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Literature Reviews

Staphylococcus aureus Vaccine Candidate from MRSA Isolates: The Prospect of a Multivalent Vaccine

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Abstract: *Staphylococcus aureus* (*S. aureus*) is a major cause various infections in humans and animals throughout the world. The increasing incidence of *S. aureus* infection, particularly methicillin-resistant *Staphylococcus aureus* (MRSA) is complicated by the antibiotic-resistance phenomenon. Thus, it requires new strategies to prevent wider scale of its incidence occurring. Preventative methods against infectious diseases through vaccination can prevent, control and reduce the severity of the disease process. Various methods have been used in the development of vaccines to prevent *S. aureus* infections, but no satisfactory results have been obtained. Recent studies suggests that multivalent vaccines based on cell-mediated immunity is expected to provide better protection against infection. The purpose of this article is to summarize the challenges of *S. aureus* infection and the current status of its potential solution with the development of a multivalent vaccine and to explain the latest approaches recommended to obtain successful development of *S. aureus* vaccine by inducing T-cells CD8+, CD4+ and Th17. Seven MRSA Indonesian origin isolates from humans and dairy-cattle were used as candidate vaccines as genotypic characterization had previously been performed (coa, eta, etb, hla, hlb, cap5, cap8, fnbA, fnbB and mecA). Vaccination can be performed with live-attenuated or killed-vaccine and could be combined with an injection of αCD40 monoclonal-antibodies (mAbs) with the aim to induce cellular-based active immunity (T CD8+, CD4+ and Th17). These isolates are expected to be safe vaccine candidates with immunogenic-protective properties based on genetic and antigenic variation to prevent and control staphylococcal infections in humans and animals.

Keywords: Antibiotic Resistant, *Staphylococcus Aureus*, Multivalent-Vaccine, Cell-Mediated Immunity, Immunization

Introduction

The incidence *Staphylococcus aureus* (*S. aureus*) infection is currently occurring at a high rate in human and animal populations in various locations within the United States of America (USA) and elsewhere around the world (Moran *et al*., 2005). *S. aureus* is known as a major cause of nosocomial and community-acquired infections and pneumonia in humans (Rubinstein *et al*., 2008; Ho and Robinson, 2009). In the dairy industry *S. aureus* is known to be a major cause of mastitis, occurring in clinical, subclinical and chronic cases (Holmes and Zadoks, 2011). The increasing incidences of *S. aureus* infection requires new strategies to prevent further infections occuring on a larger scale. This task is compounded further by the phenomenon of antibiotic resistance which is considered as the biggest barrier for controlling infectious diseases (Spellberg and Daum, 2012).

One of the methods for prevention and control of many infectious diseases is vaccination. Appropriate use of vaccinations have been known to provide satisfactory

results for the prevention, control and reduction in severity of pathology for infectious diseases in human and animal (Jansen *et al*., 2013). Therefore, further development of vaccines for infectious diseases known to be multi-drug resistant is required for future disease control. Various methods have been used to develop vaccines for the prevention of *S. aureus* infection, yet a satisfactory result is still to be obtained (Bristol-Myers Squibb, 2006; Biosynexus Incorporated, 2011; Jansen *et al*., 2013; Leitner *et al*., 2003; Martinez-Pulgarin *et al*., 2009; NABI Pharmaceuticals, 2007; Peton and Le Loir, 2013). Tekle *et al*. (2012) reports that most of the data from the current study is based on the development of methods for opsonization of antibodies against *S. aureus*. The main cause of vaccine is associated with a variety of serotypes and genotypes of the population of *S. aureus* and the development of broad-spectrum vaccines also explained by Tekle *et al*. (2012). Therefore the vaccination coverage is not sufficient to induce a protective immunogenic response that acts to prevent all *S. aureus* infections.

Antibiotic Resistance Phenomenon

The phenomenon of *S. aureus* resistance to penicillin is growing very rapidly due to the enzyme penicillinase (beta-lactamase) which is capable of hydrolyzing the beta-lactam ring from the chemical structure of penicillin. Based on this problem researchers have developed semi-syntethic penicillins that are resistant to beta-lactamase such as methicillin, nafcillin, oxacillin, dicloxallin and and flucloxacillin is mediated by penicillinase (a form of β-lactamase) production: an enzyme that cleaves the β-lactam ring of the penicillin molecule, rendering the antibiotic ineffective (Jalalpoor *et al*., 2007). Studies have shown that *S. aureus* is able to evolve to become resistant against these semi-syntethic antibiotics (Harris *et al*., 2002; Levinson and Jawetz, 2003). *S. aureus* resistant to those antibiotics, particularly methicillin are known as Methicillin-Resistant *Staphylococcus Aureus* (MRSA) (Harris *et al*., 2002). *S. aureus* which was less pathogenic and sensitive to the methicillin is known as Methicillin-Susceptible *Staphylococcus Aureus* (MSSA) (Harris *et al*., 2002). Wardenburg and Schneewind (2008) reported MRSA strains are known to be more dangerous than MSSA based on epidemiological studies related to increasing rate of morbidity and mortality, especially due to nosocomial infections (bacteremia, postoperative wound infections and pneumonia) as reported by Gastmeier *et al*. (2005) and Reed *et al*. (2005).

