% to about 70%.

The polypeptides and polypeptide-encoding DNA constructs may be formulated into a vaccine as neutral or salt forms. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the peptide) and those that are formed with

Date Reçue/Date Received 2021-01-25

inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like.

Typically, vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, including the capacity of the individual's immune system to synthesize antibodies and the degree of protection desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are of the order of several hundred micrograms of active ingredient per vaccination. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by subsequent inoculations or other administrations.

The manner of application may be varied widely. Any of the conventional methods for administration of a vaccine are applicable. These are believed to include oral application within a solid physiologically acceptable base or in a physiologically acceptable dispersion, parenterally, by injection and the like. The dosage of the vaccine will depend on the route of administration and will vary according to the size and health of the subject.

In certain instances, it will be desirable to have multiple administrations of the vaccine, *e.g.*, 2, 3, 4, 5, 6 or more administrations. The vaccinations can be at 1, 2, 3, 4, 5, 6, 7, 8, to 5, 6, 7, 8, 9 ,10, 11, 12 twelve week intervals, including all ranges there between. Periodic boosters at intervals of 1-5 years will be desirable to maintain protective levels of the antibodies. The course of the immunization may be followed by assays for antibodies against the antigens, as described in U.S. Patents 3,791,932; 4,174,384 and 3,949,064.

1. Carriers

A given composition may vary in its immunogenicity. It is often necessary therefore to boost the host immune system, as may be achieved by coupling a peptide or polypeptide to a carrier. Exemplary and preferred carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin, or rabbit serum albumin can also be used as carriers. Means for conjugating a polypeptide to a carrier protein are well known in the art and include glutaraldehyde, m-maleimidobencoyl-N-hydroxysuccinimide ester, carbodiimyde, and bis-biazotized benzidine.

2. Adjuvants

The immunogenicity of polypeptide or peptide compositions can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Suitable adjuvants include all acceptable immunostimulatory compounds, such as cytokines, toxins, or synthetic compositions. A number of adjuvants can be used to enhance an antibody response against a coagulase and or its variant, such as one or more coagulase Domains 1-2, or any other bacterial protein or combination contemplated herein. Adjuvants can (1) trap the antigen in the body to cause a slow release; (2) attract cells involved in the immune response to the site of administration; (3) induce proliferation or activation of immune system cells; or (4) improve the spread of the antigen throughout the subject's body.

Adjuvants include, but are not limited to, oil-in-water emulsions, water-in-oil emulsions, mineral salts, polynucleotides, and natural substances. Specific adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, γ-interferon, GMCSP, BCG, aluminum salts, such as aluminum hydroxide or other aluminum compound, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TDM), and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion. MHC antigens may even be used. Others adjuvants or methods are exemplified in U.S. Patents 6,814,971, 5,084,269, 6,656,462.

Various methods of achieving adjuvant affect for the vaccine includes use of agents such as aluminum hydroxide or phosphate (alum), commonly used as about 0.05 to about 0.1% solution in phosphate buffered saline, admixture with synthetic polymers of sugars (Carbopol®) used as an about 0.25% solution, aggregation of the protein in the vaccine by heat treatment with temperatures ranging between about 70° to about 101°C for a 30-second to 2-minute period, respectively. Aggregation by reactivating with pepsin-treated (Fab) antibodies to albumin; mixture with bacterial cells (e.g., *C. parvum*), endotoxins or lipopolysaccharide components of Gram-negative bacteria; emulsion in physiologically acceptable oil vehicles (*e.g.*, mannide mono-oleate (Aracel A)); or emulsion with a 20% solution of a perfluorocarbon (Fluosol-DA®) used as a block substitute may also be employed to produce an adjuvant effect.

Examples of and often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*), incomplete Freund's adjuvants, and aluminum hydroxide.

In some aspects, it is preferred that the adjuvant be selected to be a preferential inducer of either a Th1 or a Th2 type of response. High levels of Th1-type cytokines tend to favor the induction of cell mediated immune responses to a given antigen, while high levels of Th2-type cytokines tend to favor the induction of humoral immune responses to the antigen.

The distinction of Th1 and Th2-type immune response is not absolute. In reality an individual will support an immune response which is described as being predominantly Th1 or predominantly Th2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4+ T cell clones by Mosmann and Coffman (Mosmann, and Coffman, 1989). Traditionally, Th1-type responses are associated with the production of the INF-γ and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of Th1-type immune responses are not produced by T-cells, such as IL-12. In contrast, Th2-type responses are associated with the secretion of IL- 4, IL-5, IL-6, IL-10.

In addition to adjuvants, it may be desirable to co-administer biologic response modifiers (BRM) to enhance immune responses. BRMs have been shown to upregulate T cell immunity or downregulate suppresser cell activity. Such BRMs include, but are not limited to, Cimetidine (CIM; 1200 mg/d) (Smith/Kline, PA); or low-dose Cyclophosphamide (CYP; 300 mg/m^2) (Johnson/ Mead, NJ) and cytokines such as γ-interferon, IL-2, or IL-12 or genes encoding proteins involved in immune helper functions, such as B-7.

B. Lipid Components and Moieties

In certain embodiments, the present invention concerns compositions comprising one or more lipids associated with a nucleic acid or a polypeptide/peptide. A lipid is a substance that is insoluble in water and extractable with an organic solvent. Compounds other than those specifically described herein are understood by one of skill in the art as lipids, and are encompassed by the compositions and methods of the present invention. A lipid component and a non-lipid may be attached to one another, either covalently or non-covalently.

A lipid may be a naturally occurring lipid or a synthetic lipid. However, a lipid is usually a biological substance. Biological lipids are well known in the art, and include for example, neutral fats, phospholipids, phosphoglycerides, steroids, terpenes, lysolipids, glycosphingolipids, glucolipids, sulphatides, lipids with ether and ester-linked fatty acids and polymerizable lipids, and combinations thereof.

A nucleic acid molecule or a polypeptide/peptide, associated with a lipid may be dispersed in a solution containing a lipid, dissolved with a lipid, emulsified with a lipid, mixed with a lipid, combined with a lipid, covalently bonded to a lipid, contained as a suspension in a lipid or otherwise associated with a lipid. A lipid or lipid-poxvirus-associated composition of the present invention is not limited to any particular structure. For example, they may also simply be interspersed in a solution, possibly forming aggregates which are not uniform in either size or shape. In another example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. In another non-limiting example, a lipofectamine(Gibco BRL)-poxvirus or Superfect (Qiagen)-poxvirus complex is also contemplated.

In certain embodiments, a composition may comprise about 1%, about 2%, about 3%, about 4% about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or any range therebetween, of a particular lipid, lipid type, or non-lipid component such as an adjuvant, antigen, peptide, polypeptide, sugar, nucleic acid or other material disclosed herein or as would be known to one of skill in the art. In a non-limiting example, a composition may comprise about 10% to about 20% neutral lipids, and about 33% to about 34% of a cerebroside, and about 1% cholesterol. In another non-limiting example, a liposome may comprise about 4% to about 12% terpenes, wherein about 1% of the micelle is specifically lycopene, leaving about 3% to about 11% of the liposome as comprising other terpenes; and about 10% to about 35% phosphatidyl choline, and about 1% of a non-lipid component. Thus, it is contemplated that compositions of the present invention may comprise any of the lipids, lipid types or other components in any combination or percentage range.

C. Combination Therapy

The compositions and related methods of the present invention, particularly administration of a secreted virulence factor or surface protein, including a coagulase Domains 1-2 or a variant thereof, and/or other bacterial peptides or proteins to a patient/subject, may also be used in combination with the administration of traditional therapies. These include, but are not limited to, the administration of antibiotics such as streptomycin, ciprofloxacin, doxycycline, gentamycin, chloramphenicol, trimethoprim, sulfamethoxazole, ampicillin, tetracycline or various combinations of antibiotics.

In one aspect, it is contemplated that a polypeptide vaccine and/or therapy is used in conjunction with antibacterial treatment. Alternatively, the therapy may precede or follow the other agent treatment by intervals ranging from minutes to weeks. In embodiments where the other agents and/or a proteins or polynucleotides are administered separately, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the agent and antigenic composition would still be able to exert an advantageously combined effect on the subject. In such instances, it is contemplated that one may administer both modalities within about 12-24 h of each other or within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for administration significantly, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

Various combinations may be employed, for example antibiotic therapy is "A" and the immunogenic molecule given as part of an immune therapy regime, such as an antigen, is "B":

A/B/A B/A/B B/B/A A/A/B A/B/B B/A/A A/B/B/B B/A/B/B

B/B/B/A	B/B/A/B	A/A/B/B	A/B/A/B	A/B/B/A	B/B/A/A
B/A/B/A	B/A/A/B	A/A/A/B	B/A/A/A	A/B/A/A	A/A/B/A

Administration of the immunogenic compositions of the present invention to a patient/subject will follow general protocols for the administration of such compounds, taking into account the toxicity, if any, of the coagulase Domains 1-2 composition, or other compositions described herein. It is expected that the treatment cycles would be repeated as necessary. It also is contemplated that various standard therapies, such as hydration, may be applied in combination with the described therapy.

D. General Pharmaceutical Compositions

In some embodiments, pharmaceutical compositions are administered to a subject. Different aspects of the present invention involve administering an effective amount of a composition to a subject. In some embodiments of the present invention, staphylococcal antigens, members of the Ess pathway, including polypeptides or peptides of the Esa or Esx class, and/or members of sortase substrates may be administered to the patient to protect against infection by one or more staphylococcus pathogens. Alternatively, an expression vector encoding one or more such polypeptides or peptides may be given to a patient as a preventative treatment. Additionally, such compounds can be administered in combination with an antibiotic or an antibacterial. Such compositions will generally be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

In addition to the compounds formulated for parenteral administration, such as those for intravenous or intramuscular injection, other pharmaceutically acceptable forms include, *e.g.*, tablets or other solids for oral administration; time release capsules; and any other form currently used, including creams, lotions, mouthwashes, inhalants and the like.

The active compounds of the present invention can be formulated for parenteral administration, *e.g.*, formulated for injection via the intravenous, intramuscular, sub-cutaneous, or even intraperitoneal routes. The preparation of an aqueous composition that contains a compound or compounds that increase the expression of an MHC class I molecule will be known to those of skill in the art in light of the present disclosure. Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for use to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and, the preparations can also be emulsified.

Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil, or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that it may be easily injected. It also should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

The proteinaceous compositions may be formulated into a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

The carrier also can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques, which yield a powder of the active ingredient, plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Administration of the compositions according to the present invention will typically be via any common route. This includes, but is not limited to oral, nasal, or buccal administration. Alternatively, administration may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal, intranasal, or intravenous injection. In certain embodiments, a vaccine composition may be inhaled (*e.g.*, U.S. Patent 6,651,655). Such compositions would normally be administered as pharmaceutically acceptable compositions that include physiologically acceptable carriers, buffers or other excipients. As used herein, the term "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem complications commensurate with a reasonable benefit/risk ratio. The term "pharmaceutically acceptable carrier," means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a chemical agent.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous, and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in isotonic NaCl solution and either added to hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, Remington's Pharmaceutical Sciences, 1990). Some variation in dosage will necessarily occur depending on the condition of the subject. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

An effective amount of therapeutic or prophylactic composition is determined based on the intended goal. The term "unit dose" or "dosage" refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the composition calculated to produce the desired responses discussed above in association with its administration, *i.e.*, the appropriate route and regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the protection desired.

Precise amounts of the composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting dose include physical and clinical state of the subject, route of administration, intended goal of treatment (alleviation of symptoms versus cure), and potency, stability, and toxicity of the particular composition.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically or prophylactically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above.

E. *In Vitro*, *Ex Vivo*, or *In Vivo* Administration

As used herein, the term *in vitro* administration refers to manipulations performed on cells removed from or outside of a subject, including, but not limited to cells in culture. The term *ex vivo* administration refers to cells which have been manipulated *in vitro*, and are subsequently administered to a subject. The term *in vivo* administration includes all manipulations performed within a subject.

In certain aspects of the present invention, the compositions may be administered either *in vitro, ex vivo*, or *in vivo*. In certain *in vitro* embodiments, autologous B-lymphocyte cell lines are incubated with a virus vector of the instant invention for 24 to 48 hours or with a cogaulase Domains 1-2 and/or a variant thereof and/or any other composition described herein for two hours. The transduced cells can then be used for *in vitro* analysis, or alternatively for *ex vivo* administration. U.S. Patents 4,690,915 and 5,199,942, disclose methods for *ex vivo* manipulation of blood mononuclear cells and bone marrow cells for use in therapeutic applications.

F. Antibodies And Passive Immunization

Another aspect of the invention is a method of preparing an immunoglobulin for use in prevention or treatment of staphylococcal infection comprising the steps of immunizing a recipient or donor with the vaccine of the invention and isolating immunoglobulin from the recipient or donor. An immunoglobulin prepared by this method is a further aspect of the invention. A pharmaceutical composition comprising the immunoglobulin of the invention and a pharmaceutically acceptable carrier is a further aspect of the invention which could be used in the manufacture of a medicament for the treatment or prevention of staphylococcal disease. A method for treatment or prevention of staphylococcal infection comprising a step of administering to a patient an effective amount of the pharmaceutical preparation of the invention is a further aspect of the invention.

Inocula for polyclonal antibody production are typically prepared by dispersing the antigenic composition in a physiologically tolerable diluent such as saline or other adjuvants suitable for human use to form an aqueous composition. An immunostimulatory amount of inoculum is administered to a mammal and the inoculated mammal is then maintained for a time sufficient for the antigenic composition to induce protective antibodies.

The antibodies can be isolated to the extent desired by well known techniques such as affinity chromatography (Harlow and Lane, 1988). Antibodies can include antiserum preparations from a variety of commonly used animals, *e.g.* goats, primates, donkeys, swine, horses, guinea pigs, rats or man.

An immunoglobulin produced in accordance with the present invention can include whole antibodies, antibody fragments or subfragments. Antibodies can be whole immunoglobulins of any class (*e.g.,* IgG, IgM, IgA, IgD or IgE), chimeric antibodies or hybrid antibodies with dual specificity to two or more antigens of the invention. They may also be fragments (*e.g.*, F(ab')2, Fab', Fab, Fv and the like) including hybrid fragments. An immunoglobulin also includes natural, synthetic, or genetically engineered proteins that act like an antibody by binding to specific antigens to form a complex.

Date Reçue/Date Received 2021-01-25

A vaccine of the present invention can be administered to a recipient who then acts as a source of immunoglobulin, produced in response to challenge from the specific vaccine. A subject thus treated would donate plasma from which hyperimmune globulin would be obtained via conventional plasma fractionation methodology. The hyperimmune globulin would be administered to another subject in order to impart resistance against or treat staphylococcal infection. Hyperimmune globulins of the invention are particularly useful for treatment or prevention of staphylococcal disease in infants, immune compromised individuals, or where treatment is required and there is no time for the individual to produce antibodies in response to vaccination.

An additional aspect of the invention is a pharmaceutical composition comprising two of more monoclonal antibodies (or fragments thereof; preferably human or humanised) reactive against at least two constituents of the immunogenic composition of the invention, which could be used to treat or prevent infection by Gram positive bacteria, preferably staphylococci, more preferably *S. aureus* or *S. epidermidis*. Such pharmaceutical compositions comprise monoclonal antibodies that can be whole immunoglobulins of any class, chimeric antibodies, or hybrid antibodies with specificity to two or more antigens of the invention. They may also be fragments (*e.g.*, F(ab')2, Fab', Fab, Fv and the like) including hybrid fragments.

Methods of making monoclonal antibodies are well known in the art and can include the fusion of splenocytes with myeloma cells (Kohler and Milstein, 1975; Harlow and Lane, 1988). Alternatively, monoclonal Fv fragments can be obtained by screening a suitable phage display library (Vaughan *et al.*, 1998). Monoclonal antibodies may be humanized or part humanized by known methods.

VI. EXAMPLES

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

EXAMPLE 1

COAGULASES AS DETERMINANTS OF PROTECTIVE IMMUNE RESPONSES AGAINST *STAPHYLOCOCCUS AUREUS*

A. RESULTS

Antibodies against coagulase domains Rabbits were immunized with affinity purified His-tagged Coa derived from the coagulase gene of *S. aureus* Newman (Coa_{NM}). Immune serum was examined by ELISA, which revealed serum IgG antibody responses to antigen (Figs. 1A-1B). To analyze the antibody responses against specific subdomains, affinity-purified recombinant proteins ($D1_{Coa}$, $D2_{Coa}$, $D12_{Coa}$, L_{Coa} and CT_{Coa}) were subjected to ELISA (Fig. 1B). Immune serum harbored antibodies against each of the domains tested (Fig. 1B). Of note, antibodies against L_{Coa} were more abundant than antibodies that recognized the repeat domain (CT_{Coa}) (L_{Coa} vs. CT_{Coa}, $P<0.05$). Antibodies against $D12_{Coa}$ were more abundant than those that recognized the repeat domain, but this difference did not achieve statistical significance ($D12_{Coa}$ vs. CT_{Coa}, $P=0.066$). To probe the biological function of antibodies in the immune serum, the inventors used variable amounts of affinity purified Coa_{NM} antibodies to perturb the association of $D12_{Coa}$ with human prothrombin or the association of CT_{Coa} with fibrinogen (Fig. 1C). The inventors calculated that 120 nM α-Coa IgG blocked $D12_{Coa}$ binding to prothrombin, whereas 1.7 μM α-Coa IgG blocked the association of CT_{Coa} with fibrinogen (Fig. 1C).

Rabbit Coa_{NM} immune serum was subjected to affinity chromatography using either full length Coa_{NM} (α-Coa_{NM}), $D12_{Coa}$ (α-$D12_{Coa}$) or CT_{Coa} (α-CT_{Coa}). Equimolar amounts of affinity purified IgG were added to citrate-blood samples obtained from naïve BALB/c mice, which were subsequently inoculated with *S. aureus* CC8 strain Newman (Baba 2007). Compared to control samples without antibody, both α-Coa_{NM} and α-$D12_{Coa}$ IgG caused a significant delay in clotting time, whereas α-CT_{Coa} did not (Fig. 1D). Thus, rabbits respond to immunization with Coa_{NM} by generating antigen-specific IgG molecules that are predominantly directed against $D12_{Coa}$ and L_{Coa} and interfere with the clotting activity of secreted Coa. In contrast, antibodies against CT_{Coa} are generated in lesser abundance and do not interfere with *S. aureus* Newman *in vitro* coagulation of blood.

Type-specific and cross-protective inhibition of *S. aureus* coagulation To examine the ability of α-Coa_{NM} to block the coagulation of other strains isolated from human infections, antigen-specific IgG was added to citrate-blood samples from naive mice that were subsequently inoculated with *S. aureus* 85/2082 (CC8), MW2 (CC1), MSSA476 (CC1), N315 (CC5), Mu50 (CC5), MRSA252 (CC30), CowanI (CC30), WIS (CC45) and USA600 (CC45) (Table 4). Coa_{NM}-specific IgG delayed the clotting of *S. aureus* Newman (CC8), 85/2082 (CC8) and MW2 (CC1), but not of MSSA476 (CC1), N315 (CC5), Mu50 (CC1), MRSA252 (CC30), Cowan (CC3), WIS (CC45) and USA600 (CC45) (Table 4). These results suggested that antibodies against Coa_{NM} interfere not only with the coagulation of *S. aureus* strains from the same CC type (or Coa-type), but that they may also interfere with the coagulation of strains from other types (MW2 and MSSA476). The observed pattern of cross-protection is not universal, as strains from the same MLST (or Coa-type) were not affected for coagulation by antibodies against Coa_{NM}. To examine the generality of type-specific and cross-protective inhibition, $Coa_{85/2082}$, Coa_{MW2}, Coa_{N315}, $Coa_{MRSA252}$ and Coa_{WIS} were purified and rabbit immune sera were generated (Table 4). $Coa_{85/2082}$-specific IgG inhibited the coagulation of *S. aureus* Newman (CC8) and 85/2082 (CC8) and, to a lesser degree, that of N315 (CC5) and Mu50 (CC5). Antibodies directed against Coa_{N315} inhibited the clotting of *S. aureus* N315 (CC5), Mu50 (CC5), Newman (CC8) and 85/2082 (CC8) as well as MRSA252 (CC30); however, these antibodies did not affect the coagulation of *S. aureus* CowanI (the other CC30 isolate) or of CC1 and CC45 strains. Antibodies against $Coa_{MRSA252}$ inhibited clotting of *S. aureus* CC1 and CC5 strains but did not affect the clotting of the CC30 or CC45 isolates. Antibodies against the CC45 isolate (WIS) inhibited clotting of *S. aureus* CC1 strains but did not affect the clotting of CC1, CC5, CC30, or CC45 strains. In summary, coagulation of mouse blood by *S. aureus* strains was invariably inhibited by antibodies raised against the corresponding Coa (CC8, CC5, CC1 and CC30 isolates). Cross-neutralization of coagulation is observed for antibodies directed against the two coagulases from CC8 strains and for one each of the coagulase of CC1 and CC5 strains. Finally, antibodies directed against Coa from the CC1, CC5, CC8, CC30 and CC45 strains did not neutralize the clotting of *S. aureus* CC45 strains or of CowanI (CC30). We presume that blood clotting in these isolates may be dependent on another factor, for example vWbp (*vide infra*).

Table 4. Type-specific or cross-protective inhibition of staphylococcal coagulation by Coa antibodies

	Coa-specific antibodies raised against coagulases from different *S. aureus* strains

S. aureus	CC type	α-Coa_{Newman}	α-$Coa_{85/2082}$	α-Coa_{MW2}	α-Coa_{N315}	α-$Coa_{MRSA252}$	α-Coa_{WIS}
Newman	8	1.7	1.5	1.7	1.9	1.7	1.7
85/2082	8	1.5	1.8	1.3	1.5	1.6	1.4
MW2	1	1.2	1.1	1.1	0.8	1.1	1.0
MSSA476	1	1.0	1.1	1.2	0.9	1.4	1.2
N315	5	1.1	1.2	1.3	1.2	1.3	1.2
Mu50	5	1.0	1.2	1.2	1.2	1.1	0.9
MRSA252	30	0.9	1.2	1.2	1.3	1.0	0.9
CowanI	30	0.9	1.0	1.0	0.9	1.0	0.8
WIS	45	1.1	1.2	1.2	0.8	1.2	0.9
USA600	45	0.8	1.0	1.2	1.2	0.8	0.8

Coagulase antibodies and their protective effect on staphylococcal disease Purified Coa_{NM}, $D12_{Coa}$ or CT_{Coa} were emulsified and injected as a prime-booster regimen into BALB/c mice (n=10). Sera of mock (PBS) or Coa_{NM}, $D12_{Coa}$ and CT_{Coa} immunized animals were examined by ELISA for IgG responses to antigen, revealing specific immune responses in vaccinated animals but not in control mice (Figs. 2A-2B). Of note, immunization of mice with Coa_{NM} raised predominantly antibodies against $D12_{Coa}$ and, to a lesser degree, antibodies that were directed against CT_{Coa} (Fig. 2A). $D12_{Coa}$ immunization raised high titer antibodies that reacted with full length Coa_{NM} (Fig. 26A). In contrast, CT_{Coa} immunization generated weak antibody responses (Fig. 2A). Mice were challenged by intravenous injection with *S. aureus* Newman and a 10-day observation period was used to assess protection against lethal sepsis (Fig. 2B). As compared to mock immunized animals, vaccination with Coa_{NM}, $D12_{Coa}$ or CT_{Coa} resulted in increased time-to-death (Coa_{NM} vs. PBS, $P<0.001$; $D12_{Coa}$ vs. PBS, $P<0.01$; CT_{Coa} vs. PBS, $P<0.05$). Immune responses against Coa_{NM} did not significantly outperform vaccination with either $D12_{Coa}$ or CT_{Coa} in generating protection against lethal *S. aureus* challenge (Coa_{NM} vs. CT_{Coa}, $P>0.05$; $D12_{Coa}$ vs. CT_{Coa}, $P>0.05$).

Whether antibodies directed against $D12_{Coa}$ or CT_{Coa} provide protection against *S. aureus* lethal challenge was tested. Affinity purified rabbit IgG was injected into the peritoneal cavity of naïve BALB/c mice at a concentration of 5 mg/kg body weight (Fig. 2C). Twenty-four hours later, animals were challenged by intravenous injection of *S. aureus* Newman (Fig. 2C). As compared to control antibodies [IgG (α-V10) specific for the V10 plague protective antigen (DeBord 2006)], IgG directed against Coa_{NM}, $D12_{Coa}$ or CT_{Coa} each caused a delay in time-to-death for the corresponding cohort of challenged animals (all vaccines vs. PBS, $P<0.05$)(Fig. 2C). No significant differences in disease protection were detected between antibodies directed against $D12_{Coa}$, CT_{Coa} or full length Coa_{NM} (Fig. 2C). Thus, when compared to $D12_{Coa}$ and L_{Coa}, immunization with the CT_{Coa} domain elicits low antibody responses, however passive transfer of antibodies against $D12_{Coa}$ and CT_{Coa} provide similar levels of protection against *S. aureus* Newman lethal challenge. These data suggest that antibody-mediated neutralization of *S. aureus* Newman coagulase activity is not a prerequisite for disease protection. Following exposure to full length Coa_{NM}, BALB/c mice mount robust immune responses against $D12_{Coa}$ and L_{Coa}, but generate few antibodies against CT_{Coa}.

Antibodies against von-Willebrand-Factor-binding-protein domains Rabbits were immunized with affinity purified His-tagged vWbp derived from the *vwb* gene of *S. aureus* Newman ($vWbp_{NM}$). Immune serum was examined by ELISA, which revealed serum IgG antibody responses to antigen (Figs. 3A-3B). To analyze the antibody responses against specific subdomains, affinity-purified $D1_{vWbp}$, $D2_{vWbp}$, $D12_{vWbp}$, L_{vWbp} and CT_{vWbp} were subjected to ELISA (Fig. 3B). Immune serum harbored antibodies against each of the subdomains tested (Fig. 3B). Of note, antibodies against the $D1_{vWbp}$ and $D2_{vWbp}$ and were less abundant than antibodies that recognized these two domains together ($D12_{vWbp}$). Compared with immune responses against $D12_{vWbp}$, antibodies directed against the CT_{vWbp} were 30% less abundant ($D12_{vWbp}$ vs. CT_{vWbp}, $P>0.05$). To probe the biological function of antibodies in the immune serum, the inventors used variable amounts of $vWbp_{NM}$-specific IgG to perturb the association of $D12_{vWbp}$ with human prothrombin and the association of CT_{vWbp} with fibrinogen (Figs. 3C-3D). The inventors calculated that 1.3 μM α-vWbp IgG blocked $D12_{vWbp}$ binding to prothrombin, whereas 1.3 μM α-vWbp IgG blocked the association of CT_{vWbp} with fibrinogen (Fig. 3D).

Equimolar amounts of affinity purified IgG were added to citrate-blood samples obtained from naïve BALB/c mice, which were subsequently inoculated with a *coa* mutant derived from *S. aureus* Newman (Cheng 2010). Compared to control samples without antibody, both α-vWbp and α-$D12_{vWbp}$ caused small delays in clotting time, whereas α–CT_{vWbp} did not delay clotting time (Fig. 3D). Thus, rabbits respond to immunization with $vWbp_{NM}$ by generating antigen-specific IgG molecules that are directed against $D12_{vWbp}$, L_{vWbp}, and CT_{vWbp}. Antibodies against $D12_{vWbp}$ interfere with vWbp-mediated coagulation of mouse blood *in vitro*.

Antibodies against vWbp domains and their protective effect on staphylococcal disease Purified $vWbp_{NM}$, $D12_{vWbp}$ or CT_{vWbp} were emulsified and injected as a prime-booster regimen into BALB/c mice (n=10). Sera of mock (PBS) or $vWbp_{NM}$, $D12_{vWbp}$ and CT_{vWbp} immunized animals were examined by ELISA for IgG responses to antigen, revealing specific immune responses in vaccinated animals but not in control mice (Figs. 4A-4B). Of note, immunization of mice with $vWbp_{NM}$ raised predominantly antibodies against $D12_{vWbp}$ and, to a lesser degree, antibodies that were directed against CT_{vWbp} (Fig. 4A). $D12_{vWbp}$ immunization raised high titer antibodies that reacted with full length $vWbp_{NM}$ (Fig. 4A). In contrast, CT_{vWbp} immunization generated weak antibody responses (Fig. 28A). Mice were challenged by intravenous injection with *S. aureus* Newman and a 10 day observation period was used to assess protection against lethal sepsis (Fig. 4B). As compared to mock immunized animals, vaccination with $vWbp_{NM}$, $D12_{vWbp}$ or CT_{vWbp} resulted in increased time-to-death ($vWbp_{NM}$ vs. PBS, $P<0.01$; $D12_{vWbp}$ vs. PBS, $P<0.05$; CT_{vWbp} vs. PBS, $P<0.05$). Immune responses against $vWbp_{NM}$ outperformed vaccination with $D12_{vWbp}$ but not CT_{vWbp} in generating protection against lethal *S. aureus* challenge ($vWbp_{NM}$ vs. $D12_{vWbp}$, $P<0.05$; $vWbp_{NM}$ vs. CT_{vWbp}, $P>0.05$)(Fig. 4B).

Whether antibodies directed against $D12_{vWbp}$ or CT_{vWbp} provide protection against *S. aureus* lethal challenge were examined. Affinity purified rabbit IgG was injected into the peritoneal cavity of naïve BALB/c mice at a concentration of 5 mg/kg body weight (Fig. 4C). Twenty-four hours later, animals were challenged by intravenous injection of *S. aureus* Newman (Fig. 4C). As compared to control antibodies (α-V10), IgG directed against $vWbp_{NM}$, $D12_{vWbp}$ or CT_{vWbp} each caused a delay in time-to-death for the corresponding cohort of challenged animals (all vaccines vs. PBS, $P<0.05$)(Fig. 4C). No significant differences in disease protection were detected between antibodies directed against $D12_{vWbp}$, CT_{vWbp} or full length $vWbp_{NM}$ (Fig. 4C). Thus, in contrast to $D12_{vWbp}$, immunization with the CT_{vWbp} domain elicits low antibody responses. Passive transfer of antibodies against $D12_{vWbp}$ and CT_{vWbp} provide similar levels of protection against *S. aureus* Newman lethal challenge. These data suggest that antibody mediated neutralization of *S. aureus* Newman vWbp, which can occur by antibodies directed against either $D12_{vWbp}$ or CT_{vWbp}, correlates with disease protection. Following exposure to full length $vWbp_{NM}$, BALB/c mice mount robust immune responses against $D12_{vWbp}$ and L_{vWbp}, but generate few antibodies against CT_{vWbp}.

Cross-protective attributes of the Coa_{NM}/$vWbp_{NM}$ vaccine Purified recombinant Coa_{NM} and $vWbp_{NM}$ were emulsified and injected into BALB/c mice (n=10) as a prime-booster immunization regimen. Sera of mock (PBS) and Coa_{NM} /$vWbp_{NM}$ immunized animals were examined by ELISA for IgG responses to Coa_{NM} as well as $vWbp_{NM}$, which revealed antigen-specific immune responses in vaccinated but not in control mice (Fig. 5A). Intravenous injection of mice with *S. aureus* and a 10 day observation period were used to assess vaccine protection against lethal challenge with various strains (Fig. 5). As a control, Coa_{NM} /$vWbp_{NM}$ immunization raised protection against *S. aureus* Newman (CC8) (Cheng 2010) (data not shown) and USA300 (CC8), but not against MW2 (CC1) or N315 (CC5) (Figs. 5B-5D). Nevertheless, Coa_{NM} /$vWbp_{NM}$ immunization generated protection against challenge with *S. aureus* CowanI (CC30) and WIS (CC45). Taken together, these data indicate that the Coa_{NM} /$vWbp_{NM}$ vaccine provided type-specific immunity as well as cross-protection against some, but not all, coagulase type strains (Figs. 5E-5F).

