Research in Veterinary Science 100 (2015) 88–99

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Which are important targets in development of *S. aureus* mastitis vaccine?

F. Scali [a], C. Camussone [b,c], L.F. Calvinho [b,d], M. Cipolla [a], A. Zecconi [a,*]

[a] Dept. Veterinary Sciences and Public Health, Università degli Studi di Milano, Milano, Italy
[b] E.E.A. Rafaela, INTA, Santa Fe, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina
[d] Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina

ARTICLE INFO

Article history:
Received 22 December 2014
Accepted 14 March 2015

Keywords:
Staphylococcus aureus
Mastitis
Vaccine
Virulence factors
Immune response

ABSTRACT

Staphylococcus aureus represents one of the leading causes of mastitis in dairy cows worldwide. *S. aureus* IMI have variable outcomes due to virulence of the strain involved, immune defenses of the host, and by antibiotic resistance. The difficulty in eradication and the increasing concerns on antibiotics usages underscore the interest in developing new tools to control *S. aureus* mastitis. Vaccination represents one of the most studied of these tools but, so far, no vaccine seems to provide reliable protection. This review summarizes current knowledge on the major vaccine targets, including surface proteins, capsular polysaccharides, biofilm, and toxins. Finally, the present status of vaccination against *S. aureus* and the future of vaccine design were discussed, including how differences among *in vivo* models may influence vaccines development.

1. Introduction

Staphylococcus aureus can act as major pathogen in many animal species, and it represents one of the leading causes of mastitis in dairy cows worldwide. Moreover, *S. aureus* mammary gland infections may entail severe economic losses not necessarily associated with clinical signs (Halasa et al., 2007; Zecconi, 2010).

S. aureus intramammary infections (IMI) present variable outcomes due to different host–pathogen interactions. Indeed, both virulence of the strain involved and immune defenses of the host play a pivotal role (Zecconi et al., 2006). In addition, different management choices and environmental conditions can induce selective pressure on *S. aureus* and amplify differences between farms (Fox et al., 1991; Piccinini et al., 2010; Roberson et al., 1998).

Concerns on *S. aureus* mastitis are not solely limited to bovine species. Indeed, the pathogen may be a source of food poisoning due to its ability to produce enterotoxins that can still be active after heat treatments (Asao et al., 2003; Le Loir et al., 2003). Another potential source of risk for human health may arise from mastitis caused by methicillin resistant *S. aureus* (MRSA). Bovine mammary gland can harbor not only the well-known ST398 lineage of livestock-associated MRSA (LA-MRSA) (Vanderhaeghen et al., 2010), but also MRSA strains with a divergent mecA homolog, named mecA(LGA251) (Garcia-Alvarez et al., 2011). Although human infections by ST398 do not seem to be particularly severe, they may represent an important healthcare cost due to diagnostics and treatments (Holmes and Zadoks, 2011).

S. aureus has a vast array of virulence factors, which can aid the overcome or the evasion of host immune defenses and increase the severity of infections (Foster, 2005; Kim et al., 2012b; Rooijakkers et al., 2005; Zecconi and Scali, 2013). Furthermore, an impressive availability of virulence factors do not represent the only strength of *S. aureus*; indeed, the bacterium is able to resist to a large number of antibiotics, such as aminoglycosides, beta-lactams, fluoroquinolones, macrolides, and vancomycin (Holmes and Zadoks, 2011; Pantosti et al., 2007). All these characteristics facilitate the onset of chronic mastitis, and once a chronic infection has established, an effective treatment during milking has a poor chance to be successful, at least in a cost-effective way. Therefore, chronically infected cows can only be cured during the dry period; otherwise, they should be culled.

Improvements of management and udder health in several countries with well-developed dairy industry seem to have reduced prevalence of *S. aureus* mastitis. However, the pathogen is still widespread and eradication at this point is unlikely. Indeed, *S. aureus* exceptional resistance and the complex and partially unknown pathogenesis of these mastitis represent a great challenge for prevention and control programs (Zhao and Lacasse, 2008).

A strict control plan against contagious mastitis, based on segregation and single quarter milk diagnostic, can achieve eradication of *S. aureus* reducing at the same time risks for environmental or opportunistic bacterial infections (Zecconi et al., 2003, 2004).

* Corresponding author. Dept. Veterinary Sciences and Public Health – Università degli Studi di Milano, Via Celoria 10, 20133 Milano, Italy. Tel.: +39 0250318073; fax: +390250318079.
E-mail address: zecconiconsult@gmail.com (A. Zecconi).

http://dx.doi.org/10.1016/j.rvsc.2015.03.019
0034-5288/

Nevertheless, this approach is not always adoptable and other instruments to support classical control strategies should be investigated.

Over the last 40 years, vaccination has represented one of the most studied tools against *S. aureus* mastitis. Indeed, a large number of interesting data were published regarding both experimental and commercial vaccines. However, the preliminary evaluation of these studies suggests the absence of consistent methods to evaluate their efficacy. Indeed, some of them report increases in antibodies concentration or leukocytes response, others a reduction in severity of clinical mastitis and very few a decrease in new infection rate. In *S. aureus* mastitis contest this inconsistency is not a negligible problem. *S. aureus* IMI are mainly subclinical, therefore, an efficacious vaccine should reduce infection rates, while the reduction of clinical mastitis severity or an increase in immune responses are positive results, but not sufficient to define a vaccine as an effective preventive tool for *S. aureus* IMI.

The practical consequence of this is that, to date, vaccines do not provide consistent protection against *S. aureus* IMI. Furthermore, commercially available vaccines may reduce severity of clinical mastitis but they seem to be unable to prevent new infections (Middleton et al., 2009; Pereira et al., 2011; Tiwari et al., 2013; Yancey, 1999).

Despite these unsatisfactory results, the increasing concerns on antibiotics usage in food production and the need of sustainable dairy farming (Cheng et al., 2014; Trevisi et al., 2014) underscore the interest in developing new tools, including vaccines, to control widespread diseases such as *S. aureus* mastitis. Therefore, this review is focused on describing current knowledge on the most promising vaccine targets among *S. aureus* virulence factors and how they can influence the development of vaccines against *S. aureus* bovine IMI.

2. Surface protein as target of vaccination

S. aureus has at its disposal several virulence factors, among them, surface proteins carry out an essential role in colonization and infection of the host. Indeed, surface proteins are involved in both adhesion to different structures of the host (cells and extracellular matrix) and immune defenses evasion (Foster et al., 2014; Rooijakkers et al., 2005; Speziale et al., 2009; Zecconi and Scali, 2013). Four different families of *S. aureus* surface proteins can be identified on a chemical structure basis. Namely, microbial surface component recognizing adhesive matrix molecules (MSCRAMMs), Near iron transporter motif proteins (NEAT), three-helical bundle motif family, and G5–E repeat proteins (Foster et al., 2014). Surface proteins are widely investigated as potential vaccination targets because of their central role in *S. aureus* infection pathogenesis. Indeed, both active and passive immunizations (immunotherapy) showed promising results in mice models (Flock, 1999). During the last decades, various *in vivo* studies became available mainly targeting protein A (SpA), clumping factors (Clfs), and fibronectin binding proteins (FnBPs). However, these researches used mainly murine models and focused on applicability in human vaccination, whereas fewer data are available on bovine models.

2.1. Protein A

Protein A (three-helical bundle motif family) represents one of the most studied cell wall-anchored proteins and its binding properties are responsible for different virulence mechanisms. Indeed, SpA can bind several ligands, such as Fc fragment of IgG (Deisenhofer, 1981; Hjelm et al., 1975), von Willebrand factor (Hartleib et al., 2000; O'Seaghdha et al., 2006), Tumour Necrosis Factor Receptor 1 (TNFR1) (Gomez et al., 2004) and IgM (VH3 family) (Hakoda et al., 1996; Roben et al., 1995). SpA binds IgG on the bacterial surface and impairs both neutrophil phagocytosis and classical pathway complement activation (Foster et al., 2014). The binding of von Willebrand factor facilitates endocarditis and endovascular infections (Foster et al., 2014). The bond between SpA and TNFR1 plays an important role in *S. aureus* pneumonia (Gomez et al., 2004; Parker and Prince, 2012). In addition, SpA seems to induce TNR1 shedding and thus reducing TNF-α activities during systemic infections (Giai et al., 2013). Finally, the binding of VH3 IgM on humans and mice B lymphocytes surfaces may alter adaptive immune response (Kim et al., 2012b), nonetheless, specific studies in bovines are not available. Hence, among SpA functions, Fc region binding represents the most relevant mechanism of immune evasion during bovine mastitis (Zecconi and Scali, 2013).

The central role played in *S. aureus* infections explains why SpA was the first virulence factors investigated as possible target for vaccination in dairy cows. A pilot study showed that immunization against SpA could improve spontaneous cure rates of infected cows. However, vaccination did not provide protection against new infections (Pankey et al., 1985). SpA was also identified as a candidate for DNA-based vaccination in cattle; nevertheless, immune response under field conditions was inconsistent (Carter and Kerr, 2003).

Spa gene polymorphisms in *S. aureus* isolated from bovine IMI were shown to be significantly associated to specific binding of IgG and IgA obtained following mice vaccination with recombinant Efb, FnbpA and ClfA. This suggests that *spa* gene polymorphism should not be regarded solely as an epidemiological tool, but also as a means to identify the most suitable strains or gene products as vaccine candidates (Scarpa et al., 2010). However, when *S. aureus* isolates were challenged with a hyper immune serum obtained by SpA recombinant adhesin, the response observed was strongly influenced by isolates SpA gene polymorphisms (Scarpa et al., 2010). Recent studies in mice have shown that immunization via SpA_{KKAA} mutant, nontoxigenic SpA or SpA-specific monoclonal antibodies provide various degrees of protection against *S. aureus* experimental infections (Falugi et al., 2013; Kim et al., 2010a, 2012a); nonetheless, other authors did not find vaccination with SpA mutants protective against intravenous challenges (van Diemen et al., 2013). Furthermore, these researches focus on potential applications in human vaccine design and applicability in bovine models was not assessed.

2.2. Clumping factors

Clumping factors A (ClfA) and B (ClfA) are *S. aureus* MSCRAMM important for both adhesion and immune evasion. Clfs are well-known fibrinogen receptors and, in addition, they are able to facilitate bacterial invasion through binding other structures of the host (Clarke and Foster, 2006; Hauck and Ohlsen, 2006; Speziale et al., 2009). Although ClfA and ClfB share only 25% of their A region sequences (Ni Eidhin et al., 1998) and have different ligand binding sites, Clfs ligands show some remarkable chemical similarities (Ganesh et al., 2011). Clfs expression is influenced by growth phase, strains or environmental condition. Indeed, ClfB was expressed only during the exponential phase (McAleese et al., 2001; Ni Eidhin et al., 1998), while ClfA expression is possible during all phases of growth (Hartford et al., 1997). However, ClfA production showed to be significantly vary in relation to strain, microenvironment and IL-1β concentration (Kanangat et al., 2007; Nanra et al., 2009). In addition, carriage frequency of *clfA* and *clfB* genes has been shown to vary among *S. aureus* isolated from bovine IMI in different countries (Ikawaty et al., 2010; Klein et al., 2012).

ClfA bond with fibrinogen γ-chain provides adhesion to immobilized fibrinogen (McDevitt et al., 1997) and bond with soluble fibrin promotes aggregation of *S. aureus* to host platelets (Niemann et al., 2004). Moreover, interaction between ClfA and complement factor I results in an impairment of phagocytosis due to an augmented C3b degradation (Hair et al., 2008, 2010). ClfB adheres to fibrinogen via bond with fibrinogen α-chain, furthermore, ClfB promotes adhesion to host epithelial cells through binding cytokeratin 10 and

loricrin (Ganesh et al., 2011; Mulcahy et al., 2012; Ni Eidhin et al., 1998; Walsh et al., 2004).

Several studies regarding vaccination with ClfA in murine models are available in the literature and the first *in vivo* research was conducted at least 30 years ago (Espersen and Clemmensen, 1985). However, few data are available for ClfA and ClfB immunization in bovine mastitis models.

Vaccination with a ClfA mutant decreased severity of arthritis in experimentally infected mice. Moreover, immunotherapy (passive immunization) with anti-ClfA serum provided protection against experimental arthritis and reduced mortality in a murine infection model (Josefsson et al., 2001). DNA vaccines inducing immune response against not only ClfA but also fibronectin binding protein A (FnBPA) and sortase showed to decrease both arthritis severity and mortality in experimentally challenged mice (Gaudreau et al., 2007).

Useful immune response against ClfA can be stimulated with DNA vaccines in murine mastitis models too. Indeed, immunization with plasmids that encode for ClfA fibrinogen-binding region A was able to reduce severity of mastitis in mice. Nevertheless, this vaccination did not provided protection against experimental intraperitoneal infection (Brouillette et al., 2002). In addition, a DNA vaccine designed to target Clf A, FnBPA, extracellular fibrinogen binding protein (Efb), and collagen adhesin (Cna) provided significant protection against intramammary infection in mice (Castagliuolo et al., 2006). However, the efficacy of a subunit vaccine based on the same adhesion proteins as this latter one was significantly influenced by *S. aureus* virulence pattern (Scarpa et al., 2010).