According to Waters *et al.* (2011) who collected and tested a total of 136 meat and poultry samples from 5 cities in the US, encompassing 80 unique brands from 26 grocery stores *S. aureus* contamination was most common among turkey samples (77%; 20/26), followed by pork (42%; 11/26), chicken (41%; 19/46) and beef (37%; 14/38). Ninety-six percent of the *S. aureus* isolates were resistant to at least 1 antimicrobial and many resistant (intermediate or complete) to multiple clinically important antimicrobial classes such as tetracycline, ampicillin, penicillin and erythromycin. *S. aureus* also observed resistance to other important antimicrobials including dalfopristin, fluoroquinolones, oxacillin, daptomycin and vancomycin (Waters *et al*., 2011).

Burden of Disease

A map of the prevalence from MRSA infections in humans can be seen in Fig. 1, reported as by Stefani *et al*. (2012).

S. aureus infections occur world wide, although due to the variation in prevalence of the multi-drug resistance phenomenon, the severity of disease reported differs. Incidence of MRSA infections greater than 50% occur in developed countries such as United States, Japan, South Korea and Singapore as well as some countries in South America. While in some developing countries, the reported incidence of MRSA infections were at an average rate of 25-50% (Stefani *et al*., 2012). In animals *S. aureus* was found and where capable to induce many infections, from the surface of the mucosal membranes, septicaemia and infection of internal organs (Acton *et al*., 2009). In livestock animals, *S. aureus* could be found and was known as livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) and since 1970 to 2000 LA-MRSA has been isolated from animals, especially pigs, cattle and companion animals (de Neeling *et al*., 2007; Leonard and Markey, 2008; Mulders *et al*., 2010). Nagase *et al*. (2002) has reported that the incidence of infection from *S. aureus* in chickens is approximately 90%, 42% in pigs, 29% in sheep (Vautor *et al*., 2005), 14-35% in beef cattles and 35% in dairy cows (Roberson *et al*., 1994). A summary of infections in livestock and companion animal by *S. aureus* can be seen on Table 1.

Virulence Factor

S. aureus is a gram-positive, catalase positive cocci bacteria. Glucose fermentation will occur under anaerobic condition whilst mannitol acid fermentation occurs under facultative anaerobic condition (Taverna *et al*., 2007). *S. aureus* grows at an optimum temperature of 37°C, but the best formation of pigment occurs at room temperature (20-35°C) and these conditions are also optimal for blood cell lyses and plasma coagulation (Levinson and Jawetz, 2003). *S. aureus* has virulence factors which play a role in the infection process. These include polysaccharides, surface proteins such as adhesin, glycoprotein, hemagglutinin and fibronectin. A summary of the various virulence factors of *S. aureus* that play a role in the pathogenesis of many types of infections can be seen in Fig. 2, as reported by Gordon and Lowy (2008).

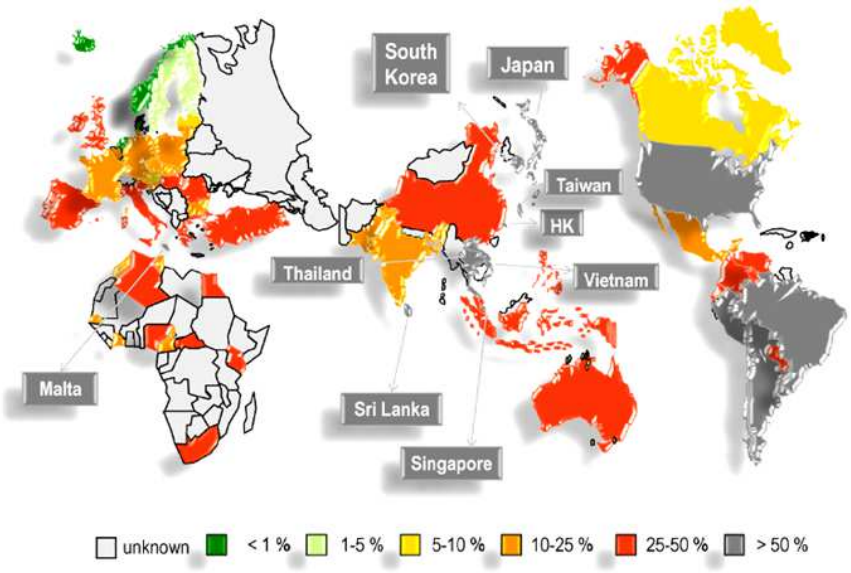

Fig. 1. Global of prevalence of MRSA infections. Adapted with permission from Stefani *et al*. (2012)

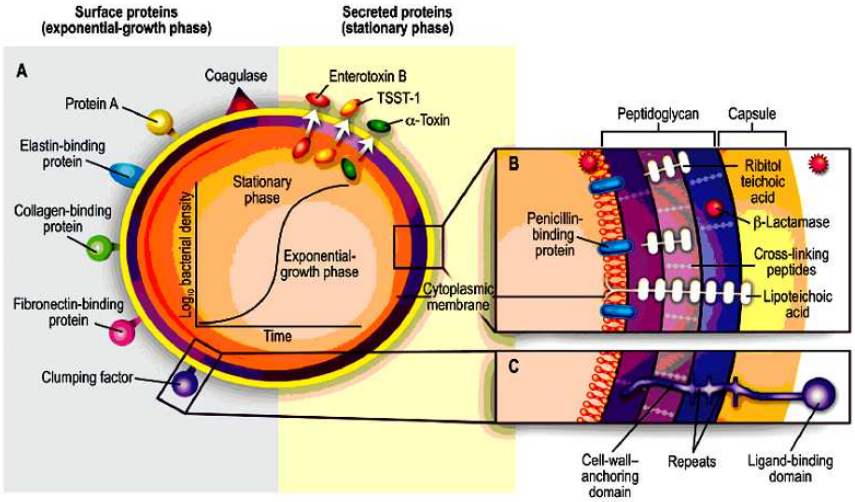

Fig. 2. Pathogenic factors from a whole cell *S. aureus* of that have structure of surface protein (part A,B,C) known to have a role in *S. aureus* pathogenicity. Adapted with permission from Gordon and Lowy (2008)