Immune responses elicited by the Coa_4/$vWbp_2$ vaccine The engineered polypeptide Coa_4 harbors the D12 domains of $Coa_{MRSA252}$, Coa_{MW2}, Coa_{N315} and full length Coa_{USA300} in addition to N-terminal His_6 and C-terminal STREP tags (Fig. 6A). Coa_4 was purified by affinity chromatography on StrepTactin-sepharose (Fig. 6B). When analyzed by Coomassie-stained SDS-PAGE, affinity purified Coa_4 was revealed as a 190 kDa polypeptide (Fig. 30B). Coa_4 encompasses the D12 domains from the most frequent coagulase-type *S. aureus* isolates from North American patients (CC1, CC5, CC8, CC30, CC45) (DeLeo 2010). The $vWbp_2$ polypeptide encompasses the D12 domain of $vWbp_{N315}$ and full length $vWbp_{USA300}$ in addition to N-terminal His_6 and C-terminal STREP tags (Fig. 6A). $vWbp_2$ was purified by affinity chromatography, which yielded a polypeptide migrating with the expected mass of 85 kDa on Coomassie-stained SDS-PAGE (Fig. 6B). Mice (n=5) were immunized with a prime-booster regimen of Coa_{NM}/$vWbp_{NM}$ or Coa_4/$vWbp_2$ and immune responses to various coagulase and von-Willebrand-Factor-binding protein types were examined by ELISA (Figs. 6C-6D). Coa_{NM}/$vWbp_{NM}$ vaccine raised antibodies in mice that bound to the coagulases from CC8 strains but displayed little cross-reactivity towards Coa_{N315}, $Coa_{MRSA252}$, Coa_{MW2} or Coa_{WIS}. By comparison, Coa_4 immunization raised higher titer antibodies not only against CC8 type coagulases, but also against the coagulases from CC1, CC5, CC30 and CC45 strains. As compared to $vWbp_{NM}$, $vWbp_2$ raised high titer antibodies against vWbp of CC5 and CC8 strains (Fig. 6D).

Cross-protective attributes of the Coa_4/$vWbp_2$ vaccine Purified recombinant Coa_4/$vWbp_2$ was emulsified and injected into BALB/c mice (n=10) using a prime-booster immunization regimen. Sera of mock (PBS) and Coa_4/$vWbp_2$ immunized animals were examined by ELISA for IgG responses to Coa_4 as well as $vWbp_2$, which revealed antigen-specific immune responses in vaccinated but not in control mice (Fig. 7A). Intravenous injection of mice with *S. aureus* and a 10 day observation period were used to assess vaccine protection against lethal challenge with various strains (Fig. 7). As expected, Coa_4/$vWbp_2$ immunization raised protection against *S. aureus* CC8 strain USA300 (Cheng 2010). Similar to Coa_{NM}/$vWbp_{NM}$ immunization, Coa_4/$vWbp_2$ vaccine raised protection against S. aureus CowanI (CC30) and WIS (CC45) challenge. Unlike Coa_{NM}/$vWbp_{NM}$, Coa_4/$vWbp_2$ protected mice against lethal challenge with either *S. aureus* N315 (CC5) or MW2 (CC1) (Figs. 7B-7D). Taken together, these data indicate that the Coa_{NM} /$vWbp_{NM}$ vaccine provided type-specific immunity as well as cross-protection against some, but not all, coagulase type strains (Figs. 7E-7F). Further, Coa_4/$vWbp_2$ vaccine protected animals against a challenge with the relevant *S. aureus* CC types isolated from North American patients with staphylococcal disease.

The inventors also examined whether Coa_4/$vWbp_2$ immunization can protect mice against staphylococcal abscess formation. BALB/c mice were immunized with a prime-booster regimen of Coa_4/$vWbp_2$ or mock control and challenged by intravenous inoculation of a sublethal dose of *S. aureus* strains USA300, N315, MW2 or CowanI. Five days after challenge, animals were euthanized, necropsied and kidneys removed. The tissues for one of the two kidneys from each mouse were fixed, thin-sectioned and stained with hematoxylin/eosin for subsequent histopathology analysis (Table 5). Tissues of the other kidneys were homogenized and spread on agar plates to enumerate the staphylococcal load as colony forming units (Table 5). Coa_4/$vWbp_2$ immunization affected the bacterial load in renal tissues of mice infected with various *S. aureus* strains, leading to a significant reduction for *S. aureus* MW2 and CowanI, but not for USA300 and N315. This is an expected result, as Coa- or vWbp-specific antibodies do not promote opsonophagocytic killing of bacteria, but interfere with staphylococcal abscess formation, thereby reducing the ability of staphylococci to replicate within the protective environment of these lesions (Cheng 2010). As compared to mock-immunized animals, Coa_4/$vWbp_2$ immunization reduced staphylococcal abscess formation in renal tissues five days following challenge with the *S. aureus* strains USA300, CowanI, MW2 or N315 (Table 5).

Table 5. Active immunization of mice with $Coa_4/vWbp_2$ and protection against challenge with *S. aureus* strains USA300, N315, MW2, or CowanI

Vaccine	Staphylococcal load in renal tissue			Abscess formation	
	[a]$\log_{10}$ CFU·g^{-1} (SEM)	[b]Significance (P value)	[c]Reduction ($\log_{10}$ CFU·g^{-1})	[d]Number of lesions	[e]Significance (P value)
S. aureus USA300					
Mock	7.31 (0.37)	-	-	8.8 (1.72)	-
$Coa_4/vWbp_2$	6.48 (0.41)	0.150	0.835	4.3 (1.11)	0.0434
S. aureus N315					
Mock	7.25 (0.13)	-	-	16.6 (1.49)	-
$Coa_4/vWbp_2$	7.10 (0.24)	0.805	0.151	11.3 (0.84)	0.0205
S. aureus MW2					
Mock	8.04 (0.25)	-	-	66.5 (8.41)	-
$Coa_4/vWbp_2$	7.25 (0.20)	0.029	0.789	27.5 (4.39)	0.0011
S. aureus CowanI					
Mock	6.94 (0.16)	-	-	7.9 (1.27)	-
$Coa_4/vWbp_2$	5.59 (0.51)	0.028	1.35	4.6 (0.73)	0.0279

Early work on coagulase demonstrated that, following *S. aureus* infection, humans as well as animals generate Coa-specific antibodies (Tager 1948; Lominski 1946). When transferred to naïve rabbits, these antibodies may neutralize *S. aureus* coagulation and, at least in some cases, may confer immunity to challenge with *S. aureus* (Lominski 1949; Lominski 1962). Active immunization of rabbits with preparations containing coagulase could prolong the life of rabbits that had been challenged by intravenous inoculation with lethal doses of *S. aureus* (Boake 1956). Comparison of different (phage-typed) *S. aureus* isolates for inhibition of plasma clotting by coagulase-antiserum revealed both phage type-specific and non-specific neutralization (Lominski 1946; Lominski 1962; Rammelkamp 1950; Duthie 1952; Harrison 1964). These data supported a general concept for the existence of serological types of Coa, which are not strictly linked to *S. aureus* phage-types (Rammelkamp 1956).

Purified coagulase toxoid, encompassing purified Coa from *S. aureus* strains M1 and Newman adsorbed to aluminum phosphate, was examined for therapeutic immunization of 71 patients with chronic furunculosis (Harrison 1963). As compared to placebo, coagulase immunization generated a rise in coagulase-specific antibody titers but failed to improve the clinical outcome of chronic furunculosis (Harrison 1963). Of note, the development of neutralizing antibodies or the possibility of type-specific immunity were not examined (Harrison 1963). Thus, although early work revealed preclinical efficacy of coagulase subunit vaccines, clinical studies failed to demonstrate efficacy in a human trial. As most of these studies were conducted from 1945-1965, one must consider the limited tools for the isolation of highly purified coagulases as well as the inability to type *S. aureus* strains or coagulase vaccine preparations on the basis of their nucleotide sequence. Further, earlier studies were conducted without knowledge of vWbp or of the molecular mechanisms of Coa- and vWbp-mediated prothrombin activation and fibrinogen cleavage (Friedrich 2003; Kroh 2009). We recently observed that both coagulases secreted by *S. aureus* Newman, Coa_{NM} and $vWbp_{NM}$, are sufficient for the ability of this strain to cause abscess formation and rapidly lethal bacteremia in mice (Cheng 2010). In active and passive immunization experiments, antibodies against both Coa_{NM} and $vWbp_{NM}$ were required to confer protection against abscess formation or lethal bacteremia (Cheng 2010). On the basis of these observations, we hypothesize that coagulases may function as protective antigens that elicit antibody responses against Coa and vWbp, which protect animals and humans against *S. aureus* disease (Cheng 2010). In agreement with this model, expression of *coa* and *vwb* is a universal trait of *S. aureus* strains (Cheng 2011). Of note, the *coa* gene of *S. aureus* isolates is variable (McCarthy 2010), with greater variation in amino acid sequence than even the tandem repeats of the protein A (*spa*) gene; the variation in *spa* is used for epidemiological typing experiments (Watanabe 2009; Koreen 2004). *S. aureus* mutants that are unable to express *coa* have not yet been isolated from humans with manifest staphylococcal disease. The *vwb* gene is less variable (McCarthy 2010). Analyzing currently available *S. aureus* genome sequences for *vwb* homology, we identified three alleles. Two of the *vwb* alleles varied in their coding sequence for the D12 domain (*S. aureus* N315 and USA300 are representatives for these alleles), whereas the third allele harbored a nucleotide deletion in codon 102, creating a frameshift that results in a nonsense mutation in codon 107 (*S. aureus* MRSA252).

Enabled by these observations, we report here that Coa and vWbp immunization of rabbits or mice generated predominantly antibodies against the D12 domain of Coa_{NM} or $vWbp_{NM}$. D12-specific antibodies neutralized the coagulase activities of *S. aureus* Newman and, when transferred to naïve animals, conferred protection against lethal bacteremia. Neutralization and disease protection of Coa_{NM}- and $vWbp_{NM}$-specific antibodies occurred in a type-specific manner, not unlike the type-specific immunity reported for *Streptococcus pyogenes* M proteins (Lancefield 1928; Lancefield 1962) or the pilus (T) antigens of *S. pyogenes* and *Streptococcus agalactiae* (Mora 2005; Nuccitelli 2011). Informed by the structural vaccinology approach for pilus antigens (Nuccitelli 2011; Schneewind 2011), we engineered two polypeptides that encompasses the D12 domains of the major Coa and vWbp types from the North American *S. aureus* isolates: CC1, CC5, CC8, CC30 and CC45 strains (Tenover 2012). The purified products, Coa_4 and $vWbp_2$, were used as antigens and elicited antibody responses against the D12 domains of every Coa and vWbp type examined. Immunization of mice with Coa_4/$vWbp_2$ provided protection against lethal bacteremia challenge with representative *S. aureus* CC1, CC5, CC8, CC30 and CC45 strains. Thus, the design criteria of the Coa_4/$vWbp_2$ vaccine, to generate universal immune responses against Coa and vWbp against clinically relevant *S. aureus*, have been met.

In addition to type-specific neutralization of Coa and vWbp via antibodies directed against the D12 domain, antibodies against the R (Coa) and CT domains (vWbp) also provided protection against *S. aureus* disease. As antibodies against the R and CT domains do not affect coagulation of fibrin via secreted Coa·prothrombin and vWbp·prothrombin complexes, we surmise that these adaptive immune mechanisms target coagulases via another mechanism. We currently do not appreciate how antibodies against the R domain of Coa or the CT domain of vWbp provide protection. It seems plausible that these antibodies may mediate Coa and vWbp removal from circulation via the binding to of immune complexes to Fc receptors on macrophages. Until the molecular mechanism of protection is revealed, the overall value of a vaccine strategy that targets the R and CT domains of Coa and vWbp cannot be appreciated.

B. MATERIALS AND METHODS

Bacterial strains and growth of cultures *S. aureus* strains were cultured on tryptic soy agar or broth at 37°C. *E. coli* strains DH5α and BL21 (DE3) were cultured on Luria Bertani agar or broth at 37°C. Ampicillin (100 μg/mL) was used for pET15b and pGEX2tk selection. Primers used for the amplification of staphylococcal DNA are found in Table 6.

Table 6. Primers used

Primer name	Sequence
F-N315coa	CGCGGATCCATAGTAACAAAGGATTATAGTAAAGAATCAAG (SEQ ID NO: 1)
R-N315coa	TCCCCCGGGTTATTTTGTTACTCTAGGCCCATA (SEQ ID NO: 2)
R-MW2coa	CGCGGATCCATAGTAACAAAGGATTATAGTGGGAAA (SEQ ID NO: 3)
R-MW2coa	TCCCCCGGGTTATTTTGTTACTCTAGGCCCATA (SEQ ID NO: 4)

F-M252coa	CGCGGATCCATAGTAACTAAAGATTATAGTAAAGAATCAAGAG (SEQ ID NO: 5)
R-M252coa	TCCCCCGGGTTATTTTGTTACTCTAGGACCATATGTC (SEQ ID NO: 6)
F-U300coa	CGCGGATCCATAGTAACAAAGGATTATAGTGGGAAAT (SEQ ID NO: 7)
R-U300coa	TCCCCCGGGTTATTTTGTTACTCTAGGCCCATA (SEQ ID NO: 8)
F-WIScoa	CGCGGATCCATAGTAACAAAGGATTATAGTGGGAAAT (SEQ ID NO: 9)
R-WIScoa	TCCCCCGGGTTATTTTGTTACTCTAGGACCATATGTC (SEQ ID NO: 10)
F-85coa	CGCGGATCCATAGTAACTAAAGATTATAGTAAAGAATCAAGAG (SEQ ID NO: 11)
R-85coa	TCCCCCGGGTTATTTTGTTACTCTAGGACCATATGTC (SEQ ID NO: 12)
F-VUSA300FL-XhoI	CCGCTCGAGGTGGTTTCTGGGGAGAAG (SEQ ID NO: 13)
R-VUSA300FL-BamHI	CGGGATCCTTATTTGCCATTATATACTTTATTGATTT (SEQ ID NO: 14)
F-VN315FL-XhoI	CCGCTCGAGGTGGTTTCTGGGGAGAAG (SEQ ID NO: 15)
R-VN315FL-BamHI	CGGGATCCTTATTTGCCATTGTATACTTTATTG (SEQ ID NO: 16)
F-CUSA300-NcoI	CATGCCATGGCCTAGGATAGTAACAAAGGATTATAGTGGGAAAT (SEQ ID NO: 17)
R-CUSA300-	CGGGATCCTTATTTTGTTACTCTAGGCCCATA (SEQ ID NO: 18)

BamHI	
F-CN315-NcoI	CATGCCATGGCTCGAGATAGTAACAAAGGATTATAGTAAAGAATC (SEQ ID NO: 19)
R-CN315-AvrII	CCTAGGCGGACCATATTGAGAAGC (SEQ ID NO: 20)
F-CMW2-NcoI	CATGCCATGGCCGCGGATAGTAACAAAGGATTATAGTGGGAAA (SEQ ID NO: 21)
R-CMW2-XhoI	GGCTCGAGTTTTTTGACAGTTTTATTTTTCCA (SEQ ID NO: 22)
F-CMRSA-NcoI	CATGCCATGGCCCGGGATAGTAACTAAAGATTATAGTAAAGAATCAAGAG (SEQ ID NO: 23)
R-CMRSA-SacII	TCCCCGCGGATTTTTGACGGTTCTTGTTTTCCAAGATT (SEQ ID NO: 24)
F-VUSA300-NcoI	CATGCCATGGCCTAGGGTGGTTTCTGGGGAGAAG (SEQ ID NO: 25)
R-VUSA300-BamHI	CGGGATCCTTATTTGCCATTATATACTTTATTGATTT (SEQ ID NO: 26)
F-VN315-NcoI	CATGCCATGGCTCGAGGTGGTTTCTGGGGAGAAG (SEQ ID NO: 27)
R-VN315-AvrII	CCTAGGTGTATTGTTAAAGTCCTTTAAATCAC (SEQ ID NO: 28)
F-His-CMRSA	CATGCCATGGGCAGCAGCCATCATCATCATCATCACAGCAGCATAGTAACTAAAGATTATAGTAAAGAATCAAGAG (SEQ ID NO: 29)
F-His-VN315	CATGCCATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGTGGTTTCTGGGGAGAAG (SEQ ID NO: 30)
R-USA300CoaStrep	CGGGATCCTTACTTCTCAAATTGAGGATGAGACCATTTTGTTACTCTAGGCCCATA (SEQ ID NO: 31)

R-USA300vwbStrep	CGGGATCCTTACTTCTCAAATTGAGGATGAGACCATTTGCCATTATATACTTTATTGATTT (SEQ ID NO: 32)

Coa_4 and $vWbp_2$ To generate the hybrid proteins, *coa* and *vwb* from strain USA300 were PCR amplified. The 5' primer included the restriction site (NcoI) to insert onto the vector (pET15b) as well as an additional restriction enzyme (AvrII) for future use. The 3' primer included the restriction site (BamHI) for vector insertion. The inserts were cloned into *E. coli* strain DH5α. In each subsequent cloning round, the D12 from the next allele was added to the vector 5' to the previous insert. In each case, the 5' primer included the vector site (NcoI) and an additional restriction enzyme site for future use. The 3' primer for each sequential insert contained the restriction site (AvrII for N315) included in the 5' primer for the previous insert. The promoter region and His tag was restored in a subsequent round of cloning, and a C-terminal STREP tag was added in another round of cloning. The entire vector was sequenced to verify DNA sequence quality. Finally, each vector was transformed into *E. coli* strain BL21 for protein expression and purification.

Protein purification *E. coli* BL21(DE3) harboring expression vectors containing *coa* from *S. aureus* Newman; *vwb* from *S. aureus* strains Newman, USA3000, and N315; or the subdomains of *coa* and *vwb*; and expression vectors containing the genetic sequence for the hybrid proteins Coa_4 and $vWbp_2$, were grown at 37°C and induced with 100 mM IPTG overnight at room temperature. Because of degradation during the purification of Coa, pGEX2tk expression vectors in *E. coli* DH5α were used to express *coa* from USA300, N315, MW2, MRSA252, 85/2082, and WIS as GST-tagged constructs. Three hours following induction, cells were centrifuged at 7,000 ×*g*, suspended in 1 × column buffer (0.1 M Tris-HCl, pH 7.5, 0.5 M NaCl) and lysed in a French pressure cell at 14,000 lb/in^2. Lysates were subjected to ultracentrifugation at 40,000 ×*g* for 30 min. The supernatant of pET15b constructs was subjected to Ni-NTA chromatography, washed with column buffer and 10 mM imidazole, and eluted with 500 mM imidazole. For strep-tagged proteins, lysate supernatants were subjected to chromatography over StrepTactin Sepharose (GE Healthcare), washed in 1× strep wash buffer (0.1 M Tris-HCl, pH 8, 0.150 M NaCl, 0.1 M EDTA), and eluted in 1× strep wash buffer containing 2.5 mM desthiobiotin. For GST-tagged proteins, the supernatant of cleared lysates was subjected to glutathione-sepharose chromatography. To remove the GST tag, following washing with column buffer, the column buffer was switched to PreScission protease cleavage buffer containing 10 mM DTT, and the column was incubated with PreScission protease (GE Healthcare) overnight at the unit definition provided by GE. Liberated protein lacking the GST tag was then collected with additional protease cleavage buffer. Eluates were dialyzed against PBS. To remove endotoxin, 1:100 Triton-X114 was added and the solution was chilled for 10 min, incubated at 37°C for 10 min, and centrifuged at 13,000 ×*g*. This was repeated twice. Supernatant was loaded onto a HiTrap desalting column to remove remnants of Triton-X114.

Rabbit antibodies Protein concentration was determined using a BCA kit (Pierce). Purity was verified by SDS-PAGE analysis and Coomassie Brilliant Blue staining. Six-month-old New-Zealand white female rabbits were immunized with 500 μg protein emulsified in CFA (Difco) for initial immunization or IFA for booster immunizations on day 24 and 48. On day 60, rabbits were bled and serum recovered for immunoblotting or passive transfer experiments. For antibody purification, recombinant His_6-Coa, His_6-vWbp, or His_6-ClfA (5 mg) was covalently linked to HiTrap NHS-activated HP columns (GE Healthcare). This antigen-matrix was then used for affinity chromatography of 10–20 mL of rabbit serum at 4°C. Charged matrix was washed with 50 column volumes of PBS, antibodies eluted with elution buffer (1 M glycine pH 2.5, 0.5 M NaCl) and immediately neutralized with 1 M Tris-HCl (pH 8.5). Purified antibodies were dialyzed overnight against PBS, 0.5 M NaCl at 4°C.

Coagulation assay Overnight cultures of staphylococcal strains were diluted 1:100 into fresh TSB and grown at 37°C until they reached an OD_{600} 0.4. One mL of culture was centrifuged, and staphylococci washed and suspended in 1 mL of sterile PBS to generate a suspension of 1×10^8 CFU/mL. Whole blood from naïve BALB/c mice was collected and sodium citrate was added to a final concentration 1% (w/v). To assess bacterial blood coagulating activity in the presence of antibodies, 10 μL of the stock bacterial culture was mixed with 10 μL of PBS containing 30 μM of anti-Coa and anti–vWbp mixture in a sterile plastic test tube (BD Falcon) and incubated for fifteen minutes. To each tube, 80 μL of anti-coagulated mouse blood in a sterile plastic test tube (BD falcon) to achieve a final concentration of 1×10^7 CFU/mL. Test tubes were incubated at 37°C and blood coagulation was verified by tipping the tubes to 45° angles at timed intervals. For human blood experiments, consenting individuals were bled for 10 mL of blood, which was treated with sodium citrate to a final concentration of 1% (w/v). The blood was then tested in the manner described above. All experiments were repeated in at least two independent experiments.

Active immunization Three week-old BALB/c mice (n=10) were injected with 50 µg protein emulsified in 60 µL incomplete Freund's adjuvant, and 40 µL complete Freund's adjuvant. Eleven days post vaccination these mice were boosted with 50 µg protein each emulsified in 100 µL incomplete Freund's adjuvant. On day 21, mice were anesthetized with ketamine/xylazine and blood was collected by retro-orbital bleeding using micro-hematocrit capillary tubes (Fisher) in Z-Gel microtubes (Sarstedt) for determining half maximal titers. Tubes were centrifuged at 10,000×g for three minutes, and serum was collected. Half maximal antibody titers were measured by enzyme-linked immunosorbant assay (ELISA).

Passive transfer of antibodies Six hours prior to infection, six week old BALB/c mice (n=10) were injected intraperitoneally with affinity purified antibodies against full-length or subdomain constructs of Coa or vWbp or of V10 (control IgG specific for the LcrV plague antigen) at a dose of 5 mg/kg body weight.

Sepsis Overnight cultures of staphylococcal strains were diluted 1:100 into fresh TSB and grown until they reached an OD_{600} of 0.4. Bacteria were centrifuged at 7,000 ×*g*, washed, and suspended in the one-tenth volume of PBS. Six week-old female BALB/c mice (n=15) (Charles River) were injected retro-orbitally with 1×10^8 CFU (*S. aureus* Newman, N315, CowanI, and WIS), 5×10^7 CFU (*S. aureus* USA300), or 2×10^8 CFU (*S. aureus* MW2) suspensions in 100 µL of PBS. Mice were monitored for survival over 10 days.

Renal abscess *S. aureus* strains were prepared as described for sepsis but following washing, bacterial pellets were resuspended in an equal volume resulting in one log fewer CFU compared to sepsis. To enumerate staphylococcal load in kidney tissue five days post-infection, mice were euthanized by CO_2 asphyxiation and kidneys were removed during necropsy. One kidney per mouse was homogenized in PBS, 1% Triton X-100. Serial dilutions of homogenate were spread on TSA and incubated for colony formation. The bacterial load in tissue was analyzed in pairwise comparisons between wild-type and mutant strains with the unpaired two-tailed Student's *t*-test. For histopathology, the alternate kidney was fixed in 10% formalin for 24 hours at room temperature. Tissues were embedded in paraffin, thin-sectioned, stained with hematoxylin and eosin, and examined by light microscopy to enumerate pathological lesions per organ. Data were analyzed in pairwise comparisons between wild-type and mutant strains with the unpaired two-tailed Student's *t*-test.

Measurement of coagulase activity 5×10^{-8} M prothrombin (Innovative Research) was pre-incubated for 10 min with an equimolar amount of functional Coa at room temperature, followed by addition of S-2238 (a chromogenic substrate) to a final concentration of 1 mM in a total reaction buffer of 100 µL PBS. The change in absorbance was measured at 450 nm for 10 minutes in a spectrophotometer, plotted as a function of time, and fit to a linear curve. The slope of the curve (dA/dt) was interpreted to be the rate of S-2238 hydrolysis, and thus reflective of enzymatic function. The assay was repeated in presence of polyclonal antibodies added at 5×10^{-9} M and data were normalized to the average activity without inhibition. All experiments were performed in triplicate.

Coagulase activity. Purified recombinant Coa or vWbp (100 nM) were mixed with human prothrombin (Innovative Research) in 1% sodium citrate /PBS. After an initial reading, fibrinogen (3 µM) (Sigma) was added and conversion of fibrinogen to fibrin was measured as an increase in turbidity at 450 nm in a plate reader (BioTek) at 2.5 min intervals. As controls, the enzymatic activity of human alpha-thrombin (Innovative Research) or prothrombin alone were measured.

Sequence Table 1:

D1-2 domains of Coa from strain MRSA252:

```
IVTKDYSKES RVNENSKYDT PIPDWYLGSI LNRLGDQIYY AKELTNKYEY     50
GEKEYKQAID KLMTRVLGED HYLLEKKKAQ YEAYKKWFEK HKSENPHSSL    100
KKIKFDDFDL YRLTKKEYNE LHQSLKEAVD EFNSEVKNIQ SKQKDLLPYD    150
EATENRVTNG IYDFVCEIDT LYAAYFNHSQ YGHNAKELRA KLDIILGDAK    200
DPVRITNERI RKEMMDDLNS IIDDFFMDTN MNRPLNITKF NPNIHDYTNK    250
PENRDNFDKL VKETREAIAN ADESWKTRTV KN (SEQ ID NO: 33)
```

D1-2 Domains of Coa from strain MW2:

```
IVTKDYSGKS QVNAGSKNGK QIADGYYWGI IENLENQFYN IFHLLDQHKY    50
AEKEYKDAVD KLKTRVLEED QYLLERKKEK YEIYKELYKK YKKENPNTQV   100
KMKAFDKYDL GDLTMEEYND LSKLLTKALD NFKLEVKKIE SENPDLKPYS   150
ESEERTAYGK IDSLVDQAYS VYFAYVTDAQ HKTEALNLRA KIDLILGDEK   200
DPIRVTNQRT EKEMIKDLES IIDDFFIETK LNRPKHITRY DGTKHDYHKH   250
KDGFDALVKE TREAVAKADE SWKNKTVKK (SEQ ID NO: 34)
```

D1-2 Domains of Coa from strain WIS:

```
IVTKDYSGKS QVNAGSKNGK QIADGYYWGI IENLENQFYN IFHLLDQHKY    50
AEKEYKDALD KLKTRVLEED QYLLERKKEK YEIYKELYKK YKKENPNTQV   100
KMKAFDKYDL GDLTMEEYND LSKLLTKALD NFKLEVKKIE SENPDLRPYS   150
ESEERTAYGK IDSLVDQAYS VYFAYVTDAQ HKTEALNLRA KIDLILGDEK   200
DPIRVTNQRT EKEMIKDLES IIDDFFIETK LNRPQHITRY DGTKHDYHKH   250
KDGFDALVKE TREAVSKADE SWKTKTVKK (SEQ ID NO: 35)
```

D1-2 Domains of Coa from strain N315:

```
IVTKDYSKES RVNEKSKKGA TVSDYYYWKI IDSLEAQFTG AIDLLEDYKY    50
GDPIYKEAKD RLMTRVLGED QYLLKKKIDE YELYKKWYKS SNKNTNMLTF   100
HKYNLYNLTM NEYNDIFNSL KDAVYQFNKE VKEIEHKNVD LKQFDKDGED   150
KATKEVYDLV SEIDTLVVTY YADKDYGEHA KELRAKLDLI LGDTDNPHKI   200
TNERIKKEMI DDLNSIIDDF FMETKQNRPN SITKYDPTKH NFKEKSENKP   250
NFDKLVEETK KAVKEADESW KNKTVKK (SEQ ID NO: 36)
```

D1-2 Domains of Coa from strain USA300:

```
IVTKDYSGKS QVNAGSKNGT LIDSRYLNSA LYYLEDYIIY AIGLTNKYEY     50
GDNIYKEAKD RLLEKVLRED QYLLERKKSQ YEDYKQWYAN YKKENPRTDL    100
KMANFHKYNL EELSMKEYNE LQDALKRALD DFHREVKDIK DKNSDLKTFN    150
AAEEDKATKE VYDLVSEIDT LVVSYYGDKD YGEHAKELRA KLDLILGDTD    200
NPHKITNERI KKEMIDDLNS IIDDFFMETK QNRPKSITKY NPTTHNYKTN    250
SDNKPNFDKL VEETKKAVKE ADDSWKKKTV KK (SEQ ID NO: 37)
```

Sequence Table No. 2

D1-2 domains of vWbp from strain N315:

VVSGEKNPYV SKALELKDKS NKSNSYENYR DSLESLISSL SFADYEKYEE 50
PEYEKAVKKY QQKFMAEDDA LKNFLNEEKK IKNADISRKS NNLLGLTHER 100
YSYIFDTLKK NKQEFLKDIE EIQLKNSDLK DFNNT (SEQ ID NO: 38)

D1-2 domains of vWbp from strain MW2:

VVSGEKNPYV SESLKLTNNK NKSRTVEEYK KSLDDLIWSF PNLDNERFDN 50
PEYKEAMKKY QQRFMAEDEA LKKFFSEEKK IKNGNTDNLD YLGLSHERYE 100
SVFNTLKKQS EEFLKEIEDI KKDNPELKDF NE (SEQ ID NO: 39)

D1-2 domains L and Fgb Domains from strain USA300

VVSGEKNPYV SESLKLTNNK NKSRTVEEYK KSLDDLIWSF PNLDNERFDN 50
PEYKEAMKKY QQRFMAEDEA LKKFFSEEKK IKNGNTDNLD YLGLSHERYE 100
SVFNTLKKQS EEFLKEIEDI KKDNPELKDF NEEEQLKCDL ELNKLENQIL 150
MLGKTFYQNY RDDVESLYSK LDLIMGYKDE ERANKKAVNK RMLENKKEDL 200
ETIIDEFFSD IDKTRPNNIP VLEDEKQEEK NHKNMAQLKS DTEAAKSDES 250
KRSKRSKRSL NTQNHKPASQ EVSEQQKAEY DKRAEERKAR FLDNQKIKKT 300
PVVSLEYDFE HKQRIDNEND KKLVVSAPTK KPTSPTTYTE TTTQVPMPTV 350
ERQTQQQIIY NAPKQLAGLN GESHDFTTTH QSPTTSNHTH NNVVEFEETS 400
ALPGRKSGSL VGISQIDSSH LTEREKRVIK REHVREAQKL VDNYKDTHSY 450
KDRINAQQKV NTLSEGHQKR FNKQINKVYN GK (SEQ ID NO: 40)

Additional sequences:

D1-2 and L Domains of Coa from strain N315:

IVTKDYSKES RVNEKSKKGA TVSDYYYWKI IDSLEAQFTG AIDLLEDYKY 50
GDPIYKEAKD RLMTRVLGED QYLLKKKIDE YELYKKWYKS SNKNTNMLTF 100
HKYNLYNLTM NEYNDIFNSL KDAVYQFNKE VKEIEHKNVD LKQFDKDGED 150
KATKEVYDLV SEIDTLVVTY YADKDYGEHA KELRAKLDLI LGDTDNPHKI 200
TNERIKKEMI DDLNSIIDDF FMETKQNRPN SITKYDPTKH NFKEKSENKP 250
NFDKLVEETK KAVKEADESW KNKTVKKYEE TVTKSPVVKE EKKVEEPQLP 300
KVGNQQEVKT TAGKAEETTQ PVAQPLVKIP QETIYGETVK GPEYPTMENK 350
TLQGEIVQGP DFLTMEQNRP SLSDNYTQPT TPNPILEGLE GSSSKLEIKP 400
QGTESTLKGI QGESSDIEVK PQATETTEAS QYGP (SEQ ID NO: 41)