Passive immunization of mice with MAbs to *S. aureus* ClfA resulted in a significant bacterial burden reduction in the mammary tissue following intramammary infection with a CP5 type strain. In addition, immunization with these MAbs combined with CP5 antibodies sterilized a significant number of experimentally infected mammary glands compared with controls, showing an additive effect (Tuchscherr et al., 2008). ClfA also showed potential in subunit vaccine design; indeed, mice vaccinated with recombinant binding region A of ClfA, seem to develop less mammary gland lesions than mice immunized with a killed *S. aureus* vaccine after an experimental intramammary infection (Gong et al., 2010).

ClfB is an important factor for adhesion to epithelial cells, particularly squamous epithelial cells located in the nares. Hence, active and passive immunization against ClfB decreases nasal colonization in mice (Schaffer et al., 2006) and reduces an important risk factor of *S. aureus* respiratory infections. Nonetheless, ClfB vaccination seems to have a neglectable role in intramammary infections.

In bovine models, immunization with a ClfA DNA vaccine was able to induce strong humoral response in cows and improve both opsonization and adherence inhibition *in vitro* (El-Din et al., 2006). Furthermore, targeting ClfA and three other adhesins (FnbpA, Efb and Cna) with a DNA vaccine stimulated significant production of Ig able to inhibit *S. aureus* adhesion to cow mammary gland epithelial cell culture (Castagliuolo et al., 2006). A DNA prime/protein boost vaccine that target both ClfA and FnBP can induce relevant immune response; however, this immunization guarantees only partial protection against intramammary challenge *in vivo* (Shkreta et al., 2004).

2.3. Fibronectin binding protein

Fibronectin binding proteins A and B (FnBPB) are members of the MSCRAMM family that interact with the extracellular matrix (ECM) and promote invasion of the host. FnBPs are able to bind fibronectin and elastin normally present within ECM (Loughman et al., 2008; Massey et al., 2001; Roche et al., 2004), furthermore, FnBPA can create a bond with fibrinogen (Wann et al., 2000). FnBPs play an important role in adhesion and invasion of epithelial cells and, thus, they are virulence factor of primary relevance in bovine mastitis (Camussone and Calvinho, 2013; Lammers et al., 1999; Shkreta et al., 2004).

In murine models, the first *in vivo* research on direct immunization against FnBPs showed underwhelming results (Espersen and Clemmensen, 1985) and passive immunization seemed to be more promising (Rozalska and Wadstrom, 1993). Nevertheless, further studies reported that vaccinated mice develop less severe infections than unvaccinated controls after experimental endocarditis (Schennings et al., 1993) and experimental mastitis (Mamo et al., 1994). In addition, inoculation of mouse mammary gland with *S. aureus* opsonized with FnBPs-antiserum seems to cause less severe mastitis than experimental infection with non-opsonized bacteria (Mamo et al., 1995). A recent study tested ligand-binding domain of FnBPs (IFnBP) as target of vaccination in a mouse mastitis model. Mice immunized against IFnBP developed less severe infection and higher interleukin-6 and interferon-gamma titers than mice vaccinated with a killed *S. aureus* (Hu et al., 2010). Data regarding bovine mastitis models are scarce. In a preliminary study, Nelson et al. (1991) immunized cows with a fusion FnBP formulated with ISCOMs, resulting in protection against mastitis following experimental challenge compared with cows in a control group. More recently, a DNA vaccine (ClfA and FnBP) described by Shkreta et al. (2004) provided only incomplete protection after experimental challenge with *S. aureus*.

2.4. Iron-regulated surface determinants

Several proteins compose the complex iron-regulated surface determinants (Isds) system. Among them, IsdA, IsdB, and IsdH are cell-wall anchored molecules of NEAT family that are showing interesting potential in vaccine design. These surface proteins are involved in *S. aureus* iron uptake systems through binding of hemoglobin and other host proteins (Clarke and Foster, 2006; Hammer and Skaar, 2011; Skaar and Schneewind, 2004; Zecconi and Scali, 2013).

In a murine model, passive immunization against IsdA and IsdB impaired *S. aureus* capability to heme scavenging and hemoglobin binding. However, administration of IsdA and IsdB antibodies did not seem to increase bacteria opsonophagocytic killing (Kim et al., 2010b).

Active immunization with recombinant IsdB was able to induce a strong antibody response in mice. IsdB specific vaccination provided significant protection in a mice septic model even when challenged with *S. aureus* clinical isolates that express different IsdB sequences (Kuklin et al., 2006). Furthermore, the protective effect of IsdB vaccination seems to be particularly correlated with Th17 cells and interleukin 17A (IL-17A) (Joshi et al., 2012).

Vaccination with a combination of IsdA, IsdB, Serin-aspartate repeat proteins D (SdrD) and E (SdrE) induced high titers of opsonophagocytic antibodies. Moreover, this vaccine has shown to provide protection in mice intraperitoneally infected with five different *S. aureus* strains (Stranger-Jones et al., 2006).

In a recent study, a chimeric bivalent vaccine of IsdB and α-hemolysin (Hla) was able to induce a higher protective immune response than immunization against the single molecules (Zuo et al., 2013). In a murine mastitis model, IsdH specific antibodies seem to decrease *S. aureus* mammary gland invasion (Ster et al., 2010).

Preliminary data on bovine identified IsdH as a possible target for vaccination. Indeed, vaccination with IsdH induces an elevated and long lasting antibody response (Ster et al., 2010), nevertheless, further research *in vivo* is needed to assess this potential.

Vaccination based on surface proteins showed interesting potential in both *in vitro* and *in vivo* models. Furthermore, different studies underlined favorable effects when immunization is stimulated against various surface proteins, even if *S. aureus* genetic variability could affect vaccine efficacy (Castagliuolo et al., 2006; Gaudreau et al., 2007; Mazzilli and Zecconi, 2010; Piccinini et al.,

2010; Scarpa et al., 2010; Shkreta et al., 2004; Stranger-Jones et al., 2006; Zuo et al., 2013).

3. Capsular polysaccharides roles in virulence and vaccination

Several strains of *S. aureus* isolated both from humans and domestic animals, cows included, are able to synthetize capsular polysaccharides (CPs) (O'Riordan and Lee, 2004). CPs impair C3 or C3b deposition and provide resistance against phagocytosis (Chavakis et al., 2007).

S. aureus CPs are produced *in vitro* in defined culture conditions (Sutra et al., 1990) and have also been detected during both acute and chronic experimental udder infections in cattle (Hensen et al., 2000). CPs are able to obstruct phagocytosis, reducing opsonophagocytic killing and decreasing respiratory burst of bovine neutrophils (Kampen et al., 2005; Sutra et al., 1990). On a serological basis it was hypothesized the existence of 11 capsular polysaccharides. However, only four of them, namely 1, 2, 5 and 8, present a recognizable chemical structure (Camussone and Calvinho, 2013). Among these CPs, CP5 and CP8 are the most represented types in *S. aureus* isolates. Interestingly, although CP5 and CP8 are two distinct serotypes, their chemical structures are almost identical (O'Riordan and Lee, 2004).

Some *S. aureus* isolates seem to have lost the ability to synthetize CPs, as they do not react with antibodies specific for serotypes 1, 2, 5 or 8. These strains are referred to as nontypeable (NT). These NT strains have been shown to react with antibodies against 336 surface polysaccharide (336PS), and can be classified as 336 serotype (Guidry et al., 1997).

Although CP5 and CP8 are the most common serotypes isolated from *S. aureus* IMI, CPs distribution vary between countries, but a relevant number of strains may not express any CPs (Camussone et al., 2012; Guidry et al., 1997; Han et al., 2000; Sordelli et al., 2000; Tollersrud et al., 2000). Even though a fair number of epidemiological studies based on serology is available, fewer data regarding genotypes distribution exist, thus, comparison between CP distributions among different countries cannot be made. CPs became molecules of interest in vaccines development since antibodies against these polysaccharides are able to induce opsonization and facilitate phagocytosis by PMN (Kampen et al., 2005; Sutra et al., 1990). Experimental injections of a CP5-protein carrier conjugate or a formaldehyde-killed CP5 *S. aureus* induced a strong antibody response in both cows and heifers (Gilbert et al., 1994; Tollersrud et al., 2001). Moreover, inoculations of CP5, CP8 or 336 polysaccharide conjugated to recombinant carrier protein and formulated with Freund's incomplete adjuvant (FICA), or the polysaccharides conjugates included in microspheres and formulated with the same adjuvant, induced a relevant IgG response in cows (O'Brien et al., 2000).

The first preliminary field results were described 30 years ago when a vaccine, composed of heat-killed *S. aureus* strain expressing CP and CP extracted from *Staphylococcus epidermidis*, was tested in two Georgia (USA) farms. Vaccinated cows showed improvement in milk quality, a smaller decrease of milk yield and a reduction of mastitis incidence (Yoshida et al., 1984), when compared to untreated controls. Further *in vivo* research revealed that, after experimental induction of *S. aureus* mastitis, cattle vaccinated with a combination of a capsulated strain and a toxoid from a beta-hemolysin producing strain emulsified with FICA developed significantly more immunoglobulin (IgG_1, IgG_2, IgM, IgA) and less IMI than unvaccinated ones (Nickerson et al., 1993).

A trivalent vaccine containing inactivated CP5, CP8 and 336 *S. aureus* strains was able to induce relevant humoral response, particularly when associated with FICA or $Al(OH)_3$. Nonetheless, administering this vaccine produced little effect on PMN phagocytosis and on CD4–CD8–lymphocytes subpopulations (Lee et al., 2005). In addition, immunization of pregnant heifers with a vaccine based on a lysate of five *S. aureus* strains containing CP5, CP8 and 336 serotypes (Lysigin®, Boehringer Ingelheim) reduced the duration of clinical symptoms and mastitis scores after challenge with a heterologous strain. However, it was unable to prevent IMI, to reduce somatic cell count (SCC), and to improve clearance rates (Middleton et al., 2006).

The strength of antibody response elicited by *S. aureus* vaccines showed to be affected by adjuvant applied (*i.e.* FICA, Al(OH)3 or mineral oil) (Lee et al., 2005; O'Brien et al., 2000; Tollersrud et al., 2001). In a recent study pregnant heifers were immunized with a formalin-killed CP5 *S. aureus* strain formulated with either $Al(OH)_3$ or immune-stimulating complexes (ISCOM matrix). Vaccine formulated with ISCOM matrix induced a significantly stronger humoral immune response (IgG and IgG2 serum levels, IgG whey levels) and improved PMN opsonic capacity (Camussone et al., 2013). A following study compared vaccination with whole-cell and lysate of a CP5 *S. aureus* strain, both formulated with ISCOM matrix; interestingly, lysate formulation stimulated a higher expression of cytokine and provided a higher and longer lasting antibody response (Camussone et al., 2014). Whey antibodies against these vaccines were able to inhibit internalization to MAC-T cells of the homologous and heterologous *S. aureus* strains and serum antibodies promoted milk macrophage phagocytosis (Renna et al., 2014). However, IgG2 was not detected in milk after vaccination and vaccine protective effects are still to be tested, with both experimental and field challenges.

4. Biofilm and immunity, relevance in bovine mastitis vaccination

Biofilms are complex agglomerates of bacteria cells, organic matrix and water. These biological interfaces promote adhesion to several surfaces and provide a protective environment against host defenses and antibiotics (Costerton et al., 1999; Donlan and Costerton, 2002; Stewart and Costerton, 2001). Other than water, organic matrix represents the major component of biofilm and it is composed by exopolysaccharides, proteins (surfactant, structural and enzymatic), extracellular DNA (eDNA), and lipids (Flemming and Wingender, 2010). A large number of bacteria are able to produce biofilm, various strains of *S. aureus* included (Hall-Stoodley et al., 2004).

Biofilms are widely studied; however, important molecular mechanisms regarding biofilm development are still unclear. Biofilm formation follows two physiologically distinct phases, named attachment and maturation. Attachment of bacteria cells to an abiotic surface is driven mostly by hydrophobic or electrostatic interactions, while adhesion to a biotic surface is facilitated by cell wall associated adhesins. Maturation comprises adhesive processes that connect bacteria during proliferation and disruptive processes that form channels in the biofilm, which are essential for nutrient transport to the deeper biofilm layers. Furthermore, detachment represents another stage crucial for bacterial dissemination during several staphylococcal infections (Otto, 2008, 2013). Molecules of particular interest involved in *S. aureus* biofilm formation are polysaccharide intercellular adhesin (PIA) also called poly-*N*-acetyl-β-(1-6)-glucosamine (PNAG), MSCRAMMs, teichoic acids, and eDNA (Gotz, 2002; Otto, 2008). Among these molecules, PIA represents one of the most studied virulence factors because of its central role in biofilm formation. Moreover, various enzymes, encoded by *ica* locus (*icaA*, *icaB*, *icaC* and *icaD*), are involved in PIA production and transportation. Nevertheless, PIA does not seem to be mandatory for biofilm formation as it was shown that in *S. aureus* strains lacking *ica* genes, and consequently unable to synthesize PIA, several MSCRAMM and secreted proteins can replace PIA functions (O'Gara, 2007; Otto, 2013).