Several of the factors are able to play multiple roles in the pathogenesis of *S. aureus*. The surface proteins are responsible for the colonization of bacteria within the host tissues and while polysaccharides and protein-A are known to inhibit phagocytosis by polymorphonuclear leukocytes. Other substances such as carotenoids, catalase and coagulase enzymes and clumping-factor are capable of supporting the bacteria for survival inside phagocytic cells, which is the key method of avoiding a host immune system response (Haraldsson and Jonsson, 1984). In addition, the production of the enzyme coagulase is a major virulence factor that distinguishes *S. aureus* from other genus *Staphylococcus* (Levinson and Jawetz, 2003; Bello and Qahtani, 2005).

Until now, the identification of genotype is the gold-standard method which produces a result with high specificity and sensitivity for the characterization of the molecular determinants of virulence for *S. aureus*. The genotyping of characters include the coa gene (encoding coagulase properties), clfA gene (encoding clumping-factor), fnbB and fnbA gene (encoding fibronectin), nuc gene (encoding the nuclease) and spa gene (IgG-binding protein) (Straub *et al*., 1999; Salasia *et al*., 2004; 2011). While the nature of the mecA gene encodes antibiotic resistance of beta-lactam (penicillin G and its semi-syntethic derivatives) and Penicillin-Binding Protein (PBP2) (Harris *et al*., 2002).

The Prospect of Multivalent Vaccine

Nowadays, there are several formulations for *S. aureus* vaccine development, although there are still obstacles. Several reasons can be considered in the form of constraints and deficiencies in vaccine development *S. aureus*. These are, but not limited to:

- Related topics to virulence factors of *S. aureus* suggested as a potential antigen for single-component vaccines appears inadequate to induce protective immunity against an infection of *S. aureus* (Pereira *et al*., 2011).
- Killed and live-attennuated vaccines known to have advantages in terms of varying the antigen and can be considered as a promising approach. The disadvantage is the need for appropriate and adequate adjuvant (for killed-vaccine) and must provide security status (for live-attennuated vaccine) (Pellegrino *et al*., 2008).

Data of *S. aureus* vaccine development during the last 10 years (2003-2013) was reported by Jansen *et al*. (2013) and Peton and Le Loir (2014). Studies showed that the development of a vaccine for *S. aureus* during the last 10 years (2003-2013) have not produced satisfactory results in terms induced protectivity after administration of these vaccines. This is caused by *S. aureus* virulence factors as well as the antigens which are highly variable and therefore the prior stimulation of the immune system must be considered with vaccine development (Spellberg and Daum, 2012). Such conditions can create barriers to understanding the complex relationship between the selection and optimization of vaccine candidates in pre-clinical and clinical trials. Moreover, vaccine development conducted to date still relies largely on humoral immunity (Bristol-Myers Squibb, 2006; Biosynexus Incorporated, 2011; NABI Biopharmaceuticals, 2012; Vaccine Research International, 2013). According to Van den Berg *et al.* (2011), immunity through neutralization by antibodies (humoral-mediated immunity) against virulence factors has not been established as an effective and protective mechanism against *S. aureus* infection. There is a possibility that a multivalent vaccine would be more effective due to its ability of inducing a cellular immune response via antigen variation. This hypothesis is supported by the theoretical strategic development of a vaccine with broad spectrum protection using multi-antigen suggested in Fig. 3, by Nagy *et al*. (2008).

A vaccine is yet to be developed from MRSA isolates resistant to the beta-lactam group of antibiotics by way of the mecA gene. The purpose for using MRSA isolate combinations of multivalent/multi-antigen at the same time is to provide a protective immunogenic response through variation of antigen contained in the vaccine, including antigens encoding resistance to penicillin (mecA gene). The method for collecting multi-isolates from both human and animal has attempted by Salasia *et al.* (2004; 2011) summarized in Table 2 includes seven MRSA isolates (carriers of mecA gene) which have been selected from 30 isolates of human and dairy cattle origin based on differences in phenotypic and genotypic characters. This hypothesis is also supported by the results of the study Garcia-Alvarez *et al*. (2011) and Holmes and Zadoks (2011) which describes the relationship of genetic similarity (including the mecA gene) of specific isolates in cattle which can also be found in humans. These isolates are expected to be used as a universal vaccine candidate for the prevention of infections by *S. aureus* in human and animal.