Full length Coa polypeptide:

Strain USA300

MKKQIISLGA LAVASSLFTW DNKADAIVTK DYSGKSQVNA GSKNGTLIDS 50
RYLNSALYYL EDYIIYAIGL TNKYEYGDNI YKEAKDRLLE KVLREDQYLL 100
ERKKSQYEDY KQWYANYKKE NPRTDLKMAN FHKYNLEELS MKEYNELQDA 150
LKRALDDFHR EVKDIKDKNS DLKTFNAAEE DKATKEVYDL VSEIDTLVVS 200
YYGDKDYGEH AKELRAKLDL ILGDTDNPHK ITNERIKKEM IDDLNSIIDD 250
FFMETKQNRP KSITKYNPTT HNYKTNSDNK PNFDKLVEET KKAVKEADDS 300
WKKKTVKKYG ETETKSPVVK EEKKVEEPQA PKVDNQQEVK TTAGKAEETT 350
QPVAQPLVKI PQGTITGEIV KGPEYPTMEN KTVQGEIVQG PDFLTMEQSG 400
PSLSNNYTNP PLTNPILEGL EGSSSKLEIK PQGTESTLKG TQGESSDIEV 450
KPQATETTEA SQYGPRPQFN KTPKYVKYRD AGTGIREYND GTFGYEARPR 500
FNKPSETNAY NVTTHANGQV SYGARPTQNK PSKTNAYNVT THGNGQVSYG 550
ARPTQNKPSK TNAYNVTTHA NGQVSYGARP TYKKPSKTNA YNVTTHADGT 600
ATYGPRVTK (SEQ ID NO: 42)

Further COA nucleic acid sequences (domains are indicated)

USA300

D1-

ATAGTAACAAAGGATTATAGTGGGAAATCACAAGTTAATGCTGGGAGTAAAAATGGG
ACATTAATAGATAGCAGATATTTAAATTCAGCTCTATATTATTTGGAAGACTATATAATTTA
TGCTATAGGATTAACTAATAAATATGAATATGGAGATAATATTTATAAAGAAGCTAAAGATA
GGTTGTTGGAAAAGGTATTAAGGGAAGATCAATATCTTTTGGAGAGAAAGAAATCTCAATAT
GAAGATTATAAACAATGGTATGCAAATTATAAAAAAGAAAATCCTCGTACAGATTTAAAAAT
GGCTAATTTTCATAAATATAATTTAGAAGAACTTTCGATGAAAGAATACAATGAACTACAGG
ATGCATTAAAGAGAGCACTGGATGATTTTCACAGAGAAGTTAAAGATATTAAGGATAAGAAT
TCAGACTTGAAAACTTTT (SEQ ID NO: 43)

D2-

AATGCAGCAGAAGAAGATAAAGCAACTAAGGAAGTATACGATCTCGTATCTGAAATT
GATACATTAGTTGTATCATATTATGGTGATAAGGATTATGGGGAGCACGCGAAAGAGTTACG
AGCAAAACTGGACTTAATCCTTGGAGATACAGACAATCCACATAAAATTACAAATGAACGTA
TTAAAAAAGAAATGATTGATGACTTAAATTCAATTATTGATGATTTCTTTATGGAAACTAAA

CAAAATAGACCGAAATCTATAACGAAATATAATCCTACAACACATAACTATAAAACAAATAGTGATAATAAACCTAATTTTGATAAATTAGTTGAAGAAACGAAAAAAGCAGTTAAAGAAGCAGATGATTCTTGGAAAAAGAAAACTGTCAAAAAA (SEQ ID NO: 44)

L-

TACGGAGAAACTGAAACAAAATCGCCAGTAGTAAAAGAAGAGAAGAAAGTTGAAGAACCTCAAGCACCTAAAGTTGATAACCAACAAGAGGTTAAAACTACGGCTGGTAAAGCTGAAGAAACAACACAACCAGTTGCACAACCATTAGTTAAAATTCCACAGGGCACAATTACAGGTGAAATTGTAAAAGGTCCGGAATATCCAACGATGGAAAATAAAACGGTACAAGGTGAAATCGTTCAAGGTCCCGATTTTCTAACAATGGAACAAAGCGGCCCATCATTAAGCAATAATTATACAAACCCACCGTTAACGAACCCTATTTTAGAAGGTCTTGAAGGTAGCTCATCTAAACTTGAAATAAAACCACAAGGTACTGAATCAACGTTAAAAGGTACTCAAGGAGAATCAAGTGATATTGAAGTTAAACCTCAAGCAACTGAAACAACAGAAGCTTCTCAATATGGTCCG (SEQ ID NO: 45)

R-

AGACCGCAATTTAACAAAACACCTAAATATGTTAAATATAGAGATGCTGGTACAGGTATCCGTGAATACAACGATGGAACATTTGGATATGAAGCGAGACCAAGATTCAATAAGCCATCAGAAACAAATGCATATAACGTAACAACACATGCAAATGGTCAAGTATCATACGGAGCTCGTCCGACACAAAACAAGCCAAGCAAAACAAACGCATATAACGTAACAACACATGGAAACGGCCAAGTATCATATGGCGCTCGCCCAACACAAAACAAGCCAAGCAAAACAAATGCATACAACGTAACAACACATGCAAACGGTCAAGTGTCATACGGAGCTCGCCCGACATACAAGAAGCCAAGTAAAACAAATGCATACAATGTAACAACACATGCAGATGGTACTGCGACATATGGGCCTAGAGTAACAAAATAA (SEQ ID NO: 46)

N315

D1-

ATGAAAAAGCAAATAATTTCGCTAGGCGCATTAGCAGTTGCATCTAGCTTATTTACATGGGATAACAAAGCAGATGCGATAGTAACAAAGGATTATAGTAAAGAATCAAGAGTGAATGAGAAAAGTAAAAAGGGAGCTACTGTTTCAGATTATTACTATTGGAAAATAATTGATAGTTTAGAGGCACAATTTACTGGAGCAATAGACTTATTGGAAGATTATAAATATGGAGATCCTATCTAT

AAAGAAGCGAAAGATAGATTGATGACAAGAGTATTAGGAGAAGACCAGTATTTATTAAAGAAAAAGATTGATGAATATGAGCTTTATAAAAAGTGGTATAAAAGTTCAAATAAGAACACTAATATGCTTACTTTCCATAAATATAATCTTTACAATTTAACAATGAATGAATATAACGATATTTTTAACTCTTTGAAAGATGCAGTTTATCAATTTAATAAAGAAGTTAAAGAAATAGAGCATAAAAATGTTGACTTGAAGCAGTTT (SEQ ID NO: 47)

D2-

GATAAAGATGGAGAAGACAAGGCAACTAAAGAAGTTTATGACCTTGTTTCTGAAATTGATACATTAGTTGTAACTTATTATGCTGATAAGGATTATGGGGAGCATGCGAAAGAGTTACGAGCAAAACTGGACTTAATCCTTGGAGATACAGACAATCCACATAAAATTACAAATGAGCGTATAAAAAAAGAAATGATCGATGACTTAAATTCAATTATAGATGATTTCTTTATGGAGACTAAACAAAATAGACCGAATTCTATAACAAAATATGATCCAACAAAACACAATTTTAAAGAGAAGAGTGAAAATAAACCTAATTTTGATAAATTAGTTGAAGAAACAAAAAAAGCAGTTAAAGAAGCAGACGAATCTTGGAAAAATAAAACTGTCAAAAAA (SEQ ID NO: 48)

L-

TACGAGGAAACTGTAACAAAATCTCCTGTTGTAAAAGAAGAGAAGAAAGTTGAAGAACCTCAATTACCTAAAGTTGGAAACCAGCAAGAGGTTAAAACTACGGCTGGTAAAGCTGAAGAAACAACACAACCAGTGGCACAGCCATTAGTAAAAATTCCACAAGAAACAATCTATGGTGAAACTGTAAAAGGTCCAGAATATCCAACGATGGAAAATAAAACGTTACAAGGTGAAATCGTTCAAGGTCCCGATTTTCTAACAATGGAACAAAACAGACCATCTTTAAGCGATAATTATACTCAACCGACGACACCGAACCCTATTTTAGAAGGTCTTGAAGGTAGCTCATCTAAACTTGAAATAAAACCACAAGGTACTGAATCAACGTTGAAAGGTATTCAAGGAGAATCAAGTGATATTGAAGTTAAACCTCAAGCAACTGAAACAACAGAAGCTTCTCAATATGGTCCG (SEQ ID NO: 49)

R-

AGACCGCAATTTAACAAAACACCTAAGTATGTGAAATATAGAGATGCTGGTACAGGTATCCGTGAATACAACGATGGAACATTTGGATATGAAGCGAGACCAAGATTCAACAAGCCAAGTGAAACAAATGCATACAACGTAACGACAAATCAAGATGGCACAGTATCATACGGAGCTCGCCCAACACAAAACAAGCCAAGTGAAACAAACGCATATAACGTAACAACACATGCAAATGGTCAAGTATCATACGGTGCTCGCCCAACACAAAAAAAGCCAAGCAAAACAAATGCATACAACGTAACAACACATGCAAATGGTCAAGTATCATATGGCGCTCGCCCGACACAAAAAAAGCCAAGCAAAA

CAAATGCATATAACGTAACAACACATGCAAATGGTCAAGTATCATACGGAGCTCGCCCGACA TACAAGAAGCCAAGCGAAACAAATGCATACAACGTAACAACACATGCAAATGGTCAAGTATC ATATGGCGCTCGCCCGACACAAAAAAAGCCAAGCGAAACAAACGCATATAACGTAACAACAC ATGCAGATGGTACTGCGACATATGGGCCTAGAGTAACAAAATAA (SEQ ID NO: 50)

Strain MW2

D1-

ATGAAAAAGCAAATAATTTCGCTAGGCGCATTAGCAGTTGCATCTAGCTTATTTACA TGGGATAACAAAGCAGATGCGATAGTAACAAAGGATTATAGTGGGAAATCACAAGTTAATGC TGGGAGTAAAAATGGGAAACAAATTGCAGATGGATATTATTGGGGAATAATTGAAAATCTAG AAAACCAGTTTTACAATATTTTTCATTTACTGGATCAGCATAAATATGCAGAAAAAGAATAT AAAGATGCAGTAGATAAATTAAAAACTAGAGTTTTAGAGGAAGACCAATACCTGCTAGAAAG AAAAAAAGAAAAATACGAAATTTATAAAGAACTATATAAAAAATACAAAAAAGAGAATCCTA ATACTCAAGTTAAAATGAAAGCATTTGATAAATACGATCTTGGCGATTTAACTATGGAAGAA TACAATGACTTATCAAAATTATTAACAAAAGCATTGGATAACTTTAAGTTAGAAGTAAAGAA AATTGAATCAGAGAATCCAGATTTAAAACCATAT (SEQ ID NO: 51)

D2-

TCTGAAAGCGAAGAAAGAACAGCATATGGTAAAATAGATTCACTTGTTGATCAAGCATATAG TGTATATTTTGCCTACGTTACAGATGCACAACATAAAACAGAAGCATTAAATCTTAGGGCGA AAATTGATTTGATTTTAGGTGATGAAAAAGATCCAATTAGAGTTACGAATCAACGTACTGAA AAAGAAATGATTAAAGATTTAGAATCTATTATTGATGATTTCTTCATTGAAACCAAGTTGAA TAGACCTAAACACATTACTAGGTATGATGGAACTAAACATGATTACCATAAACATAAAGATG GATTTGATGCTCTAGTTAAAGAAACAAGAGAAGCGGTTGCAAAGGCTGACGAATCTTGGAAA AATAAAACTGTCAAAAAA (SEQ ID NO: 52)

L-

TACGAGGAAACTGTAACAAAATCTCCAGTTGTAAAAGAAGAGAAGAAAGTTGAAGAA CCTCAATCACCTAAATTTGATAACCAACAAGAGGTTAAAATTACAGTTGATAAAGCTGAAGA

AACAACACAACCAGTGGCACAGCCATTAGTTAAAATTCCACAGGGCACAATTACAGGTGAAATTGTAAAAGGTCCGGAATATCCAACGATGGAAAATAAAACGTTACAAGGTGAAATCGTTCAAGGTCCAGATTTCCCAACAATGGAACAAAACAGACCATCTTTAAGCGATAATTATACTCAACCGACGACACCGAACCCTATTTTAGAAGGTCTTGAAGGTAGCTCATCTAAACTTGAAATAAAACCACAAGGTACTGAATCAACGTTAAAAGGTACTCAAGGAGAATCAAGTGATATTGAAGTTAAACCTCAAGCATCTGAAACAACAGAAGCATCACATTATCCAGCAAGACCTCAATTTAACAAAACACCTAAATATGTTAAATATAGAGATGCTGGTACAGGTATCCGTGAATACAACGATGGAACATTTGGATATGAA (SEQ ID NO: 53)

R-

GCGAGACCAAGATTCAATAAGCCATCAGAAACAAACGCATACAACGTAACGACAAATCAAGATGGCACAGTAACATATGGCGCTCGCCCAACACAAAACAAACCAAGCAAAACAAATGCATACAACGTAACAACACATGCAAATGGTCAAGTATCATATGGCGCTCGCCCGACACAAAACAAGCCAAGCAAAACAAATGCATATAACGTAACAACACATGCAAATGGTCAAGTATCATACGGAGCTCGCCCGACACAAAACAAGCCAAGCAAAACAAATGCATATAACGTAACAACACACGCAAACGGTCAAGTGTCATACGGAGCTCGCCCGACATACAAGAAGCCAAGTAAAACAAATGCATACAATGTAACAACACATGCAGATGGTACTGCGACATATGGGCCTAGAGTAACAAAATAA (SEQ ID NO: 54)

Strain MRSA252

D1-

ATGAAAAAGCAAATAATTTCGCTAGGCGCATTAGCAGTTGCATCTAGCTTATTTACATGGGATAACAAAGCAGATGCGATAGTAACTAAAGATTATAGTAAAGAATCAAGAGTGAATGAGAACAGTAAATACGATACACCAATTCCAGATTGGTATCTAGGTAGTATTTTAAACAGATTAGGGGATCAAATATACTACGCTAAGGAATTAACTAATAAATACGAATATGGTGAGAAAGAGTATAAGCAAGCGATAGATAAATTGATGACTAGAGTTTTGGGAGAAGATCATTATCTATTAGAAAAAAAGAAAGCACAATATGAAGCATACAAAAAAATGGTTTGAAAAACATAAAAGTGAAAATCCACATTCTAGTTTAAAAAAGATTAAATTTGACGATTTTGATTTATATAGATTAACGAAGAAAGAATACAA

TGAGTTACATCAATCATTAAAAGAAGCTGTTGATGAGTTTAATAGTGAAGTGAAAAATATTC AATCTAAACAAAAGGATTTATTACCTTAT (SEQ ID NO: 55)

D2-

GATGAAGCAACTGAAAATCGAGTAACAAATGGAATATATGATTTTGTTTGCGAGATTGACAC ATTATACGCAGCATATTTTAATCATAGCCAATATGGTCATAATGCTAAAGAATTAAGAGCAA AGCTAGATATAATTCTTGGTGATGCTAAAGATCCTGTTAGAATTACGAATGAAAGAATAAGA AAAGAAATGATGGATGATTTAAATTCTATTATTGATGATTTCTTTATGGATACAAACATGAA TAGACCATTAAACATAACTAAATTTAATCCGAATATTCATGACTATACTAATAAGCCTGAAA ATAGAGATAACTTCGATAAATTAGTCAAAGAAACAAGAGAAGCAATCGCAAACGCTGACGAA TCTTGGAAAACAAGAACCGTCAAAAAT (SEQ ID NO: 56)

L-

TACGGTGAATCTGAAACAAAATCTCCTGTTGTAAAAGAAGAGAAGAAAGTTGAAGAACCTCA ATTACCTAAAGTTGGAAACCAGCAAGAGGATAAAATTACAGTTGGTACAACTGAAGAAGCAC CATTACCAATTGCGCAACCACTAGTTAAAATTCCACAGGGCACAATTCAAGGTGAAATTGTA AAAGGTCCGGAATATCTAACGATGGAAAATAAAACGTTACAAGGTGAAATCGTTCAAGGTCC AGATTTCCCAACAATGGAACAAAACAGACCATCTTTAAGCGATAATTATACTCAACCGACGA CACCGAACCCTATTTTAAAAGGTATTGAAGGAAACTCAACTAAACTTGAAATAAAACCACAA GGTACTGAATCAACGTTAAAAGGTACTCAAGGAGAATCAAGTGATATTGAAGTTAAACCTCA AGCAACTGAAACAACAGAAGCATCACATTATCCAGCGAGACCTCAATTTAACAAAACACCTA AGTATGTGAAATATAGAGATGCTGGTACAGGTATCCGTGAATACAACGATGGAACATTTGGA TATGAA (SEQ ID NO: 57)

R-

GCGAGACCAAGATTCAACAAGCCAAGCGAAACAAATGCATACAACGTAACGACAAATCAAGA TGGCACAGTATCATATGGCGCTCGCCCGACACAAAACAAGCCAAGCGAAACAAACGCATATA ACGTAACAACACATGCAAACGGCCAAGTATCATACGGAGCTCGTCCGACACAAAACAAGCCA AGCGAAACGAACGCATATAACGTAACAACACATGCAAACGGTCAAGTGTCATACGGAGCTCG CCCAACACAAAACAAGCCAAGTAAAACAAATGCATACAATGTAACAACACATGCAGATGGTA CTGCGACATATGGTCCTAGAGTAACAAAATAA (SEQ ID NO: 58)

Strain WIS

D1-

ATAGTAACAAAGGATTATAGTGGGAAATCACAAGTTAATGCTGGGAGTAAAAATGGGAAACAAATTGCAGATGGATATTATTGGGGAATAATTGAAAATCTAGAGAACCAGTTTTACAATATTTTTCATTTATTGGATCAGCATAAATATGCAGAAAAAGAATATAAAGATGCATTAGATAAATTAAAAACTAGAGTTTTAGAGGAAGACCAATACCTGCTAGAAAGAAAAAAAGAAAAATACGAAATTTATAAAGAACTATATAAAAAATACAAAAAAGAGAATCCTAATACTCAGGTTAAAATGAAAGCATTTGATAAATACGATCTTGGCGATTTAACTATGGAAGAATACAATGACTTATCAAAATTATTAACAAAAGCATTGGATAACTTTAAGTTAGAAGTAAAGAAAATTGAATCAGAGAATCCAGATTTAAGACCATAT (SEQ ID NO: 59)

D2-

TCTGAAAGTGAAGAGAGAACAGCATATGGTAAAATAGATTCACTTGTTGATCAAGCATATAGTGTATATTTTGCCTACGTTACAGATGCTCAACATAAAACAGAAGCATTAAATCTTAGGGCAAAAATA**GA**TTT**GA**TTTTAGGT**GA**T**GA**AAAA**GA**TCCAATTA**GA**GT**GA**C**GA**ATCAACGTACT**GA**AAAA**GA**AAT**GA**TTAAAGATTTAGAATCTATTATTGATGATTTCTTCATTGAAACAAAGTTGAATAGACCTCAACACATTACTAGATATGATGGAACTAAACATGATTACCATAAACATAAAGATGGATTTGATGCTTTAGTTAAAGAAACAAGAGAAGCGGTTTCTAAGGCTGACGAATCTTGGAAAACTAAAACTGTCAAAAAA (SEQ ID NO: 60)

L-

TACGGGGAAACTGAAACAAAATATCCTGTTGTAAAAGAAGAGAAGAAAGTTGAAGAACCTCAATCACCTAAAGTTTCTGAAAAAGTGGATGTTCAGGAAACGGTTGGTACAACTGAAGAAGCACCATTACCAATTGCGCAACCACTAGTTAAATTACCACAAATTGGGACTCAAGGCGAAATTGTAAAAGGTCCCGACTATCCAACTATGGAAAATAAAACGTTACAAGGTGTAATTGTTCAAGGTCCAGATTTCCCAACAATGGAACAAAACAGACCATCTTTAAGTGACAATTATACACAACCATCTGTGACTTTACCGTCAATTACAGGTGAAAGTACACCAACGAACCCTATTTTAAAAGGTATTGAAGGAAACTCATCTAAACTTGAAATAAAACCACAAGGTACTGAATCAACGTTGAAAGGTATTCAAGGAGAATCAAGTGATATTGAAGTTAAACCTCAAGCAACTGAAACAACAGAAGCATCACATTATCCAGCGAGACCGCAATTTAACAAAACACCTAAATATGTGAAATATAGAGATGCTGGTACAGGTATTCGTGAATACAACGATGGAACTTTTGGATATGAA (SEQ ID NO: 61)

R-

GCGAGACCAAGATTCAACAAGCCATCAGAAACAAACGCATACAACGTAACGACAAATCAAGATGGCACAGTATCATATGGGGCTCGCCCAACACAAAACAAGCCAAGCAAAACAAATGCATATAACGTAACAACACATGCAAACGGCCAAGTATCATATGGCGCTCGCCCGACATACAACAAGCCAAGTGAAACAAATGCATACAACGTAACGACAAATCGAGATGGCACAGTATCATATGGCGCTCGCCCGACACAAAACAAGCCAAGCGAAACGAATGCATATAACGTAACAACACACGGAAATGGCCAAGTATCATATGGCGCTCGTCCGACACAAAAGAAGCCAAGCAAAACAAATGCATATAACGTAACAACACATGCAAACGGCCAAGTATCATATGGCGCTCGTCCGACATACAACAAGCCAAGTAAAACAAATGCATACAATGTAACAACACATGCAGATGGTACTGCGACATATGGTCCTAGAGTAACAAAATAA (SEQ ID NO: 62)

MU50

D1-

GATTGGGCAATTACATTTTGGAGGAATTAAAAAATTATGAAAAAGCAAATAATTTCGCTAGGCGCATTAGCAGTTGCATCTAGCTTATTTACATGGGATAACAAAGCAGATGCGATAGTAACAAAGGATTATAGTAAAGAATCAAGAGTGAATGAGAAAAGTAAAAAGGGAGCTACTGTTTCAGATTATTACTATTGGAAAATAATTGATAGTTTAGAGGCACAATTTACTGGAGCAATAGACTTATTGGAAGATTATAAATATGGAGATCCTATCTATAAAGAAGCGAAAGATAGATTGATGACAAGAGTATTAGGAGAAGACCAGTATTTATTAAAGAAAAAGATTGATGAATATGAGCTTTATAAAAAGTGGTATAAAAGTTCAAATAAGAACACTAATATGCTTACTTTCCATAAATATAATCTTTACAATTTAACAATGAATGAATATAACGATATTTTTAACTCTTTGAAAGATGCAGTTTATCAATTTAATAAAGAAGTTAAAGAAATAGAGCATAAAAATGTTGACTTGAAGCAGTTT (SEQ ID NO: 63)

D2-

GATAAAGATGGAGAAGACAAGGCAACTAAAGAAGTTTATGACCTTGTTTCTGAAATTGATACATTAGTTGTAACTTATTATGCTGATAAGGATTATGGGGAGCATGCGAAAGAGTTACGAGCAAAACTGGACTTAATCCTTGGAGATACAGACAATCCACATAAAATTACAAATGAGCGTATAAAAAAAGAAATGATCGATGACTTAAATTCAATTATAGATGATTTCTTTATGGAGACTAAA

CAAAATAGACCGAATTCTATAACAAAATATGATCCAACAAAACACAATTTTAAAGAGAAGAG
TGAAAATAAACCTAATTTTGATAAATTAGTTGAAGAAACAAAAAAAGCAGTTAAAGAAGCAG
ACGAATCTTGGAAAAATAAAACTGTCAAAAAA (SEQ ID NO: 64)

L-

TACGAGGAAACTGTAACAAAATCTCCTGTTGTAAAAGAAGAGAAGAAAGTTGAAGAACCTCA
ATTACCTAAAGTTGGAAACCAGCAAGAGGTTAAAACTACGGCTGGTAAAGCTGAAGAAACAA
CACAACCAGTGGCACAGCCATTAGTAAAAATTCCACAAGAAACAATCTATGGTGAAACTGTA
AAAGGTCCAGAATATCCAACGATGGAAAATAAAACGTTACAAGGTGAAATCGTTCAAGGTCC
CGATTTTCTAACAATGGAACAAAACAGACCATCTTTAAGCGATAATTATACTCAACCGACGA
CACCGAACCCTATTTTAGAAGGTCTTGAAGGTAGCTCATCTAAACTTGAAATAAAACCACAA
GGTACTGAATCAACGTTGAAAGGTATTCAAGGAGAATCAAGTGATATTGAAGTTAAACCTCA
AGCAACTGAAACAACAGAAGCTTCTCAATATGGTCCG (SEQ ID NO: 65)

R-

AGACCGCAATTTAACAAAACACCTAAGTATGTGAAATATAGAGATGCTGGTACAGGTATCCG
TGAATACAACGATGGAACATTTGGATATGAAGCGAGACCAAGATTCAACAAGCCAAGTGAAA
CAAATGCATACAACGTAACGACAAATCAAGATGGCACAGTATCATACGGAGCTCGCCCAACA
CAAAACAAGCCAAGTGAAACAAACGCATATAACGTAACAACACATGCAAATGGTCAAGTATC
ATACGGTGCTCGCCCAACACAAAAAAAGCCAAGCAAAACAAATGCATACAACGTAACAACAC
ATGCAAATGGTCAAGTATCATATGGCGCTCGCCCGACACAAAAAAAGCCAAGCAAAACAAAT
GCATATAACGTAACAACACATGCAAATGGTCAAGTATCATACGGAGCTCGCCCGACATACAA
GAAGCCAAGCGAAACAAATGCATACAACGTAACAACACATGCAAATGGTCAAGTATCATATG
GCGCTCGCCCGACACAAAAAAAGCCAAGCGAAACAAACGCATATAACGTAACAACACATGCA
GATGGTACTGCGACATATGGGCCTAGAGTAACAAAATAA (SEQ ID NO: 66)

85/2082

D1-

ATAGTAACTAAAGATTATAGTAAAGAATCAAGAGTGAATGAGAACAGTAAATACGATACACC
AATTCCAGATTGGTATCTAGGTAGTATTTTAAACAGATTAGGGGATCAAATATACTACGCTA
AGGAATTAACTAATAAATACGAATATGGTGAGAAAGAGTATAAGCAAGCGATAGATAAATTG

ATGACTAGAGTTTTGGGAGAAGATCATTATCTATTAGAAAAAAAGAAAGCACAATATGAAGCATACAAAAAATGGTTTGAAAAACATAAAAGTGAAAATCCACATTCTAGTTTAAAAAAGATTAAATTTGACGATTTTGATTTATATAGATTAACGAAGAAAGAATACAATGAGTTACATCAATCATTAAAAGAAGCTGTTGATGAGTTTAATAGTGAAGTGAAAAATATTCAATCTAAACAAAAGGATTTATTACCTTAT (SEQ ID NO: 67)

D2-

GATGAAGCAACTGAAAATCGAGTAACAAATGGAATATATGATTTTGTTTGCGAGATTGACACATTATACGCAGCATATTTTAATCATAGCCAATATGGTCATAATGCTAAAGAATTAAGAGCAAAGCTAGATATAATTCTTGGTGATGCTAAAGATCCTGTTAGAATTACGAATGAAAGAATAAGAAAAGAAATGATGGATGATTTAAATTCTATTATTGATGATTTCTTTATGGATACAAACATGAATAGACCATTAAACATAACTAAATTTAATCCGAATATTCATGACTATACTAATAAGCCTGAAAATAGAGATAACTTCGATAAATTAGTCAAAGAAACAAGAGAAGCAGTCGCAAACGCTGACGAATCTTGGAAAACAAGAACCGTCAAAAAT (SEQ ID NO: 68)

L-

TACGGTGAATCTGAAACAAAATCTCCTGTTGTAAAAGAAGAGAAGAAAGTTGAAGAACCTCAATTACCTAAAGTTGGAAACCAGCAAGAGGATAAAATTACAGTTGGTACAACTGAAGAAGCACCATTACCAATTGCGCAACCACTAGTTAAAATTCCACAGGGCACAATTCAAGGTGAAATTGTAAAAGGTCCGGAATATCTAACGATGGAAAATAAAACGTTACAAGGTGAAATCGTTCAAGGTCCAGATTTCCCAACAATGGAACAAAACAGACCATCTTTAAGCGATAATTATACTCAACCGACGACACCGAACCCTATTTTAAAAGGTATTGAAGGAAACTCAACTAAACTTGAAATAAAACCACAAGGTACTGAATCAACGTTAAAAGGTACTCAAGGAGAATCAAGTGATATTGAAGTTAAACCTCAAGCAACTGAAACAACAGAAGCATCACATTATCCAGCGAGACCTCAATTTAACAAAACACCTAAGTATGTGAAATATAGAGATGCTGGTACAGGTATCCGTGAATACAACGATGGAACATTTGGATATGAA (SEQ ID NO: 69)

R-

GCGAGACCAAGATTCAACAAGCCAAGCGAAACAAATGCATACAACGTAACGACAAATCAAGATGGCACAGTATCATATGGCGCTCGCCCGACACAAAACAAACCAAGCGAAACAAATGCATACAACGTAACAACACATGCAAACGGCCAAGTATCATATGGCGCCCGCCCAACATACAAGAAGCCAAGCGAAACAAACGCATACAACGTAACGACAAATCAAGATGGCACAGTATCATATGGCGCTCGCCCGACACAAAACAAGCCAAGCGAAACAAACGCATATAACGTAACAACACATGCAAACGGCC

AAGTATCATACGGAGCTCGTCCGACACAAAACAAGCCAAGCGAAACGAACGCATATAACGTA
ACAACACATGCAAACGGTCAAGTGTCATACGGAGCTCGCCCAACACAAAACAAGCCAAGTAA
AACAAATGCATACAATGTAACAACACATGCAGATGGTACTGCGACATATGGTCCTAGAGTAA
CAAAATAA (SEQ ID NO: 70)

Newman

D1-

atgaaaaagcaaataatttcgctaggcgcattagcagttgcatctagcttatttacatggga
taacaaagcagatgcgatagtaacaaaggattatagtgggaaatcacaagttaatgctggga
gtaaaaatgggacattaatagatagcagatatttaaattcagctctatattatttggaagac
tatataatttatgctataggattaactaataaatatgaatatggagataatatttataaaga
agctaaagataggttgttggaaaaggtattaagggaagatcaatatcttttggagagaaaga
aatctcaatatgaagattataaacaatggtatgcaaattataaaaaagaaaatcctcgtaca
gatttaaaaatggctaattttcataaatataatttagaagaactttcgatgaaagaatacaa
tgaactacaggatgcattaaagagagcactggatgattttcacagagaagttaaagatatta
aggataagaattcagacttgaaaactttt (SEQ ID NO: 71)

D2-

aatgcagcagaagaagataaagcaactaaggaagtatacgatctcgtatctgaaattgatac
attagttgtatcatattatggtgataaggattatggggagcacgcgaaagagttacgagcaa
aactggacttaatccttggagatacagacaatccacataaaattacaaatgaacgtattaaa
aaagaaatgattgatgacttaaattcaattattgatgatttctttatggaaactaaacaaaa
tagaccgaaatctataacgaaatataatcctacaacacataactataaaacaaatagtgata
ataaacctaattttgataaattagttgaagaaacgaaaaaagcagttaaagaagcagatgat
tcttggaaaaagaaaactgtcaaaaaa (SEQ ID NO: 72)

L-

tacggagaaactgaaacaaaatcgccagtagtaaaagaagagaagaaagttgaagaacctca
agcacctaaagttgataaccaacaagaggttaaaactacggctggtaaagctgaagaaacaa
cacaaccagttgcacaaccattagttaaaattccacagggcacaattacaggtgaaattgta
aaaggtccggaatatccaacgatggaaaataaaacggtacaaggtgaaatcgttcaaggtcc

cgattttctaacaatggaacaaagcggcccatcattaagcaataattatacaaacccaccgt
taacgaaccctattttagaaggtcttgaaggtagctcatctaaacttgaaataaaaccacaa
ggtactgaatcaacgttaaaaggtactcaaggagaatcaagtgatattgaagttaaacctca
agcaactgaaacaacagaagcttctcaatatggtccg (SEQ ID NO: 73)