Depending on studies and geographical areas, reported frequency of *S. aureus* biofilm-producing isolates from bovine IMI varies from

41 to 80% (Coelho et al., 2011; Dhanawade et al., 2010; Fox et al., 2005; Oliveira et al., 2007; Vasudevan et al., 2003).

Although biofilm roles in *S. aureus* IMI raised great interest during the last years, few data on vaccination regarding ruminant mastitis models *in vivo* are available. An *in vivo* study on ovine mastitis model was carried out using a *S. aureus* biofilm-producing bacterin, crude bacterial extracts, or purified PNGA, in different vehicles. Sheep immunized with the bacterin yielded high antibody titers against PIA. After experimental induced mastitis, immunized sheep developed lower infection levels and less mammary lesions than unvaccinated controls (Perez et al., 2009). Immunization with bacterins associated with high or low content of extracellular biofilm matrix, named by the authors "slime associated antigenic complex" (SAAC), formulated with an oil based adjuvant, was tested in an *in vivo* bovine mastitis model. Cows vaccinated with bacterin containing high concentration of SAAC developed stronger antibody response and less severe clinical symptoms than both unvaccinated and immunized with bacterin containing low concentration of SAAC (Prenafeta et al., 2010). This vaccine named Startvac® (Hipra, S.A., Spain) is commercially available in many countries and showed to reduce severity of experimental clinical mastitis (Prenafeta et al., 2010). Efficacy of this vaccine was estimated in two herds based on infection transmission and infection duration parameters, yielding around 45% reduction of the basic reproduction ratio of *S. aureus* (Schukken et al., 2014). However, significant differences in efficacy were detected between farms, possibly associated with management practices that are known to affect infection dynamics and could thus have contributed to the overall observed efficacy.

5. *S. aureus* toxins activities and potential application in vaccine design

S. aureus virulence factors involved in adhesion and immune evasion play a pivotal role in host invasion. Nevertheless, virulence factors activities are not limited to immune-modulation and adherence, indeed, *S. aureus* can synthetize and secrete several toxins that cause direct damage on different host structures. Various cells and molecules of the host can be harmed by *S. aureus* depending on the type of toxin secreted. Specifically, *S. aureus* toxins are able to damage cell membranes, induce gastro-enteric and systemic symptoms due to alterations of specific receptors, or enzymatically degrade diverse host molecules (DuMont and Torres, 2014; Otto, 2014). The relevance of Hla and Hlb as virulence factors of *S. aureus* causing IMI has been well documented (Camussone and Calvinho, 2013; Kerro Dego et al., 2002; Zecconi and Scali, 2013).

Although toxins as potential targets of vaccination are less investigated than surface proteins, several research studies on active and passive immunization are available. Among *S. aureus* toxins, Hla represents the most studied and different vaccination strategies against this molecule have shown various degrees of effectiveness (Berube and Wardenburg, 2013). Other than alpha-toxin, beta-toxin (Hlb), Panton–Valentine leukocidin (PVL), superantigen and superantigen-like proteins (SSLs) may represent interesting targets for bovine vaccination.

5.1. Hemolysins

S. aureus Hla is a pore-forming toxin that specifically recognizes ADAM10 receptor. Hence, interactions between Hla and ADAM10 seem to be required in order to create pores within the host cells membrane, at least at low doses (Berube and Wardenburg, 2013; Otto, 2014).

In murine models, various *in vivo* studies highlight the relevance of Hla as vaccination target in diverse infection models. Both passive immunization against Hla and vaccination with an Hla mutant are able to induce high titers of specific IgG and seem to provide various degrees of protection against mouse lungs infection (Ragle and Bubeck Wardenburg, 2009; Wardenburg and Schneewind, 2008). Moreover, a recently designed Hla subunit vaccine seems to confer significant protection against experimental pneumonia or septicemia in mice (Adhikari et al., 2012). Both passive and active immunization against Hla reduce severity of skin and corneal infections in murine models (Hume et al., 2000; Kennedy et al., 2010; Tkaczyk et al., 2012). A vaccine, based on a *S. aureus* Hla isolate of bovine IMI, showed to provide protection in mice challenged with homologous or heterologous strains in a septic arthritis model (Leitner et al., 2003).

In small ruminants models, a preliminary study in a sheep mastitis model reported that vaccination with killed *S. aureus* cell-toxoid or with the same live strain was unable to protect ewes against experimental induced IMI (Watson and Lee, 1978). A later study described that association of killed *S. aureus* cells, Hla-toxoid and Hlb-toxoid formulated with an oil-based adjuvant generated a detectable immune response in sheep (Tollersrud et al., 2002).

In bovines, vaccination with proper doses of Hla-toxoid and *S. aureus* bacterin induces high titers of specific IgG for several weeks (Opdebeeck and Norcross, 1982). Furthermore, active immunization against Hla can trigger recruitment of both neutrophil and lymphocytes in milk (Herbelin et al., 1997; Riollet et al., 2000).

Although various *in vitro* researches in bovine mammary gland models are available, specific experimental data on *in vivo* models are scarce. Bovine mammary gland epithelial cells cultures exposed to Hla show alteration of mitochondrial transmembrane potential, increase of ROS production, and more DNA fragmentation (Seol et al., 2010). Hla also facilitates adherence of *S. aureus* to bovine mammary epithelial cells *in vitro* (Cifrian et al., 1995). However, anti-Hla serum alone does not seem to obstruct bacterial adhesion, whereas an antiserum to both Hla and Hlb seems to decrease this adhesion (Cifrian et al., 1996b). Moreover, Hla antiserum seems to have anti-cytotoxic properties *in vitro*, particularly when an association of anti-Hla and anti-Hlb antibodies is used (Cifrian et al., 1996b).

Hla vaccination in lactating cows can trigger a fast and imposing neutrophil recruitment into mammary gland. In addition, milk of immunized bovines shows relevant bactericidal activity *in vitro* (Herbelin et al., 1997). Vaccination with autogenous toxoid-bacterin (α- and β-hemolytic *S. aureus* strains) emulsified with FICA has shown to reduce SCC and increase cure rates in quarters already infected with the same strain (Hwang et al., 2000), nonetheless, no information is available on heterologous strains.

S. aureus beta-toxin is a cytotoxic hemolysin classified as a sphingomyelinase C, a group of hydrolase enzymes. Hlb can increase cellular permeability and can induce cellular destruction via hydrolysis of sphingomyelin, a sphingolipid normally present in host cells membranes (Bownik and Siwicki, 2008; Dinges et al., 2000; Otto, 2014). *In vitro*, Hlb showed to improve *S. aureus* adhesion to bovine mammary epithelial cells and to induce erythrocytes lysis, however, cytotoxic effect on epithelial cells seems to be less stronger than Hla (Cifrian et al., 1996a). In addition, recent studies reported an interesting interaction of Hlb with other virulence factors such as catalase (CatA) and superantigens. Indeed, association of the superantigen toxic shock syndrome toxin-1 (TSST-1) and Hlb can cause alteration of proliferating lymphocytes *in vitro* (Huseby et al., 2007). A *S. aureus* double mutant, deprived of both Hlb and CatA, survives significantly longer within bovine mammary epithelial cells *in vitro* than both a wild strains and a mutant without Hlb only. Furthermore, this double mutant seems to be less virulent in different ovine (IMI and subcutaneous) and murine (intraperitoneal and subcutaneous) experimental infections (Martinez-Pulgarin et al., 2009).

Although Hlb does not raise an interest as target of vaccination comparable to Hla, some data in bovine models are available. Hlb antiserum provides moderate reduction of cytotoxicity and adherence of *S. aureus* to mammary epithelial cell culture, whereas Hla

and Hlb serum shows better results (Cifrian et al., 1996b). *In vivo* studies on an inactivated vaccine, associated with Hla and Hlb toxoids, did not resulted in a relevant antibody response against Hlb (Nordhaug et al., 1994b). Additionally, the vaccine was unable to significantly prevent subclinical IMI (Nordhaug et al., 1994a).

5.2. Toxins

Staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin-1 are identified as superantigens because they can trigger T-cells proliferation without antigen presentation, and thus, lead to a massive release of cytokines (Larkin et al., 2009). Early studies suggested a potential role of SEs as vaccination targets (Bavari et al., 1996, 1999; Nilsson et al., 1999; Woody et al., 1997).

Nasal or oral vaccination with an attenuated staphylococcal enterotoxin B (SEB) decreases mortality and toxic shock in a mouse sepsis model (Stiles et al., 2001). In a recent study, passive immunization with SEB-specific monoclonal antibody showed to improve survival and to reduce infection level in a mouse sepsis model (Varshney et al., 2013). A recombinant bovine variant of staphylococcal enterotoxin C (SEC) was able to stimulate a neutralizing antibody response in both rabbit and mice (Uhl et al., 2004). Moreover, mice immunized with non-toxic SEC developed inferior level of infection and mortality when experimentally challenged (Hu et al., 2005). Immunization against TSST-1 protected rabbits from a lethal dose of TSST-1 and lipopolysaccharide (LPS) (Gampfer et al., 2002). Furthermore, vaccination with an attenuated TSST-1 or with a specific fusion protein (glutathione S-transferase and mutant TSST-1) reduced infection levels and septic death in mice (Cui et al., 2005; Hu et al., 2003).

Researches on superantigens in vaccine design started in relatively recent times, and thus, specific studies in bovine mastitis models are scarcely available. A recombinant SEC-mutant vaccine reduced both SCC and infection levels in experimentally induced bovine subclinical mastitis (Chang et al., 2008). In addition, cows immunized with a fusion protein (glutathione S-transferase and mutant TSST-1) seem to develop lower SCC than control cows when challenged with *S. aureus* (Cui et al., 2010).

Superantigen-like proteins are a group of *S. aureus* virulence factors with different and not always clear functions but frequently related to immune evasion. SSLs present a chemical structure similar to SEs and TSST-1, however, they are not able to bind major histocompatibility complex (MHC) class II and do not have superantigen activity (Al-Shangiti et al., 2004; Fraser and Proft, 2008). Emerging roles of SSLs in immune modulation and evasion may suggest important future applications of SLLs in vaccine design. Indeed, both SSL5 and SSL7 impede neutrophil activities: SSL5 obstructs neutrophils rolling (Bestebroer et al., 2007) while SSL7 hinders neutrophil recognition and complement functions via binding IgA and C5 (Bestebroer et al., 2010; Lorenz et al., 2013). SSL3 seems to have a significant role in immune evasion due to its binding to Toll-like receptor 2 (Bardoel et al., 2012; Yokoyama et al., 2012). SSL10 binds various host molecules such as IgG (Itoh et al., 2010; Patel et al., 2010), phosphatidylserine (Itoh et al., 2012), prothrombin and blood coagulation factor Xa (Itoh et al., 2013b). Hence, SSL10 seems to inhibit blood coagulation and classical complement pathway cascade. SSL11 seems to improve *S. aureus* survival through binding to leukocytes, IgA and P-selectin glycoprotein ligand-1 (Chung et al., 2007). SSL4 and SSL8 seem also to bind diverse host molecules (Hermans et al., 2012; Itoh et al., 2013a) but their functions are still to be clarified. Little is known about the other SSLs. Therefore, further studies on SSLs may help to better understand *S. aureus* immune evasion intricate mechanisms and, hopefully, to counteract these mechanism.

Panton–Valentine leucocidin (PVL) is a two-component toxin able to induce leucocytes lysis. *S. aureus* secretes two separate components, LukS-PV and LukF-PV, these components polymerize and create pores on neutrophils membrane (Kaneko and Kamio, 2004). A recent study described that a LukS-PV subunit vaccine protected mice against experimental pneumonia, when administered intranasally, while a LukS-PV and LukF-PV subunit vaccine administered subcutaneously provided protection against intradermal challenge. Interestingly, intranasal and subcutaneous vaccinations did not induce protection against intradermal and lung challenges, respectively (Brown et al., 2009). Moreover, both passive and active immunizations against LukS-PV and LukF-PV provided protection in a mouse sepsis model (Karauzum et al., 2013). To date, there are few data available regarding PVL vaccination in bovine models. An early study was carried out vaccinating with formalinized crude leucocidin, containing trace amounts of Hla and Hlb activity, adjuvanted with FICA to determine the optimal production of antileucocidin antibodies in milk of lactating cows (Loeffler et al., 1988). However, the potential of PVL as vaccination target was not further explored.