Vaccination Based on Cell-Mediated Immunity

A study conducted by Proctor (2012) suggested that vaccinations based on cell-mediated immunity will lead to better protection against infection of *S. aureus*. Proctor also reported (2012) about the components that have been implicated in the mechanism of immunity for the vaccine is the combination of cellular and humoral immunity, these are the T-helper 17 cell (Th17) and interleukin-17 (IL-17). The Th17 cell function is to release IL-17 which plays a role in the movement and activation of neutrophils. While the role and ability of the neutrophils is enhanced by antibodies to kill *S. aureus*, the antibody alone without the action of the neutrophils, is known to be insufficient to provide protective effects (Jansen *et al*., 2013). In addition, several studies have developed a vaccine for *S. aureus* which is able to induce CD8+T cell (Rolph and Kaufmann, 2001) and CD4+ (Lawrence *et al*., 2012) as the main axis in cellular immunity.

Th17 plays role in cell-mediated immunity through the release of IL-17 and is a potential inducer of neutrophils chemotaxis to the site of infection and also enhances the functional activity of neutrophils (Fischer, 2008; Lin *et al*., 2009; Spellberg *et al*., 2008). Disorders of the Th17 axis will result in a delay and reduction in the number and phagocytic ability of neutrophils, which will result in the persistence of bacteria and lead to chronic inflammation (Romagnani *et al*., 2009). The results of the study conducted by Schmaler *et al.* (2011) showed in mice, administration of a vaccine of heat-Killed *Staphylococcus Aureus* (HKSA) is able to stimulate Th17 well as IL-17, through the presentation of staphylococcal lipoproteins by activating Toll -Like Receptos (TLR-2/MyD88). The study also showed that T lymphocytes are required to provide protection against infection of *S. aureus*; they are able to decrease the susceptibility of animal models (mice) by increasing the level of cytokines induced by superantigens, therefore lowering the risk of death.

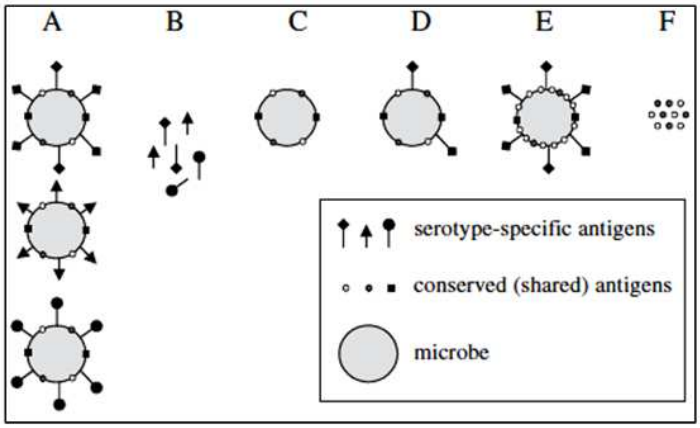

Fig. 3. The theoritical strategic development of vaccine with broad spectrum. Adapted with permission from Nagy *et al*. (2008)

Table 1. Variety of infections caused by *S. aureus* in animals

Species	Host	Infections	References
Staphylococcus aureus	Cattle	Mastitis, impetigo	Leitner *et al*. (2011)
			Leitner *et al*. (2011)
	Sheep	Mastitis, dermatitis, mild folliculitis	
	Goat	Mastitis botryomycosis	Leitner *et al*. (2011)
	Pig	Mastitis, castration wounds	Pellerin, 2010
	Horse	Dermatitis, urinary tract infections, abscesses	Pellerin, 2010
	Cat	Dermatitis	Pellerin, 2010
	Dog	Dermatitis	Pellerin, 2010
	Fowl (turkey)	Arthritis, septicemia	Pellerin, 2010

Table 2. Distribution of various virulence determinant of carrier of the *mecA* gene (MRSA strains) isolates from humans and animals based on AFLP analysis

No	Code of Isolates	AFLP Pattern/ class	Gene	*coa*	*eta*	*etb*	*hla*	*hlb*	*cap5*	*cap8*	*fnb*A	*fnb*B	*mecA*
1	SU2	O/IV	*She*	2	+	-	+	+	+	+	+	-	+
2	SU24	S/V	-	2	+	-	+	-	+	+	-	+	+
3	SU25	E/I	-	2	+	-	+	-	+	+	-	+	+
4	169	L/III	*She*	2	+	-	+	-	-	+	+	-	+
5	179	F/II	*See*	3	+	+	+	+	+	+	-	+	+
6	199	U/VI	*See*	3	-	-	+	+	-	+	+	-	+
7	1091	W/VII	-	1	+	-	+	-	-	+	+	+	+

Amplified Fragment Length Polymorphism (AFLP); genes encoding coagulase (*coa*); exfoliative toxins A (*eta*) and B (*etb*); alpha-hemolysin (*hla*); beta-hemolysin (*hlb*); capsular polysaccharide 5 (*cap5*) and 8 (*cap8*); fibronectin binding protein A (*fnbA*) and B (*fnbB*); penicillin binding protein 2A (*mecA*). Adapted with permission from Salasia *et al*. (2004; 2011)

Gjertsson *et al*. (2004) reported that the results of research in the septic arthritis mouse model due to *S. aureus* infection showed that the Ig G and B is not necessarily needed for the healing process. Lin *et al*. (2009) also reported that in mice which have a damaged immune response specific to Th17 cells and IL-17 are known become more sensitive to infection by *S. aureus*, indicating that Th17 and neutrophils have an important role in animal models in the protection from infectiom. Wallemacq *et al*. (2012) reported the results from a recent study showed that vaccination using *Heat-Killed S. Aureus* (HKSA) in conjunction with αCD40 monoclonal antibodies (mAbs) administered intra-mammary are able to induce immunity against *S. aureus* mastitis.