R-
agaccgcaatttaacaaaacacctaaatatgttaaatatagagatgctggtacaggtatccg
tgaatacaacgatggaacatttggatatgaagcgagaccaagattcaataagccatcagaaa
caaatgcatataacgtaacaacacatgcaaatggtcaagtatcatacggagctcgtccgaca
tacaagaagccaagcgaaacgaatgcatacaatgtaacaacacatgcaaacggccaagtatc
atacggagctcgtccgacacaaaacaagccaagcaaaacaaacgcatataacgtaacaacac
atggaaacggccaagtatcatatggcgctcgcccaacacaaaacaagccaagcaaaacaaat
gcatacaacgtaacaacacatgcaaacggtcaagtgtcatacggagctcgcccgacatacaa
gaagccaagtaaaacaaatgcatacaatgtaacaacacatgcagatggtactgcgacatatg
ggcctagagtaacaaaataa (SEQ ID NO: 74)

Full length vWbp polypeptide from strain USA 300

mknkllvlsl galcvsqiwe snrasavvsg eknpyvsesl kltnnknksr
tveeykksld dliwsfpnld nerfdnpeyk eamkkyqqrf maedealkkf
fseekkikng ntdnldylgl sheryesvfn tlkkqseefl keiedikkdn
pelkdfneee qlkcdlelnk lenqilmlgk tfyqnyrddv eslyskldli
mgykdeeran kkavnkrmle nkkedletii deffsdidkt rpnnipvled
ekqeeknhkn maqlksdtea aksdeskrsk rskrslntqn hkpasqevse
qqkaeydkra eerkarfldn qkikktpvvs leydfehkqr idnendkklv
vsaptkkpts pttytetttq vpmptverqt qqqiiynapk qlaglngesh
dftttthqspt tsnhthnnvv efeetsalpg rksgslvgis qidsshlter
ekrvikrehv reaqklvdny kdthsykdri naqqkvntls eghqkrfnkq
inkvyngk (SEQ ID NO: 75)

Additional vWbp Sequences:

USA300

GTGGTTTCTGGGGAGAAGAATCCATATGTATCTGAGTCGTTGAAACTGACTAATAATAAAAATAAATC
TAGAACAGTAGAAGAGTATAAGAAAAGCTTGGATGATTTAATATGGTCCTTTCCAAACTTAGATAATGAAAGAT

TTGATAATCCTGAATATAAAGAAGCTATGAAAAAATATCAACAGAGATTTATGGCTGAAGATGAGGCTTTGAAG AAATTTTTTAGTGAAGAGAAAAAAATAAAAAATGGAAATACTGATAATTTAGATTATCTAGGATTATCTCATGA AAGATATGAAAGTGTATTTAATACTTTGAAAAAACAAAGTGAGGAGTTCTTAAAAGAAATTGAAGATATAAAAA AAGATAACCCTGAATTGAAAGACTTTAATGAAGAGGAGCAATTAAAGTGCGACTTAGAATTAAACAAATTAGAA AATCAGATATTAATGTTAGGTAAAACATTTTATCAAAACTATAGAGATGATGTTGAAAGTTTATATAGTAAGTT AGATTTAATTATGGGATATAAAGATGAAGAAAGAGCAAATAAAAAAGCAGTTAACAAAAGGATGTTAGAAAATA AAAAAGAAGACTTAGAAACCATAATTGATGAATTTTTTAGTGATATAGATAAAACAAGACCTAATAATATTCCT GTTTTAGAAGATGAAAAACAAGAAGAGAAAAATCATAAAAATATGGCTCAATTAAAATCTGACACTGAAGCAGC AAAAAGTGATGAATCAAAAAGAAGCAAGAGAAGTAAAAGAAGTTTAAATACTCAAAATCACAAACCTGCATCTC AAGAAGTTTCTGAACAACAAAAAGCTGAATATGATAAAAGAGCAGAAGAAAGAAAAGCGAGATTTTTGGATAAT CAAAAAATTAAGAAAACACCTGTAGTGTCATTAGAATATGATTTTGAGCATAAACAACGTATTGACAACGAAAA CGACAAGAAACTTGTGGTTTCTGCACCAACAAAGAAACCAACATCACCGACTACATATACTGAAACAACGACAC AGGTACCAATGCCTACAGTTGAGCGTCAAACTCAGCAACAAATTATTTATAATGCACCAAAACAATTGGCTGGA TTAAATGGTGAAAGTCATGATTTCACAACAACGCATCAATCACCAACAACTTCAAATCACACGCATAATAATGT TGTTGAATTTGAAGAAACGTCTGCTTTACCTGGTAGAAAATCAGGATCACTGGTTGGTATAAGTCAAATTGATT CTTCTCATCTAACTGAACGTGAGAAGCGTGTAATTAAGCGTGAACACGTTAGAGAAGCTCAAAAGTTAGTTGAT AATTATAAAGATACACATAGTTATAAAGACCGAATAAATGCACAACAAAAAGTAAATACTTTAAGTGAAGGTCA TCAAAAACGTTTTAATAAACAAATCAATAAAGTATATAATGGCAAATAA (SEQ ID NO: 76)

N315

GTGGTTTCTGGGGAGAAGAATCCATATGTATCAAAAGCTTTAGAATTGAAAGATAAAAGTAATAAATC CAATTCTTACGAAAATTATAGAGATAGTTTAGAAAGTTTGATTTCATCATTATCTTTTGCTGATTATGAAAAAT ATGAAGAGCCAGAATATGAAAAGGCTGTAAAAAAATATCAACAAAAATTTATGGCTGAAGATGATGCATTAAAA AATTTTTTAAATGAAGAAAAGAAGATAAAAAATGCAGATATTAGCAGAAAATCGAATAATTTATTAGGTTTAAC ACATGAAAGATATTCTTATATTTTTGATACATTAAAGAAAAATAAACAAGAGTTTTTAAAAGATATTGAAGAAA TACAACTGAAAAATAGTGATTTAAAGGACTTTAACAATACAGAGCAACATAATGCCGACGTAGAAATAAACAAT TTAGAAAATAAAGTATTAATGGTAGGGTATACATTCTATAATACAAATAAGGACGAAGTTGAAGAATTATATAG TGAGTTAGATTTGATTGTTGGAGAAGTTCAAGATAAGTCGGATAAAAAAAGAGCAGTAAATCAAAGGATGTTAA ATAGAAAAAAAGAGGATTTAGAATTTATTATAGATAAATTTTTTAAAAAAATTCAACAAGAACGTCCAGAGAGT ATACCAGCATTAACTAGTGAAAAAAATCATAATCAGACTATGGCATTAAAGTTAAAAGCAGATACAGAAGCTGC TAAAAATGACGTATCAAAAAGAAGTAAAAGAAGTTTAAATACTCAAAATAATAAATCTACAACACAAGAAATTT CTGAAGAACAAAAAGCTGAATATCAAAGAAAGTCAGAGGCATTAAAAGAAAGATTTATAAACAGACAAAAATCT AAAAATGAGTCTGTGGTTTCACTAATCGATGACGAAGACGACAACGAAAACGACAGGCAACTTGTGGTTTCTGC GCCATCAAAGAAACCAACAACACCGACTACATATACTGAAACAACGACTCAGGTACCAATGCCTACAGTTGAGC GTCAAACTCAGCAACAAATCGTTTACAAAACACCAAAACCATTAGCTGGATTAAATGGTGAAAGTCATGATTTC ACAACAACGCATCAATCACCAACAACTTCAAATCATACGCATAATAATGTTGTTGAATTTGAAGAAACGTCTGC TTTACCTGGTAGAAAATCAGGATCACTGGTTGGTATAAGTCAAATTGATTCTTCTCATCTAACTGAACGTGAGA AGCGTGTAATCAAGCGTGAACACGTTAGAGAAGCTCAAAAGTTAGTTGATAATTATAAAGATACACATAGTTAT

AAAGACCGATTAAATGCACAACAAAAAGTAAATACTTTAAGTGAAGGTCATCAAAAACGTTTTAATAAACAAATCAATAAAGTATACAATGGCAAATAA (SEQ ID NO: 77)

MRSA252

GTGGTTTCTGGGGAGGAGAATCCATATAAATCTGAGTCATTGAAATTAAATGGGAAAAGAAGTACTACAATAACTAGTGATAAATATGAAGAAAATTTAGATATGTTAATATCGTCATTATCATTTGCAGATTATGAAAAATATGAGGAACCAGAATACAAAGAAGCAGTTAAAAAGTATCAACAAAAATTTATGGCTGAAGATGATGCATTAAAAAATTTTTTAGTGAAGAGAAAAAAAATAAAAAATAGAAATACTAATACATCAAATTATCTGGGATTAACACACGAAAGATATGAGTCAATTTATAATTCATTAAAAAATCATCGTGAAGAATTTTCAAAAGAAATCGAAGAAATTAATAATAAAAATCCAGTGTTAAAAGAATATAACAATGAGGAACAAACTAAAGCTGATACGGAATTAAACACTCTTGAAAATCAAGTACTAATGATAGGTTATACATTTTATCACTCGAATAAAAATGAAGTAGAAGATTTATATAACAAATTAGATATGATTCTTGGTTATAAAGATGAAGAGAGAAAAAAGAAGAGGGCTACCAATCAAAGAATGTTCAATAATAAAAAAGAGGATTTAGAAACTATTATTGATGAATTCTTTGGAGAAATTGGACAACAAAGGCCAACATCTATACCAACATTAGCGCCTAAAGAAGAAAAAGAAACAAATATAAAAAATGCAAATAAATTAAAATCTGACACTGAAGCAGCAAAAAATGATGAAGCAAAAAGAAGTTTAAATACCCACAATCACAAATCTGTATCTCAAGAAGTCTCTGAACAACAAAAAGCTGACTACGAAAGAAAAGCTGAAGAAAGAAAAGCGAGATTTTTAGATAAGCAAAAAAATAAGAAAACTCCTGTAGTTTCATTAGAATATGATTTTGAACATAAACAACGTGTTGACAACGAAAACGACAAGCAACTTGTGGTTTCTGAGCCATCAAAGAAACCAACAACACCGCCTACATACACTGAAACAACCACACAGCTACCAATGCCTACAGTTGAGCGTCAAACACAGCAACAAATCGTTTACAAAGCACCAAAACCATTAGCTGGATTAAATGGTGAAAGTCATGATTTCACAACAACGCATCAATCACCAACTACTTCAAATCACACGCATAATCATCTTATTGAAATTGAAGAAACATCTGCTTTACCTGGTAGAAAGACAGGTTCATTGGTTGGTTTGAGTCAAATTGATTCTTCGCATTTAACTGAACGTGAGAAGCGCGTGATTAAACGTGAACACGTGAGAGAAGCTCAAAAGTTAGTTGATAATTATAAAGATACACATAGTTATAAAGACCGATTAAATGCCCAACAAAAAGTAAATACTTTAAGTGCAGGTCATCAAAAACGTTTTAATAAACAAATTAATAAAGTATATAATGGCAAATAATTAATGCATGGCTGCAAAGGAAATAATGAGTTTGCCGTAAAAATAACAACATTTTAAACTAGCAATAAATAATATCAAAGTCATCATTTCAATGATGCAATCTAGTATAGTCCACATTCTAAACAGGTGTGGACTATTACTTTTTTCACTTTATATTACGAAAAAATTATTATGCTTAACTATCAATATCAATAATTAATTTTAAGCTGAAAAACAATAAAAATGTTAAGACAACGTTTACTTCAAGTTAATTATTATACTGAAAATTCTGGTATATAATGCTGTTAGTGAATATAACAGGAAAATTAAATTGGTTATGATATTGAGTCTATATAAAGGAGAAATAACAGATGAAAAAGAAATTATTAGTTTTAACTATGAGCACGCTATTTGCTACACAATTTATGAATTCAAATCACGCTAATGCATCAACAGAAAGTGTTGATAAAAACTTTGTAGTTCCAGAATCGGGTATTAATAAAATTATTCCAACTTACGATGAATTTAAAAAAGCACCAAAAGTAAATGTTAGTAATTTAGCTGACAACAAAAACTTTGTAGCTTCTGAAGATAAATTGAATAAGATTGCAGATCCATCGGCAGCTAGTAAAATTGTAGATAAAAACTTTGCCGTACCAGAATCAAAATTAGGAATCATTGTACCAGAGTATAAAGAAATCAATAATCGAGTGAATGTAACAACAAACAATCCAGCTTCAAAACAAGTTGACAAGCAAATTGTTGCTAAAGACCCAGAGGTGAATAGATTTATTACGCAAAATAAAGTAAACCATCGTTTCATTACTACGCAAACCCACTATAAGAAAGTTATTACTTCATACAAATCAACACATGTACATAAACATGTAAACCATGCAACATCTTCTATCCATCATCACTTTACTATTAAACCATCAGAAGCACCTAGATATACACACCCATCTCAATCTCAATCGTTAATTATAAATCATCATTTTGCAGTTCCTGGATACCATGGTCATAAAGTTGTAACACCAGGACAAGCTAGTATTAGAATTCATCACTTTTGTGCTGTACCTCAAATAAATAGTTTTAAGGTCA

TTCCATCATATGGTCACAATTCACATCGTATGCATGTACCAAGTTTCCAAAATAACACAACAGCAACACATCAA
AATGCAAAAGTAAATAAAACTTATAACTATAAATATTTTTATACTTATAAAGTAGTCAAAGGTGTAAAAAAACA
TTTCTCATTTTCAAAATCACATGGTTGTAAAATTGTTAAACCAGCATTAAACATCAAAAATGTAAATTATCAAT
ATGCTGTTCCAAGTAATAGCCCTACACACGTTGTTCCTGAGTTTCAGGGTATCTTACCAGCACCACGAGTATAA
AAATTGACATTAAGTTTACGAGATATGATAAATACCTATTATTTTAAACATAGTCTGCAATCTATGAGGTTGTA
GGCTATGTTTTTTGCAGTTTATCAATAAACACCCATCAACAAATTATACCGTTTTTCTACTTTAAAAGTTGGAA
GTAACATAATCTTAAATAAATATATTATTAATTAAGATAAATATAAGACTCGAGATTATTGTTAATAGTTTGTT
CATCGCAAGTTAATTATTGTTTCTAAAATATTGGTATATAATTTTCAATGGCGAAGAAAACAGGTAAAAAAGT
CGGTTTTTAAATCAAAGCAAATAAGGAGTAAAAAATGAAAAGGAAAGTACTAGTATTAACAATGGGCGTACTTT
GTGCGACACAATTATGGCAAACGAATAATGCAAAAGCTTTAGTGACAGAGAGTGGCGTTAATGATACTAAGCAA
TTTACTGAAGTAACATCGGAAGAAAAAGTTATAAAAGATGCTATTTCGAAAGTCAATGAAAGCTTTATTTACTA
TCCCCAAAATGATTTGAAGGGATTAGGTGGAGAACACAACGATTACGAAAAAATTACATATAGCACTTCTTCTA
ATAATGTTTTAGAATTATCAATGAGTTCAAAATACGTAGGCGGTAAATCAGGAGCTATGGTTGGTTATAGTGAA
ATTTACTCATCACATTTCACAGACCGCGACAAACGTGCTATCAGACGTGATCATGTTAAAGAAGCACAAAACTT
GATTAATGATTATAAATATACGCAAATATATGAAGACTTTGCTAAAGCTACTGCAAAGGTAAGTACACTTAGTC
AGTCTCACCAAAATTATTTAAATAAACAAATTGATAAAGTGAATAATAAGATAGAGAAAACTGAAAAACGCTAA
(SEQ ID NO: 78)

MW2

D1D2-
GTGGTTTCTGGGGAGAAGAATCCATATGTATCTGAGTCGTTGAAACTGACTAATAATAAAAATAAATCTAGAAC
AGTAGAAGAGTATAAGAAAAGCTTGGATGATTTAATATGGTCCTTTCCAAACTTAGATAATGAAAGATTTGATA
ATCCTGAATATAAAGAAGCTATGAAAAAATATCAACAGAGATTTATGGCTGAAGATGAGGCTTTGAAGAAATTT
TTTAGTGAAGAGAAAAAAATAAAAAATGGAAATACTGATAATTTAGATTATCTAGGATTATCTCATGAAAGATA
TGAAAGTGTATTTAATACTTTGAAAAAACAAAGTGAGGAGTTCTTAAAAGAAATTGAAGATATAAAAAAAGATA
ACCCTGAATTGAAAGACTTTAATGAATAG (SEQ ID NO: 79)

>USA300_vWbp

VVSGEKNPYVSESLKLTNNKNKSRTVEEYKKSLDDLIWSFPNLDNERFDNPEYKEAMKKYQQRFMAED
EALKKFFSEEKKIKNGNTDNLDYLGLSHERYESVFNTLKKQSEEFLKEIEDIKKDNPELKDFNEEEQLKCDLEL
NKLENQILMLGKTFYQNYRDDVESLYSKLDLIMGYKDEERANKKAVNKRMLENKKEDLETIIDEFFSDIDKTRP
NNIPVLEDEKQEEKNHKNMAQLKSDTEAAKSDESKRSKRSKRSLNTQNHKPASQEVSEQQKAEYDKRAEERKAR
FLDNQKIKKTPVVSLEYDFEHKQRIDNEND (SEQ ID NO: 80)

KKLVVSAPTKKPTSPTTYTETTTQVPMPTVERQTQQQIIYNAPKQLAGLNGESHDFTTTHQSPTTSNH
THNNVVEFEETSALPGRKSGSLVGISQIDSSHLTEREKRVIKREHVREAQKLVDNYKDTHSYKDRINAQQKVNT
LSEGHQKRFNKQINKVYNGK (SEQ ID NO: 81)

>N315_vWbp

VVSGEKNPYVSKALELKDKSNKSNSYENYRDSLESLISSLSFADYEKYEEPEYEKAVKKYQQKFMAED DALKNFLNEEKKIKNADISRKSNNLLGLTHERYSYIFDTLKKNKQEFLKDIEEIQLKNSDLKDFNNTEQHNADV EINNLENKVLMVGYTFYNTNKDEVEELYSELDLIVGEVQDKSDKKRAVNQRMLNRKKEDLEFIIDKFFKKIQQE RPESIPALTSEKNHNQTMALKLKADTEAAKNDVSKRSKRSLNTQNNKSTTQEISEEQKAEYQRKSEALKERFIN RQKSKNESVVSLIDDEDDNENDRQLVVSAP (SEQ ID NO: 82)

SKKPTTIPTTYTETTTQVPMPTVERQTQQQIVYKTPKPLAGLNGESHDFTTTHQSPTTSNHTHNNVVEF EETSALPGRKSGSLVGISQIDSSHLTEREKRVIKREHVREAQKLVDNYKDTHSYKDRLNAQQKVNTLSEGHQKR FNKQINKVYNGK (SEQ ID NO: 83)

>MRSA252_vWbp

VVSGEENPYKSESLKLNGKRSTTITSDKYEENLDMLISSLSFADYEKYEEPEYKEAVKKYQQKFMAED DALKNFLVKRKK (SEQ ID NO: 84)

>MW2_vWbp

VVSGEKNPYVSESLKLTNNKNKSRTVEEYKKSLDDLIWSFPNLDNERFDNPEYKEAMKKYQQRFMAED EALKKFFSEEKKIKNGNTDNLDYLGLSHERYESVFNTLKKQSEEFLKEIEDIKKDNPELKDFNE (SEQ ID NO: 85)

>Newman_vWbp

VVSGEKNPYVSESLKLTNNKNKSRTVEEYKKSLDDLIWSFPNLDNERFDNPEYKEAMKKYQQRFMAED EALKKFFSEEKKIKNGNTDNLDYLGLSHERYESVFNTLKKQSEEFLKEIEDIKKDNPELKDFNEEEQLKCDLEL NKLENQILMLGKTFYQNYRDDVESLYSKLDLIMGYKDEERANKKAVNKRMLENKKEDLETIIDEFFSDIDKTRP NNIPVLEDEKQEEKNHKNMAQLKSDTEAAKSDESKRSKRSKRSLNTQNHKPASQEVSEQQKAEYDKRAEERKAR FLDNQKIKKTPVVSLEYDFEHKQRIDNEND (SEQ ID NO: 86)

KKLVVSAPTKKPTSPTTYTETTTQVPMPTVERQTQQQIIYNAPKQLAGLNGESHDFTTTHQSPTTSNHTHNNVV EFEETSALPGRKSGSLVGISQIDSSHLTEREKRVIKREHVREAQKLVDNYKDTHSYKDRINAQQKVNTLSEGHQ KRFNKQINKVYNGK (SEQ ID NO: 87)

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein.

U.S. Patent 3,791,932

U.S. Patent 3,949,064

U.S. Patent 4,174,384

U.S. Patent 4,338,298

U.S. Patent 4,356,170

U.S. Patent 4,367,110

U.S. Patent 4,372,945

U.S. Patent 4,452,901

U.S. Patent 4,474,757

U.S. Patent 4,554,101

U.S. Patent 4,578,770

U.S. Patent 4,596,792

U.S. Patent 4,599,230

U.S. Patent 4,599,231

U.S. Patent 4,601,903

U.S. Patent 4,608,251

U.S. Patent 4,683,195

U.S. Patent 4,683,202

U.S. Patent 4,684,611

U.S. Patent 4,690,915

U.S. Patent 4,690,915

U.S. Patent 4,748,018

U.S. Patent 4,800,159

U.S. Patent 4,879,236

U.S. Patent 4,952,500

U.S. Patent 5,084,269

U.S. Patent 5,199,942

U.S. Patent 5,221,605

U.S. Patent 5,238,808

Date Reçue/Date Received 2021-01-25

U.S. Patent 5,302,523
U.S. Patent 5,310,687
U.S. Patent 5,322,783
U.S. Patent 5,384,253
U.S. Patent 5,464,765
U.S. Patent 5,512,282
U.S. Patent 5,512,282
U.S. Patent 5,538,877
U.S. Patent 5,538,880
U.S. Patent 5,548,066
U.S. Patent 5,550,318
U.S. Patent 5,563,055
U.S. Patent 5,580,859
U.S. Patent 5,589,466
U.S. Patent 5,591,616
U.S. Patent 5,610,042
U.S. Patent 5,620,896
U.S. Patent 5,648,240
U.S. Patent 5,656,610
U.S. Patent 5,702,932
U.S. Patent 5,736,524
U.S. Patent 5,780,448
U.S. Patent 5,789,215
U.S. Patent 5,801,234
U.S. Patent 5,840,846
U.S. Patent 5,843,650
U.S. Patent 5,846,709
U.S. Patent 5,846,783
U.S. Patent 5,849,497
U.S. Patent 5,849,546
U.S. Patent 5,849,547
U.S. Patent 5,858,652
U.S. Patent 5,866,366
U.S. Patent 5,871,986

U.S. Patent 5,916,776

U.S. Patent 5,922,574

U.S. Patent 5,925,565

U.S. Patent 5,925,565

U.S. Patent 5,928,905

U.S. Patent 5,928,906

U.S. Patent 5,932,451

U.S. Patent 5,935,819

U.S. Patent 5,935,825

U.S. Patent 5,939,291

U.S. Patent 5,942,391

U.S. Patent 5,945,100

U.S. Patent 5,958,895

U.S. Patent 5,981,274

U.S. Patent 5,994,624

U.S. Patent 6,00,8341

U.S. Patent 6,288,214

U.S. Patent 6,294,177

U.S. Patent 6,651,655

U.S. Patent 6,656,462

U.S. Patent 6,733,754

U.S. Patent 6,756,361

U.S. Patent 6,770,278

U.S. Patent 6,793,923

U.S. Patent 6,814,971

U.S. Patent 6,936,258

U.S. Patent Appln. 2002/0169288

U.S. Patent Appln. 2003/0153022

Abdallah *et al.*, *Mol. Microbiol.*, 62, 667-679, 2006.

Abdallah *et al.*, *Nat. Rev. Microbiol.*, 5, 883-891, 2007.

Adams & Bird, *Nephrology*. 14:462-470, 2009.

Albus, *et al.*, *Infect Immun.* 59: 1008-1014, 1991.

An, *J. Virol.*, 71(3):2292-302, 1997.

Anavi, Sc. thesis from the department of Molecular Microbiology and Biotechnology of the Tel-Aviv University, Israel, 1998.

Andersen *et al.*, *J. Immunol.*, 154, 3359-3372, 1995.

Andersen, *et al., Biol Chem.* 390:1279-1283, 2009.

Angel *et al.*, *Cell*, 49:729, 1987b.

Angel *et al.*, *Mol. Cell. Biol.*, 7:2256, 1987a.

Archer, *Clin. Infect. Dis.*, 26, 1179-1181, 1998.

Ariens, *et al., Blood.* 96:988-995, 2000.

Arrecubieta, *et al., J Infect Dis.* 198: 571-575, 2008.

Atchison and Perry, *Cell*, 46:253, 1986.

Atchison and Perry, *Cell*, 48:121, 1987.

Ausubel *et al., In: Current Protocols in Molecular Biology,* John, Wiley & Sons, Inc, New York, 1996.

Baba *et al., J. Bacteriol.* 190:300-310, 2007.

Baba, *et al., Lancet.* 359: 1819-1827, 2002.

Baddour, *et al., J Infect Dis.* 165: 749-753, 1992.

Baddour, *et al., J Med Microbiol.* 41:259-263, 1994.

Bae and Schneewind, *Plasmid*, 55:58-63, 2006.

Bae *et al., Proc. Natl. Acad. Sci. USA*, 101, 12312-12317, 2004.

Balaban, *et al., Science.* 280:438-440, 1998.

Banerji *et al.*, *Cell*, 27(2 Pt 1):299-308, 1981.

Banerji *et al.*, *Cell*, 33(3):729-740, 1983.

Barany and Merrifield, In: *The Peptides,* Gross and Meienhofer (Eds.), Academic Press, NY, 1-284, 1979.

Behring. *Deutsche Medzinische Wochenschrift.* 16:1145-8, 1890.

Bellus, *J. Macromol. Sci. Pure Appl. Chem.*, A31(1): 1355-1376, 1994.

Berger, *J Pathol Bacteriol.* 55, 1943.

Berkhout *et al., Cell*, 59:273-282, 1989.

Birch-Hirschfeld, *Klinische Woschenschrift.* 13:331, 1934.

Bjerketorp, *et al., FEMS Microbiol Lett.* 234:309-314, 2004.

Bjerketorp, *et al., Microbiol.* 148:2037-2044, 2002.

Blanar *et al., EMBO J.*, 8:1139, 1989.

Blomback, *et al., Nature.* 275:501-505, 1978.

Boake, *J Immunol.* 76:89-96, 1956.

Bodine and Ley, *EMBO J.*, 6:2997, 1987.

Borrebaeck, In: *Antibody Engineering--A Practical Guide*, W. H. Freeman and Co., 1992.

Boshart *et al.*, *Cell*, 41:521, 1985.

Bosze *et al.*, *EMBO J.*, 5(7):1615-1623, 1986.

Boucher and Corey. *Clin. Infect. Dis.* 46:S334-S349, 2008.

Braddock *et al.*, *Cell*, 58:269, 1989.

Brown *et al.*, *Biochemistry*, 37:4397-4406, 1998.

Bubeck Wardenburg and Schneewind. *J. Exp. Med.* 205:287-294, 2008.

Bubeck-Wardenburg *et al.*, *Infect. Immun.* 74:1040-1044, 2007..

Bubeck-Wardenburg *et al.*, *Proc. Natl. Acad. Sci. USA*, 103:13831-13836, 2006.

Bulla and Siddiqui, *J. Virol.*, 62:1437, 1986.

Burke *et al.*, , *J. Inf. Dis.*, 170:1110-1119, 1994.

Burlak *et al.*, *Cell Microbiol.*, 9:1172-1190, 2007.

Burts and Missiakas, *Mol. Microbiol.*, 69:736-46, 2008.

Burts *et al.*, *Proc. Natl. Acad. Sci. USA*, 102:1169-1174, 2005.

Cadness-Graves, *et al., Lancet.* 2:736-738, 1943.

Camargo & Gilmore, *J Bacteriol.* 190:2253-2256, 2008.

Campbell and Villarreal, *Mol. Cell. Biol.*, 8:1993, 1988.

Campere and Tilghman, *Genes and Dev.*, 3:537, 1989.

Campo *et al.*, *Nature*, 303:77, 1983.

Carbonelli *et al.*, *FEMS Microbiol. Lett.*, 177(1):75-82, 1999.

Cawdery, *et al., British J Exp Pathol.* 50:408-412, 1969.

Cedergren *et al.*, *Protein Eng.*, 6:441-448, 1993..

Celander and Haseltine, *J. Virology*, 61:269, 1987.

Celander *et al.*, *J. Virology*, 62:1314, 1988.

Cespedes *et al.*, *J. Infect. Dis.* 191(3):444-52, 2005.

Chambers & Deleo. *Nature Rev Microbiol.* 7: 629-641, 2009.

Champion *et al.*, *Science*, 313:1632-1636, 2006.

Chandler *et al.*, *Cell*, 33:489, 1983.

Chandler *et al.*, *Proc. Natl. Acad. Sci. USA*, 94(8):3596-601, 1997.

Chang *et al.*, *Lancet.*, 362(9381):362-369, 2003.

Chang *et al.*, *Mol. Cell. Biol.*, 9:2153, 1989.

Chapman, *et al., J Bacteriol.* 28:343-363, 1934.

Chatterjee *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:9114, 1989.

Chen and Okayama, *Mol. Cell Biol.*, 7(8):2745-2752, 1987.

Cheng, *et al., Trends Microbiol.* 19: 225-232, 2011.

Cheng *et al., FASEB J.*, 23:1-12, 2009.

Cheng, *et al., FASEB J.* 23(10):3393-3404, 2009.

Cheng, *et al., PLoS Pathogens.* 6, 2010.

Cheung, *et al., Infect Immun.* 63:1914-1920, 1995.

Choi *et al., Cell*, 53:519, 1988.

Chu, *et al., Am J Med.* 118:1416, 2005.

Clarke, *et al., Ad Microbial Phys.* 51:187-224, 2006.

Cocea, *Biotechniques*, 23(5):814-816, 1997.

Cohen *et al., J. Cell. Physiol.*, 5:75, 1987.

Cosgrove *et al., Infect. Control Hosp. Epidemiol.* 26:166-174, 2005..

Costa *et al., Mol. Cell. Biol.*, 8:81, 1988.

Crawley, *et al., J Thrombosis Haemostasis.* 5 Suppl 1:95-101, 2007.

Cripe *et al., EMBO J.*, 6:3745, 1987.

Culotta and Hamer, *Mol. Cell. Biol.*, 9:1376, 1989.

Dalbey and Wickner, *J. Biol. Chem.*, 260:15925-15931, 1985.

Dandolo *et al., J. Virology,* 47:55-64, 1983.

de Haas , *et al., J Exp Med.* 199:687-695, 2004.

De Villiers *et al., Nature,* 312(5991):242-246, 1984.

DeBord *et al., Infect. Immun.*, 74:4910-4914, 2006.

DeDent, *et al., Sem Immunopathol.* 34: 317-333, 2012.

DeDent *et al., EMBO J.* 27:2656-2668, 2008.

DeDent *et al., J. Bacteriol.* 189:4473-4484, 2007.

Deisenhofer *et al., Hoppe-Seyh Zeitsch. Physiol. Chem.* 359:975-985, 1978..

Deisenhofer, *Biochemistry* 20:2361-2370, 1981.

Deivanayagam, *et al., EMBO J.* 21:6660-6672, 2002.

DeLeo, *et al., Lancet.* 375: 1557-1568, 2010.

Delvaeye & Conway, *Blood.* 114:2367-2374, 2009.

Deschamps *et al., Science*, 230:1174-1177, 1985.