6. Discussion

Despite a large number of research work has been devoted in the last 50 years to develop *S. aureus* vaccines both in human and in veterinary medicine, very few products are commercially available. Indeed, in human medicine, the search for vaccines against *S. aureus* human infections employed enormous resources, yet, still no vaccine passed phase 3 trials. Despite the inferior amount of resources invested and less number of studies involved, at least two commercial vaccines are available for cattle: namely, Lysigin® (Boehringer Ingelheim, Germany), available in North America since the 1980s, and the recent Startvac® (Hipra S.a., Spain) available in EU since 2009. Important differences between human and bovine in both *S. aureus* infections and vaccination objectives are at the base of this difference. Vaccination design for bovine species has one major advantage; indeed, *S. aureus* IMI one of the most frequent diseases in dairy cows and an economically relevant target for vaccination. On the other hand, vaccination in humans should take account of diverse severe *S. aureus* infections, *e.g.*, pneumonia, endocarditis, cutaneous infections, septicemia, implant infections and osteomyelitis. In addition, vaccines must provide protection for patients at different ages and conditions such as metabolic diseases, immunodeficiency and other chronic disorders.

Another advantage, regarding vaccine development in bovine, is represented by a relatively simpler procedure for marketing authorization. A vaccine designed for human, in order to demand approval, should follow a strict procedure and requires three phases of clinical trials. Rather, vaccine development for cattle follows simplified field trials, without the three-phase procedure, thus speeding up considerably the process.

Finally, vaccination against *S. aureus* in bovine and human presents different purposes. *S. aureus* infections represent a major risk for human health because of their potential severity. Hence, a vaccine able to prevent severe diseases and to reduce transmission may be considered effective. In the case of bovine, on the contrary, *S. aureus* IMI are frequently subclinical and their impact is essentially economic. Therefore, vaccination should impede colonization and infection of mammary gland instead of prevention of clinical forms. Specifically, the two commercial vaccines currently available for mastitis control do not seem to prevent new IMI and their overall efficacy is still controversial.

The reasons why these relatively poor results have been obtained are different, and related to both bacteria and host. In the first case one major problem is represented by the large genetic variability observed in *S. aureus* (Delgado et al., 2011; Fournier et al., 2008; Haveri et al., 2008; Piccinini et al., 2010; Reinoso et al., 2008; Scarpa et al., 2010; Zecconi et al., 2005). Furthermore, improper antigen selection may significantly impair efficacy of vaccination.

Appropriate antigen selection seems to be a major obstacle for the development of effective vaccines for dairy cows, and a general consensus on the antigen targets to achieve is still missing. Several research suggest that targeting specific antigens is the most efficient method to develop efficacious vaccine. However, other studies suggest that *S. aureus* milk isolates have a large polymorphism and regional patterns, suggesting the importance to develop vaccine based on antigens common to different isolates.

S. aureus has a tremendous amount of virulence factors and selecting a proper combination of antigens is extremely hard. These antigens should not stimulate only humoral response but also cell-mediated and innate immunity. Furthermore, antigen selection seems to represent a critical limitation in human vaccines too (Botelho-Nevers et al., 2013; Daum and Spellberg, 2012; Pier, 2013; Proctor, 2012).

Other than the aforementioned gaps of knowledge about virulence factors, principal problems linked to antigen selection are variability in antigen expression and host immune response against antigen combination. MSCRAMMs, CP and biofilm expression may vary significantly among strains isolated from mastitis in different countries, additionally, even expression of Hla and Hlb may present variations (Aarestrup et al., 1999). Diverse virulence factor expression may alter host immune response, *e.g. S. aureus* that express CP seem able to mask ClfA (Risley et al., 2007) and thus ClfA efficacy as vaccination target may reduce. Moreover, monoclonal antibodies for CP and PNAG showed relevant interference activities if combined together (Skurnik et al., 2010). On the other hand, ClfA covalently linked with dPNAG provided an increased immune response and promising results in a murine model compared with mice vaccinated with mixture of ClfA and dPNAG (Maira-Litran et al., 2012).

To fill these latter gaps, the wider availability of technologies such as genomics, proteomics and serum proteomic analysis (SERPA) are opening a new frontier in assessing the most appropriate targets in developing vaccines and this will help future research in this field (Otto et al., 2014; Seyffert et al., 2012; Taverna et al., 2007; Tedeschi et al., 2009).

On the host side, the role of udder immune defenses, their efficiency and the factors that can impair them should be considered (Gilbert et al., 2013; Mazzilli and Zecconi, 2010; Piccinini et al., 2005; Zecconi et al., 2006), in addition to host genetic variability. However, the most important obstacles in vaccine development are gaps in the knowledge on the interaction between *S. aureus* virulence factors and host immune response. Indeed, the differences observed in the different vaccination trials, in addition to the large variations observed related to the different antigen(s) applied, make hard to identify if failures in achieving a protective immune response are due to an insufficient capability of vaccines to induce it or to host's failure to mount a response. Therefore, not only some of the virulence factor functions are still to be clarified, but also host immune response needs additional investigations. In example, a remarkable number of studies on *S. aureus* virulence factors are available. However, several functions of these factors are still unknown. SSLs family, identified 15 years ago (Williams et al., 2000), represents an emblematic example of these lacks of knowledge. Although the role of the 14 SSL proteins in *S. aureus* infections is not always clear, they seem to be involved in immune evasion, but very few studies investigate them in relation to vaccine development.

Most vaccine trials carried out so far have addressed humoral immune response following vaccination and although several experimental and commercial vaccines were able to generate significant antibody titers in serum and milk, protection afforded was limited (Middleton et al., 2009; Pereira et al., 2011). Cellular immune responses are known to play a key role against facultative intracellular pathogens. However, there is very limited information about the protective cellular immunity in the bovine mammary gland following bacterial infection (Bharathan and Mullarky, 2011). Achieving a better understanding of protective cellular immunity in the mammary gland coupled to the use of novel antigen delivery systems that can modulate the immune response against selected candidate antigens that stimulate both humoral and cellular reactions can potentially enhance the limited protection obtained so far with *S. aureus* mastitis vaccines.

Among the problems to be solved to develop an efficacious vaccine there is the selection of the proper animal model and, consequently, the assessment on the immune response elicited. Murine immunology is relatively well known and a vast amount of data are available. Relevant studies exist on bovine immune response against *S. aureus* too, nonetheless, data available are far less than in mice. As stressed on various chapters of this review, *in vivo* studies on mice reported numerous promising vaccine candidates that were not tested or underperformed in bovine models. Gaps in bovine mammary gland immunity knowledge and differences in immune response against *S. aureus* in mice and bovine could have significantly contributed to the past failures. In addition, the same problems may be among the main causes of clinical trial failures in humans (Fowler and Proctor, 2014).

Mice models were identified as viable *in vivo* models for mastitis more than 40 years ago (Chandler, 1970). Moreover, murine models are considered reliable to investigate *S. aureus* bovine IMI pathogenesis and host immune response (Brouillette and Malouin, 2005; Notebaert and Meyer, 2006). However, some relevant differences between cattle and mice exist other than anatomical and immunological ones. A major drawback of murine models is the impossibility of a SCC evaluation during milking which cannot be replaced by a histopathological exam (Notebaert and Meyer, 2006). Furthermore, murine and bovine IMI present a diverse pathological and clinical evolution. *S. aureus* mastitis in mice is severe and acute processes can lead to death in less than 72 hours. Therefore, in order to induce a chronic infection, mice can be pre-emptively immunized with endotoxin (Brouillette and Malouin, 2005).

Ovine models may represent an interesting alternative to murine models. Mastitis is the leading cause of SCC increase in sheep milk while parity and stage of lactation seem to play a less important role than in bovine (Ariznabarreta et al., 2002; Paape et al., 2007; Souza et al., 2012).

Although *S. aureus* seems to be a frequent cause of clinical mastitis in ewes (Kirk et al., 1996; Mork et al., 2007), this agent can be isolated from ovine subclinical mastitis too (Batavani et al., 2003; Fthenakis, 1994). Therefore, *S. aureus* mastitis in dairy sheep presents a more similar evolution to bovine IMI than mice ones, particularly in natural conditions. In addition, a genotyping comparison study of *S. aureus* strains from ovine and bovine IMI in Norway found scarce difference and limited host specificity among the majority of the isolates (Mork et al., 2005). Nevertheless, other authors found relevant variance among ruminant strains from different countries (France, Belgium, the US and Brazil) and pointed out a possible host specificity (Alves et al., 2009). Despite ovine mastitis may represent a promising *in vivo* model for bovine IMI, important limits to this approach should be considered. Specifically, costs are lower than bovine models but still elevated and groups of sheep genetically homogeneous are difficult to create.

7. Conclusion

This review was focused on describing current knowledge on the most promising vaccine targets among *S. aureus* virulence factors and how they can influence the development of vaccine against *S. aureus* bovine IMI. A better knowledge on the interactions between virulence factors and host, specifically in the area of immune responses, will allow developing new and more efficacious tools to control this disease.

Moreover, the available genetic methods that allow to express different antigens in non-conventional hosts such as plants (Festa

et al., 2013) will allow to increase the availability of these products also in area where conventional vaccination is not easy to perform. This aspect coupled with the increasing concerns on antibiotics usage in food production (Cheng et al., 2014; Trevisi et al., 2014) underscores, furthermore, the interest in developing new vaccines, to control widespread diseases such as *S. aureus* mastitis.

References

Aarestrup, F.M., Larsen, H.D., Eriksen, N.H.R., Elsberg, C.S., Jensen, N.E., 1999. Frequency of alpha- and beta-haemolysin in Staphylococcus aureus of bovine and human origin – a comparison between pheno- and genotype and variation in phenotypic expression. APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica 107, 425–430.

Adhikari, R.P., Karauzum, H., Sarwar, J., Abaandou, L., Mahmoudieh, M., Boroun, A.R., et al., 2012. Novel structurally designed vaccine for S. aureus alpha-hemolysin: protection against bacteremia and pneumonia. PLoS ONE 7, e38567.

Al-Shangiti, A.M., Naylor, C.E., Nair, S.P., Briggs, D.C., Henderson, B., Chain, B.M., 2004. Structural relationships and cellular tropism of staphylococcal superantigen-like proteins. Infection and Immunity 72, 4261–4270.

Alves, P.D.D., McCulloch, J.A., Even, S., Le Marechal, C., Thierry, A., Grosset, N., et al., 2009. Molecular characterisation of Staphylococcus aureus strains isolated from small and large ruminants reveals a host rather than tissue specificity. Veterinary Microbiology 137, 190–195.

Ariznabarreta, A., Gonzalo, C., San Primitivo, F., 2002. Microbiological quality and somatic cell count of ewe milk with special reference to staphylococci. Journal of Dairy Science 85, 1370–1375.

Asao, T., Kumeda, Y., Kawai, T., Shibata, T., Oda, H., Haruki, K., et al., 2003. An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. Epidemiology and Infection 130, 33–40.

Bardoel, B.W., Vos, R., Bouman, T., Aerts, P.C., Bestebroer, J., Huizinga, E.G., et al., 2012. Evasion of Toll-like receptor 2 activation by staphylococcal superantigen-like protein 3. Journal of Molecular Medicine 90, 1109–1120.

Batavani, R.A., Mortaz, E., Falahian, K., Dawoodi, M.A., 2003. Study on frequency, etiology and some enzymatic activities of subclinical ovine mastitis in Urmia, Iran. Small Ruminant Research 50, 45–50.

Bavari, S., Dyas, B., Ulrich, R.G., 1996. Superantigen vaccines: a comparative study of genetically attenuated receptor-binding mutants of staphylococcal enterotoxin A. The Journal of Infectious Diseases 174, 338–345.

Bavari, S., Ulrich, R.G., LeClaire, R.D., 1999. Cross-reactive antibodies prevent the lethal effects of Staphylococcus aureus superantigens. The Journal of Infectious Diseases 180, 1365–1369.

Berube, B.J., Wardenburg, J.B., 2013. Staphylococcus aureus alpha-toxin: nearly a century of intrigue. Toxins 5, 1140–1166.

Bestebroer, J., Poppelier, M.J.J.G., Ulfman, L.H., Lenting, P.J., Denis, C.V., van Kessel, K.P.M., et al., 2007. Staphylococcal superantigen-like 5 binds PSGL-1 and inhibits P-selectin-mediated neutrophil rolling. Blood 109, 2936–2943.

Bestebroer, J., Aerts, P.C., Rooijakkers, S.H.M., Pandey, M.K., Kohl, J., van Strijp, J.A.G., et al., 2010. Functional basis for complement evasion by staphylococcal superantigen-like 7. Cellular Microbiology 12, 1506–1516.

Bharathan, M., Mullarky, I.K., 2011. Targeting mucosal immunity in the battle to develop a mastitis vaccine. Journal of Mammary Gland Biology and Neoplasia 16 (4), 409–419.