Induced immunity is based on cell-mediated immunity, which is an increase of CD8+ cytotoxic T lymphocytes (CTLs) specific antigen. The same thing was reported by Rolph and Kaufmann (2001), Chen *et al*. (2001) and Field *et al*. (2007) which uses a combination of CD40 and vaccine of heat-killed *Listeria mononcytogenes* and *Leishmania major*. The development of vaccines based on cell-mediated immunity was also reported by Lawrence *et al*. (2012) in a vaccination using HKSA. It was able to induce cellular immunity, particularly CD4+ T lymphocytes induced by superantigens found in isolates of *S. aureus* (strain Newman). The action of CD4+ is urgently needed to promote interferon-gamma (IFN-γ) activities which have

functions to mediate B lymphocyte transformation into an active form to produce IgG antibodies and activate phagocytic cells (mononuclear and polymorphonuclear) to eliminate *S. aureus* (Lawrence *et al*., 2012).

Producing Vaccine Candidate

The initial step for making the vaccine candidate can be done by various methods including live-attenuated vaccine (Watson, 1984; Pridgeon and Klesius, 2013; Pridgeon *et al*., 2013) or killed-vaccine (Leitner *et al*., 2011; Wallemacq *et al*., 2012). The route of administration of the vaccines that have been developed and have been through clinical trial stage in humans should refer to the guidelines by the Centers of Disease Control and Prevention (CDC) under the supervision of the U.S. Department of Health. Research in animal models have used varied routes of vaccine administration, intra-nasal (Hu *et al*., 2006), sub-cutaneous (Tollersrud *et al*., 2001; Leitner *et al*., 2003) intra-muscular (Senna *et al*., 2003), intra-mammary (Kauf *et al*., 2007; Wallemacq *et al*., 2012), as well as intra-peritoneal (Hu *et al*., 2010). Various routes of *S. aureus* vaccine administration in humans and animals should be adapted to the type of vaccine used (live-attenuated or killed) as well as by type of infection (local or systemic). If the satisfactory results can be obtained from clinical trials related to the development of a vaccine, then further evaluation for the consideration of mass production with the aim of commercializing the product should be conducted. This can be done through cooperation with industrial stakeholders and research institutions who play a role in the development and production of vaccines.

Conclusion

The increasing incidence of *S. aureus* infection, particularly MRSA which is compounded by the phenomenon of antibiotic resistance is regarded as one of the biggest barriers in the control of infectious diseases. Thus, it requires new strategies to prevent a higher rate of incidence. Various methods of vaccine development for the prevention of *S. aureus* infection has been undertaken, but have not obtained satisfactory results. Vaccine development conducted to date is still largely relying on humoral immunity and have not developed vaccine isolates from MRSA that is resistant to the beta-lactam antibiotic group, encoded by the mecA gene. Thus vaccines have not been able to induce a protective and active cellular based immunity. Recent studies suggests that vaccinations based on cell-mediated immunity are expected to provide better protection against infection. Furthermore, the development of a vaccine candidate based on multi-isolates of *S. aureus* (including MRSA strain) that induce cellular immunity are expected to give satisfactory results for the development of broad-spectrum (universal) vaccine to prevent *S. aureus* infections.

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Author's Contribution

Novra Arya Sandi: Participated in all aquisition of literatures, reviewed the literatures, coordinated and contributed the writing of the manuscript.

Tenri Ashari Wanahari: Participated in all aquisition of literatures, reviewed the literatures and contributed the writing of the manuscript.

Isabel MacPhillamy: Provided the technical guidance in literature review and contributed of the writing of the manuscript.

Siti Isrina Oktavia Salasia: Reviewed the literatures and provided the technical guidance in literature review.

Basofi Ashari Mappakaya: Contributed the writing of the manuscript.

Asmarani Kusumawati: Provided the technical guidance in literature review.

Ethics

This study was approved by Research Ethics Commitee, Faculty of Veterinary Medicine, Gadjah Mada University, Indonesia.

References

Acton, D.S., M.J. Plat-Sinnige, W.W. Vande, G.N. de and B.A. Van, 2009. Intestinal carriage of *Staphylococcus aureus*: How does its frequency compare with that of nasal carriage and what is its clinical impact? Eur. J. Clin. Microbiol. Infect. Dis., 28: 115-127. DOI: 10.1007/s10096-008-0602-7

Bello, C.S. and A. Qahtani, 2005. Pitfalls in the routine diagnosis of *Staphylococcus aureus*. Afr. J. Biotechnol., 4: 83-86. DOI: 10.5897/AJB2005.000-3014

Chen, G., P.A. Darrah and D.M. Mosser, 2001. Vaccination against the intracellular pathogens *Leishmania major* and *L. Amazonensis* by directing CD40 ligand to macrophages. Infect. Immun., 69: 3255-3263. DOI: 10.1128/IAI.69.5.3255-3263.2001

de Neeling, A.J., M.J.M. van den Broekb, E.C. Spalburga, M.G. van Santen-Verheuvel and W.D.C. Dam-Deisz *et al.*, 2007. High prevalence of methicillin-resistant *Staphylococcus aureus* in pigs. Vet. Microbiol., 122: 366-772. DOI: 10.1016/j.vetmic.2007.01.027