Devereux *et al., Nucl. Acid Res.*, 12:387-395, 1984.

Diep *et al., J. Infect. Dis.*, 193:1495-1503, 2006a.

Diep *et al., Lancet.*, 367:731-739, 2006b.

Dinges *et al., Clin. Microbiol. Rev.*, 13:16-34, 2000.

Donahue, *et al., PNAS USA.* 91: 12178-12182, 1994.

Doolittle, *Blood Rev.* 17: 33-41, 2003.

Duthie and Lorenz, *J. Gen. Microbiol.*, 6:95-107, 1952.

Duthie, *J Gen Microbiol.* 6: 95-107, 1952.

Duthie, *J Gen Microbiol.* 10:427-436, 1954.

Edbrooke *et al.*, *Mol. Cell. Biol.*, 9:1908, 1989.

Edlund *et al.*, *Science*, 230:912-916, 1985.

Ekstedt & Yotis, *J Bacteriol.* 80:496-500, 1960.

Ekstedt and Yotis, *Ann. N.Y. Acad. Sci.*, 80:496-500, 1960.

Emorl and Gaynes, *Clin. Microbiol. Rev.*, 6:428-442, 1993.

Enright, *et al., J Clin Microbiol.* 38: 1008-1015, 2000.

EP 0786519

EP 497524

EP 497525

Epitope Mapping Protocols In: *Methods in Molecular Biology*, Vol. 66, Morris (Ed.), 1996.

Etz, *et al., PNAS USA.* 99:6573-6578, 2002.

Fattom, *et al., Vaccine.* 23: 656-663, 2004.

Fechheimer, *et al.*, *Proc Natl. Acad. Sci. USA,* 84:8463-8467, 1987.

Feng and Holland, *Nature*, 334:6178, 1988.

Ferry, *et al., Curr Infect Dis Report.* 7:420-428, 2005.

Field and Smith, *J. Comp. Pathol.*, 55:63, 1945.

Firak and Subramanian, *Mol. Cell. Biol.*, 6:3667, 1986.

Fitzgerald, *et al., Nature Rev Microbiol.* 4:445-457, 2006.

Foecking and Hofstetter, *Gene*, 45(1):101-105, 1986.

Fortune *et al.*, *Proc Natl. Acad. Sci. USA,* 102:10676-10681, 2005.

Foster, *Nat. Rev. Microbiol.*, 3:948-958, 2005.

Fournier *et al.*, *Infect. Immun.*, 45:87-93, 1984.

Fowler, *et al., New England J Med.* 355: 653-665, 2006.

Fowler, *et al., JAMA.* 293: 3012-3021, 2005.

Fraley *et al.*, *Proc. Natl. Acad. Sci. USA,* 76:3348-3352, 1979.

Friedrich, *et al., Nature.* 425:535-539, 2003.

Fujita *et al.*, *Cell,* 49:357, 1987.

Gailani & Renne, *Arteriosclerosis, Thrombosis & Vascular Biol.* 27:2507-2513, 2007.

Ganesh, *et al., PLoS Pathogens.* 4: e1000226, 2008.

GB Appln. 2 202 328

Geoghegan, *et al., J Biol Chem.* 285: 6208-6216, 2010.

Gilles *et al., Cell*, 33:717, 1983.

Gloss *et al., EMBO J.*, 6:3735, 1987.

Godbout *et al., Mol. Cell. Biol.,* 8:1169, 1988.

Gomez *et al., EMBO J.* 26:701-709, 2007..

Gomez *et al., J. Biol. Chem.* 281:20190-20196, 2006..

Gomez *et al., Nature Med.* 10:842-8, 2004.

Gong, *et al., Clim Vacc Immunol.* CVI 17: 1746-1752, 2010.

Gonzalez, *et al., CMLS.* 65:493-507, 2008.

Goodbourn and Maniatis, *Proc. Natl. Acad. Sci. USA,* 85:1447, 1988.

Goodbourn *et al., Cell*, 45:601, 1986.

Goodyear and Silverman, *J. Exp. Med.,* 197:1125-1139, 2003.

Goodyear, *et al., PNAS USA.* 101:11392-11397, 2004.

Gopal, *Mol. Cell Biol.*, 5:1188-1190, 1985.

Gouda *et al., Biochemistry*, 31(40):9665-72, 1992.

Gouda *et al., Biochemistry*, 37:129-36, 1998.

Graham and Van Der Eb, *Virology*, 52:456-467, 1973.

Graille *et al., Proc. Nat. Acad. Sci. USA* 97:5399-5404, 2000.

Gravenkemper, *et al., J Bacteriol.* 89:1005-1010, 1965.

Greene *et al., Immunology Today,* 10:272, 1989

Grosschedl and Baltimore, *Cell*, 41:885, 1985.

Guinn *et al., Mol. Microbiol.*, 51:359-370, 2004.

Guss *et al., Eur. J. Biochem.* 138:413-420, 1984.

Hair, *et al., Infect Immun.* 78: 1717-1727, 2010.

Hale & Smith, *Br J Exp Pathol.* 26: 209-216, 1945.

Hall, *et al., Infect Immun.* 71: 6864-6870, 2003.

Haraldsson & Jonsson, *J Comp Pathol.* 94:183-196, 1984.

Harland and Weintraub, *J. Cell Biol.*, 101(3):1094-1099, 1985.

Harlow *et al., Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., Chapter 8, 1988.

Harrison, *Br Med J.* 2: 149-152, 1963.

Harrison, *J Pathol Bacteriol.* 87: 145-150, 1964.

Harro, *et al., Clin Vacc Immun.* CVI 17: 1868-1874, 2010.

Hartleib, *et al., Blood.* 96:2149-2156, 2000.

Harvey *et al., Proc. Natl. Acad. Sci. USA*, 83:1084-1088, 1986.

Haslinger and Karin, *Proc. Natl. Acad. Sci. USA*, 82:8572, 1985.

Hauber and Cullen, *J. Virology*, 62:673, 1988.

Hauel, *et al., J Med Chem.* 45: 1757-1766, 2002.

Hawiger, *et al., Nature.* 258: 643-645, 1975.

Hawiger, *et al., Biochem.* 21: 1407-1413, 1982.

Heilmann, *et al., J Infect Dis.* 186: 32-39, 2002.

Hen *et al., Nature*, 321:249, 1986.

Hendrix, *et al., J Biol Chem.* 258:3637-3644, 1983.

Hensel *et al., Lymphokine Res.*, 8:347, 1989.

Herold, *et al., JAMA.* 279:593-598, 1998.

Herr and Clarke, *Cell*, 45:461, 1986.

Hijikata-Okunomiya, *J Thrombosis Haemostasis.* 1: 2060-2061, 2003.

Hirochika *et al., J. Virol.*, 61:2599, 1987.

Hirsch *et al., Mol. Cell. Biol.*, 10:1959, 1990.

Holbrook *et al., Virology*, 157:211, 1987.

Horlick and Benfield, *Mol. Cell. Biol.*, 9:2396, 1989.

Hsu *et al., Proc. Natl. Acad. Sci. USA,* 100:12420–12425, 2003.

Huang *et al., Cell,* 27:245, 1981.

Huber-Lang, *et al., Nature Med.* 12:682-687, 2006.

Hug *et al., Mol. Cell. Biol.*, 8:3065, 1988.

Hussain, *et al., J Bacteriol.* 183: 6778-6786, 2001.

Huston *et al.*, In: *Methods in Enzymology*, Langone (Ed.), Academic Press, NY, 203:46-88, 1991.

Hwang *et al., Mol. Cell. Biol.*, 10:585, 1990.

Imagawa *et al., Cell*, 51:251, 1987.

Imbra and Karin, *Nature*, 323:555, 1986.

Imler *et al., Mol. Cell. Biol.,* 7:2558, 1987.

Imperiale and Nevins, *Mol. Cell. Biol.*, 4:875, 1984.

Innis *et al., Proc Natl Acad Sci USA*, 85(24):9436-9440, 1988.

Inoshima, *et al., Nat Med.* 17(10):1310-4, 2011.

Inouye and Inouye, *Nucleic Acids Res.*, 13: 3101-3109, 1985.

Jakobovits *et al., Mol. Cell. Biol.,* 8:2555, 1988.

Jameel and Siddiqui, *Mol. Cell. Biol.*, 6:710, 1986.

Jansson *et al.*, *FEMS Immunol. Med. Microbiol.* 20:69-78 1998.

Jaynes *et al.*, *Mol. Cell. Biol.*, 8:62, 1988.

Jensen, *Acta Path. Microbiol. Scandin.* 44:421-428, 1958.

Jensen, *APMIS: acta pathol, microbiol et immunol Scandinavica.* 115: 533-539, 2007.

Johnson *et al.*, *Methods in Enzymol.*, 203:88-99, 1991.

Johnson *et al.*, *Mol. Cell. Biol.*, 9:3393, 1989.

Johnstone & Smith, *Nature.* 178:982-983, 1956.

Jones, *Carb. Research*, 340:1097-1106, 2005.

Jonsson *et al.*, *Oral Dis.*, 8(3):130-140, 2002.

Jonsson, *et al.*, *Infect Immun.* 49: 765-769, 1985.

Josefsson, *et al.*, *J Infect Dis.* 184: 1572-1580, 2001.

Josefsson, *et al.*, *PLoS One.* 3: e2206, 2008.

Joyce *et al.*, *Carbohydrate Research* 338:903-922 (2003

Kadesch and Berg, *Mol. Cell. Biol.*, 6:2593, 1986.

Kaeppler *et al.*, *Plant Cell Rep.*, 8:415–418, 1990.

Kaida, *et al.*, *J Biochem.* 102: 1177-1186, 1987.

Kallen, *et al.*, *Ann Emmergency Med.* 53: 358-365, 2009.

Kaneda *et al.*, *Science*, 243:375-378, 1989.

Kanemitsu, *et al.*, *Microbiol Immunol.* 45: 23-27, 2001.

Kang, *et al.*, *Am J Infection Control.* 40:416-20, 2011.

Kantyka, *et al.*, *Adv Exp Med Biol.* 712:1-14, 2011.

Kapral, *J Bacteriol.* 92:1188-1195, 1966.

Karesh. Pediatric Focused Safety Review: Argatroban. Pediatric Advisory Committee Meeting, 2009.

Karin *et al.*, *Mol. Cell. Biol.*, 7:606, 1987.

Katinka *et al.*, *Cell*, 20:393, 1980.

Kato *et al*, *J. Biol. Chem.*, 266:3361-3364, 1991.

Kawabata, *et al.*, *J Biochem.* 97: 1073-1078, 1985.

Kawabata, *et al.*, *J Biochem.* 97:325-331, 1985.

Kawabata, *et al.*, *J Biochem.* 98:1603-1614, 1985.

Kawamoto *et al.*, *Mol. Cell. Biol.*, 8:267, 1988.

Kennedy, *et al.*, *J Infect Dis.* 202: 1050-1058, 2010.

Kennedy *et al.*, *Proc. Natl. Acad. Sci. USA* 105:1327-1332, 2008.

Kernodle, *J Infect Dis.* 203: 1692-1693; author reply 1693-1694, 2011.

Kiledjian *et al.*, *Mol. Cell. Biol.*, 8:145, 1988.

Kim, *et al., Vaccine.* 28: 6382-6392, 2001.

Kim, *et al., FASEB J.* 25: 3605-3612, 2011.

Kim, *et al., J Exp Med.* 207:1863-1870, 2010.

Kinoshita, *et al., Microbiol Immunol.* 52: 334-348, 2008.

Klamut *et al.*, *Mol. Cell. Biol.*, 10:193, 1990.

Klevens *et al., Clin. Infect. Dis.*, 2008; 47:927-30, 2008.

Klevens *et al.*, *JAMA,* 298:1763-1771, 2007.

Kluytmans, *et al., Clin Microbiol Rev.* 10: 505-520, 1997.

Koch *et al.*, *Mol. Cell. Biol.*, 9:303, 1989.

Kohler and Milstein, *Nature* 256:495-497 (1975

Kolle & Otto. *Z Hygiene.* 41, 1902.

Kollman, *et al., Biochemistry.* 48:3877-3886, 2009.

Konings, *et al., Blood.* 118(14):3942-51, 2011.

Kopec, *et al., Thrombosis et diathesis haemorrhagica.* 18:475-486, 1967.

Koreen, *et al., J Clin Microbiol.* 42: 792-799, 2004.

Krarup, *et al., PLoS One.* 2:e623, 2007.

Kriegler and Botchan, *In: Eukaryotic Viral Vectors,* Gluzman (Ed.), Cold Spring Harbor: Cold Spring Harbor Laboratory, NY, 1982.

Kriegler and Botchan, *Mol. Cell. Biol.*, 3:325, 1983.

Kriegler *et al.*, *Cell*, 38:483, 1984a.

Kriegler *et al.*, *Cell,* 53:45, 1988.

Kriegler *et al.*, *In: Cancer Cells 2/Oncogenes and Viral Genes,* Van de Woude *et al.* eds, Cold Spring Harbor, Cold Spring Harbor Laboratory, 1984b.

Kroh, *et al., PNAS USA.* 106:7786-7791, 2009.

Kuehnert, *et al., J Infect Dis.* 193: 172-179, 2006.

Kuhl *et al.*, *Cell,* 50:1057, 1987.

Kuklin *et al., Infect. Immun.*, 74:2215-23, 2006.

Kunz *et al.*, *Nucl. Acids Res.*, 17:1121, 1989.

Kuroda *et al., Lancet.*, 357:1225–1240, 2001.

Kwiecinski, *et al., J Infect Dis.* 202:1041-1049, 2010.

Kyte and Doolittle, *J. Mol. Biol.,* 157(1):105-132, 1982.

Lack, *Nature.* 161:559, 1948.

Lagergard *et al., Eur. J. Clin. Microbiol. Infect. Dis.*, 11:341-5, 1992.

Lam, *et al., J Bacteriol.* 86:611-615, 1963.

Lam, *et al., J Bacteriol.* 86:87-91, 1963.

Lancefield, *J Immunol.* 89: 307-313, 1962.

Lancefield, *J Exp Med.* 47: 91-103, 1928.

Larsen *et al., Proc Natl. Acad. Sci. USA.*, 83:8283, 1986, 1963.

Laspia *et al., Cell*, 59:283, 1989.

Latimer *et al., Mol. Cell. Biol.,* 10:760, 1990.

Lattar, *et al., Infect Immun.* 77:1968-1975, 2009.

Lee *et al., Nature*, 294:228, 1981.

Lee *et al., Nucleic Acids Res.,* 12:4191-206, 1984.

Lee, *et al., J Infect Dis.* 156: 741-750, 1987.

Lee, *Trends Microbiol.* 4(4):162-166, 1996.

Levenson *et al., Hum. Gene Ther.*, 9(8):1233-1236, 1998.

Levine MM, editor. New generation vaccines. 4th ed. New York: Informa Healthcare USA. xxvii, 1011, 2010

Levinson *et al., Nature*, 295:79, 1982.

Lin *et al., Mol. Cell. Biol.*, 10:850, 1990.

Lin, *et al., J Bacteriol.* 176: 7005-7016, 1994.

Liu, *et al., Clin Infect Dis.* 52: 285-292, 2011.

Loeb, *J Med Res.* 10:407-419, 1903.

Lominski, *J Gen Microbiol.* 3: ix, 1949.

Lominski & Roberts, *J Pathol Bacteriol.* 58: 187-199, 1946.

Lominski, *et al., Lancet.* 1: 1315-1318, 1962.

Loof, *et al., Blood.* 118:2589-98, 2011.

Lorand, *Arteriosclerosis, Thrombosis, and Vascular Biol.* 20:2-9, 2000.

Lord, *Arteriosclerosis, Thrombosis & Vascular Biol.* 31:494-499, 2011.

Lowy, *N Engl J Med.* 339:520-532, 1998.

Luria *et al., EMBO J.*, 6:3307, 1987.

Lusky and Botchan, *Proc. Natl. Acad. Sci. USA*, 83:3609, 1986.

Lusky *et al., Mol. Cell. Biol.,* 3:1108, 1983.

Macejak and Sarnow, *Nature*, 353:90-94, 1991.

MacGurn *et al., Mol. Microbiol.*, 57:1653-1663, 2005.

Mainiero, *et al., J Bacteriol.* 192: 613-623, 2010.

Maira-Litran *et al.*, *Infect. Immun.*, 70:4433-4440, 2002.

Maira-Litran *et al.*, *Vaccine*, 22:872-879, 2004.

Majors and Varmus, *Proc. Natl. Acad. Sci. USA*, 80:5866, 1983.

Markwardt F (1955) Untersuchungen √°ber Hirudin: naturwiss. F, 1955.

Markwardt, *Untersuchungen über Hirudin. Naturwissenschaften*, 41:537-538, 1955.

Mazmanian *et al.*, *Mol. Microbiol.*, 40(5):1049-1057, 2001.

Mazmanian *et al.*, *Proc. Natl. Acad. Sci. USA*, 97:5510–5515, 2000.

Mazmanian *et al.*, *Science*, 285(5428):760-3, 1999.

Mazmanian, *et al.*, *Science*. 299:906-909, 2003.

McAdow , *et al.*, *PLoS Pathogens*. 7: e1002307, 2011.

McAdow, *et al.*, *J Inn Immun*. 4: 141-148, 2012.

McAdow, *et al.*, Coagulases as determinants of protective immune responses against *Staphylococcus aureus*. In preparation, 2012.

McAleese, *et al.*, *Microbiology*. 149:99-109, 2003.

McCarthy & Lindsay, *BMC Microbiol*. 10: 173, 2010.

McDevitt, *et al.*, *Mol Microbiol*. 16: 895-907, 1995.

McDevitt, *et al.*, *Euro J Biochem/FEBS*. 247: 416-424, 1997.

McDevitt, *et al.*, *Mol Microbiol*. 11:237-248, 1994.

McLaughlin *et al.*, *PLoS Pathog.*, 3:e105, 2007.

McNeall *et al.*, *Gene*, 76:81, 1989.

Melles, *et al.*, *FEMS Immunol Med Microbiol*. 52:287-292, 2008.

Mernaugh *et al.*, In: *Molecular Methods in Plant Pathology*, Singh *et al.* (Eds.), CRC Press Inc., Boca Raton, FL, 359-365, 1995.

Merrifield, *Science*, 232(4748):341-347, 1986.

Miksicek *et al.*, *Cell*, 46:203, 1986.

Mora, *et al.*, *PNAS USA*. 102: 15641-15646, 2005.

Mordacq and Linzer, *Genes and Dev.*, 3:760, 1989.

Moreau *et al.*, *Carbohydrate Res.*, 201:285-297, 1990.

Moreau *et al.*, *Nucl. Acids Res.*, 9:6047, 1981.

Moreillon, *et al.*, *Infect Immun*. 63:4738-4743, 1995.

Mosmann and Coffman, *Ann. Rev. Immunol.*, 7:145-173, 1989.

Much, *Biochem Z*. 14:143-155, 1908.

Muesing *et al.*, *Cell*, 48:691, 1987.

Musher *et al.*, *Medicine (Baltimore)*, 73:186-208, 1994.

Mutch, *et al., Blood.* 115:3980-3988, 2010.

Navarre and Schneewind, *J. Biol. Chem.*, 274:15847-15856, 1999.

Na'was T, *et al., J Clin Immunol.* 36:414-420, 1998.

Needleman & Wunsch, *J. Mol. Biol.*, 48:443, 1970.

Ng *et al., Nuc. Acids Res.*, 17:601, 1989.

Ni Eidhin, *et al., Mol Microbiol.* 30:245-257, 1998.

Nicolau and Sene, *Biochim. Biophys. Acta,* 721:185-190, 1982.

Nicolau *et al., Methods Enzymol.*, 149:157-176, 1987.

Niemann, *et al., Circulation.* 110:193-200, 2004.

Nilsson, *et al., J Clin Invest.* 101: 2640-2649, 1998.

Noble, *et al., FEMS Microbiol Lett.* 72:195-198, 1992.

Novick, *Mol. Microbiol.*, 48:1429–1449, 2003.

Nuccitelli, *et al., PNAS USA.* 108: 10278-10283, 2011.

O'Seaghdha *et al., FEBS J.* 273:4831-4841, 2006..

O'Brien, *et al., Mol Microbiol.* 44:1033-1044, 2002.

Omirulleh *et al., Plant Mol. Biol.,* 21(3):415-28, 1993.

Ondek *et al., EMBO J.*, 6:1017, 1987.

Ornitz *et al., Mol. Cell. Biol.*, 7:3466, 1987.

O'Seaghdha, *et al. FEBS J.* 273: 4831-4841, 2006.

Pallen, *Trends Microbiol.,* 10:209-212, 2002.

Palma, *et al., Infect Immun.* 64: 5284-5289, 1996.

Palma, *et al., J Biol Chem.* 276: 31691-31697, 2001.

Palma, *et al., J Biol Chem.* 273: 13177-13181, 1998.

Palmiter *et al., Nature*, 300:611, 1982.

Palmqvist *et al., Microbes. Infect.*, 7:1501-11, 2005.

Palmqvist, *et al., Microbes Infect.* 6: 188-195, 2004.

Panizzi *et al., J. Biol. Chem.*, 281:1179-1187, 2006.

Panizzi, *et al., Cell Mol Life Sci.* 61: 2793-2798, 2004.

Panizzi, *et al., Nat Med.* 17: 1142-1146, 2011.

Patel, *et al., Infect Contr Hosp Epidemiol.* 32: 881-888, 2011.

Paul-Satyaseela, *et al., Epidemiol Infect.* 132:831-838, 2004.

PCT Appln. PCT/US89/01025

PCT Appln. WO 00/02523

PCT Appln. WO 00/12132

PCT Appln. WO 00/12689

PCT Appln. WO 00/15238

PCT Appln. WO 01/34809

PCT Appln. WO 01/60852

PCT Appln. WO 01/98499

PCT Appln. WO 02/059148

PCT Appln. WO 02/094868

PCT Appln. WO 03/53462

PCT Appln. WO 04/43407

PCT Appln. WO 06/032472

PCT Appln. WO 06/032475

PCT Appln. WO 06/032500

PCT Appln. WO 07/113222

PCT Appln. WO 07/113223

PCT Appln. WO 94/09699

PCT Appln. WO 95/06128

PCT Appln. WO 95/08348

PCT Appln. WO 98/57994

Pearson & Lipman, *Proc. Natl. Acad. Sci. USA*, 85:2444, 1988.

Pech *et al.*, *Mol. Cell. Biol.*, 9:396, 1989.

Pelletier and Sonenberg, *Nature*, 334(6180):320-325, 1988.

Perez-Stable and Constantini, *Mol. Cell. Biol.*, 10:1116, 1990.

Peterson, *et al., Infect Immun.* 15:760-764, 1997.

Phonimdaeng, *et al., J Gen Microbiol.* 134:75-83, 1988.

Phonimdaeng, *et al., Mol Microbiol.* 4:393-404, 1990.

Picard and Schaffner, *Nature*, 307:83, 1984.

Pinkert *et al.*, *Genes and Dev.*, 1:268, 1987.

Plotkin, SA, Orenstein WA, editors (2004) Vaccines. 4th ed. Philadelphia, Pa.: Saunders. xxi, 1662 p. p.2004

Ponta *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:1020, 1985.

Porton *et al.*, *Mol. Cell. Biol.*, 10:1076, 1990.

Potrykus *et al.*, *Mol. Gen. Genet.*, 199(2):169-177, 1985.

Powers, *et al., J Infect Dis.* Doi: 10.1093/infdis/jis192, 2012.

Procyk & Blomback, *Biochemistry.* 29:1501-1507, 1990.

Projan, *et al., Curr Opin Pharmacol.* 6: 473-479, 2006.

Pugsley, *Microbiol. Rev.*, 57:50-108, 1993.

Pym *et al., Mol. Microbiol.*, 46;709-717, 2002.

Pym *et al., Nat. Med.*, 9:533-539, 2003.

Que, *et al., Infect Immun.* 68:3516-3522, 2000.

Queen and Baltimore, *Cell*, 35:741, 1983.

Quinn *et al., Mol. Cell. Biol.*, 9:4713, 1989.

Rammelkamp, *et al., J Exp Med.* 91: 295-307, 1950.

Rammelkamp, *et al., Ann NY Acad Sci.* 65: 144-151, 1956.

Redondo *et al., Science*, 247:1225, 1990.

Reisman and Rotter, *Mol. Cell. Biol.*, 9:3571, 1989.

Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1289-1329, 1990.

Resendez Jr. *et al., Mol. Cell. Biol.*, 8:4579, 1988.

Rijken & Lijnen, *J Thrombosis & Haemostasis.* 7:4-13, 2009.

Ripe *et al., Mol. Cell. Biol.*, 9:2224, 1989.

Rippe, *et al., Mol. Cell Biol.*, 10:689-695, 1990.

Rittling *et al., Nuc. Acids Res.*, 17:1619, 1989.

Roben *et al., J. Immunol.* 154:6437-6445, 1995.

Rogers, *et al., Ann NY Acad Sci.* 128: 274-284, 1965.

Rooijakkers, *et al., Nature Immunol.* 6:920-927, 2005.

Rosen *et al., Cell,* 41:813, 1988.

Rothfork, *et al., J Immunol.* 171: 5389-5395, 2003.

Ryan, K. J., & Ray, C. G. (Eds.). Sherris Medical Microbiology: An Introduction to Infectious Disease. (Fourth Edition. ed.). New York.: McGraw-Hill, 2004.

Sakai *et al., Genes and Dev.,* 2:1144, 1988.

Sakharov, *et al. J Biol Chem.* 271:27912-27918, 1996.

Salid-Salim *et al., Infect. Control Hosp. Epidemiol.* 24:451-455, 2003.

Sambrook *et al., In: Molecular cloning,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001.

Saravolatz, *et al., Ann Int Med.* 97:325-329, 1982.

Sawai, *et al., Infect Immunity.* 65:466-471, 1997.

Schaffner *et al., J. Mol. Biol.*, 201:81, 1988.

Schneewind *et al., Cell* 70:267-281, 1992.

Schneewind *et al., EMBO*, 12:4803-4811, 1993.

Schneewind & Missiakas, *PNAS USA.* 108: 10029-10030, 2011.

Schneewind, *et al., Science.* 268:103-106, 1995.

Searle *et al., Mol. Cell. Biol.*, 5:1480, 1985.

Seki, *et al., Microbiol Immunol.* 33:981-990, 1989.

Sharp and Marciniak, *Cell*, 59:229, 1989.

Shaul and Ben-Levy, *EMBO J.*, 6:1913, 1987.

Shaw *et al., Microbiology*, 150:217-228, 2004.

Sheagren, *N. Engl. J. Med.* 310:1368-1373, 1984.

Sherman *et al., Mol. Cell. Biol.*, 9:50, 1989.

Shinefield, *et al., New England J Med.* 346: 491-496, 2002.

Shopsin *et al., J. Clin. Microbiol.*, 37:3556-63, 1999.

Sibbald *et al., Microbiol. Mol Biol. Rev.*, 70:755-788, 2006.

Sievert, *et al., Clin Infect Dis.* 46:668-674, 2008.

Silberman, *et al., Brit J Haemotol.* 24: 101-113, 1973.

Silverman and Goodyear. *Nat. Rev. Immunol.*, 6:465-75, 2006.

Sjodahl, *Eur. J. Biochem.* 73:343-351, 1977.

Sjoquist *et al., Eur. J. Biochem.* 30:190-194, 1972.

Sleigh and Lockett, *J. EMBO*, 4:3831, 1985.

Smith & Waterman, *Adv. Appl. Math.*, 2:482, 1981.

Smith & Johnstone. *Nature.* 178: 982-983, 1956.

Smith *et al., Brit. J. Exp. Pathol.*, 28:57, 1947.

Sorensen *et al., Infect. Immun.*, 63:1710–1717, 1995.

Soulier, *et al., Thrombosis et diathesis haemorrhagica.* 17:321-334, 1967.

Spalholz *et al., Cell*, 42:183, 1985.

Spandau and Lee, *J. Virology*, 62:427, 1988.

Spandidos and Wilkie, *EMBO J.*, 2:1193, 1983.

Spink, *et al., J Clin Invest.* 21:353-356, 1942.

Stanley *et al., Proc. Natl. Acad. Sci. USA*, 100:13001–13006, 2003.

Stephens and Hentschel, *Biochem. J.*, 248:1, 1987.

Stewart and Young, In: *Solid Phase Peptide Synthesis*, 2d. ed., Pierce Chemical Co., 1984.

Stranger-Jones *et al., Proc. Nat. Acad. Sci. USA*, 103:16942-16947, 2006.

Streitfeld, *et al., Nature.* 184(Suppl 21): 1665-1666, 1959.

Strong, *et al., Biochem.* 21: 1414-1420, 1982.

Stuart *et al., Nature*, 317:828, 1985.

Studier *et al.*, *Methods Enzymol.* 185:60-89 1990.

Stutzmann, *et al., Infect Immun.* 69:657-664, 2001.

Sullivan and Peterlin, *Mol. Cell. Biol.*, 7:3315, 1987.

Sutter, *et al., FEMS Immunol Med Microbiol.* 63:16-24, 2011.

Swartzendruber and Lehman, *J. Cell. Physiology*, 85:179, 1975.

Tager & Drumman, *Ann NY Acad Sci.* 128: 92-111, 1965.

Tager & Hales, *J Immunol.* 60: 1-9, 1948.

Takebe *et al.*, *Mol. Cell. Biol.*, 8:466, 1988.

Tam *et al.*, *J. Am. Chem. Soc.*, 105:6442, 1983.

Tavernier *et al.*, *Nature,* 301:634, 1983.

Taylor and Kingston, *Mol. Cell. Biol.*, 10:165, 1990a.

Taylor and Kingston, *Mol. Cell. Biol.*, 10:176, 1990b.

Taylor *et al.*, *J. Biol. Chem.*, 264:15160, 1989.

Tenover, *et al., Antimicrob Agents Chemother.* 56: 1324-1330, 2012.

Thammavongsa, *et al., J Exp Med.* 206:2417-2427, 2009.

Thiesen *et al.*, *J. Virology,* 62:614, 1988.

Thomson *et al.*, *J. Immunol.,* 157(2):822-826, 1996.

Tigges *et al.*, *J. Immunol.,* 156(10):3901-3910, 1996.

Ton-That *et al.*, *Proc. Natl. Acad. Sci. USA*, 96(22):12424-9, 1999.

Treisman, *Cell*, 42:889, 1985.

Tronche *et al.*, *Mol. Biol. Med.*, 7:173, 1990.

Trudel and Constantini, *Genes and Dev.*, 6:954, 1987.

Tuchscherr, *et al., Infect Immun.* 73:7932-7937, 2005.

Tyndell *et al.*, *Nuc. Acids. Res.*, 9:6231, 1981.

Uhlen *et al.*, *J. Biol. Chem.* 259:1695-1702 and 13628 (Corr.) 1984.

Umeda, *et al., J Bacteriol.* 141:838-844, 1980.

van den Ent and Lowe, *FEBS Lett.*, 579:3837-3841, 2005.

van Wely *et al.*, *FEMS Microbiol. Rev.*, 25:437-454, 2001.

Vanassche, *et al., J Clin Microbiol.* 48: 4248-4250, 2010.

Vanassche, *et al., J Thrombosis Haemostasis.* 9: 2436-2446, 2011.

Vanassche, *et al., J Thrombosis Haemostasis.* 107, 2012.

Vannice and Levinson, *J. Virology*, 62:1305, 1988.

Vasseur *et al.*, *Proc Natl. Acad. Sci. USA*, 77:1068, 1980.

Vaughan, *et al.*, *Nat. Biotech.* 16; 535-539, 1998.

Walker & Nesheim, *J Biol Chem.* 274:5201-5212, 1999.

Walsh, *Science.* 261: 308-309, 1993.

Walsh, *et al., Microbiol.* 154: 550-558, 2008.

Walsh, *Am J Med.* 4:782-782, 2010.

Wang and Calame, *Cell*, 47:241, 1986.

Wang, *et al., PLoS Pathogens.* 6:e1000763, 2010.

Ware, *et al., Protein Sci.* 8: 2663-2671, 1999.