Botelho-Nevers, E., Verhoeven, P., Paul, S., Grattard, F., Pozzetto, B., Berthelot, P., et al., 2013. Staphylococcal vaccine development: review of past failures and plea for a future evaluation of vaccine efficacy not only on staphylococcal infections but also on mucosal carriage. Expert Review of Vaccines 12, 1249–1259.

Bownik, A., Siwicki, A.K., 2008. Effects of staphylococcal hemolysins on the immune system of vertebrates. Central European Journal of Immunology 33, 87–90.

Brouillette, E., Malouin, F., 2005. The pathogenesis and control of Staphylococcus aureus-induced mastitis: study models in the mouse. Microbes and Infection 7, 560–568.

Brouillette, E., Lacasse, P., Shkreta, L., Belanger, J., Grondin, G., Diarra, M.S., et al., 2002. DNA immunization against the clumping factor A (ClfA) of Staphylococcus aureus. Vaccine 20, 2348–2357.

Brown, E.L., Dumitrescu, O., Thomas, D., Badiou, C., Koers, E.M., Choudhury, P., et al., 2009. The Panton-Valentine leukocidin vaccine protects mice against lung and skin infections caused by Staphylococcus aureus USA300. Clinical Microbiology and Infection 15, 156–164.

Camussone, C., Rejf, P., Pujato, N., Schwab, A., Marcipar, I., Calvinho, L.F., 2012. Genotypic and phenotypic detection of capsular polysaccharides in Staphylococcus aureus isolated from bovine intramammary infections in Argentina. Brazilian Journal of Microbiology 43, 1010–1014.

Camussone, C.M., Calvinho, L.F., 2013. Virulence factors of Staphylococcus aureus associated with intramammary infections in cows: relevance and role as immunogens. Revista Argentina de Microbiologia 45, 119–130.

Camussone, C.M., Veaute, C.M., Porporatto, C., Morein, B., Marcipar, I.S., Calvinho, L.F., 2013. Immune response of heifers against a Staphylococcus aureus CP5 whole cell vaccine formulated with ISCOMATRIX adjuvant. Journal of Dairy Research 80, 72–80.

Camussone, C.M., Veaute, C.M., Pujato, N., Morein, B., Marcipar, I.S., Calvinho, L.F., 2014. Immune response of heifers against a Staphylococcus aureus CP5 whole cell and lysate vaccine formulated with ISCOM matrix adjuvant. Research in Veterinary Science 96, 86–94.

Carter, E.W., Kerr, D.E., 2003. Optimization of DNA-based vaccination in cows using green fluorescent protein and protein A as a prelude to immunization against staphylococcal mastitis. Journal of Dairy Science 86, 1177–1186.

Castagliuolo, I., Piccinini, R., Beggiao, E., Palu, G., Mengoli, C., Ditadi, F., et al., 2006. Mucosal genetic immunization against four adhesins protects against Staphylococcus aureus-induced mastitis in mice. Vaccine 24, 4393–4402.

Chandler, R.L., 1970. Experimental bacterial mastitis in the mouse. Journal of Medical Microbiology 3, 273–282.

Chang, B.S., Moon, J.S., Kang, H.M., Kim, Y.I., Lee, H.K., Kim, J.D., et al., 2008. Protective effects of recombinant staphylococcal enterotoxin type C mutant vaccine against experimental bovine infection by a strain of Staphylococcus aureus isolated from subclinical mastitis in dairy cattle. Vaccine 26, 2081–2091.

Chavakis, T., Preissner, K.T., Herrmann, M., 2007. The anti-inflammatory activities of Staphylococcus aureus. Trends in Immunology 28, 408–418.

Cheng, G.Y., Hao, H.H., Xie, S.Y., Wang, X., Dai, M.H., Huang, L.L., et al., 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Frontiers in Microbiology 5, 217. doi:10.3389/fmicb.2014.00217.

Chung, M.C., Wines, B.D., Baker, H., Langley, R.J., Baker, E.N., Fraser, J.D., 2007. The crystal structure of staphylococcal superantigen-like protein 11 in complex with sialyl Lewis X reveals the mechanism for cell binding and immune inhibition. Molecular Microbiology 66, 1342–1355.

Cifrian, E., Guidry, A.J., Obrien, C.N., Marquardt, W.W., 1995. Effect of alpha-toxin and capsular exopolysaccharide on the adherence of staphylococcus-aureus to cultured teat, ductal and secretory mammary epithelial-cells. Research in Veterinary Science 58, 20–25.

Cifrian, E., Guidry, A.J., Bramley, A.J., Norcross, N.L., BastidaCorcuera, F.D., Marquardt, W.W., 1996a. Effect of staphylococcal beta toxin on the cytotoxicity, proliferation and adherence of Staphylococcus aureus to bovine mammary epithelial cells. Veterinary Microbiology 48, 187–198.

Cifrian, E., Guidry, A.J., OBrien, C.N., Marquardt, W.W., 1996b. Effect of antibodies to staphylococcal alpha and beta toxins and Staphylococcus aureus on the cytotoxicity for and adherence of the organism to bovine mammary epithelial cells. American Journal of Veterinary Research 57, 1308–1311.

Clarke, S.R., Foster, S.J., 2006. Surface adhesins of Staphylococcus aureus. Advances in Microbial Physiology 51, 187–225.

Coelho, S.M.O., Pereira, I.A., Soares, L.C., Pribul, B.R., Souza, M.M.S., 2011. Short communication: profile of virulence factors of Staphylococcus aureus isolated from subclinical bovine mastitis in the state of Rio de Janeiro, Brazil. Journal of Dairy Science 94, 3305–3310.

Costerton, J.W., Stewart, P.S., Greenberg, E.P., 1999. Bacterial biofilms: a common cause of persistent infections. Science 284, 1318–1322.

Cui, J.C., Hu, D.L., Lin, Y.C., Qian, A.D., Nakane, A., 2005. Immunization with glutathione S-transferase and mutant toxic shock syndrome toxin 1 fusion protein protects against Staphylococcus aureus infection. FEMS Immunology and Medical Microbiology 45, 45–51.

Cui, J.C., Zhang, B.J., Lin, Y.C., Wang, Q.K., Qian, A.D., Nakane, A., et al., 2010. Protective effect of glutathione S-transferase-fused mutant staphylococcal enterotoxin C against Staphylococcus aureus-induced bovine mastitis. Veterinary Immunology and Immunopathology 135, 64–70.

Daum, R.S., Spellberg, B., 2012. Progress toward a staphylococcus aureus vaccine. Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America 54, 560–567.

Deisenhofer, J., 1981. Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from Staphylococcus aureus at 2.9- and 2.8-A resolution. Biochemistry 20, 2361–2370.

Delgado, S., Garcia, P., Fernandez, L., Jimenez, E., Rodriguez-Banos, M., del Campo, R., et al., 2011. Characterization of Staphylococcus aureus strains involved in human and bovine mastitis. FEMS Immunology and Medical Microbiology 62, 225–235.

Dhanawade, N.B., Kalorey, D.R., Srinivasan, R., Barbuddhe, S.B., Kurkure, N.V., 2010. Detection of intercellular adhesion genes and biofilm production in Staphylococcus aureus isolated from bovine subclinical mastitis. Veterinary Research Communications 34, 81–89.

Dinges, M.M., Orwin, P.M., Schlievert, P.M., 2000. Exotoxins of Staphylococcus aureus. Clinical Microbiology Reviews 13, 16–34.

Donlan, R.M., Costerton, J.W., 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. Clinical Microbiology Reviews 15, 167–193.

DuMont, A.L., Torres, V.J., 2014. Cell targeting by the Staphylococcus aureus pore-forming toxins: it's not just about lipids. Trends in Microbiology 22, 21–27.

El-Din, A.N.M.N., Shkreta, L., Talbot, B.G., Diarra, M.S., Lacasse, P., 2006. DNA immunization of dairy cows with the clumping factor A of Staphylococcus aureus. Vaccine 24, 1997–2006.

Espersen, F., Clemmensen, I., 1985. Immunization of mice with the fibronectin-binding protein and clumping factor from staphylococcus-aureus - antibody-response and resistance against intraperitoneal infection. Acta Pathologica, Microbiologica, et Immunologica Scandinavica. Section C, Immunology 93, 53–58.

Falugi, F., Kim, H.K., Missiakas, D.M., Schneewind, O., 2013. Role of protein A in the evasion of host adaptive immune responses by staphylococcus aureus. mBio 4, e00575–13.

Festa, M., Brun, P., Piccinini, R., Castagliuolo, I., Basso, B., Zecconi, A., 2013. Staphylococcus aureus Efb protein expression in Nicotiana tabacum and immune response to oral administration. Research in Veterinary Science 94, 484–489.