Fischer, A., 2008. Human immunodeficiency: Connecting STAT3, Th17 and human mucosal immunity. Immunol Cell Biol., 86: 549-551. DOI: 10.1038/icb.2008.52

Field, A.E., S. Wagage, S.M. Conrad and D.M. Mosser, 2007. Reduced pathology following infection with transgenic *Leishmania major* expressing murine CD40 ligand. Infect. Immun., 75: 3140-3149. DOI: 10.1128/IAI.00160-07

Garcia-Alvarez, L., M.T. Holden, H. Lindsay, C.R. Webb and D.F. Brown, 2011. Metichillin-resistant *Staphylococcus aureus* with a novel mecA homologue in human and bovine populations in the UK and Denmark: A descriptive study. Lancet. Infect. Dis., 11: 595-603. DOI: 10.1016/S1473-3099(11)70126-8

Gastmeier, P., D. Sohr, C. Geffers, M. Behnke and F. Daschner *et al*., 2005. Mortality risk factors with nosocomial *Staphylococcus aureus* infections in intensive care units: Results from the German nosocomial infection surveillance system (KISS). Infection, 33: 50-55. DOI: 10.1007/s15010-005-3186-5

Gordon, R.J. and F.D. Lowy, 2008. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clin. Infect. Dis., 46: 350-359. DOI: 10.1086/533591

Gjertsson, I., L. Nitschke and A. Tarkowski, 2004. The role of B cell CD22 expression in *Staphylococcus aureus* arthritis and sepsis. Microbes. Infect., 6: 377-82. DOI: 10.1016/j.micinf.2003.12.013

Haraldsson, I. and O. Jonsson, 1984. Histopathology and pathogenesis of mouse mastitis induced with *Staphylococcus aureus* mutans. J. Comp. Path., 94: 183-189. DOI: 10.1016/0021-9975(84)90039-2

Harris, L.G., S.J. Foster and R.G. Richards, 2002. An introduction to *Staphylococcus aureus* and techniques for identifying and quantifying *S. aureus* adhesins in relation to adhesion to biomaterials: Review. Eur. Cell. Mater., 31: 39-60. PMID: 14562246

Ho, K.M. and J.O. Robinson, 2009. Risk factors and outcomes of methicillin-resistant *Staphylococcus aureus* bacteraemia in critically ill patients: A case control study. Anaesth. Intensive Care, 37: 457-463. PMID: 19499868

Holmes, M.A. and R.N. Zadoks, 2011. Methicillin resistant *S. aureus* in human and bovine mastitis. J. Mammary Gland Biol. Neoplasia, 16: 373-382. DOI: 10.1007/s10911-011-9237-x

Hu, D.L., K. Omoe, K. Narita, J.C. Cui and S. Kunihiro *et al*., 2006. Intranasal vaccination with a double mutant of staphylococcal enterotoxin C provides protection against *Staphylococcus aureus* Infection. Microbes Infect., 8: 2841-2848. DOI: 10.1016/j.micinf.2006.09.001

Hu, C., R. Gong, A. Guo and H. Chen, 2010. Protective effect of ligand-binding domain of fibronectin-binding protein on mastitis induced by *Staphylococcus aureus* in mice. Vaccine, 28: 4038-4044. DOI: 10.1016/j.vaccine.2010.04.017

Jansen, K.U., D.Q. Girgenti, I.L. Scully and A.S. Anderson, 2013. Vaccine review: *Staphyloccocus aureus* vaccines: Problems and prospects. Vaccine, 31: 2723-2730. DOI: 10.1016/j.vaccine.2013.04.002

Jalalpoor, S.H, K.R. Kasra, A. Noohi and E.H. Zarkesh, 2007. Study of β-lactamase and S-layer production in some of isolated pathogen bacteria from clinical and environmental hospital samples. MSc Thesis, Iran, Tehran, Islamic Azad University Science and Research Branch Tehran, pp: 169-207.

Kauf, A.C.W., B.T. Vinyard and D.D. Bannerman, 2007. Effect of intramammary infusion of bacterial lipopolysaccharide on experimentally induced *Staphylococcus aureus* intramammary infection. Res. Vet. Sci., 82: 39-46. DOI: 10.1016/j.rvsc.2006.05.006

Lawrence, P.K., B. Rokbi, N. Arnaud-Barbe, E.L. Sutten and J. Norimine, 2012. CD4 T Cell antigens from *Staphylococcus aureus* newman strain identified following immunization with heat-killed bacteria. Clin. Vaccine Immunol., 19: 477-489. DOI: 10.1128/CVI.05642-11

Leitner, G., N. Yadlin, E. Lubashevsy, E. Ezra and A. Glickman *et al*., 2003. Development of a *Staphylococcus aureus* vaccine against mastitis in dairy cows. II. Field trial. Vet. Immunol. Immunopathol., 93: 153-158. DOI: 10.1016/S0165-2427(03)00062-X

Leitner, G., O. Krifucks, M.D. Kiran and N. Balaban, 2011. Vaccine development for the prevention of staphylococcal mastitis in dairy cows. Vet. Immunol. Immunopathol., 142: 25-35. DOI: 10.1016/j.vetimm.2011.03.023

Leonard, F.C. and B.K. Markey, 2008. Methicillin-resistant *Staphylococcus aureus* in animals: A review. Vet. J., 175: 27-36. DOI: 10.1016/j.tvjl.2006.11.008

Levinson, W. and E. Jawetz, 2003. Medical Microbiology and Immunology: Examination and Board Review. 7th Edn, McGraw-Hill Companies Inc., Singapore, ISBN: 10- 0838562620.