Watanabe, *et al., PLoS One.* S, 4: e5714, 2009.

Watanabe, *et al., J Bacteriol.* 187:3698-3707, 2005.

Weber *et al., Cell*, 36:983, 1984.

Weems, *et al., Antimicrob Agents Chemother.* 50: 2751-2755, 2006.

Weidenmaier, *et al., Nature Med.* 10:243-245, 2004.

Weigel, *et al., Science.* 302:1569-1571, 2003.

Weinberger *et al. Mol. Cell. Biol.*, 8:988, 1984.

Weiss *et al., J. Antimicrob. Chemother.*, 53(3):480-6, 2004.

Wilke, *et al., PNAS USA.* 107:13473-13478, 2010.

Winoto and Baltimore, *Cell,* 59:649, 1989.

Wolberg, *Haemophilia.* 16 Suppl 3:7-12, 2010.

Wong *et al., Gene*, 10:87-94, 1980.

Xu *et al., J. Infect. Dis.*, 189:2323-2333, 2004.

Xu *et al., Mol. Microbiol.*, 66(3):787-800, 2007.

Yang, *et al., PNAS USA.* 97:14156-14161, 2000.

Yeaman, *et al., Antimicrobial Agents Chemotherapy.* 36:1665-1670, 1992.

Yutzey *et al. Mol. Cell. Biol.*, 9:1397, 1989.

Zajdel, *Thrombosis Red.* 6:501-510, 1975.

CLAIMS

1. An immunogenic composition comprising a pharmaceutically accepted carrier and at least two different staphylococcal coagulase Domains 1-2, wherein each of the at least two Domains 1-2 is 80% identical in sequence to a Domains 1-2 in SEQ ID NOs: 33-41 and wherein at least one Domain 1-2 is comprised in a less than full-length coagulase protein that lacks an L, R, or Fgb Domain.

2. The composition of claim 1, wherein one of the Domains 1-2 is from a *S. aureus* Newman, 85/2082, MW2, MSSA476, N315, Mu50, MRSA252, CowanI, WIS or USA300 strain.

3. The composition of any one of claims 1 and 2, wherein one of the Domains 1-2 is a Coa Domains 1-2 at least 80% identical in sequence to a SEQ ID NO identified in SEQ ID NOs: 33-37.

4. The composition of any one of claims 1-3, wherein one of the Domains 1-2 is a vWbp Domains 1-2 at least 80% identical in sequence to a SEQ ID NO identified in SEQ ID NOs: 38-41.

5. The composition of any one of claims 1-4, wherein the Domains 1-2 are at least 85%, 90% or 95% identical to an amino acid sequence of SEQ ID NOs: 33-41.

6. The composition of any one of claims 1-5, wherein one of the Domains 1-2 is a vWbp Domains 1-2 from a *S. aureus* N315 or USA300.

7. The composition of any one of claims 1-6, wherein one of the Domains 1-2 is a Coa Domains 1-2 and comprises an L or R Domain from a staphylococcal Coa protein.

8. The composition of any one of claims 1-7, wherein one of the Domains 1-2 is a vWbp Domains 1-2 and comprises an L or Fgb domain from a staphylococcal vWbp protein.

9. The composition of any one of claims 1-8, comprising at least three, four or five different staphylococcal coagulase Domains 1-2.

Date reçu/Date Received 2020-04-14

10. The composition of claim 9, comprising at least four different staphylococcal coagulase Domains 1-2 wherein the different Domains 1-2 are staphylococcal Coa Domains 1-2 from strains MRSA252, MW2, N315 and USA300.

11. The composition of any one of claims 1-10, wherein the at least two different staphylococcal coagulase Domains 1-2 are comprised in a fusion protein.

12. The composition of any one of claims 1-11, further comprising one or more additional staphylococcal antigen(s).

13. The composition of claim 12, wherein the additional staphylococcal antigen(s) is Emp, EsxA, EsxB, EsaC, Eap, Ebh, EsaB, Coa, vWbp, vWh, Hla, SdrC, SdrD, SdrE, IsdA, IsdB, IsdC, ClfA, ClfB, SasF and/or a nontoxigenic SpA.

14. The composition of any one of claims 1-13, further comprising an adjuvant.

15. A recombinant polypeptide comprising at least two different staphylococcal coagulase Domains 1-2 wherein each of the two sequences of the Domains 1-2 are at least 80% identical in sequence to a SEQ ID NO in SEQ ID NOs: 33-41 and wherein at least one Domain 1-2 is comprised in a less than full-length coagulase protein that lacks an L, R, or Fgb Domain.

16. A polynucleotide molecule comprising a nucleic acid sequence encoding a recombinant polypeptide of claim 15.

17. An expression vector comprising a nucleic acid sequence encoding a recombinant polypeptide of claim 15 operably linked to an expression control sequence.

18. A host cell comprising the expression vector of claim 17.

19. Use of a composition of any one of claims 1 -14, a recombinant polypeptide of claim 15 or an expression vector of claim 17, in the preparation of a medicament for the treatment or prevention of a staphylococcal infection in a subject.

20. The use of claim 19, wherein the subject is a human.

Date reçu/Date Received 2020-04-14

21. The use of claim 19 or 20, wherein the subject had been tested for staphyloccal infection.

22. The use of any one of claims 19 to 21, wherein the medicament is for multiple time administration.

23. The use of any one of claims 19 to 22, wherein the composition or polypeptide is formulated for intravenous, intramuscular, intravascular, intratracheal, intrathecal, intraocular, intraperitoneal, topical, oral, injection, infusion, or bolus administration.

24. The use of any one of claims 19 to 23, wherein the medicament is a liquid, solid, gel, tablet, pill, semi-solid, cream, ointment, pessiary, or suppository.

25. The use of any one of claims 19 to 24, wherein the medicament is for administration with one or more antibiotics.

26. The use of any one of claims 19 to 25, wherein the infection is a drug-resistant infection.

27. The use of claim 26, wherein the drug-resistant infection is methicillin-resistant.

28. The use of any one of claims 19 to 27, wherein the subject has been identified as having a Staphylococcal infection.

29. The use of any one of claims 19 to 28, wherein the medicament is for administration within 1 week of the subject being determined to have a staphyloccal infection.

30. The use of any one of claims 19 to 29, wherein the subject is immune deficient, is immunocompromised, is hospitalized, is undergoing an invasive medical procedure, has a respiratory infection, is infected with influenza virus or is on a respirator.

31. The use of any one of claims 19 to 30, further comprising use of testing of the subject to measure response to the medicament.

32. The use of any one of claims 19 to 31, wherein the subject exhibits a skin abscess, a boil, or a furuncle.

Date reçu/Date Received 2020-04-14

33. A method of manufacturing an immunogenic composition comprising mixing at least two different staphylococcal coagulase Domains 1-2, wherein the sequences of the two different Domains 1-2 are 80% identical to a Domain 1-2 sequence in SEQ ID NOs: 33-41 and wherein at least one Domain 1-2 is comprised in a less than full-length coagulase protein that lacks an L, R, or Fgb Domain.

34. The composition of any one of claims 1-14, the recombinant polypeptide of claim 15 or the expression vector of claim 17 for use in the manufacture of a medicament for treating or preventing a staphylococcal infection in a subject.

35. The composition, recombinant polypeptide, or expression vector for use of claim 34, wherein the subject is a human.

36. The composition, recombinant polypeptide, or expression vector for use of claim 34 or 35, wherein the subject had been tested for staphyloccal infection.

37. The composition, recombinant polypeptide, or expression vector for use of any one of claims 34 to 36, wherein the medicament is for multiple time administration.

38. The composition, recombinant polypeptide, or expression vector for use of any one of claims 34 to 37, wherein the composition or polypeptide is formulated for intravenous, intramuscular, intravascular, intratracheal, intrathecal, intraocular, intraperitoneal, topical, oral, infusion, or bolus administration.

39. The composition, recombinant polypeptide, or expression vector for use of any one of claims 34 to 37, wherein the medicament is a liquid, solid, gel, tablet, pill, semi-solid, cream, ointment, pessiary, or suppository.

40. The composition, recombinant polypeptide, or expression vector for use of any one of claims 34 to 39, wherein the medicament is for administration with one or more antibiotics.

41. The composition, recombinant polypeptide, or expression vector for use of any one of claims 34 to 40, wherein the infection is a drug-resistant infection.

42. The composition, recombinant polypeptide, or expression vector for use of claim 41, wherein the drug-resistant infection is methicillin-resistant.

Date reçu/Date Received 2020-04-14

43. The composition, recombinant polypeptide, or expression vector for use of any one of claims 34 to 42, wherein the subject has been identified as having a Staphylococcal infection.

44. The composition, recombinant polypeptide, or expression vector for use of any one of claims 34 to 43, wherein the medicament is for administration within 1 week of the subject being determined to have a staphyloccal infection.

45. The composition, recombinant polypeptide, or expression vector for use of any one of claims 34 to 44, wherein the subject is immune deficient, is immunocompromised, is hospitalized, is undergoing an invasive medical procedure, has a respiratory infection, is infected with influenza virus or is on a respirator.

46. The composition, recombinant polypeptide, or expression vector for use of any one of claims 34 to 45, further comprising use of testing of the subject to measure response to the medicament.

47. The composition, recombinant polypeptide, or expression vector for use of any one of claims 34 to 45, wherein the subject exhibits a skin abscess, a boil, or a furuncle.

48. An immunogenic composition comprising at least two different staphylococcal coagulase Domains 1-2 from a staphylococcal Coa or vWbp protein, wherein at least one Domain 1-2 is comprised in a less than full-length coagulase protein that lacks an L, R, or Fgb Domain.

49. An immunogenic composition of claim 1, wherein a first Domains 1-2 is 80% identical in sequence to a Coa Domains 1-2 from a first strain and is comprised in a less than full-length coagulase protein that lacks an L and R domain and a second Domains 1-2 is 80% identical in sequence to a vWbp Domains 1-2 from a second strain and is comprised in a less than full-length coagulase protein that lacks an L and Fgb domain.

Date reçu/Date Received 2020-04-14

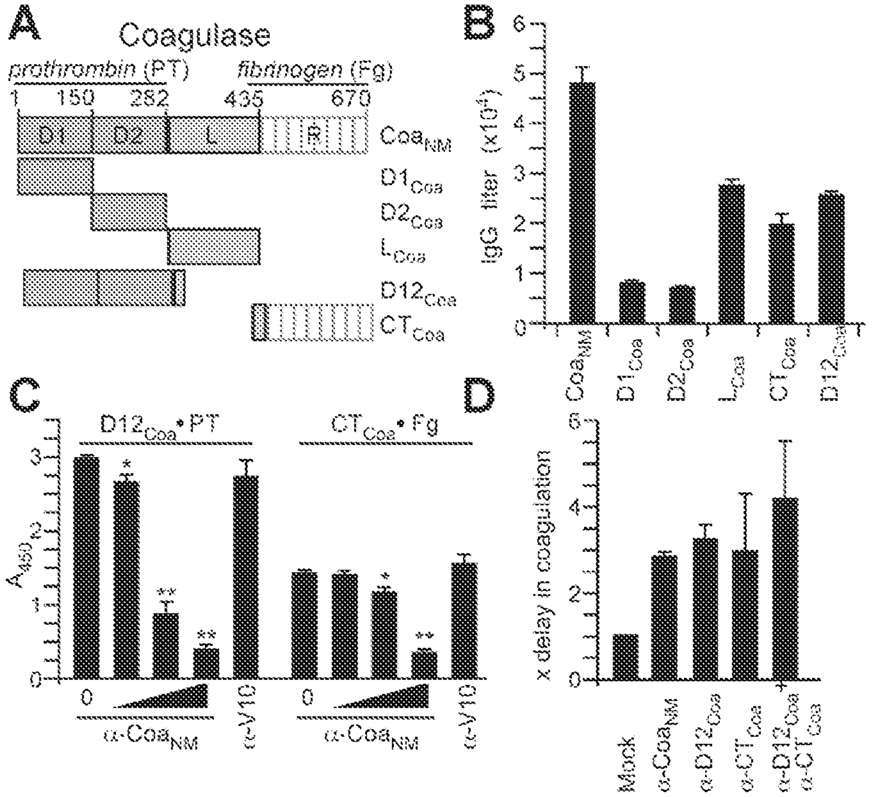
A
Coagulase
prothrombin (PT)
fibrinogen (Fg)
1
150
282
435
670
D1
D2
L
R
Coa$_{NM}$
D1$_{Coa}$
D2$_{Coa}$
L$_{Coa}$
D12$_{Coa}$
CT$_{Coa}$
B
IgG titer (x10^4)
C
D12$_{Coa}$•PT
CT$_{Coa}$•Fg
A$_{450}$
α-Coa$_{NM}$
α-V10
D
x delay in coagulation
Mock
α-Coa$_{NM}$
α-D12$_{Coa}$
α-CT$_{Coa}$
α-D12$_{Coa}$ + α-CT$_{Coa}$

FIG. 1

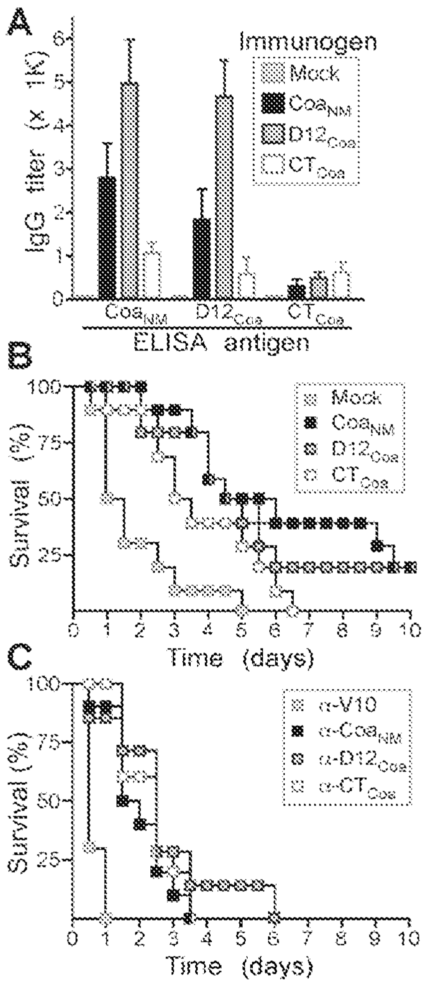
A
Immunogen
Mock
Coa_{NM}
$D12_{Coa}$
CT_{Coa}
IgG titer (x 1K)
Coa_{NM}
$D12_{Coa}$
CT_{Coa}
ELISA antigen
B
Survival (%)
Time (days)
C
α-V10
α-Coa_{NM}
α-$D12_{Coa}$
α-CT_{Coa}
Survival (%)
Time (days)

FIG. 2

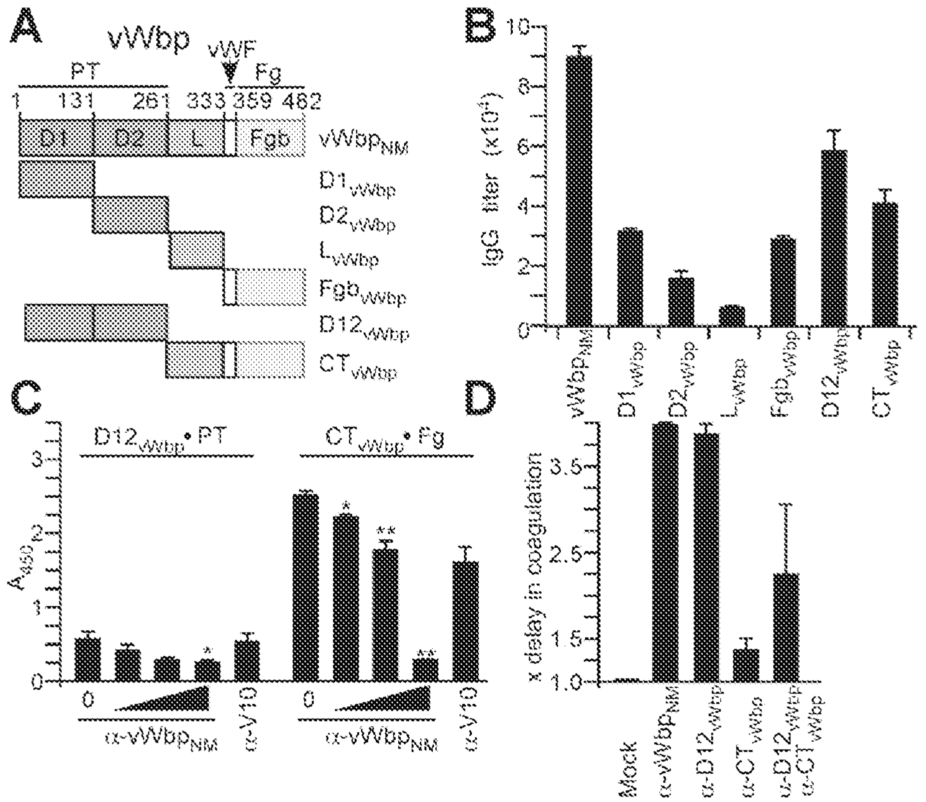
A
vWbp
vWF
PT
Fg
1 131 261 333 359 482
D1
D2
L
Fgb
vWbp$_{NM}$
D1$_{vWbp}$
D2$_{vWbp}$
L$_{vWbp}$
Fgb$_{vWbp}$
D12$_{vWbp}$
CT$_{vWbp}$
B
IgG titer (x10^4)
C
D12$_{vWbp}$• PT
CT$_{vWbp}$• Fg
A$_{450\ nm}$
α-vWbp$_{NM}$
α-V10
D
x delay in coagulation
Mock
α-vWbp$_{NM}$
α-D12$_{vWbp}$
α-CT$_{vWbp}$
α-D12$_{vWbp}$ α-CT$_{vWbp}$

FIG. 3

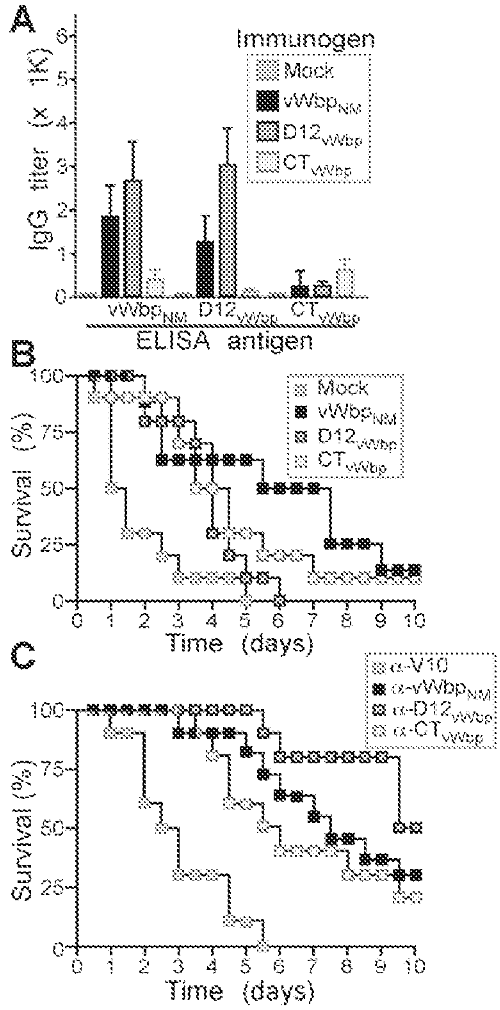
A
Immunogen
Mock
vWbp$_{NM}$
D12$_{vWbp}$
CT$_{vWbp}$
IgG titer (x 1K)
vWbp$_{NM}$ D12$_{vWbp}$ CT$_{vWbp}$
ELISA antigen
B
Mock
vWbp$_{NM}$
D12$_{vWbp}$
CT$_{vWbp}$
Survival (%)
Time (days)
C
α-V10
α-vWbp$_{NM}$
α-D12$_{vWbp}$
α-CT$_{vWbp}$
Survival (%)
Time (days)

FIG. 4

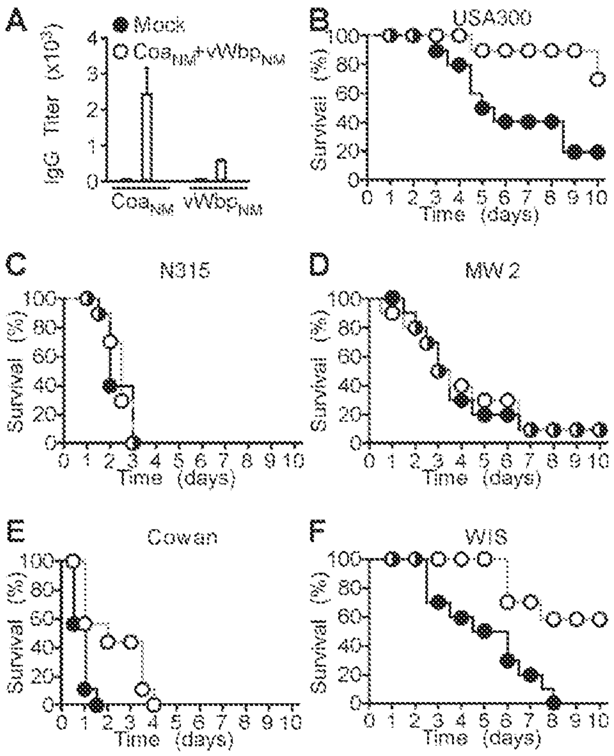
A
Mock
Coa$_{NM}$+vWbp$_{NM}$
IgG Titer (x10^3)
Coa$_{NM}$
vWbp$_{NM}$
B
USA300
Survival (%)
Time (days)
C
N315
Survival (%)
Time (days)
D
MW2
Survival (%)
Time (days)
E
Cowan
Survival (%)
Time (days)
F
WIS
Survival (%)
Time (days)

FIG. 5

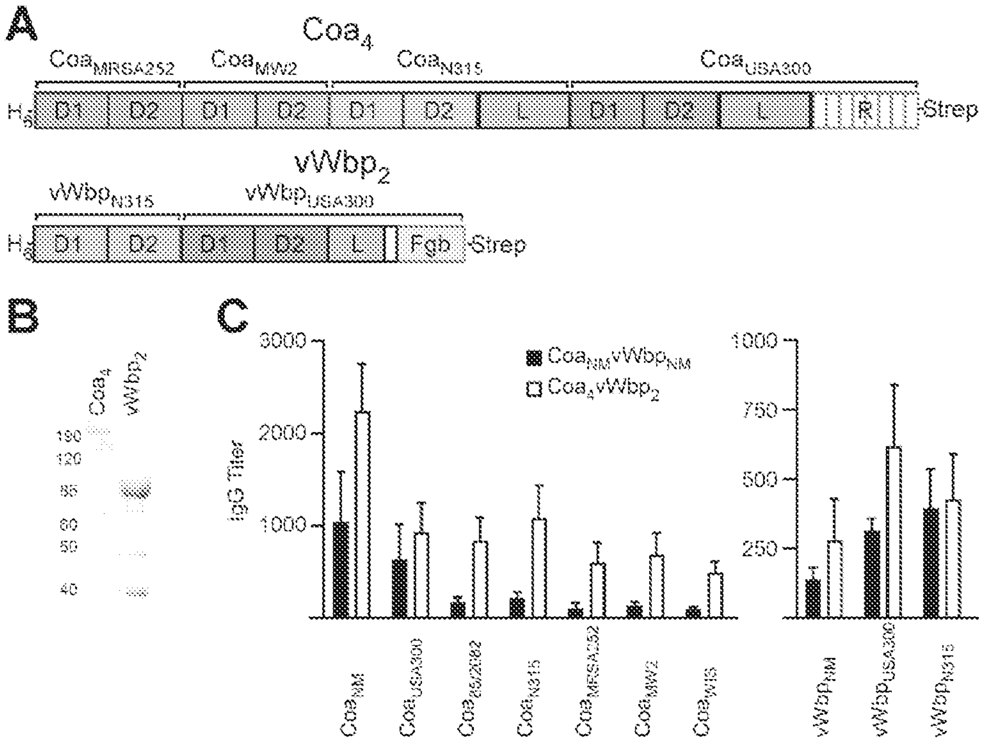
A
Coa$_4$
Coa$_{MRSA252}$
Coa$_{MW2}$
Coa$_{N315}$
Coa$_{USA300}$
H$_6$
D1
D2
L
R
Strep
vWbp$_2$
vWbp$_{N315}$
vWbp$_{USA300}$
Fgb
B
Coa$_4$
vWbp$_2$
190
120
85
60
50
40
C
Coa$_{NM}$vWbp$_{NM}$
Coa$_4$vWbp$_2$
IgG Titer
Coa$_{NM}$
Coa$_{USA300}$
Coa$_{85/2082}$
Coa$_{N315}$
Coa$_{MRSA252}$
Coa$_{MW2}$
Coa$_{WIS}$
vWbp$_{NM}$
vWbp$_{USA300}$
vWbp$_{N315}$

FIG. 6

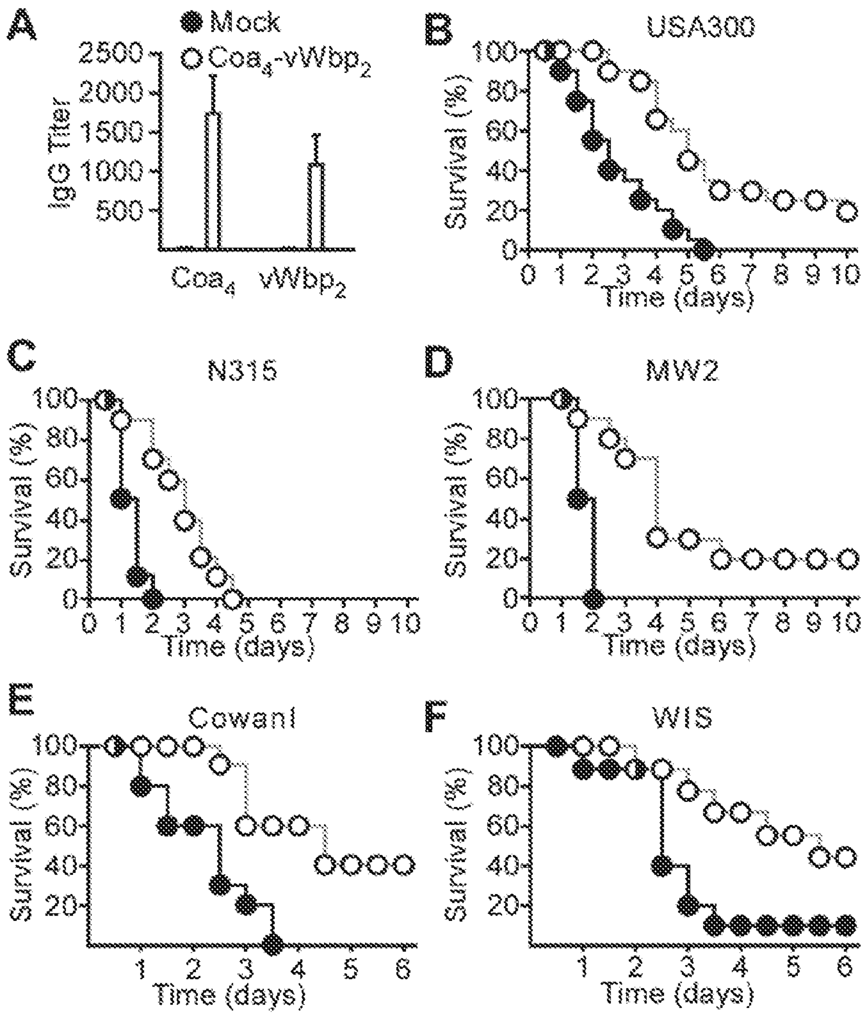
A
Mock
Coa4-vWbp2
IgG Titer
2500
2000
1500
1000
500
Coa4
vWbp2
B
USA300
C
N315
D
MW2
E
CowanI
F
WIS
Survival (%)
Time (days)