Flemming, H.C., Wingender, J., 2010. The biofilm matrix. Nature Reviews. Microbiology 8, 623–633.
Flock, J.I., 1999. Extracellular-matrix-binding proteins as targets for the prevention of Staphylococcus aureus infections. Molecular Medicine Today 5, 532–537.
Foster, T.J., 2005. Immune evasion by staphylococci. Nature Reviews. Microbiology 3, 948–958.
Foster, T.J., Geoghegan, J.A., Ganesh, V.K., Hook, M., 2014. Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus. Nature Reviews. Microbiology 12, 49–62.
Fournier, C., Kuhnert, P., Frey, J., Miserez, R., Kirchhofer, M., Kaufmann, T., et al., 2008. Bovine Staphylococcus aureus: association of virulence genes, genotypes and clinical outcome. Research in Veterinary Science 85, 439–448.
Fowler, V.G., Proctor, R.A., 2014. Where does a Staphylococcus aureus vaccine stand? Clinical Microbiology and Infection 20, 66–75.
Fox, L.K., Gershman, M., Hancock, D.D., Hutton, C.T., 1991. Fomites and reservoirs of staphylococcus-aureus causing intramammary infections as determined by phage typing – the effect of milking time hygiene practices. Cornell Veterinarian 81, 183–193.
Fox, L.K., Zadoks, R.N., Gaskins, C.T., 2005. Biofilm production by Staphylococcus aureus associated with intramammary infection. Veterinary Microbiology 107, 295–299.
Fraser, J.D., Proft, T., 2008. The bacterial superantigen and superantigen-like proteins. Immunological Reviews 225, 226–243.
Fthenakis, G.C., 1994. Prevalence and etiology of subclinical mastitis in ewes of Southern Greece. Small Ruminant Research 13, 293–300.
Gampfer, J., Thon, V., Gulle, H., Wolf, H.M., Eibl, M.M., 2002. Double mutant and formaldehyde inactivated TSST-1 as vaccine candidates for TSST-1-induced toxic shock syndrome. Vaccine 20, 1354–1364.
Ganesh, V.K., Barbu, E.M., Deivanayagam, C.C.S., Le, B., Anderson, A.S., Matsuka, Y.V., et al., 2011. Structural and biochemical characterization of staphylococcus aureus clumping factor B/ligand interactions. The Journal of Biological Chemistry 286, 25963–25972.
Garcia-Alvarez, L., Holden, M.T., Lindsay, H., Webb, C.R., Brown, D.F., Curran, M.D., et al., 2011. Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. The Lancet Infectious Diseases 11, 595–603.
Gaudreau, M.C., Lacasse, P., Talbot, B.G., 2007. Protective immune responses to a multi-gene DNA vaccine against Staphylococcus aureus. Vaccine 25, 814–824.
Giai, C., Gonzalez, C., Ledo, C., Garofalo, A., Di Genaro, M.S., Sordelli, D.O., et al., 2013. Shedding of tumor necrosis factor receptor 1 induced by protein A decreases tumor necrosis factor alpha availability and inflammation during systemic Staphylococcus aureus infection. Infection and Immunity 81, 4200–4207.
Gilbert, F.B., Poutrel, B., Sutra, L., 1994. Immunogenicity in cows of staphylococcus-aureus type 5 capsular polysaccharide-ovalbumin conjugate. Vaccine 12, 369–374.
Gilbert, F.B., Cunha, P., Jensen, K., Glass, E.J., Foucras, G., Robert-Granie, C., et al., 2013. Differential response of bovine mammary epithelial cells to Staphylococcus aureus or Escherichia coli agonists of the innate immune system. Veterinary Research 44, 40.
Gomez, M.I., Lee, A., Reddy, B., Muir, A., Soong, G., Pitt, A., et al., 2004. Staphylococcus aureus protein A induces airway epithelial inflammatory responses by activating TNFR1. Nature Medicine 10, 842–848.
Gong, R., Hu, C.M., Xu, H.Y., Guo, A.Z., Chen, H.C., Zhang, G.Z., et al., 2010. Evaluation of clumping factor a binding region A in a subunit vaccine against staphylococcus aureus-induced mastitis in mice. Clinical and Vaccine Immunology 17, 1746–1752.
Gotz, F., 2002. Staphylococcus and biofilms. Molecular Microbiology 43, 1367–1378.
Guidry, A., Fattom, A., Patel, A., O'Brien, C., 1997. Prevalence of capsular serotypes among Staphylococcus aureus isolates from cows with mastitis in the United States. Veterinary Microbiology 59, 53–58.
Hair, P.S., Ward, M.D., Semmes, O.J., Foster, T.J., Cunnion, K.M., 2008. Staphylococcus aureus clumping factor A binds to complement regulator factor I and increases factor I cleavage of C3b. The Journal of Infectious Diseases 198, 125–133.
Hair, P.S., Echague, C.G., Sholl, A.M., Watkins, J.A., Geoghegan, J.A., Foster, T.J., et al., 2010. Clumping factor A interaction with complement factor I increases C3b cleavage on the bacterial surface of Staphylococcus aureus and decreases complement-mediated phagocytosis. Infection and Immunity 78, 1717–1727.
Hakoda, M., Kamatani, N., Hayashimoto-Kurumada, S., Silverman, G.J., Yamanaka, H., Terai, C., et al., 1996. Differential binding avidities of human IgM for staphylococcal protein A derive from specific germ-line VH3 gene usage. The Journal of Immunology 157, 2976–2981.
Halasa, T., Huijps, K., Osteras, O., Hogeveen, H., 2007. Economic effects of bovine mastitis and mastitis management: a review. The Veterinary Quarterly 29, 18–31.
Hall-Stoodley, L., Costerton, J.W., Stoodley, P., 2004. Bacterial biofilms: from the natural environment to infectious diseases. Nature Reviews. Microbiology 2, 95–108.
Hammer, N.D., Skaar, E.P., 2011. Molecular mechanisms of staphylococcus aureus iron acquisition. Annual Review of Microbiology 65, 129–147.
Han, H.R., Pak, S.I., Guidry, A., 2000. Prevalence of capsular polysaccharide (CP) types of Staphylococcus aureus isolated from bovine mastitic milk and protection of S-aureus infection in mice with CP vaccine. Journal of Veterinary Medical Science 62, 1331–1333.
Hartford, O., Francois, P., Vaudaux, P., Foster, T.J., 1997. The dipeptide repeat region of the fibrinogen-binding protein (clumping factor) is required for functional expression of the fibrinogen-binding domain on the Staphylococcus aureus cell surface. Molecular Microbiology 25, 1065–1076.
Hartleib, J., Kohler, N., Dickinson, R.B., Chhatwal, G.S., Sixma, J.J., Hartford, O.M., et al., 2000. Protein A is the von Willebrand factor binding protein on Staphylococcus aureus. Blood 96, 2149–2156.
Hauck, C.R., Ohlsen, K., 2006. Sticky connections: extracellular matrix protein recognition and integrin-mediated cellular invasion by Staphylococcus aureus. Current Opinion in Microbiology 9, 5–11.
Haveri, M., Hovinen, M., Roslof, A., Pyorala, S., 2008. Molecular types and genetic profiles of staphylococcus aureus strains isolated from bovine intramammary infections and extramammary sites. Journal of Clinical Microbiology 46, 3728–3735.
Hensen, S.M., Pavicic, M., Lohuis, J., de Hoog, J.A.M., Poutrel, B., 2000. Location of Staphylococcus aureus within the experimentally infected bovine udder and the expression of capsular polysaccharide type 5 in situ. Journal of Dairy Science 83 (9), 1966–1975.
Herbelin, C., Poutrel, B., Gilbert, F.B., Rainard, P., 1997. Immune recruitment and bactericidal activity of neutrophils in milk of cows vaccinated with staphylococcal alpha-toxin. Journal of Dairy Science 80, 2025–2034.
Hermans, S.J., Baker, H.M., Sequeira, R.P., Langley, R.J., Baker, E.N., Fraser, J.D., 2012. Structural and functional properties of Staphylococcal Superantigen-Like Protein 4. Infection and Immunity 80, 4004–4013.
Hjelm, H., Sjodahl, J., Sjoquist, J., 1975. Immunologically active and structurally similar fragments of protein A from Staphylococcus aureus. European Journal of Biochemistry 57, 395–403.
Holmes, M.A., Zadoks, R.N., 2011. Methicillin resistant S. aureus in human and bovine mastitis. Journal of Mammary Gland Biology and Neoplasia 16, 373–382.
Hu, C.M., Gong, R., Guo, A.Z., Chen, H.C., 2010. Protective effect of ligand-binding domain of fibronectin-binding protein on mastitis induced by Staphylococcus aureus in mice. Vaccine 28, 4038–4044.
Hu, D.L., Omoe, K., Sasaki, S., Sashinami, H., Sakuraba, H., Yokomizo, Y., et al., 2003. Vaccination with nontoxic mutant toxic shock syndrome toxin 1 protects against Staphylococcus aureus infection. The Journal of Infectious Diseases 188, 743–752.
Hu, D.L., Cui, J.C., Omoe, K., Sashinami, H., Yokomizo, Y., Shinagawa, K., et al., 2005. A mutant of staphylococcal enterotoxin C devoid of bacterial superantigenic activity elicits a Th2 immune response for protection against Staphylococcus aureus infection. Infection and Immunity 73, 174–180.
Hume, E.B.H., Dajcs, J.J., Moreau, J.M., O'Callaghan, R.J., 2000. Immunization with alpha-toxin toxoid protects the cornea against tissue damage during experimental Staphylococcus aureus keratitis. Infection and Immunity 68, 6052–6055.
Huseby, M., Shi, K., Brown, C.K., Digre, J., Mengistu, F., Seo, K.S., et al., 2007. Structure and biological activities of beta toxin from Staphylococcus aureus. Journal of Bacteriology 189, 8719–8726.
Hwang, C.Y., Pak, S.I., Han, H.R., 2000. Effects of autogenous toxoid-bacterin in lactating cows with Staphylococcus aureus subclinical mastitis. Journal of Veterinary Medical Science 62, 875–880.
Ikawaty, R., Brouwer, E.C., Duijkeren, E.V., Mevius, D., Verhoef, J., Fluit, A.C., 2010. Virulence factors of genotyped bovine mastitis Staphylococcus aureus isolates in The Netherlands. International Journal of Dairy Science 5, 60–70.
Itoh, S., Hamada, E., Kamoshida, G., Yokoyama, R., Takii, T., Onozaki, K., et al., 2010. Staphylococcal superantigen-like protein 10 (SSL10) binds to human immunoglobulin G (IgG) and inhibits complement activation via the classical pathway. Molecular Immunology 47, 932–938.
Itoh, S., Yokoyama, R., Murase, C., Takii, T., Tsuji, T., Onozaki, K., 2012. Staphylococcal superantigen-like protein 10 binds to phosphatidylserine and apoptotic cells. Microbiology and Immunology 56, 363–371.
Itoh, S., Yamaoka, N., Kamoshida, G., Takii, T., Tsuji, T., Hayashi, H., et al., 2013a. Staphylococcal superantigen-like protein 8 (SSL8) binds to tenascin C and inhibits tenascin C-fibronectin interaction and cell motility of keratinocytes. Biochemical and Biophysical Research Communications 433, 127–132.
Itoh, S., Yokoyama, R., Kamoshida, G., Fujiwara, T., Okada, H., Takii, T., et al., 2013b. Staphylococcal superantigen-like protein 10 (SSL10) inhibits blood coagulation by binding to prothrombin and factor Xa via their gamma-carboxyglutamic acid (Gla) domain. The Journal of Biological Chemistry 288, 21569–21580.
Josefsson, E., Hartford, O., O'Brien, L., Patti, J.M., Foster, T., 2001. Protection against experimental Staphylococcus aureus arthritis by vaccination with clumping factor A, a novel virulence determinant. The Journal of Infectious Diseases 184, 1572–1580.
Joshi, A., Pancari, G., Cope, L., Bowman, E.P., Cua, D., Proctor, R.A., et al., 2012. Immunization with Staphylococcus aureus iron regulated surface determinant B (IsdB) confers protection via Th17/IL17 pathway in a murine sepsis model. Human Vaccines & Immunotherapeutics 8.
Kampen, A.H., Tollersrud, T., Lund, A., 2005. Staphylococcus aureus capsular polysaccharide types 5 and 8 reduce killing by bovine neutrophils in vitro. Infection and Immunity 73, 1578–1583.
Kanangat, S., Postlethwaite, A., Cholera, S., Williams, L., Schaberg, D., 2007. Modulation of virulence gene expression in Staphylococcus aureus by interleukin-1beta: novel implications in bacterial pathogenesis. Microbes and Infection 9, 408–415.
Kaneko, J., Kamio, Y., 2004. Bacterial two-component and hetero-heptameric pore-forming cytolytic toxins: structures, pore-forming mechanism, and organization of the genes. Bioscience Biotechnology and Biochemistry 68, 981–1003.
Karauzum, H., Adhikari, R.P., Sarwar, J., Devi, V.S., Abaandou, L., Haudenschild, C., et al., 2013. Structurally designed attenuated subunit vaccines for S. aureus LukS-PV and LukF-PV confer protection in a mouse bacteremia model. PLoS ONE 8.
Kennedy, A.D., Wardenburg, J.B., Gardner, D.J., Long, D., Whitney, A.R., Braughton, K.R., et al., 2010. Targeting of alpha-hemolysin by active or passive immunization decreases severity of USA300 skin infection in a mouse model. The Journal of Infectious Diseases 202, 1050–1058.