Lin, L., A.S. Ibrahim and X. Xu, 2009. Th1-Th17 cells mediate protective adaptive immunity against *Staphylococcus aureus* and *Candida albicans* infection in mice. PLoS Pathog., 5: 1000-1003. DOI: 10.1371/journal.ppat.1000703

Moran, G.J., R.N. Amii, F.M. Abrahamian and D.A. Talan, 2005. Methicillin-resistant *Staphylococcus aureus* in community-acquired skin infections. Emerg. Infect. Dis., 11: 928-930. DOI: 10.3201/eid1106.040641

Mulders, M.N., A.P.J. Haenen, P.L. Geenen, P.C. Vesseur and E.S. Poldervaart, 2010. Prevalence of livestock-associated MRSA in broiler flocks and risk factors for slaughterhouse personnel in the Netherlands. Epidemiol. Infect., 138: 743-755. DOI: 10.1017/S0950268810000075

Nagy, G., L. Emody and G. Pal, 2008. Strategies for the development of vaccines conferring broad-spectrum protection. Int. J. Med. Microbiol., 298: 379-395. DOI: 10.1016/j.ijmm.2008.01.012

Pellegrino, M., J. Giraudo, C. Raspanti, R. Nagel and L. Odierno *et al*., 2008. Experimental trial in heifers vaccinated with *Staphylococcus aureus* avirulent mutant against bovine mastitis. Vet. Microbiol., 127: 186-90. DOI: 10.1016/j.vetmic.2007.07.028

Pereira, U.P., D.G. Oliveira, L.R. Mesquita, G.M. Costa and L.J. Pereira, 2011. Efficacy of *Staphylococcus aureus* vaccines for bovine mastitis: A systematic review. Vet. Microbiol., 148: 17-24. DOI: 10.1016/j.vetmic.2010.10.003

Peton, V. and Y. Le Loir, 2014. *Staphylococcus aureus* in veterinary medicine. Infect. Genet. Evol., 21: 602-615. DOI: 10.1016/j.meegid.2013.08.011

Pridgeon, J.W. and K.H. Klesius, 2013. Development of live attenuated *Streptococcus agalactiae* as potential vaccines by selecting for resistance to sparfloxacin. Vaccine, 31: 2705-2712. DOI: 10.1016/j.vaccine.2013.03.066

Pridgeon, J.W., P.H. Klesius and M. Yildirim-Aksoy, 2013. Attempt to develop live attenuated bacterial vaccines by selecting resistance to gossypol, proflavine hemisulfate, novobiocin, or ciprofloxacin. Vaccine, 31: 2222-2230. PMID: 23499519

Proctor, R., 2012. Review: Is there a future for a *Staphylococcus aureus* vaccine?. Vaccine, 30: 2921-2927. DOI: 10.1016/j.vaccine.2011.11.006

Pellerin, J.L., 2010. Infections à *Staphylococcus aureus* Chez l'animal (Hors Mammites). In: *Staphylococcus aureus*, Le Loir, Y. and M. Gautier (Eds.), Lavoisier, Paris, pp: 203-210.

Reed, S.D., J.Y. Friedman and J.J. Engemann, 2005. Costs and outcomes among hemodialysis-dependent patients with methicillin-resistant or methicillin-susceptible *Staphylococcus aureus* bacteremia. Infect. Control Hosp. Epidemiol., 26: 175-183. DOI: 10.1086/502523

Roberson, J.R., L.K. Fox, D.D. Hancock, J.M. Gay and T.E. Besser, 1994. Ecology of *Staphylococcus aureus* isolated from various sites on dairy farms. J. Dairy Sci., 77: 3354-3364. PMID: 7814712

Rolph, M.S. and S.H. Kaufmann, 2001. CD40 signaling converts a minimally immunogenic antigen into a potent vaccine against the intracellular pathogen *Listeria monocytogenes*. J. Immunol., 166: 5115-5121. DOI: 10.4049/jimmunol.166.8.5115

Romagnani, S., E. Maggi, F. Liotta, L. Cosmi and F. Annunziato, 2009. Properties and origin of human Th17 cells. Mol. Immunol., 47: 3-7. DOI: 10.1016/j.molimm.2008.12.019

Rubinstein, E., M.H. Kollef and D. Nathwani, 2008. Pneumonia caused by methicillin-resistant *Staphylococcus aureus.* Clin. Infect. Dis., 46: 378-385. PMID: 18462093

Salasia, S.I. O., Khusnan and S. Artanto, 2004. Distribution of cap5 and cap8 genes of *Staphylococcus aureus* isolated from subclinical mastitis cows in Central Java, Indo. J. Biotechnol., 681: 701-705.