FIG. 7

Alignment of Coa from five S. aureus strains

```
USA300_Coa          ATGAAAAAGCAAATAATTTCGCTAGGCGCATTAGCAGTTGCATCTAGCTTATTTACATGG 60
N315_Coa            ATGAAAAAGCAAATAATTTCGCTAGGCGCATTAGCAGTTGCATCTAGCTTATTTACATGG 60
MRSA252_Coa         ATGAAAAAGCAAATAATTTCGCTAGGCGCATTAGCAGTTGCATCTAGCTTATTTACATGG 60
MW2_Coa             ATGAAAAAGCAAATAATTTCGCTAGGCGCATTAGCAGTTGCATCTAGCTTATTTACATGG 60
WIS_Coa             ------------------------------------------------------------


USA300_Coa          GATAACAAAGCAGATGCGATAGTAACAAAGGATTATAGTGGGAAATCACAAGTTAATGCT 120
N315_Coa            GATAACAAAGCAGATGCGATAGTAACAAAGGATTATAGTAAAGAATCAAGAGTGAATGAG 120
MRSA252_Coa         GATAACAAAGCAGATGCGATAGTAACTAAAGATTATAGTAAAGAATCAAGAGTGAATGAG 120
MW2_Coa             GATAACAAAGCAGATGCGATAGTAACAAAGGATTATAGTGGGAAATCACAAGTTAATGCT 120
WIS_Coa             ------------------ATAGTAACAAAGGATTATAGTGGGAAATCACAAGTTAATGCT 42
                                      ******** ** *********    *****  *** ****

USA300_Coa          GGGAGTAAAAATGGGAC-ATTAAT---AGATAGCAGATATTTAAATTCAGCTCTATATTA 176
N315_Coa            AAAAGTAAAAAGGGAGCTACTGTTTC-AGATTATTACTATTGGAAAATAATT---GATAG 176
MRSA252_Coa         AACAGTAAATACGATAC-ACCAATTCCAGATTG---GTATCTAGGTAGTATTTTAAACAG 176
MW2_Coa             GGGAGTAAAAATGGGAA-ACAAATTGCAGATGGATATTATTGGGGAATAATT---GAAAA 176
WIS_Coa             GGGAGTAAAAATGGGAA-ACAAATTGCAGATGGATATTATTGGGGAATAATT---GAAAA 98
                       ****** * *     *    *   ****      ***           *    *

USA300_Coa          TTTGGAAGACTATATAATTTAT----GCTATAGGATTAACTAATAAATATGAATATGGAG 232
N315_Coa            TTTAGAGG----CACAATTTACTGGAGCAATAGACTTATTGGAAGATTATAAATATGGAG 232
MRSA252_Coa         ATTAGGGGATCAAATATACTAC----GCTAAGGAATTAACTAATAAATACGAATATGGTG 232
MW2_Coa             TCTAGAAAACCA---GTTTTAC-AATATTTTTCATTTACTGGATCAGCATAAATATGCAG 232
WIS_Coa             TCTAGAGAACCA---GTTTTAC-AATATTTTTCATTTATTGGATCAGCATAAATATGCAG 154
                      * *              **              ***    *  *  *  ******  *

USA300_Coa          ATAATATTTATAAAGAAGCTAAAGATAGGTTGTTGGAAAAGGTATTAAGGGAAGATCAAT 292
N315_Coa            ATCCTATCTATAAAGAAGCGAAAGATAGATTGATGACAAGAGTATTAGGAGAAGACCAGT 292
MRSA252_Coa         AGAAAGAGTATAAGCAAGCGATAGATAAATTGATGACTAGAGTTTTGGGAGAAGATCATT 292
MW2_Coa             AAAAAGAATATAAAGATGCAGTAGATAAATTAAAAACTAGAGTTTTAGAGGAAGACCAAT 292
WIS_Coa             AAAAAGAATATAAAGATGCATTAGATAAATTAAAAACTAGAGTTTTAGAGGAAGACCAAT 214
                    *       *****  * **   *****  **       *  ** **    ***** ** *

USA300_Coa          ATCTTTTGGAGAGAAAGAAATCTCAATATGAAGATTATAAACAATGGTATGCAAATTATA 352
N315_Coa            ATTTATTAAAGAAAAAGATTGATGAATATGAGCTTTATAAAAAGTGGTATAAAAGTT-CA 351
MRSA252_Coa         ATCTATTAGAAAAAAAGAAAGCACAATATGAAGCATACAAAAAATGGTTTGAAAAACATA 352
MW2_Coa             ACCTGCTAGAAAGAAAAAAAGAAAAATACGAAATTTATAAAGAACTATATAAAAAATACA 352
WIS_Coa             ACCTGCTAGAAAGAAAAAAAGAAAAATACGAAATTTATAAAGAACTATATAAAAAATACA 274
                    *  *  *  * * *** *      **** **    ** *** *    * *  **     *

USA300_Coa          AAAAAGAAAATCCTCGTACAGATTTAAAAATGGCTAATTTTCATAAATATAATTTAGAAG 412
N315_Coa            AATAAGAACACT--------------AATATGCTTACTTTCCATAAATATAATCTTTACA 397
MRSA252_Coa         AAAGTGAAAATCCACATTCTAGTTTAAAAAGATTAAATTTGACGATTTTGATTTATATA 412
MW2_Coa             AAAAAGAGAATCCTAATACTCAAGTTAAAATGAAAGCATTTGATAAATACGATCTTGGCG 412
WIS_Coa             AAAAAGAGAATCCTAATACTCAGGTTAAAATGAAAGCATTTGATAAATACGATCTTGGCG 334
                    **   **  *                **  * *      **  *  * *   ** *

USA300_Coa          AACTTTCGATGAAAGAATACAATGAACTACAGGATGCATTAAAGAGAGCACTGGATGATT 472
N315_Coa            ATTTAACAATGAATGAATATAACGATATTTTTAACTCTTTGAAAGATGCAGTTTATCAAT 457
MRSA252_Coa         GATTAACGAAGAAAGAATACAATGAGTTACATCAATCATTAAAAGAAGCTGTTGATGAGT 472
MW2_Coa             ATTTAACTATGGAAGAATACAATGACTTATCAAAATTATTAACAAAAGCATTGGATAACT 472
WIS_Coa             ATTTAACTATGGAAGAATACAATGACTTATCAAAATTATTAACAAAAGCATTGGATAACT 394
                       *  * * * * ***** ** **  *     *    ** *     **  *  ** * *

USA300_Coa          TTCACAGAGAAGTTAAAGATATTAAGGATAAGAATTCAGACTTGAAAACTTTTAATGCAG 532
N315_Coa            TTAATAAAGAAGTTAAAGAAATAGAGCATAAAAATGTTGACTTGAAGCAGTTTGATAAAG 517
MRSA252_Coa         TTAATAGTGAAGTGAAAAATATTCAATCTAAACAAAAGGATTTATTACCTTATGATGAAG 532
MW2_Coa             TTAAGTTAGAAGTAAAGAAAATTGAATCAGAGAATCCAGATTTAAAACCATATTCTGAAA 532
WIS_Coa             TTAAGTTAGAAGTAAAGAAAATTGAATCAGAGAATCCAGATTTAAGACCATATTCTGAAA 454
                    ** *    ***** **  * **  *     *  *    ** **       * *  *  *

USA300_Coa          CAGAAGAAGATAAAGCAACTAAGGAAGTATACGATCTCGTATCTGAAATTGATACATTAG 592
N315_Coa            ATGGAGAAGACAAGGCAACTAAAGAAGTTTATGACCTTGTTTCTGAAATTGATACATTAG 577
MRSA252_Coa         CAACTGAAAATCGAGTAACAAATGGAATATATGATTTTGTTTGCGAGATTGACACATTAT 592
MW2_Coa             GCGAAGAAAGAACAGCATATGGTAAAATAGATTCACTTGTTGATCAAGCATATAGTGTAT 592
WIS_Coa             GTGAAGAGAGAACAGCATATGGTAAAATAGATTCACTTGTTGATCAAGCATATAGTGTAT 514
                         **       * *        * *  *     * **     *     * *   **

USA300_Coa          TTGTATCATATTATGGTGATAAGGATTATGGGGAGCACGCGAAAGAGTTACGAGCAAAAC 652
```

```
N315_Coa        TTGTAACTTATTATGCTGATAAGGATTATGGGGAGCATGCGAAAGAGTTACGAGCAAAAC 637
MRSA252_Coa     ACGCAGCATATTTTAATCATAGCCAATATGGTCATAATGCTAAAGAATTAAGAGCAAAGC 652
MW2_Coa         ATTTTGCCTACGTTACAGATGCACAACATAAAACAGAAGCATTAAATCTTAGGGCGAAAA 652
WIS_Coa         ATTTTGCCTACGTTACAGATGCTCAACATAAAACAGAAGCATTAAATCTTAGGGCAAAAA 574
                      * **   *    **    *  **       * **   * *  *  * ** **

USA300_Coa      TGGACTTAATCCTTGGAGATACAGACAATCCACATAAAATTACAAATGAACGTATTAAAA 712
N315_Coa        TGGACTTAATCCTTGGAGATACAGACAATCCACATAAAATTACAAATGAGCGTATAAAAA 697
MRSA252_Coa     TAGATATAATTCTTGGTGATGCTAAAGATCCTGTTAGAATTACGAATGAAAGAATAAGAA 712
MW2_Coa         TTGATTTGATTTTAGGTGATGAAAAAGATCCAATTAGAGTTACGAATCAACGTACTGAAA 712
WIS_Coa         TAGATTTGATTTTAGGTGATGAAAAAGATCCAATTAGAGTGACGAATCAACGTACTGAAA 634
                * **  * **  * ** ***    *  ****   ** * * ** *** *  * *    **

USA300_Coa      AAGAAATGATTGATGACTTAAATTCAATTATTGATGATTTCTTTATGGAAACTAAACA-A 771
N315_Coa        AAGAAATGATCGATGACTTAAATTCAATTATAGATGATTTCTTTATGGAGACTAAACA-A 756
MRSA252_Coa     AAGAAATGATGGATGATTTAAATTCTATTATTGATGATTTCTTTATGGATAC-AAACATG 771
MW2_Coa         AAGAAATGATTAAAGATTTAGAATCTATTATTGATGATTTCTTCATTGAAACCAAGTT-G 771
WIS_Coa         AAGAAATGATTAAAGATTTAGAATCTATTATTGATGATTTCTTCATTGAAACAAAGTT-G 693
                **********  * ** *** * ** ***** *********** ** ** ** **

USA300_Coa      AATAGACCGAAATCTATAACGAAATATAATCCTACAACACATAACTATAAAACAAATAGT 831
N315_Coa        AATAGACCGAATTCTATAACAAAATATGATCCAACAAAACACAATTTTAAAGAGAAGAGT 816
MRSA252_Coa     AATAGACCATTAAACATAACTAAATTTAATCCGAATATTCATGACTATACTAATAAGCCT 831
MW2_Coa         AATAGACCTAAACACATTACTAGGTATGATGGAACTAAACATGATTACCA---------T 822
WIS_Coa         AATAGACCTCAACACATTACTAGATATGATGGAACTAAACATGATTACCA---------T 744
                ********       ** ** *  * * **   *  *  **  * *             *

USA300_Coa      GATAATAAACCTAATTTTGATAAATTAGTTGAAGAAACGAAAAAAGCAGTTAAAGAAGCA 891
N315_Coa        GAAAATAAACCTAATTTTGATAAATTAGTTGAAGAAACAAAAAAAGCAGTTAAAGAAGCA 876
MRSA252_Coa     GAAAATAGAGATAACTTCGATAAATTAGTCAAAGAAACAAGAGAAGCAATCGCAAACGCT 891
MW2_Coa         AAACATAAAGATGGATTTGATGCTCTAGTTAAAGAAACAAGAGAAGCGGTTGCAAAGGCT 882
WIS_Coa         AAACATAAAGATGGATTTGATGCTTTAGTTAAAGAAACAAGAGAAGCGGTTTCTAAGGCT 804
                 *  *** *  *   ** ***    ****  ******* * * ****  *     * **

USA300_Coa      GATGATTCTTGGAAAAAGAAAACTGTCAAAAAATACGGAGAAACTGAAACAAAATCGCCA 951
N315_Coa        GACGAATCTTGGAAAAATAAAACTGTCAAAAAATACGAGGAAACTGTAACAAAATCTCCT 936
MRSA252_Coa     GACGAATCTTGGAAAACAAGAACCGTCAAAAATTACGGTGAATCTGAAACAAAATCTCCT 951
MW2_Coa         GACGAATCTTGGAAAAATAAAACTGTCAAAAAATACGAGGAAACTGTAACAAAATCTCCA 942
WIS_Coa         GACGAATCTTGGAAAACTAAAACTGTCAAAAAATACGGGGAAACTGAAACAAAATATCCT 864
                ** ** **********  * *** ******** ****  *** *** ********  **

USA300_Coa      GTAGTAAAAGAAGAGAAGAAAGTTGAAGAACCTCAAGCACCTAAAGTTGATAACCAACAA 1011
N315_Coa        GTTGTAAAAGAAGAGAAGAAAGTTGAAGAACCTCAATTACCTAAAGTTGGAAACCAGCAA 996
MRSA252_Coa     GTTGTAAAAGAAGAGAAGAAAGTTGAAGAACCTCAATTACCTAAAGTTGGAAACCAGCAA 1011
MW2_Coa         GTTGTAAAAGAAGAGAAGAAAGTTGAAGAACCTCAATCACCTAAATTTGATAACCAACAA 1002
WIS_Coa         GTTGTAAAAGAAGAGAAGAAAGTTGAAGAACCTCAATCACCTAAAGTTTCTGAAAAAGTG 924
                ** *********************************  ******* **    *  *

USA300_Coa      GAGGTTAAAACTACGGCTGGTAAAGCTGAAGAAACAACACAACCAGTTGCACAACCATTA 1071
N315_Coa        GAGGTTAAAACTACGGCTGGTAAAGCTGAAGAAACAACACAACCAGTGGCACAGCCATTA 1056
MRSA252_Coa     GAGGATAAAATTACAGTTGGTACAACTGAAGAAGCACCATTACCAATTGCGCAACCACTA 1071
MW2_Coa         GAGGTTAAAATTACAGTTGATAAAGCTGAAGAAACAACACAACCAGTGGCACAGCCATTA 1062
WIS_Coa         GATGTTCAGGAAACGGTTGGTACAACTGAAGAAGCACCATTACCAATTGCGCAACCACTA 984
                ** * * *    ** * ** ** * ******** ** **  **** * ** ** *** **

USA300_Coa      GTTAAAATTCCACAGGGCACAATTACAGGTGAAATTGTAAAAGGTCCGGAATATCCAACG 1131
N315_Coa        GTAAAAATTCCACAAGAAACAATCTATGGTGAAACTGTAAAAGGTCCAGAATATCCAACG 1116
MRSA252_Coa     GTTAAAATTCCACAGGGCACAATTCAAGGTGAAATTGTAAAAGGTCCGGAATATCTAACG 1131
MW2_Coa         GTTAAAATTCCACAGGGCACAATTACAGGTGAAATTGTAAAAGGTCCGGAATATCCAACG 1122
WIS_Coa         GTTAAATTACCACAAATTGGGACTCAAGGCGAAATTGTAAAAGGTCCCGACTATCCAACT 1044
                ** *** * *****       *     ** **** *********** ** **** ***

USA300_Coa      ATGGAAAATAAAACGGTACAAGGTGAAATCGTTCAAGGTCCCGATTTTCTAACAATGGAA 1191
N315_Coa        ATGGAAAATAAAACGTTACAAGGTGAAATCGTTCAAGGTCCCGATTTTCTAACAATGGAA 1176
MRSA252_Coa     ATGGAAAATAAAACGTTACAAGGTGAAATCGTTCAAGGTCCAGATTTCCCAACAATGGAA 1191
MW2_Coa         ATGGAAAATAAAACGTTACAAGGTGAAATCGTTCAAGGTCCAGATTTCCCAACAATGGAA 1182
WIS_Coa         ATGGAAAATAAAACGTTACAAGGTGTAATTGTTCAAGGTCCAGATTTCCCAACAATGGAA 1104
                *************** ********* *** *********** ***** * **********

USA300_Coa      CAAAGCGGCCCATCATTAAGCAATAATTATACAAACCCA--------------------- 1230
N315_Coa        CAAAACAGACCATCTTTAAGCGATAATTATACTCAACCG--------------------- 1215
MRSA252_Coa     CAAAACAGACCATCTTTAAGCGATAATTATACTCAACCG--------------------- 1230
```

```
MW2_Coa         CAAAACAGACCATCTTTAAGCGATAATTATACTCAACCG--------------------- 1221
WIS_Coa         CAAAACAGACCATCTTTAAGTGACAATTATACACAACCATCTGTGACTTTACCGTCAATT 1164
                **** * * ***** *****  * ********   * **

USA300_Coa      ------------CCGTTAACGAACCCTATTTTAGAAGGTCTTGAAGGTAGCTCATCTAAA 1278
N315_Coa        ------------ACGACACCGAACCCTATTTTAGAAGGTCTTGAAGGTAGCTCATCTAAA 1263
MRSA252_Coa     ------------ACGACACCGAACCCTATTTTAAAAGGTATTGAAGGAAACTCAACTAAA 1278
MW2_Coa         ------------ACGACACCGAACCCTATTTTAGAAGGTCTTGAAGGTAGCTCATCTAAA 1269
WIS_Coa         ACAGGTGAAAGTACACCAACGAACCCTATTTTAAAAGGTATTGAAGGAAACTCATCTAAA 1224
                             *   * ************* ***** ******* * **** *****

USA300_Coa      CTTGAAATAAAACCACAAGGTACTGAATCAACGTTAAAAGGTACTCAAGGAGAATCAAGT 1338
N315_Coa        CTTGAAATAAAACCACAAGGTACTGAATCAACGTTGAAAGGTATTCAAGGAGAATCAAGT 1323
MRSA252_Coa     CTTGAAATAAAACCACAAGGTACTGAATCAACGTTAAAAGGTACTCAAGGAGAATCAAGT 1338
MW2_Coa         CTTGAAATAAAACCACAAGGTACTGAATCAACGTTAAAAGGTACTCAAGGAGAATCAAGT 1329
WIS_Coa         CTTGAAATAAAACCACAAGGTACTGAATCAACGTTGAAAGGTATTCAAGGAGAATCAAGT 1284
                *********************************** ******* ***************

USA300_Coa      GATATTGAAGTTAAACCTCAAGCAACTGAAACAACAGAAGCTTCTCAATATGGTCCGAGA 1398
N315_Coa        GATATTGAAGTTAAACCTCAAGCAACTGAAACAACAGAAGCTTCTCAATATGGTCCGAGA 1383
MRSA252_Coa     GATATTGAAGTTAAACCTCAAGCAACTGAAACAACAGAAGCATCACATTATCCAGCGAGA 1398
MW2_Coa         GATATTGAAGTTAAACCTCAAGCATCTGAAACAACAGAAGCATCACATTATCCAGCAAGA 1389
WIS_Coa         GATATTGAAGTTAAACCTCAAGCAACTGAAACAACAGAAGCATCACATTATCCAGCGAGA 1344
                ************************ *************** ** ** ***     * ***

USA300_Coa      CCGCAATTTAACAAAACACCTAAATATGTTAAATATAGAGATGCTGGTACAGGTATCCGT 1458
N315_Coa        CCGCAATTTAACAAAACACCTAAGTATGTGAAATATAGAGATGCTGGTACAGGTATCCGT 1443
MRSA252_Coa     CCTCAATTTAACAAAACACCTAAGTATGTGAAATATAGAGATGCTGGTACAGGTATCCGT 1458
MW2_Coa         CCTCAATTTAACAAAACACCTAAATATGTTAAATATAGAGATGCTGGTACAGGTATCCGT 1449
WIS_Coa         CCGCAATTTAACAAAACACCTAAATATGTGAAATATAGAGATGCTGGTACAGGTATTCGT 1404
                ** ******************** ***** ************************** ***

USA300_Coa      GAATACAACGATGGAACATTTGGATATGAAGCGAGACCAAGATTCAATAAGCCA------ 1512
N315_Coa        GAATACAACGATGGAACATTTGGATATGAAGCGAGACCAAGATTCAACAAGCCAAGTGAA 1503
MRSA252_Coa     GAATACAACGATGGAACATTTGGATATGAAGCGAGACCAAGATTCAACAAGCCAAG---- 1514
MW2_Coa         GAATACAACGATGGAACATTTGGATATGAAGCGAGACCAAGATTCAATAAGCCATCAGAA 1509
WIS_Coa         GAATACAACGATGGAACTTTTGGATATGAAGCGAGACCAAGATTCAACAAGCCATCAGAA 1464
                ***************** ***************************** ******

USA300_Coa      --------------------------TCA------------------------------- 1515
N315_Coa        ACAAATGCATACAACGTAACGACAAATCAAGATGGCACAGTATCATACGGAGCTCGCCCA 1563
MRSA252_Coa     ---------------------------C-------------------------------- 1515
MW2_Coa         ACAAACGCATACAACGTAACGACAAATCAAGATGGCACAGTAACATATGGCGCTCGCCCA 1569
WIS_Coa         ACAAACGCATACAACGTAACGACAAATCAAGATGGCACAGTATCATATGGGGCTCGCCCA 1524
                                           *

USA300_Coa      ------------------GAAACAAATGCATATAACGTAACAACACATGCAAATGGTCAA 1557
N315_Coa        ACACAAAACAAGCCAAGTGAAACAAACGCATATAACGTAACAACACATGCAAATGGTCAA 1623
MRSA252_Coa     ------------------GAAACAAATGCATACAACGTAACGACAAATCAAGATGGCACA 1557
MW2_Coa         ACACAAAACAAACCAAGCAAAACAAATGCATACAACGTAACAACACATGCAAATGGTCAA 1629
WIS_Coa         ACACAAAACAAGCCAAGCAAAACAAATGCATATAACGTAACAACACATGCAAACGGCCAA 1584
                                   ******* ***** ******** *** **  * * **    *

USA300_Coa      GTATCATACGGAGCTCGTCCGACA------------------------------------ 1581
N315_Coa        GTATCATACGGTGCTCGCCCAACA------------------------------------ 1647
MRSA252_Coa     GTATCATATGGCGCTCGCCCGACA------------------------------------ 1581
MW2_Coa         GTATCATATGGCGCTCGCCCGACA------------------------------------ 1653
WIS_Coa         GTATCATATGGCGCTCGCCCGACATACAACAAGCCAAGTGAAACAAATGCATACAACGTA 1644
                ******** ** ***** ** ***

USA300_Coa      ---------------------------------------------CAAAACAAGCCAAGC 1596
N315_Coa        ---------------------------------------------CAAAAAAAGCCAAGC 1662
MRSA252_Coa     ---------------------------------------------CAAAACAAGCCAAGC 1596
MW2_Coa         ---------------------------------------------CAAAACAAGCCAAGC 1668
WIS_Coa         ACGACAAATCGAGATGGCACAGTATCATATGGCGCTCGCCCGACACAAAACAAGCCAAGC 1704
                                                             ***** *********

USA300_Coa      AAAACAAACGCATATAACGTAACAACACATGGAAACGGCCAAGTATCATATGGCGCTCGC 1656
N315_Coa        AAAACAAATGCATACAACGTAACAACACATGCAAATGGTCAAGTATCATATGGCGCTCGC 1722
MRSA252_Coa     GAAACAAACGCATATAACGTAACAACACATGCAAACGGCCAAGTATCATACGGAGCTCGT 1656
MW2_Coa         AAAACAAATGCATATAACGTAACAACACATGCAAATGGTCAAGTATCATACGGAGCTCGC 1728
WIS_Coa         GAAACGAATGCATATAACGTAACAACACACGGAAATGGCCAAGTATCATATGGCGCTCGT 1764
```

```
                     **** ** ***** ************** * *** ** *********** ** *****

USA300_Coa          CCAACACAAAACAAGCCAAGCAAAACAAATGCATACAACGTAACAACACATGCAAACGGT 1716
N315_Coa            CCGACACAAAAAAAGCCAAGCAAAACAAATGCATATAACGTAACAACACATGCAAATGGT 1782
MRSA252_Coa         CCGACACAAAACAAGCCAAGCGAAACGAACGCATATAACGTAACAACACATGCAAACGGT 1716
MW2_Coa             CCGACACAAAACAAGCCAAGCAAAACAAATGCATATAACGTAACAACACACGCAAACGGT 1788
WIS_Coa             CCGACACAAAAGAAGCCAAGCAAAACAAATGCATATAACGTAACAACACATGCAAACGGC 1824
                    ** ******** ********* **** ** ***** ************* ***** **

USA300_Coa          CAAGTGTCATACGGAGCTCGCCCGACATACAAGAAGCCAAGTAAAACAAATGCATACAAT 1776
N315_Coa            CAAGTATCATACGGAGCTCGCCCGACATACAAGAAGCCAAGCGAAACAAATGCATACAAC 1842
MRSA252_Coa         CAAGTGTCATACGGAGCTCGCCCAACACAAAACAAGCCAAGTAAAACAAATGCATACAAT 1776
MW2_Coa             CAAGTGTCATACGGAGCTCGCCCGACATACAAGAAGCCAAGTAAAACAAATGCATACAAT 1848
WIS_Coa             CAAGTATCATATGGCGCTCGTCCGACATACAACAAGCCAAGTAAAACAAATGCATACAAT 1884
                    ***** ***** ** ***** ** *** * ** ********  ***************

USA300_Coa          GTAACAACACATGCA--------------------------------------------- 1791
N315_Coa            GTAACAACACATGCAAATGGTCAAGTATCATATGGCGCTCGCCCGACACAAAAAAAGCCA 1902
MRSA252_Coa         GTAACAACACATGCA--------------------------------------------- 1791
MW2_Coa             GTAACAACACATGCA--------------------------------------------- 1863
WIS_Coa             GTAACAACACATGCA--------------------------------------------- 1899
                    ***************

USA300_Coa          ------------------------------------GATGGTACTGCGACATATGGGCCT 1815
N315_Coa            AGCGAAACAAACGCATATAACGTAACAACACATGCAGATGGTACTGCGACATATGGGCCT 1962
MRSA252_Coa         ------------------------------------GATGGTACTGCGACATATGGTCCT 1815
MW2_Coa             ------------------------------------GATGGTACTGCGACATATGGGCCT 1887
WIS_Coa             ------------------------------------GATGGTACTGCGACATATGGTCCT 1923
                                                        ******************** ***

USA300_Coa          AGAGTAACAAAATAA 1830
N315_Coa            AGAGTAACAAAATAA 1977
MRSA252_Coa         AGAGTAACAAAATAA 1830
MW2_Coa             AGAGTAACAAAATAA 1902
WIS_Coa             AGAGTAACAAAATAA 1938
                    ***************
```

FIG. 8A

```
USA300_D1D2      IVTKDYSGKSQVNAGSKNGTLIDSRYLNSALYYLEDYIIYAIGLTNKYEYGDNIYKEAKD 60
Newman_D1D2      IVTKDYSGKSQVNAGSKNGTLIDSRYLNSALYYLEDYIIYAIGLTNKYEYGDNIYKEAKD 60
N315_D1D2        IVTKDYSKESRVNEKSKKGATVSDYYYWKIIDSLEAQFTGAIDLLEDYKYGDPIYKEAKD 60
MU50_D1D2        IVTKDYSKESRVNEKSKKGATVSDYYYWKIIDSLEAQFTGAIDLLEDYKYGDPIYKEAKD 60
MRSA252_D1D2     IVTKDYSKESRVNENSKYDTPIPDWYLGSILNRLGDQIYYAKELTNKYEYGEKEYKQAID 60
85/2082_D1D2     IVTKDYSKESRVNENSKYDTPIPDWYLGSILNRLGDQIYYAKELTNKYEYGEKEYKQAID 60
MW2_D1D2         IVTKDYSGKSQVNAGSKNGKQIADGYYWGIIENLENQFYNIFHLLDQHKYAEKEYKDAVD 60
WIS_D1D2         IVTKDYSGKSQVNAGSKNGKQIADGYYWGIIENLENQFYNIFHLLDQHKYAEKEYKDALD 60
                 ******* :*:**  ** .  : . *     :  *   :      * :.::*.:  **:* *

USA300_D1D2      RLLEKVLREDQYLLERKKSQYEDYKQWYANYKKENPRTDLKMANFHKYNLEELSMKEYNE 120
Newman_D1D2      RLLEKVLREDQYLLERKKSQYEDYKQWYANYKKENPRTDLKMANFHKYNLEELSMKEYNE 120
N315_D1D2        RLMTRVLGEDQYLLKKKIDEYELYKKWYKSSNK-----NTNMLTFHKYNLYNLTMNEYND 115
MU50_D1D2        RLMTRVLGEDQYLLKKKIDEYELYKKWYKSSNK-----NTNMLTFHKYNLYNLTMNEYND 115
MRSA252_D1D2     KLMTRVLGEDHYLLEKKKAQYEAYKKWFEKHKSENPHSSLKKIKFDDFDLYRLTKKEYNE 120
85/2082_D1D2     KLMTRVLGEDHYLLEKKKAQYEAYKKWFEKHKSENPHSSLKKIKFDDFDLYRLTKKEYNE 120
MW2_D1D2         KLKTRVLEEDQYLLERKKEKYEIYKELYKKYKKENPNTQVKMKAFDKYDLGDLTMEEYND 120
WIS_D1D2         KLKTRVLEEDQYLLERKKEKYEIYKELYKKYKKENPNTQVKMKAFDKYDLGDLTMEEYND 120
                 :*   :** **:***::*  :** **: : . :.       . :    *..::*  *: :***:

USA300_D1D2      LQDALKRALDDFHREVKDIKDKNSDLKTFNAAEEDKATKEVYDLVSEIDTLVVSYYGDKD 180
Newman_D1D2      LQDALKRALDDFHREVKDIKDKNSDLKTFNAAEEDKATKEVYDLVSEIDTLVVSYYGDKD 180
N315_D1D2        IFNSLKDAVYQFNKEVKEIEHKNVDLKQFDKDGEDKATKEVYDLVSEIDTLVVTYYADKD 175
MU50_D1D2        IFNSLKDAVYQFNKEVKEIEHKNVDLKQFDKDGEDKATKEVYDLVSEIDTLVVTYYADKD 175
MRSA252_D1D2     LHQSLKEAVDEFNSEVKNIQSKQKDLLPYDEATENRVTNGIYDFVCEIDTLYAAYFNHSQ 180
85/2082_D1D2     LHQSLKEAVDEFNSEVKNIQSKQKDLLPYDEATENRVTNGIYDFVCEIDTLYAAYFNHSQ 180
MW2_D1D2         LSKLLTKALDNFKLEVKKIESENPDLKPYSESEERTAYGKIDSLVDQAYSVYFAYVTDAQ 180
WIS_D1D2         LSKLLTKALDNFKLEVKKIESENPDLRPYSESEERTAYGKIDSLVDQAYSVYFAYVTDAQ 180
                 : . *. *: :*: ***.*: :: **  :.    *  .    : .:* :   ::  :*   . :

USA300_D1D2      YGEHAKELRAKLDLILGDTDNPHKITNERIKKEMIDDLNSIIDDFFMETKQNRPKSITKY 240
Newman_D1D2      YGEHAKELRAKLDLILGDTDNPHKITNERIKKEMIDDLNSIIDDFFMETKQNRPKSITKY 240
N315_D1D2        YGEHAKELRAKLDLILGDTDNPHKITNERIKKEMIDDLNSIIDDFFMETKQNRPNSITKY 235
MU50_D1D2        YGEHAKELRAKLDLILGDTDNPHKITNERIKKEMIDDLNSIIDDFFMETKQNRPNSITKY 235
MRSA252_D1D2     YGHNAKELRAKLDIILGDAKDPVRITNERIRKEMMDDLNSIIDDFFMDTNMNRPLNITKF 240
85/2082_D1D2     YGHNAKELRAKLDIILGDAKDPVRITNERIRKEMMDDLNSIIDDFFMDTNMNRPLNITKF 240
MW2_D1D2         HKTEALNLRAKIDLILGDEKDPIRVTNQRTEKEMIKDLESIIDDFFIETKLNRPKHITRY 240
WIS_D1D2         HKTEALNLRAKIDLILGDEKDPIRVTNQRTEKEMIKDLESIIDDFFIETKLNRPQHITRY 240
                 :   .* :****:*:**** .:* ::**:* .***:.**:*******::*: ***  **::

USA300_D1D2      NPTTHNYKTNSDNKPNFDKLVEETKKAVKEADDSWKKKTVKK 282
Newman_D1D2      NPTTHNYKTNSDNKPNFDKLVEETKKAVKEADDSWKKKTVKK 282
N315_D1D2        DPTKHNFKEKSENKPNFDKLVEETKKAVKEADESWKNKTVKK 277
MU50_D1D2        DPTKHNFKEKSENKPNFDKLVEETKKAVKEADESWKNKTVKK 277
MRSA252_D1D2     NPNIHDYTNKPENRDNFDKLVKETREAIANADESWKTRTVKN 282
85/2082_D1D2     NPNIHDYTNKPENRDNFDKLVKETREAVANADESWKTRTVKN 282
MW2_D1D2         DGTKHDYHK---HKDGFDALVKETREAVAKADESWKNKTVKK 279
WIS_D1D2         DGTKHDYHK---HKDGFDALVKETREAVSKADESWKTKTVKK 279
                 : . *::      :: .** **:**::*: :**:***.:***:
```