Kerro Dego, O., van Dijk, J.E., Nederbragt, H., 2002. Factors involved in the early pathogenesis of bovine Staphylococcus aureus mastitis with emphasis on bacterial adhesion and invasion. A review. The Veterinary Quarterly 24 (4), 181–198.
Kim, H.K., Cheng, A.G., Kim, H.Y., Missiakas, D.M., Schneewind, O., 2010a. Nontoxigenic protein A vaccine for methicillin-resistant Staphylococcus aureus infections in mice. Journal of Experimental Medicine 207, 1863–1870.
Kim, H.K., DeDent, A., Cheng, A.G., McAdow, M., Bagnoli, F., Missiakas, D.M., et al., 2010b. IsdA and IsdB antibodies protect mice against Staphylococcus aureus abscess formation and lethal challenge. Vaccine 28, 6382–6392.
Kim, H.K., Emolo, C., DeDent, A.C., Falugi, F., Missiakas, D.M., Schneewind, O., 2012a. Protein A-specific monoclonal antibodies and prevention of staphylococcus aureus disease in mice. Infection and Immunity 80, 3460–3470.
Kim, H.K., Thammavongsa, V., Schneewind, O., Missiakas, D., 2012b. Recurrent infections and immune evasion strategies of Staphylococcus aureus. Current Opinion in Microbiology 15, 92–99.
Kirk, J.H., Glenn, J.S., Maas, J.P., 1996. Mastitis in a flock of milking sheep. Small Ruminant Research 22, 187–191.
Klein, R.C., Fabres-Klein, M.H., Brito, M., Fietto, L.G., Ribon, A.D.B., 2012. Staphylococcus aureus of bovine origin: genetic diversity, prevalence and the expression of adhesin-encoding genes. Veterinary Microbiology 160 (1–2), 183–188.
Kuklin, N.A., Clark, D.J., Secore, S., Cook, J., Cope, L.D., McNeely, T., et al., 2006. A novel Staphylococcus aureus vaccine: iron surface determinant B induces rapid antibody responses in rhesus macaques and specific increased survival in a murine S-aureus sepsis model. Infection and Immunity 74, 2215–2223.
Lammers, A., Nuijten, P.J.M., Smith, H.E., 1999. The fibronectin binding proteins of Staphylococcus aureus are required for adhesion to and invasion of bovine mammary gland cells. FEMS Microbiology Letters 180, 103–109.
Larkin, E.A., Carman, R.J., Krakauer, T., Stiles, B.G., 2009. Staphylococcus aureus: the toxic presence of a pathogen extraordinaire. Current Medicinal Chemistry 16, 4003–4019.
Le Loir, Y., Baron, F., Gautier, M., 2003. Staphylococcus aureus and food poisoning. Genetics and Molecular Research 2, 63–76.
Lee, J.W., O'Brien, C.N., Guidry, A.J., Paape, M.J., Shafer-Weaver, K.A., Zhao, X., 2005. Effect of a trivalent vaccine against Staphylococcus aureus mastitis lymphocyte subpopulations, antibody production, and neutrophil phagocytosis. Canadian Journal of Veterinary Research 69, 11–18.
Leitner, G., Krifucks, O., Glickman, A., Younis, A., Saran, A., 2003. Staphylococcus aureus strains isolated from bovine mastitis: virulence, antibody production and protection from challenge in a mouse model. FEMS Immunology and Medical Microbiology 35, 99–106.
Loeffler, D.A., Norcross, N.L., Opdebeeck, J.P., 1988. Determination by enzyme-linked immunosorbent assay of the optimal dose of staphylococcal leukocydin for systemic immunization of dairy cows. American Journal of Veterinary Research 49 (9), 1452–1455.
Lorenz, N., Clow, F., Radcliff, F.J., Fraser, J.D., 2013. Full functional activity of SSL7 requires binding of both complement C5 and IgA. Immunology and Cell Biology 91, 469–476.
Loughman, A., Sweeney, T., Keane, F.M., Pietrocola, G., Speziale, P., Foster, T.J., 2008. Sequence diversity in the A domain of Staphylococcus aureus fibronectin-binding protein A. BMC Microbiology 8.
Maira-Litran, T., Bentancor, L.V., Bozkurt-Guzel, C., O'Malley, J.M., Cywes-Bentley, C., Pier, G.B., 2012. Synthesis and evaluation of a conjugate vaccine composed of Staphylococcus aureus poly-N-acetyl-glucosamine and clumping factor A. PLoS ONE 7, e43813.
Mamo, W., Jonsson, P., Flock, J.I., Lindberg, M., Muller, H.P., Wadstrom, T., et al., 1994. Vaccination against Staphylococcus aureus mastitis: immunological response of mice vaccinated with fibronectin-binding protein (FnBP-A) to challenge with S. aureus. Vaccine 12, 988–992.
Mamo, W., Jonsson, P., Muller, H.P., 1995. Opsonization of Staphylococcus aureus with a fibronectin-binding protein antiserum induces protection in mice. Microbial Pathogenesis 19, 49–55.
Martinez-Pulgarin, S., Dominguez-Bernal, G., Orden, J.A., de la Fuente, R., 2009. Simultaneous lack of catalase and beta-toxin in Staphylococcus aureus leads to increased intracellular survival in macrophages and epithelial cells and to attenuated virulence in murine and ovine models. Microbiology-Sgm 155, 1505–1515.
Massey, R.C., Kantzanou, M.N., Fowler, T., Day, N.P.J., Schofield, K., Wann, E.R., et al., 2001. Fibronectin-binding protein A of Staphylococcus aureus has multiple, substituting, binding regions that mediate adherence to fibronectin and invasion of endothelial cells. Cellular Microbiology 3, 839–851.
Mazzilli, M., Zecconi, A., 2010. Assessment of epithelial cells' immune and inflammatory response to Staphylococcus aureus when exposed to a macrolide. Journal of Dairy Research 77, 404–410.
McAleese, F.M., Walsh, E.J., Sieprawska, M., Potempa, J., Foster, T.J., 2001. Loss of clumping factor B fibrinogen binding activity by Staphylococcus aureus involves cessation of transcription, shedding and cleavage by metalloprotease. The Journal of Biological Chemistry 276, 29969–29978.
McDevitt, D., Nanavaty, T., HousePompeo, K., Bell, E., Turner, N., McIntire, L., et al., 1997. Characterization of the interaction between the Staphylococcus aureus clumping factor (ClfA) and fibrinogen. European Journal of Biochemistry 247, 416–424.
Middleton, J.R., Ma, J., Rinehart, C.L., Taylor, V.N., Luby, C.D., Steevens, B.J., 2006. Efficacy of different Lysigin formulations in the prevention of Staphylococcus aureus intramammary infection in dairy heifers. Journal of Dairy Research 73, 10–19.
Middleton, J.R., Luby, C.D., Adams, D.S., 2009. Efficacy of vaccination against staphylococcal mastitis: a review and new data. Veterinary Microbiology 134, 192–198.
Mork, T., Tollersrud, T., Kvitle, B., Jorgensen, H.J., Waage, S., 2005. Comparison of Staphylococcus aureus genotypes recovered from cases of bovine, ovine, and caprine mastitis. Journal of Clinical Microbiology 43, 3979–3984.
Mork, T., Waage, S., Tollersrud, T., Kvitle, B., Sviland, S., 2007. Clinical mastitis in ewes; bacteriology, epidemiology and clinical features. Acta Veterinaria Scandinavica 49.
Mulcahy, M.E., Geoghegan, J.A., Monk, I.R., O'Keeffe, K.M., Walsh, E.J., Foster, T.J., et al., 2012. Nasal colonisation by staphylococcus aureus depends upon clumping factor B binding to the squamous epithelial cell envelope protein loricrin. PLoS Pathogens 8, e1003092.
Nanra, J.S., Timofeyeva, Y., Buitrago, S.M., Sellman, B.R., Dilts, D.A., Fink, P., et al., 2009. Heterogeneous in vivo expression of clumping factor A and capsular polysaccharide by Staphylococcus aureus: implications for vaccine design. Vaccine 27, 3276–3280.
Nelson, L., Flock, J.I., Hook, M., Lindberg, M., Muller, H.P., Wadstrom, T., 1991. Adhesins in Staphylococcal Mastitis as Vaccine Components. Rijksuniversiteit Gent, Ghent, pp. 111–125.
Ni Eidhin, D., Perkins, S., Francois, P., Vaudaux, P., Hook, M., Foster, T.J., 1998. Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of Staphylococcus aureus. Molecular Microbiology 30, 245–257.
Nickerson, S.C., Owens, W.E., Boddie, R.L., 1993. Effect of a Staphylococcus aureus bacterin on serum antibody, new infection, and mammary histology in nonlactating dairy cows. Journal of Dairy Science 76, 1290–1297.
Niemann, S., Spehr, N., Van Aken, H., Morgenstern, E., Peters, G., Herrmann, M., et al., 2004. Soluble fibrin is the main mediator of Staphylococcus aureus adhesion to platelets. Circulation 110, 193–200.
Nilsson, I.M., Verdrengh, M., Ulrich, R.G., Bavari, S., Tarkowski, A., 1999. Protection against Staphylococcus aureus sepsis by vaccination with recombinant staphylococcal enterotoxin A devoid of superantigenicity. The Journal of Infectious Diseases 180, 1370–1373.
Nordhaug, M.L., Nesse, L.L., Norcross, N.L., Gudding, R., 1994a. A field trial with an experimental vaccine against staphylococcus-aureus mastitis in cattle .1. clinical-parameters. Journal of Dairy Science 77, 1267–1275.
Nordhaug, M.L., Nesse, L.L., Norcross, N.L., Gudding, R., 1994b. A field trial with an experimental vaccine against staphylococcus-aureus mastitis in cattle .2. antibody-response. Journal of Dairy Science 77, 1276–1284.
Notebaert, S., Meyer, E., 2006. Mouse models to study the pathogenesis and control of bovine mastitis. A review. The Veterinary Quarterly 28, 2–13.
O'Brien, C.N., Guidry, A.J., Fattom, A., Shepherd, S., Douglass, L.W., Westhoff, D.C., 2000. Production of antibodies to Staphylococcus aureus serotypes 5, 8, and 336 using poly(DL-lactide-co-glycolide) microspheres. Journal of Dairy Science 83, 1758–1766.
O'Gara, J.P., 2007. ica and beyond: biofilm mechanisms and regulation in Staphylococcus epidermidis and Staphylococcus aureus. FEMS Microbiology Letters 270, 179–188.
Oliveira, M., Nunes, S.F., Carneiro, C., Bexiga, R., Bernardo, F., Vilela, C.L., 2007. Time course of biofilm formation by Staphylococcus aureus and Staphylococcus epidermidis mastitis isolates. Veterinary Microbiology 124, 187–191.
Opdebeeck, J.P., Norcross, N.L., 1982. Antibody response in lacteal secretions of cows after immunization with various concentrations of staphylococcal and streptococcal antigens. American Journal of Veterinary Research 43, 1770–1775.
O'Riordan, K., Lee, J.C., 2004. Staphylococcus aureus capsular polysaccharides. Clinical Microbiology Reviews 17, 218–234.
O'Seaghdha, M., van Schooten, C.J., Kerrigan, S.W., Emsley, J., Silverman, G.J., Cox, D., et al., 2006. Staphylococcus aureus protein A binding to von Willebrand factor A1 domain is mediated by conserved IgG binding regions. FEBS Journal 273, 4831–4841.
Otto, A., van Dijl, J.M., Hecker, M., Becher, D., 2014. The Staphylococcus aureus proteome. International Journal of Medical Microbiology 304, 110–120.
Otto, M., 2008. Staphylococcal biofilms. Current Topics in Microbiology and Immunology 322, 207–228.
Otto, M., 2013. Staphylococcal infections: mechanisms of biofilm maturation and detachment as critical determinants of pathogenicity. Annual Review of Medicine 64, 175–188.
Otto, M., 2014. Staphylococcus aureus toxins. Current Opinion in Microbiology 17, 32–37.
Paape, M.J., Wiggans, G.R., Bannerman, D.D., Thomas, D.L., Sanders, A.H., Contreras, A., et al., 2007. Monitoring goat and sheep milk somatic cell counts. Small Ruminant Research 68, 114–125.
Pankey, J.W., Boddie, N.T., Watts, J.L., Nickerson, S.C., 1985. Evaluation of protein-a and a commercial bacterin as vaccines against staphylococcus-aureus mastitis by experimental challenge. Journal of Dairy Science 68, 726–731.
Pantosti, A., Sanchini, A., Monaco, M., 2007. Mechanisms of antibiotic resistance in Staphylococcus aureus. Future Microbiology 2, 323–334.
Parker, D., Prince, A., 2012. Immunopathogenesis of Staphylococcus aureus pulmonary infection. Seminars in Immunopathology 34, 281–297.
Patel, D., Wines, B.D., Langley, R.J., Fraser, J.D., 2010. Specificity of staphylococcal superantigen-like protein 10 toward the human IgG1 Fc domain. Journal of Immunology 184, 6283–6292.
Pereira, U.P., Oliveira, D.G.S., Mesquita, L.R., Costa, G.M., Pereira, U., 2011. Efficacy of Staphylococcus aureus vaccines for bovine mastitis: a systematic review. Veterinary Microbiology 148, 117–124.