Salasia, S.I.O., S. Tato, N. Sugiyono, D. Ariyanti and F. Prabawati, 2011. Genotypic characterization of *Staphylococcus aureus* isolated from bovines, humans and food in Indonesia. J. Vet. Sci., 12: 353-361. DOI: 10.4142/jvs.2011.12.4.353

Schmaler, M., N. Jann, F. Ferracin and R. Landmann, 2011. T and B cells are not required for clearing *Staphylococcus aureus* in systemic infection despite a strong TLR2-MyD88-dependent T cell activation. J. Immunol., 186: 43-52. DOI: 10.4049/jimmunol.1001407

Senna, J.P.M., D.M. Roth, J.S. Oliveira, D.C. Machado and D.S. Santos, 2003. Protective immune response against methicillin resistant *Staphylococcus aureus* in a murine model using a DNA vaccine approach. Vaccine, 21: 2661-2666. DOI: 10.1016/S0264-410X(02)00738-7

Spellberg, B. and R. Daum, 2012. Development of a vaccine against *Staphylococcus aureus*: A review. Semin Immunopathol., 34: 335-348. DOI: 10.1007/s00281-011-0293-5

Spellberg, B., A.S. Ibrahim and M.R. Yeaman, 2008. The antifungal vaccine derived from the recombinant n terminus of Als3p protects mice against the bacterium *Staphylococcus aureus*. Infect Immun., 76: 4574-4580. DOI: 10.1128/IAI.00700-08

Stefani, S., D.R. Chung, J.A. Lindsayc, A.W. Friedrichd and A.M. Kearnse *et al*., 2012. Meticillin-Resistant *Staphylococcus Aureus* (MRSA): Global epidemiology and harmonisation of typing methods. Int. J. Antimicrob. Agents, 39: 273-282. DOI: 10.1016/j.ijantimicag.2011.09.030

Straub, J.A., C. Hertel and W.P. Hammes, 1999. A 23S rDNA-targeted polymerase chain reaction-based system for detection of *Staphylococcus aureus* in meat starter cultures and dairy products. J. Food Prot., 62: 1150-1156. PMID: 10528718

Taverna, F., N. Armando, P. Renata, Z. Alfonso and N. Simona *et al*., 2007. Characterization of cell wall associated proteins of a *Staphylococcus aureus* isolated from bovine mastitis case by a proteomic approach. J. Vet. Microbiol., 119: 240-247. DOI: 10.1016/j.vetmic.2006.09.007

Tekle, T.I., K.M. Nielsen, J. Liu, M.M. Pettigrew and L.A. Meyers *et al*., 2012. Controlling antimicrobial resistance through targeted, vaccine-induced replacement of strains. PLoS ONE., 7: 50688. DOI: 10.1371/journal.pone.0050688

Tollersrud, T., L. Zernichow, R.A. Svein, K. Kenny and A. Lund, 2001. *Staphylococcus aureus* capsular polysaccharide type 5 conjugate and whole cell vaccines stimulate antibody responses in cattle. Vaccine, 19: 3896-3903. DOI: 10.1016/S0264-410X(01)00124-4

Van den Berg, S., M.G. Bowden, T. Bosma, G. Buist and J.M. van Dijl *et al.*, 2011. A multiplex assay for the quantification of antibody responses in *Staphylococcus aureus* infections in mice. J. Immunol. Methods, 365: 142-148. DOI: 10.1016/j.jim.2010.12.013

Vautor, E., G. Abadie, J.M. Guibert, N. Chevalier and M. Pepin, 2005. Nasal carriage of *Staphylococcus aureus* in dairy sheep. Vet. Microbiol., 106: 235-239. DOI: 10.1016/j.vetmic.2004.11.019

Wallemacq, H., D. Bedoret, J. Pujol, C. Desmet and P.V. Drion *et al*., 2012. CD40 triggering induces strong cytotoxic T lymphocyte responses to heat-killed *Staphylococcus aureus* immunization in mice: A new vaccine strategy for staphylococcal mastitis. Vaccine, 30: 2116-2124. DOI: 10.1016/j.vaccine.2012.01.039

Wardenburg, J.B. and O. Schneewind, 2008. Vaccine protection against *Staphylococcus aureus* pneumonia. J. Exp. Med., 205: 287-294. DOI: 10.1084/jem.20072208

Watson, D.L., 1984. Evaluation of attenuated, live staphylococcal mastitis vaccine in lactating heifers. J. Dairy Sci., 67: 2608-2613. DOI: 10.3168/jds.S0022-0302(84)81620-3

Bristol-Myers Squibb, 2006. Safety and efficacy of veronate® versus placebo in preventing nosocomial staphylococcal sepsis in premature infants. National Library of Medicine United States.

Biosynexus Incorporated, 2011. Safety and efficacy of pagibaximab injection in very low birth weight neonates for prevention of staphylococcal sepsis. National Library of Medicine United States.

Martinez-Pulgarin, S., G. Dominguez-Bernal, J.A. Orden and F.R. de la, 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, 155: 1505-1515. DOI: 10.1099/mic.0.025544-0

NABI Biopharmaceuticals, 2012. Safety study of an intravenous *Staphylococcus Aureus* immune globulin (human), [altastaph] in low-birth-weight-neonates. National Library of Medicine United States.

Nagase, N., A. Sasaki, K. Yamashita, A. Shimizu and Y. Wakita *et al*., 2002. Isolation and species distribution of staphylococci from animal and human skin. J. Vet. Med. Sci., 64: 245-250. DOI: 10.1292/jvms.64.245

Vaccine Research International, PLC., 2013. Phase I Trial. Birmingham, United Kingdom.

Waters, A.E., T. Contene-Cuomo, J. Buchhagen, C.M. Liu and L. Watson *et al*., 2011. Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. Clin. Infect. Dis., 52: 1227-1230. DOI: 10.1093/cid/cir181