FIG. 8B

Alignment of vwb from strains investigated

```
USA300_vwb      ------------------------------------------------------------
Newman_vwb      TTGAAAAATAAATTGCTAGTTTTATCATTGGGAGCATTATGTGTATCACAAATTTGGGAA 60
MW2_vwb         TTGAAAAATAAATTGCTAGTTTTATCATTGGGAGCATTATGTGTATCACAAATTTGGGAA 60
MRSA252_vwb     TTGAAAAATAAATTGCTAGTTTTATCATTGGGAGCATTATGTGTATCACAAATTTGGGAA 60
N315_vwb        TTGAAAAATAAATTGCTAGTTTTATCATTGGGAGCATTATGTGTATCACAAATTTGGGAA 60


USA300_vwb      ------------------GTGGTTTCTGGGGAGAAGAATCCATATGTATCTGAGTCGTTG 42
Newman_vwb      AGTAATCGTGCGAGTGCAGTGGTTTCTGGGGAGAAGAATCCATATGTATCTGAGTCGTTG 120
MW2_vwb         AGTAATCGTGCGAGTGCAGTGGTTTCTGGGGAGAAGAATCCATATGTATCTGAGTCGTTG 120
MRSA252_vwb     AGCAATCGTGCGAGTGCAGTGGTTTCTGGGGAGGAGAATCCATATAAATCTGAGTCATTG 120
N315_vwb        AGTAATCATGCGAGTGCAGTGGTTTCTGGGGAGAAGAATCCATATGTATCAAAAGCTTTA 120
                                  *************** ***********  ***  *  * **

USA300_vwb      AAACTGACTAATAATAAAAATAAATCTAGAAC-AGTAGAAGAGTATAAGAAAAGCTTGGA 101
Newman_vwb      AAACTGACTAATAATAAAAATAAATCTAGAAC-AGTAGAAGAGTATAAGAAAAGCTTGGA 179
MW2_vwb         AAACTGACTAATAATAAAAATAAATCTAGAAC-AGTAGAAGAGTATAAGAAAAGCTTGGA 179
MRSA252_vwb     AAATTAAATGGGAAAAGAAGTACTACAATAACTAGT-GATAAATATGAAGAAAATTTAGA 179
N315_vwb        GAATTGAAAGATAAAAGTAATAAATCCAATTCTTAC-GAAAATTATAGAGATAGTTTAGA 179
                 ** * *     ** *  * **   * *   *     **  * ***    * *  ** **

USA300_vwb      TGATTTAATATGGTCCTTTCCAAACTTAGATAATGAAAGATTTGATAATCCTGAATATAA 161
Newman_vwb      TGATTTAATATGGTCCTTTCCAAACTTAGATAATGAAAGATTTGATAATCCTGAATATAA 239
MW2_vwb         TGATTTAATATGGTCCTTTCCAAACTTAGATAATGAAAGATTTGATAATCCTGAATATAA 239
MRSA252_vwb     TATGTTAATATCGTCATTATCATTTGCAGATTATGAAAAATATGAGGAACCAGAATACAA 239
N315_vwb        AAGTTTGATTTCATCATTATCTTTTGCTGATTATGAAAAATATGAAGAGCCAGAATATGA 239
                    ** ** *  ** **  *       *** ****** ** ***  * ** *****  *

USA300_vwb      AGAAGCTATGAAAAAATATCAACAGAGATTTATGGCTGAAGATGAGGCTTTGAAGAAATT 221
Newman_vwb      AGAAGCTATGAAAAAATATCAACAGAGATTTATGGCTGAAGATGAGGCTTTGAAGAAATT 299
MW2_vwb         AGAAGCTATGAAAAAATATCAACAGAGATTTATGGCTGAAGATGAGGCTTTGAAGAAATT 299
MRSA252_vwb     AGAAGCAGTTAAAAAGTATCAACAAAAATTTATGGCTGAAGATGATGCATT-AAAAAATT 298
N315_vwb        AAAGGCTGTAAAAAAATATCAACAAAAATTTATGGCTGAAGATGATGCATTAAAAAATTT 299
                * * **  * ***** ******** * ****************** ** ** ** ** **

USA300_vwb      TTTTAGTGAAGAGAAAAAAATAAAAAATGGAAATACTGATA---ATTTAGATTATCTA-- 276
Newman_vwb      TTTTAGTGAAGAGAAAAAAATAAAAAATGGAAATACTGATA---ATTTAGATTATCTA-- 354
MW2_vwb         TTTTAGTGAAGAGAAAAAAATAAAAAATGGAAATACTGATA---ATTTAGATTATCTA-- 354
MRSA252_vwb     TTTTAGTGAAGAGAAAAAAATAAAAAATAGAAATACTAATA---CATCAAATTATCTG-- 353
N315_vwb        TTTAAATGAAGAAAAGAAGATAAAAAATGCAGATATTAGCAGAAAATCGAATAATTTATT 359
                *** * ****** ** ** *********  * *** *   *     *   ** ** *

USA300_vwb      -GGATTATCTCATGAAAGATATGAAAGTGTATTTAATACTTTGAAAAAACAAAGTGAGGA 335
Newman_vwb      -GGATTATCTCATGAAAGATATGAAAGTGTATTTAATACTTTGAAAAAACAAAGTGAGGA 413
MW2_vwb         -GGATTATCTCATGAAAGATATGAAAGTGTATTTAATACTTTGAAAAAACAAAGTGAGGA 413
MRSA252_vwb     -GGATTAACACACGAAAGATATGAGTCAATTTATAATTCATTAAAAAATCATCGTGAAGA 412
N315_vwb        AGGTTTAACACATGAAAGATATTCTTATATTTTTGATACATTAAAGAAAAATAAACAAGA 419
                 ** *** * ** *********       * * * ** * ** ** **  *     * **

USA300_vwb      GTTCTTAAAAGAAATTGAAGATATAAAAAAAGATAACCCTGAATTGAAAGACTTTAATGA 395
Newman_vwb      GTTCTTAAAAGAAATTGAAGATATAAAAAAAGATAACCCTGAATTGAAAGACTTTAATGA 473
MW2_vwb         GTTCTTAAAAGAAATTGAAGATATAAAAAAAGATAACCCTGAATTGAAAGACTTTAATGA 473
MRSA252_vwb     ATTTTCAAAAGAAATCGAAGAAATTAATAATAAAAATCCAGTGTTAAAAGAATATAACAA 472
N315_vwb        GTTTTTAAAAGATATTGAAGAAATACAACTGAAAAATAGTGATTTAAAGGACTTTAACAA 479
                 ** * ****** ** ***** **  *     * **    *  ** ** ** * ***  *

USA300_vwb      AGAGGAGCAATTAAAGTGCGACTTAGAATTAAACAAATTAGAAAATCAGATATTAATGTT 455
Newman_vwb      AGAGGAGCAATTAAAGTGCGACTTAGAATTAAACAAATTAGAAAATCAGATATTAATGTT 533
MW2_vwb         ATAG-------------------------------------------------------- 477
MRSA252_vwb     TGAGGAACAAACTAAAGCTGATACGGAATTAAACACTCTTGAAAATCAAGTACTAATGAT 532
N315_vwb        TACAGAGCAACATAATGCCGACGTAGAAATAAACAATTTAGAAAATAAAGTATTAATGGT 539


USA300_vwb      AGGTAAAACATTTTATCAAAACTATAGAGATGATGTTGAAAGTTTATATAGTAAGTTAGA 515
Newman_vwb      AGGTAAAACATTTTATCAAAACTATAGAGATGATGTTGAAAGTTTATATAGTAAGTTAGA 593
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AGGTTATACATTTTATCACTCGAATAAAAATGAAGTAGAAGATTTATATAACAAATTAGA 592
N315_vwb        AGGGTATACATTCTATAATACAAATAAGGACGAAGTTGAAGAATTATATAGTGAGTTAGA 599
```

```
USA300_vwb      TTTAATTATGGATATAAAGATGAAGAAAGA---GCAAATAAAAAAGCAGTTAACAAAAG 572
Newman_vwb      TTTAATTATGGATATAAAGATGAAGAAAGA---GCAAATAAAAAAGCAGTTAACAAAAG 650
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     TATGATTCTTGGTTATAAAGATGAAGAGAGA---AAAAAGAAGAGGGCTACCAATCAAAG 649
N315_vwb        TTTGATTGTTGGA---GAAGTTCAAGATAAGTCGGATAAAAAAAGAGCAGTAAATCAAAG 656

USA300_vwb      GATGTTAGAAAATAAAAAAGAAGACTTAGAAACCATAATTGATGAATTTTTTAGTGATAT 632
Newman_vwb      GATGTTAGAAAATAAAAAAGAAGACTTAGAAACCATAATTGATGAATTTTTTAGTGATAT 710
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AATGTTCAATAATAAAAAAGAGGATTTAGAAACTATTATTGATGAATTCTTTGGAGAAAT 709
N315_vwb        GATGTTAAATAGAAAAAAAGAGGATTTAGAATTTATTATAGATAAATTTTTTAAAAAAAT 716

USA300_vwb      AGATAAAACAAGACCTAATAA-TATTCCTGTTTTAGAAGATGAAAAACAAGAAGAGAAAA 691
Newman_vwb      AGATAAAACAAGACCTAATAA-TATTCCTGTTTTAGAAGATGAAAAACAAGAAGAGAAAA 769
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     TGG-ACAACAAAGGCCAACATCTATACCAACATTAGCGCCTAAAGAAGAAAAAGAAACAA 768
N315_vwb        TCA-ACAAGAACGTCCAGAGAGTATACCAGCATTAACTAGTGAAAAA-AATCATAATCAG 774

USA300_vwb      ATCATAAAAATATGGCTCAATTAAAATCTGACACTGAAGCAGCAAAAAGTGATGAATCAA 751
Newman_vwb      ATCATAAAAATATGGCTCAATTAAAATCTGACACTGAAGCAGCAAAAAGTGATGAATCAA 829
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     ATATAAAAAATGCAAATAAATTAAAATCTGACACTGAAGCAGCAAAAAATGATGAAGCAA 828
N315_vwb        ACTATGGCATTA-----AAGTTAAAAGCAGATACAGAAGCTGCTAAAAATGACGTATCAA 829

USA300_vwb      AAAGAAGCAAGAGAAGTAAAAGAAGTTTAAATACTCAAAATCACAAACCTGCATCTCAAG 811
Newman_vwb      AAAGAAGCAAGAGAAGTAAAAGAAGTTTAAATACTCAAAATCACAAACCTGCATCTCAAG 889
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AAAGAAG------------------TTTAAATACCCACAATCACAAATCTGTATCTCAAG 870
N315_vwb        AAAGAAG---------TAAAAGAAGTTTAAATACTCAAAATAATAAATCTACAACACAAG 880

USA300_vwb      AAGTTTCTGAACAACAAAAAGCTGAATATGATAAAAGAGCAGAAGAAAGAAAAGCGAGAT 871
Newman_vwb      AAGTTTCTGAACAACAAAAAGCTGAATATGATAAAAGAGCAGAAGAAAGAAAAGCGAGAT 949
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AAGTCTCTGAACAACAAAAAGCTGACTACGAAAGAAAAGCTGAAGAAAGAAAAGCGAGAT 930
N315_vwb        AAATTTCTGAAGAACAAAAAGCTGAATATCAAAGAAAGTCAGAGGCATTAAAAGAAAGAT 940

USA300_vwb      TTTTGGATAATCAAAAAATTAAGAAAACACCTGTAGTGTCATTAGAATATGATTTTGAGC 931
Newman_vwb      TTTTGGATAATCAAAAAATTAAGAAAACACCTGTAGTGTCATTAGAATATGATTTTGAGC 1009
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     TTTTAGATAAGCAAAAAAATAAGAAAACTCCTGTAGTTTCATTAGAATATGATTTTGAAC 990
N315_vwb        TTATAAACAGACAAAAATCTAAAAATGAGTCTGTGGTTTCACTAA---------TCGATG 991

USA300_vwb      ATAAACAACGTATTGACAACGAAAACGACAAGAAACTTGTGGTTTCTGCACCAACAAAGA 991
Newman_vwb      ATAAACAACGTATTGACAACGAAAACGACAAGAAACTTGTGGTTTCTGCACCAACAAAGA 1069
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     ATAAACAACGTGTTGACAACGAAAACGACAAGCAACTTGTGGTTTCTGAGCCATCAAAGA 1050
N315_vwb        ACGAAGA------CGACAACGAAAACGACAGGCAACTTGTGGTTTCTGCGCCATCAAAGA 1045

USA300_vwb      AACCAACATCACCGACTACATATACTGAAACAACGACACAGGTACCAATGCCTACAGTTG 1051
Newman_vwb      AACCAACATCACCGACTACATATACTGAAACAACGACACAGGTACCAATGCCTACAGTTG 1129
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AACCAACAACACCGCCTACATACACTGAAACAACCACACAGCTACCAATGCCTACAGTTG 1110
N315_vwb        AACCAACAACACCGACTACATATACTGAAACAACGACTCAGGTACCAATGCCTACAGTTG 1105

USA300_vwb      AGCGTCAAACTCAGCAACAAATTATTTATAATGCACCAAAACAATTGGCTGGATTAAATG 1111
Newman_vwb      AGCGTCAAACTCAGCAACAAATTATTTATAATGCACCAAAACAATTGGCTGGATTAAATG 1189
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AGCGTCAAACACAGCAACAAATCGTTTACAAAGCACCAAAACCATTAGCTGGATTAAATG 1170
N315_vwb        AGCGTCAAACTCAGCAACAAATCGTTTACAAAACACCAAAACCATTAGCTGGATTAAATG 1165

USA300_vwb      GTGAAAGTCATGATTTCACAACAACGCATCAATCACCAACAACTTCAAATCACACGCATA 1171
Newman_vwb      GTGAAAGTCATGATTTCACAACAACGCATCAATCACCAACAACTTCAAATCACACGCATA 1249
```

```
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     GTGAAAGTCATGATTTCACAACAACGCATCAATCACCAACTACTTCAAATCACACGCATA 1230
N315_vwb        GTGAAAGTCATGATTTCACAACAACGCATCAATCACCAACAACTTCAAATCATACGCATA 1225

USA300_vwb      ATAATGTTGTTGAATTTGAAGAAACGTCTGCTTTACCTGGTAGAAAATCAGGATCACTGG 1231
Newman_vwb      ATAATGTTGTTGAATTTGAAGAAACGTCTGCTTTACCTGGTAGAAAATCAGGATCACTGG 1309
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     ATCATCTTATTGAAATTGAAGAAACATCTGCTTTACCTGGTAGAAAGACAGGTTCATTGG 1290
N315_vwb        ATAATGTTGTTGAATTTGAAGAAACGTCTGCTTTACCTGGTAGAAAATCAGGATCACTGG 1285

USA300_vwb      TTGGTATAAGTCAAATTGATTCTTCTCATCTAACTGAACGTGAGAAGCGTGTAATTAAGC 1291
Newman_vwb      TTGGTATAAGTCAAATTGATTCTTCTCATCTAACTGAACGTGAGAAGCGTGTAATTAAGC 1369
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     TTGGTTTGAGTCAAATTGATTCTTCGCATTTAACTGAACGTGAGAAGCGCGTGATTAAAC 1350
N315_vwb        TTGGTATAAGTCAAATTGATTCTTCTCATCTAACTGAACGTGAGAAGCGTGTAATCAAGC 1345

USA300_vwb      GTGAACACGTTAGAGAAGCTCAAAAGTTAGTTGATAATTATAAAGATACACATAGTTATA 1351
Newman_vwb      GTGAACACGTTAGAGAAGCTCAAAAGTTAGTTGATAATTATAAAGATACACATAGTTATA 1429
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     GTGAACACGTGAGAGAAGCTCAAAAGTTAGTTGATAATTATAAAGATACACATAGTTATA 1410
N315_vwb        GTGAACACGTTAGAGAAGCTCAAAAGTTAGTTGATAATTATAAAGATACACATAGTTATA 1405

USA300_vwb      AAGACCGAATAAATGCACAACAAAAAGTAAATACTTTAAGTGAAGGTCATCAAAAACGTT 1411
Newman_vwb      AAGACCGAATAAATGCACAACAAAAAGTAAATACTTTAAGTGAAGGTCATCAAAAACGTT 1489
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AAGACCGATTAAATGCCCAACAAAAAGTAAATACTTTAAGTGCAGGTCATCAAAAACGTT 1470
N315_vwb        AAGACCGATTAAATGCACAACAAAAAGTAAATACTTTAAGTGAAGGTCATCAAAAACGTT 1465

USA300_vwb      TTAATAAACAAATCAATAAAGTATATAATGGCAAATAA---------------------- 1449
Newman_vwb      TTAATAAACAAATCAATAAAGTATATAATGGCAAATAA---------------------- 1527
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     TTAATAAACAAATTAATAAAGTATATAATGGCAAATAATTAATGCATGGCTGCAAAGGAA 1530
N315_vwb        TTAATAAACAAATCAATAAAGTATACAATGGCAAATAA---------------------- 1503

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     ATAATGAGTTTGCCGTAAAAATAACAACATTTTAAACTAGCAATAAATAATATCAAAGTC 1590
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     ATCATTTCAATGATGCAATCTAGTATAGTCCACATTCTAAACAGGTGTGGACTATTACTT 1650
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     TTTTCACTTTATATTACGAAAAAATTATTATGCTTAACTATCAATATCAATAATTAATTT 1710
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     TAAGCTGAAAAACAATAAAAATGTTAAGACAACGTTTACTTCAAGTTAATTATTATACTG 1770
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AAAATTCTGGTATATAATGCTGTTAGTGAATATAACAGGAAAATTAAATTGGTTATGATA 1830
```

```
N315_vwb       ------------------------------------------------------------

USA300_vwb     ------------------------------------------------------------
Newman_vwb     ------------------------------------------------------------
MW2_vwb        ------------------------------------------------------------
MRSA252_vwb    TTGAGTCTATATAAAGGAGAAATAACAGATGAAAAAGAAATTATTAGTTTTAACTATGAG 1890
N315_vwb       ------------------------------------------------------------

USA300_vwb     ------------------------------------------------------------
Newman_vwb     ------------------------------------------------------------
MW2_vwb        ------------------------------------------------------------
MRSA252_vwb    CACGCTATTTGCTACACAATTTATGAATTCAAATCACGCTAATGCATCAACAGAAAGTGT 1950
N315_vwb       ------------------------------------------------------------

USA300_vwb     ------------------------------------------------------------
Newman_vwb     ------------------------------------------------------------
MW2_vwb        ------------------------------------------------------------
MRSA252_vwb    TGATAAAAACTTTGTAGTTCCAGAATCGGGTATTAATAAAATTATTCCAACTTACGATGA 2010
N315_vwb       ------------------------------------------------------------

USA300_vwb     ------------------------------------------------------------
Newman_vwb     ------------------------------------------------------------
MW2_vwb        ------------------------------------------------------------
MRSA252_vwb    ATTTAAAAAAGCACCAAAAGTAAATGTTAGTAATTTAGCTGACAACAAAAACTTTGTAGC 2070
N315_vwb       ------------------------------------------------------------

USA300_vwb     ------------------------------------------------------------
Newman_vwb     ------------------------------------------------------------
MW2_vwb        ------------------------------------------------------------
MRSA252_vwb    TTCTGAAGATAAATTGAATAAGATTGCAGATCCATCGGCAGCTAGTAAAATTGTAGATAA 2130
N315_vwb       ------------------------------------------------------------

USA300_vwb     ------------------------------------------------------------
Newman_vwb     ------------------------------------------------------------
MW2_vwb        ------------------------------------------------------------
MRSA252_vwb    AAACTTTGCCGTACCAGAATCAAAATTAGGAATCATTGTACCAGAGTATAAAGAAATCAA 2190
N315_vwb       ------------------------------------------------------------

USA300_vwb     ------------------------------------------------------------
Newman_vwb     ------------------------------------------------------------
MW2_vwb        ------------------------------------------------------------
MRSA252_vwb    TAATCGAGTGAATGTAACAACAAACAATCCAGCTTCAAAACAAGTTGACAAGCAAATTGT 2250
N315_vwb       ------------------------------------------------------------

USA300_vwb     ------------------------------------------------------------
Newman_vwb     ------------------------------------------------------------
MW2_vwb        ------------------------------------------------------------
MRSA252_vwb    TGCTAAAGACCCAGAGGTGAATAGATTTATTACGCAAAATAAAGTAAACCATCGTTTCAT 2310
N315_vwb       ------------------------------------------------------------

USA300_vwb     ------------------------------------------------------------
Newman_vwb     ------------------------------------------------------------
MW2_vwb        ------------------------------------------------------------
MRSA252_vwb    TACTACGCAAACCCACTATAAGAAAGTTATTACTTCATACAAATCAACACATGTACATAA 2370
N315_vwb       ------------------------------------------------------------

USA300_vwb     ------------------------------------------------------------
Newman_vwb     ------------------------------------------------------------
MW2_vwb        ------------------------------------------------------------
MRSA252_vwb    ACATGTAAACCATGCAACATCTTCTATCCATCATCACTTTACTATTAAACCATCAGAAGC 2430
N315_vwb       ------------------------------------------------------------
```

```
USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     ACCTAGATATACACACCCATCTCAATCTCAATCGTTAATTATAAATCATCATTTTGCAGT 2490
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     TCCTGGATACCATGGTCATAAAGTTGTAACACCAGGACAAGCTAGTATTAGAATTCATCA 2550
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     CTTTTGTGCTGTACCTCAAATAAATAGTTTTAAGGTCATTCCATCATATGGTCACAATTC 2610
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     ACATCGTATGCATGTACCAAGTTTCCAAAATAACACAACAGCAACACATCAAAATGCAAA 2670
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AGTAAATAAAACTTATAACTATAAATATTTTTATACTTATAAAGTAGTCAAAGGTGTAAA 2730
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AAAACATTTCTCATTTTCAAAATCACATGGTTGTAAAATTGTTAAACCAGCATTAAACAT 2790
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     CAAAAATGTAAATTATCAATATGCTGTTCCAAGTAATAGCCCTACACACGTTGTTCCTGA 2850
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     GTTTCAGGGTATCTTACCAGCACCACGAGTATAAAAATTGACATTAAGTTTACGAGATAT 2910
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     GATAAATACCTATTATTTTAAACATAGTCTGCAATCTATGAGGTTGTAGGCTATGTTTTT 2970
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     TGCAGTTTATCAATAAACACCCATCAACAAATTATACCGTTTTTCTACTTTAAAAGTTGG 3030
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
```

```
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AAGTAACATAATCTTAAATAAATATATTATTAATTAAGATAAATATAAGACTCGAGATTA 3090
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     TTGTTAATAGTTTGTTCATCGCAAGTTAATTATTGTTTCTAAAATATTGGTATATAATTT 3150
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     TCAATGGCGAAGAAAACAGGGTAAAAAAGTCGGTTTTTAAATCAAAGCAAATAAGGAGTA 3210
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AAAAATGAAAAGGAAAGTACTAGTATTAACAATGGGCGTACTTTGTGCGACACAATTATG 3270
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     GCAAACGAATAATGCAAAAGCTTTAGTGACAGAGAGTGGCGTTAATGATACTAAGCAATT 3330
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     TACTGAAGTAACATCGGAAGAAAAAGTTATAAAAGATGCTATTTCGAAAGTCAATGAAAG 3390
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     CTTTATTTACTATCCCCAAAATGATTTGAAGGGATTAGGTGGAGAACACAACGATTACGA 3450
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AAAAATTACATATAGCACTTCTTCTAATAATGTTTTAGAATTATCAATGAGTTCAAAATA 3510
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     CGTAGGCGGTAAATCAGGAGCTATGGTTGGTTATAGTGAAATTTACTCATCACATTTCAC 3570
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
MRSA252_vwb     AGACCGCGACAAACGTGCTATCAGACGTGATCATGTTAAAGAAGCACAAAACTTGATTAA 3630
N315_vwb        ------------------------------------------------------------

USA300_vwb      ------------------------------------------------------------
Newman_vwb      ------------------------------------------------------------
MW2_vwb         ------------------------------------------------------------
```

```
MRSA252_vwb      TGATTATAAATATACGCAAATATATGAAGACTTTGCTAAAGCTACTGCAAAGGTAAGTAC 3690
N315_vwb         ------------------------------------------------------------

USA300_vwb       ------------------------------------------------------------
Newman_vwb       ------------------------------------------------------------
MW2_vwb          ------------------------------------------------------------
MRSA252_vwb      ACTTAGTCAGTCTCACCAAAATTATTTAAATAAACAAATTGATAAAGTGAATAATAAGAT 3750
N315_vwb         ------------------------------------------------------------

USA300_vwb       ----------------------
Newman_vwb       ----------------------
MW2_vwb          ----------------------
MRSA252_vwb      AGAGAAAACTGAAAAACGCTAA 3772
N315_vwb         ----------------------
```

FIG. 9A

```
MW2_vWbp          VVSGEKNPYVSESLKLTNNKNKSRTVEEYKKSLDDLIWSFPNLDNERFDNPEYKEAMKKY 60
Newman_vWbp       VVSGEKNPYVSESLKLTNNKNKSRTVEEYKKSLDDLIWSFPNLDNERFDNPEYKEAMKKY 60
USA300_vWbp       VVSGEKNPYVSESLKLTNNKNKSRTVEEYKKSLDDLIWSFPNLDNERFDNPEYKEAMKKY 60
N315_vWbp         VVSGEKNPYVSKALELKDKSNKSNSYENYRDSLESLISSLSFADYEKYEEPEYEKAVKKY 60
MRSA252_vWbp      VVSGEENPYKSESLKLNGKRSTTITSDKYEENLDMLISSLSFADYEKYEEPEYKEAVKKY 60
                  *****:*** *::*:*..: ..: : ::*...*: ** *:.   * *::::***::*:***

MW2_vWbp          QQRFMAEDEALKKFFSEEKKIKNGNTDN--LDYLGLSHERYESVFNTLKKQSEEFLKEIE 118
Newman_vWbp       QQRFMAEDEALKKFFSEEKKIKNGNTDN--LDYLGLSHERYESVFNTLKKQSEEFLKEIE 118
USA300_vWbp       QQRFMAEDEALKKFFSEEKKIKNGNTDN--LDYLGLSHERYESVFNTLKKQSEEFLKEIE 118
N315_vWbp         QQKFMAEDDALKNFLNEEKKIKNADISRKSNNLLGLTHERYSYIFDTLKKNKQEFLKDIE 120
MRSA252_vWbp      QQKFMAEDDALKNFLVKRKK---------------------------------------- 80
                  **:*****:***:*: :.**

MW2_vWbp          DIKKDNPELKDFNE---------------------------------------------- 132
Newman_vWbp       DIKKDNPELKDFNEEEQLKCDLELNKLENQILMLGKTFYQNYRDDVESLYSKLDLIMGYK 178
USA300_vWbp       DIKKDNPELKDFNEEEQLKCDLELNKLENQILMLGKTFYQNYRDDVESLYSKLDLIMGYK 178
N315_vWbp         EIQLKNSDLKDFNNTEQHNADVEINNLENKVLMVGYTFYNTNKDEVEELYSELDLIVGEV 180
MRSA252_vWbp      ------------------------------------------------------------

MW2_vWbp          ------------------------------------------------------------
Newman_vWbp       DEERANKKAVNKRMLENKKEDLETIIDEFFSDIDKTRPNNIPVLEDEKQEEKNHKNMAQL 238
USA300_vWbp       DEERANKKAVNKRMLENKKEDLETIIDEFFSDIDKTRPNNIPVLEDEKQEEKNHKNMAQL 238
N315_vWbp         QDKSDKKRAVNQRMLNRKKEDLEFIIDKFFKKIQQERPESIPALTSEKN--HNQTMALKL 238
MRSA252_vWbp      ------------------------------------------------------------

MW2_vWbp          ------------------------------------------------------------
Newman_vWbp       KSDTEAAKSDESKRSKRSKRSLNTQNHKPASQEVSEQQKAEYDKRAEERKARFLDNQKIK 298
USA300_vWbp       KSDTEAAKSDESKRSKRSKRSLNTQNHKPASQEVSEQQKAEYDKRAEERKARFLDNQKIK 298
N315_vWbp         KADTEAAKNDVSKRSKRS---LNTQNNKSTTQEISEEQKAEYQRKSEALKERFINRQKSK 295
MRSA252_vWbp      ------------------------------------------------------------

MW2_vWbp          ------------------------------------------------------------
Newman_vWbp       KTPVVSLEYDFEHKQRIDNENDKKLVVSAPTKKPTSPTTYTETTTQVPMPTVERQTQQQI 358
USA300_vWbp       KTPVVSLEYDFEHKQRIDNENDKKLVVSAPTKKPTSPTTYTETTTQVPMPTVERQTQQQI 358
N315_vWbp         NESVVSLIDDED------DNENDRQLVVSAPSKKPTTPTTYTETTTQVPMPTVERQTQQQI 350
MRSA252_vWbp      ------------------------------------------------------------

MW2_vWbp          ------------------------------------------------------------
Newman_vWbp       IYNAPKQLAGLNGESHDFTTTHQSPTTSNHTHNNVVEFEETSALPGRKSGSLVGISQIDS 418
USA300_vWbp       IYNAPKQLAGLNGESHDFTTTHQSPTTSNHTHNNVVEFEETSALPGRKSGSLVGISQIDS 418
N315_vWbp         VYKTPKPLAGLNGESHDFTTTHQSPTTSNHTHNNVVEFEETSALPGRKSGSLVGISQIDS 410
MRSA252_vWbp      ------------------------------------------------------------

MW2_vWbp          ------------------------------------------------------------
Newman_vWbp       SHLTEREKRVIKREHVREAQKLVDNYKDTHSYKDRINAQQKVNTLSEGHQKRFNKQINKV 478
USA300_vWbp       SHLTEREKRVIKREHVREAQKLVDNYKDTHSYKDRINAQQKVNTLSEGHQKRFNKQINKV 478
N315_vWbp         SHLTEREKRVIKREHVREAQKLVDNYKDTHSYKDRLNAQQKVNTLSEGHQKRFNKQINKV 470
MRSA252_vWbp      ------------------------------------------------------------

MW2_vWbp          ----
Newman_vWbp       YNGK 482
USA300_vWbp       YNGK 482
N315_vWbp         YNGK 474
MRSA252_vWbp      ----
```

FIG. 9B

```
USA300_vWbp     VVSGEKNPYVSESLKLTNNKNKSRTVEEYKKSLDDLIWSFPNLDNERFDNPEYKEAMKKY 60
Newman_vWbp     VVSGEKNPYVSESLKLTNNKNKSRTVEEYKKSLDDLIWSFPNLDNERFDNPEYKEAMKKY 60
N315_vWbp       VVSGEKNPYVSKALELKDKSNKSNSYENYRDSLESLISSLSFADYEKYEEPEYEKAVKKY 60
                ***********::*:*.::.***.: *:*:.**:.** *:.   * *::::***::*:***

USA300_vWbp     QQRFMAEDEALKKFFSEEKKIKNGNTDN--LDYLGLSHERYESVFNTLKKQSEEFLKEIE 118
Newman_vWbp     QQRFMAEDEALKKFFSEEKKIKNGNTDN--LDYLGLSHERYESVFNTLKKQSEEFLKEIE 118
N315_vWbp       QQKFMAEDDALKNFLNEEKKIKNADISRKSNNLLGLTHERYSYIFDTLKKNKQEFLKDIE 120
                **:*****:***:*:.*******.: ..    : ***:****. :*:****:.:****:**

USA300_vWbp     DIKKDNPELKDFNEEEQLKCDLELNKLENQILMLGKTFYQNYRDDVESLYSKLDLIMGYK 178
Newman_vWbp     DIKKDNPELKDFNEEEQLKCDLELNKLENQILMLGKTFYQNYRDDVESLYSKLDLIMGYK 178
N315_vWbp       EIQLKNSDLKDFNNTEQHNADVEINNLENKVLMVGYTFYNTNKDEVEELYSELDLIVGEV 180
                :*: .*.:*****: ** :.*:*:*:***::**:* ***:. :*:**.***:****:*

USA300_vWbp     DEERANKKAVNKRMLENKKEDLETIIDEFFSDIDKTRPNNIPVLEDEKQEEKNHKNMAQL 238
Newman_vWbp     DEERANKKAVNKRMLENKKEDLETIIDEFFSDIDKTRPNNIPVLEDEKQEEKNHKNMAQL 238
N315_vWbp       QDKSDKKRAVNQRMLNRKKEDLEFIIDKFFKKIQQERPESIPALTSEKN--HNQTMALKL 238
                :::  :*:***:***:.****** ***:**..*:: **:.**.* .**:  :*:.   :*

USA300_vWbp     KSDTEAAKSDESKRSKRSKRSLNTQNHKPASQEVSEQQKAEYDKRAEERKARFLDNQKIK 298
Newman_vWbp     KSDTEAAKSDESKRSKRSKRSLNTQNHKPASQEVSEQQKAEYDKRAEERKARFLDNQKIK 298
N315_vWbp       KADTEAAKNDVSKRSKRS---LNTQNNKSTTQEISEEQKAEYQRKSEALKERFINRQKSK 295
                *:******.* *******   *****:*.::**:**:*****:::*  * **::.** *

USA300_vWbp     KTPVVSLEYDFEHKQRIDNENDKKLVVSAPTKKPTSPTTYTETTTQVPMPTVERQTQQQI 358
Newman_vWbp     KTPVVSLEYDFEHKQRIDNENDKKLVVSAPTKKPTSPTTYTETTTQVPMPTVERQTQQQI 358
N315_vWbp       NESVVSLIDDED------DNENDRQLVVSAPSKKPTTPTTYTETTTQVPMPTVERQTQQQI 350
                : .****  * :      *****::******:****:***********************

USA300_vWbp     IYNAPKQLAGLNGESHDFTTTHQSPTTSNHTHNNVVEFEETSALPGRKSGSLVGISQIDS 418
Newman_vWbp     IYNAPKQLAGLNGESHDFTTTHQSPTTSNHTHNNVVEFEETSALPGRKSGSLVGISQIDS 418
N315_vWbp       VYKTPKPLAGLNGESHDFTTTHQSPTTSNHTHNNVVEFEETSALPGRKSGSLVGISQIDS 410
                :*::** *****************************************************

USA300_vWbp     SHLTEREKRVIKREHVREAQKLVDNYKDTHSYKDRINAQQKVNTLSEGHQKRFNKQINKV 478
Newman_vWbp     SHLTEREKRVIKREHVREAQKLVDNYKDTHSYKDRINAQQKVNTLSEGHQKRFNKQINKV 478
N315_vWbp       SHLTEREKRVIKREHVREAQKLVDNYKDTHSYKDRLNAQQKVNTLSEGHQKRFNKQINKV 470
                ***********************************:************************

USA300_vWbp     YNGK 482
Newman_vWbp     YNGK 482
N315_vWbp       YNGK 474
                ****
```

FIG. 9C