Perez, M.M., Prenafeta, A., Valle, J., Penades, J., Rota, C., Solano, C., et al., 2009. Protection from Staphylococcus aureus mastitis associated with poly-N-acetyl beta-1,6 glucosamine specific antibody production using biofilm-embedded bacteria. Vaccine 27, 2379–2386.
Piccinini, R., Binda, E., Belotti, M., Casirani, G., Zecconi, A., 2005. Comparison of blood and milk non-specific immune parameters in heifers after calving in relation to udder health. Veterinary Research 36, 747–757.
Piccinini, R., Borromeo, V., Zecconi, A., 2010. Relationship between S. aureus gene pattern and dairy herd mastitis prevalence. Veterinary Microbiology 145, 100–105.
Pier, G.B., 2013. Will there ever be a universal Staphylococcus aureus vaccine? Human Vaccines & Immunotherapeutics 9, 1865–1876.
Prenafeta, A., March, R., Foix, A., Casals, I., Costa, L., 2010. Study of the humoral immunological response after vaccination with a Staphylococcus aureus biofilm-embedded bacterin in dairy cows: possible role of the exopolysaccharide specific antibody production in the protection from Staphylococcus aureus induced mastitis. Veterinary Immunology and Immunopathology 134, 208–217.
Proctor, R.A., 2012. Challenges for a universal staphylococcus aureus vaccine. Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America 54, 1179–1186.
Ragle, B.E., Bubeck Wardenburg, J., 2009. Anti-alpha-hemolysin monoclonal antibodies mediate protection against Staphylococcus aureus pneumonia. Infection and Immunity 77, 2712–2718.
Reinoso, E.B., El-Sayed, A., Laemmler, C., Bogni, C., Zschoeck, M., 2008. Genotyping of Staphylococcus aureus isolated from humans, bovine subclinical mastitis and food samples in Argentina. Microbiological Research 163, 314–322.
Renna, M.S., Pereyra, E.A.L., Baravalle, C., Camussone, C.M., Dallard, B.E., Marcipar, I.S., et al., 2014. Functional role of antibodies generated in heifers through immunization with Staphylococcus aureus vaccines in invasion and phagocytosis assays. FEMS Microbiology Letters 360 (1), 62–69.
Riollet, C., Rainard, P., Poutrel, B., 2000. Kinetics of cells and cytokines during immune-mediated inflammation in the mammary gland of cows systemically immunized with Staphylococcus aureus alpha-toxin. Inflammation Research 49, 486–496.
Risley, A.L., Loughman, A., Cywes-Bentley, C., Foster, T.J., Lee, J.C., 2007. Capsular polysaccharide masks clumping factor A-mediated adherence of Staphylococcus aureus to fibrinogen and platelets. The Journal of Infectious Diseases 196, 919–927.
Roben, P.W., Salem, A.N., Silverman, G.J., 1995. VH3 family antibodies bind domain D of staphylococcal protein A. The Journal of Immunology 154, 6437–6445.
Roberson, J.R., Fox, L.K., Hancock, D.D., Gay, J.M., Besser, T.E., 1998. Sources of intramammary infections from Staphylococcus aureus in dairy heifers at first parturition. Journal of Dairy Science 81, 687–693.
Roche, F.M., Downer, R., Keane, F., Speziale, P., Park, P.W., Foster, T.J., 2004. The N-terminal A domain of fibronectin-binding proteins A and B promotes adhesion of Staphylococcus aureus to elastin. The Journal of Biological Chemistry 279, 38433–38440.
Rooijakkers, S.H.M., van Kessel, K.P.M., van Strijp, J.A.G., 2005. Staphylococcal innate immune evasion. Trends in Microbiology 13, 596–601.
Rozalska, B., Wadstrom, T., 1993. Protective opsonic activity of antibodies against fibronectin-binding proteins (Fnbps) of staphylococcus-aureus. Scandinavian Journal of Immunology 37, 575–580.
Scarpa, M., Piccinini, R., Brun, P., Grillo, A., Palu, G., Mengoli, C., et al., 2010. Relationship between virulence factor genes in bovine Staphylococcus aureus subclinical mastitis isolates and binding to anti-adhesin antibodies. Journal of Dairy Research 77, 159–167.
Schaffer, A.C., Solinga, R.M., Cocchiaro, J., Portoles, M., Kiser, K.B., Risley, A., et al., 2006. Immunization with Staphylococcus aureus clumping factor B, a major determinant in nasal carriage, reduces nasal colonization in a murine model. Infection and Immunity 74, 2145–2153.
Schennings, T., Heimdahl, A., Coster, K., Flock, J.I., 1993. Immunization with fibronectin-binding protein from staphylococcus-aureus protects against experimental endocarditis in rats. Microbial Pathogenesis 15, 227–236.
Schukken, Y.H., Bronzo, V., Locatelli, C., Pollera, C., Rota, N., Casula, A., et al., 2014. Efficacy of vaccination on Staphylococcus aureus and coagulase-negative staphylococci intramammary infection dynamics in 2 dairy herds. Journal of Dairy Science 97 (8), 5250–5264.
Seol, J.W., Kang, S.J., Park, S.Y., 2010. Silver ion treatment of primary cultured bovine mammary gland epithelial cell (BMEC) damage from Staphylococcus aureus-derived alpha-toxin. Veterinary Research Communications 34, 33–42.
Seyffert, N., Le Marechal, C., Jardin, J., McCulloch, J.A., Rosado, F.R., Miyoshi, A., et al., 2012. Staphylococcus aureus proteins differentially recognized by the ovine immune response in mastitis or nasal carriage. Veterinary Microbiology 157, 439–447.
Shkreta, L., Talbot, B.G., Diarra, M.S., Lacasse, P., 2004. Immune responses to a DNA/protein vaccination strategy against Staphylococcus aureus induced mastitis in dairy cows. Vaccine 23, 114–126.
Skaar, E.P., Schneewind, O., 2004. Iron-regulated surface determinants (Isd) of Staphylococcus aureus: stealing iron from heme. Microbes and Infection 6, 390–397.
Skurnik, D., Merighi, M., Grout, M., Gadjeva, M., Maira-Litran, T., Ericsson, M., et al., 2010. Animal and human antibodies to distinct Staphylococcus aureus antigens mutually neutralize opsonic killing and protection in mice. The Journal of Clinical Investigation 120, 3220–3233.
Sordelli, D.O., Buzzola, F.R., Gomez, M.I., Steele-Moore, L., Berg, D., Gentilini, E., et al., 2000. Capsule expression by bovine isolates of Staphylococcus aureus from Argentina: genetic and epidemiologic analyses. Journal of Clinical Microbiology 38, 846–850.
Souza, F.N., Blagitz, M.G., Penna, C.F.A.M., Della Libera, A.M.M.P., Heinemann, M.B., Cerqueira, M.M.O.P., 2012. Somatic cell count in small ruminants: friend or foe? Small Ruminant Research 107, 65–75.
Speziale, P., Pietrocola, G., Rindi, S., Provenzano, M., Provenza, G., Di Poto, A., et al., 2009. Structural and functional role of Staphylococcus aureus surface components recognizing adhesive matrix molecules of the host. Future Microbiology 4, 1337–1352.
Ster, C., Beaudoin, F., Diarra, M.S., Jacques, M., Malouin, F., Lacasse, P., 2010. Evaluation of some Staphylococcus aureus iron-regulated proteins as vaccine targets. Veterinary Immunology and Immunopathology 136, 311–318.
Stewart, P.S., Costerton, J.W., 2001. Antibiotic resistance of bacteria in biofilms. Lancet 358, 135–138.
Stiles, B.G., Garza, A.R., Ulrich, R.G., Boles, J.W., 2001. Mucosal vaccination with recombinantly attenuated staphylococcal enterotoxin B and protection in a murine model. Infection and Immunity 69, 2031–2036.
Stranger-Jones, Y.K., Bae, T., Schneewind, O., 2006. Vaccine assembly from surface proteins of Staphylococcus aureus. Proceedings of the National Academy of Sciences of the United States of America 103, 16942–16947.
Sutra, L., Rainard, P., Poutrel, B., 1990. Phagocytosis of mastitis isolates of staphylococcus-aureus and expression of type-5 capsular polysaccharide are influenced by growth in the presence of milk. Journal of Clinical Microbiology 28, 2253–2258.
Taverna, F., Negri, A., Piccinini, R., Zecconi, A., Nonnis, S., Ronchi, S., et al., 2007. Characterization of cell wall associated proteins of a Staphylococcus aureus isolated from bovine mastitis case by a proteomic approach. Veterinary Microbiology 119, 240–247.
Tedeschi, G., Taverna, F., Negri, A., Piccinini, R., Nonnis, S., Ronchi, S., et al., 2009. Serological proteome analysis of Staphylococcus aureus isolated from sub-clinical mastitis. Veterinary Microbiology 134, 388–391.
Tiwari, J.G., Babra, C., Tiwari, H.K., Williams, V., Wet, S.D., Gibson, J., et al., 2013. Trends in therapeutic and prevention strategies for management of bovine mastitis: an overview. Journal of Vaccines and Vaccination 4, 176. doi:10.4172/2157-7560.1000176.
Tkaczyk, C., Hua, L., Varkey, R., Shi, Y., Dettinger, L., Woods, R., et al., 2012. Identification of anti-alpha toxin monoclonal antibodies that reduce the severity of staphylococcus aureus dermonecrosis and exhibit a correlation between affinity and potency. Clinical and Vaccine Immunology 19, 377–385.
Tollersrud, T., Kenny, K., Reitz, A.J., Lee, J.C., 2000. Genetic and serologic evaluation of capsule production by bovine mammary isolates of Staphylococcus aureus and other Staphylococcus spp. from Europe and the United States. Journal of Clinical Microbiology 38, 2998–3003.
Tollersrud, T., Zernichow, L., Andersen, S.R., Kenny, K., Lund, A., 2001. Staphylococcus aureus capsular polysaccharide type 5 conjugate and whole cell vaccines stimulate antibody responses in cattle. Vaccine 19, 3896–3903.
Tollersrud, T., Norstebo, P.E., Engvik, J.P., Andersen, S.R., Reitan, L.J., Lund, A., 2002. Antibody responses in sheep vaccinated against Staphylococcus aureus mastitis: a comparison of two experimental vaccines containing different adjuvants. Veterinary Research Communications 26, 587–600.
Trevisi, E., Zecconi, A., Cogrossi, S., Razzuoli, E., Grossi, P., Amadori, M., 2014. Strategies for reduced antibiotic usage in dairy cattle farms. Research in Veterinary Science 96, 229–233.
Tuchscherr, L.P.N., Buzzola, F.R., Alvarez, L.P., Lee, J.C., Sordelli, D.O., 2008. Antibodies to capsular polysaccharide and clumping factor a prevent mastitis and the emergence of unencapsulated and small-colony variants of staphylococcus aureus in mice. Infection and Immunity 76 (12), 5738–5744.
Uhl, M.V.D., Bottecchia, R.J., Azevedo-Silva, J., Antonio, D.L., Vieira-Da-Motta, O., Mittmann, J., et al., 2004. Suitability of a recombinant Staphylococcus aureus enterotoxin C bovine variant for immunodiagnostics and therapeutic vaccine development. Vaccine 22, 4191–4202.
van Diemen, P.M., Yamaguchi, Y., Paterson, G.K., Rollier, C.S., Hill, A.V., Wyllie, D.H., 2013. Irradiated wild-type and Spa mutant Staphylococcus aureus induce anti-S. aureus immune responses in mice which do not protect against subsequent intravenous challenge. Pathogens and Disease 68, 20–26.
Vanderhaeghen, W., Hermans, K., Haesebrouck, F., Butaye, P., 2010. Methicillin-resistant Staphylococcus aureus (MRSA) in food production animals. Epidemiology and Infection 138, 606–625.
Varshney, A.K., Wang, X.B., Scharff, M.D., Maclntyre, J., Zollner, R.S., Kovalenko, O.V., et al., 2013. Staphylococcal enterotoxin B-specific monoclonal antibody 20b1 successfully treats diverse staphylococcus aureus infections. The Journal of Infectious Diseases 208, 2058–2066.
Vasudevan, P., Nair, M.K.M., Annamalai, T., Venkitanarayanan, K.S., 2003. Phenotypic and genotypic characterization of bovine mastitis isolates of Staphylococcus aureus for biofilm formation. Veterinary Microbiology 92, 179–185.
Walsh, E.J., O'Brien, L.M., Liang, X.W., Hook, M., Foster, T.J., 2004. Clumping factor B, a fibrinogen-binding MSCRAMM (microbial surface components recognizing adhesive matrix molecules) adhesin of Staphylococcus aureus, also binds to the tail region of type I cytokeratin 10. The Journal of Biological Chemistry 279, 50691–50699.
Wann, E.R., Gurusiddappa, S., Hook, M., 2000. The fibronectin-binding MSCRAMM FnbpA of Staphylococcus aureus is a bifunctional protein that also binds to fibrinogen. The Journal of Biological Chemistry 275, 13863–13871.
Wardenburg, J.B., Schneewind, O., 2008. Vaccine protection against Staphylococcus aureus pneumonia. Journal of Experimental Medicine 205, 287–294.
Watson, D.L., Lee, C.G., 1978. Immunity to experimental staphylococcal mastitis–comparison of live and killed vaccines. Australian Veterinary Journal 54, 374–378.

Williams, R.J., Ward, J.M., Henderson, B., Poole, S., O'Hara, B.P., Wilson, M., et al., 2000. Identification of a novel gene cluster encoding staphylococcal exotoxin-like proteins: characterization of the prototypic gene and its protein product, SET1. Infection and Immunity 68, 4407–4415.

Woody, M.A., Krakauer, T., Stiles, B.G., 1997. Staphylococcal enterotoxin B mutants (N23K and F44S): biological effects and vaccine potential in a mouse model. Vaccine 15, 133–139.

Yancey, R.J., 1999. Vaccines and diagnostic methods for bovine mastitis: fact and fiction. Advances in Veterinary Medicine 41, 257–273.

Yokoyama, R., Itoh, S., Kamoshida, G., Takii, T., Fujii, S., Tsuji, T., et al., 2012. Staphylococcal superantigen-like protein 3 binds to the toll-like receptor 2 extracellular domain and inhibits cytokine production induced by staphylococcus aureus, cell wall component, or lipopeptides in murine macrophages. Infection and Immunity 80, 2816–2825.

Yoshida, K., Ichiman, Y., Narikawa, S., Evans, W.B., 1984. Staphylococcal capsular vaccine for preventing mastitis in two herds in Georgia. Journal of Dairy Science 67, 620–627.

Zecconi, A., 2010. Staphylococcus aureus mastitis: what we need to know to control them. Israel Journal of Veterinary Medicine 65, 93–99.

Zecconi, A., Scali, F., 2013. Staphylococcus aureus virulence factors in evasion from innate immune defenses in human and animal diseases. Immunology Letters 150, 12–22.

Zecconi, A., Piccinini, R., Fox, L.K., 2003. Epidemiologic study of intramammary infections with Staphylococcus aureus during a control program in nine commercial dairy herds. Journal of the American Veterinary Medical Association 223, 684–688.

Zecconi, A., Piccinini, R., Fox, L.K., 2004. Epidemiological study of non-contagious intramammary infections in nine commercial dairy herds following a Staphylococcus aureus control programme. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 51, 333–336.

Zecconi, A., Binda, E., Borromeo, V., Piccinini, R., 2005. Relationship between some Staphylococcus aureus pathogenic factors and growth rates or somatic cell counts. The Journal of Dairy Research 72, 203–208.

Zecconi, A., Cesaris, L., Liandris, E., Daprà, V., Piccinini, R., 2006. Role of several Staphylococcus aureus virulence factors on the inflammatory response in bovine mammary gland. Microbial Pathogenesis 40, 177–183.

Zhao, X., Lacasse, P., 2008. Mammary tissue damage during bovine mastitis: causes and control. Journal of Animal Science 86, 57–65.

Zuo, Q.F., Yang, L.Y., Feng, Q., Lu, D.S., Dong, Y.D., Cai, C.Z., et al., 2013. Evaluation of the protective immunity of a novel subunit fusion vaccine in a murine model of systemic MRSA infection. PLoS ONE 8, e81212.