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 occurring 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).

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American Journal of Infectious Diseases

Literature Reviews

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Article history
Received: 19-05-2015
Revised: 14-08-2015
Accepted: 19-08-2015

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Introduction

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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 \textit{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 \textit{S. aureus}. The main cause of vaccine is associated with a variety of serotypes and genotypes of the population of \textit{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 \textit{S. aureus} infections.

\textbf{Antibiotic Resistance Phenomenon}

The phenomenon of \textit{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-synthentic penicillins that are resistant to beta-lactamase such as methicillin, nafcillin, oxacillin, dicloxacillin and flucoxacinil is mediated by penicillinase (a form of beta-lactamase) production: an enzyme that cleaves the beta-lactam ring of the penicillin molecule, rendering the antibiotic ineffective (Jalalpoor et al., 2007). Studies have shown that \textit{S. aureus} is able to evolve to become resistant against these semi-synthetic antibiotics (Harris et al., 2002; Levinson and Jawetz, 2003). \textit{S. aureus} resistant to those antibiotics, particularly methicillin are known as Methicillin-Resistant \textit{Staphylococcus Aureus} (MRSA) (Harris et al., 2002). \textit{S. aureus} which was less pathogenic and sensitive to the methicillin is known as Methicillin-Susceptible \textit{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 \textit{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 \textit{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. \textit{S. aureus} also observed resistance to other important antimicrobials including dalfopristin, fluoroquinolones, oxacillin, daptomycin and vancomycin (Waters et al., 2011).

\textbf{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).

\textit{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 \textit{S. aureus} was found and where capable to induce many infections, from the surface of the mucosal membranes, septicemia and infection of internal organs (Acton et al., 2009). In livestock animals, \textit{S. aureus} could be found and was known as livestock-associated methicillin-resistant \textit{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 \textit{S. aureus} in chickens is approximately 90%, 42% in pigs, 29% in sheep (Vautor et al., 2005), 14-35% in beef cattle and 35% in dairy cows (Roberson et al., 1994). A summary of infections in livestock and companion animal by \textit{S. aureus} can be seen on Table 1.

\textbf{Virulence Factor}

\textit{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). \textit{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). \textit{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 \textit{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 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-synthetic 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-attenuated 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-attenuated 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 character. 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 Receptors (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 theoretical 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) |
|                     | Sheep    | Mastitis, dermatitis, mild folliculitis | Leitner et al. (2011) |
|                     | 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 | Pattern/ class | Gene | coa | eta | eth | hla | hlb | cap5 | cap8 | fnbA | fnbB | mecA |
|----|------------------|----------------|------|-----|-----|-----|-----|-----|------|------|------|------|------|
| 1  | SU2              | O/IV           | She  | 2   | +   | -   | +   | +   | +    | +    | -    | +    | +    |
| 2  | SU24             | S/N            | -    | 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 infection. Wallenaar 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 monocytogenes* 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., 2003; Leitner 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), subcutaneous (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.

Acknowledgment

We acknowledge to Tri Untari, DVM, MSc PhD for provided the general support.

Funding Information

This work was partly funded by a grant from Directorate General of Higher Education (DIKTI), Ministry of Education and Culture of Indonesia.

Author’s Contribution

Novra Arya Sandi: Participated in all acquisition of literatures, reviewed the literatures, coordinated and contributed the writing of the manuscript.
Tenri Ashari Wanahari: Participated in all acquisition of literatures, reviewed the literatures and contributed the writing of the manuscript.
Isabel MacPhlially: 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 Committee, Faculty of Veterinary Medicine, Gadjah Mada University, Indonesia.

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