

Review

Secretome of *Staphylococcus aureus*

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Presence of secreted proteins is the key for sustenance of *Staphylococcus aureus* strains in the host system. General secretory (Sec) system is a well known and major secretion system that is responsible for the transportation of exoproteins across the bacterial cytoplasmic membrane and cell wall. In order to define the secretome of *S. aureus*, the availability of combination of two-dimensional gel electrophoresis (2-DE) with the latest advanced techniques (MALDI-TOF and ESI-MS/MS) has provided a powerful tool for the investigation of a large number of protein spots with the aim of identifying new drug candidates and discovering new diagnostics tools. In this paper, we present a short review regarding *S. aureus* general secretory system and role of secreted protein in host cells, and discuss the proteomic, together with genomics of *S. aureus*.

Key words: *Staphylococcus aureus*, secretome, general secretory system, pathogenesis, proteomic.

INTRODUCTION

Staphylococcus aureus is one of the dangerous opportunistic human pathogen that involve in community- and hospital- acquired infections (Chambers, 2001; Diekema et al., 2001; Vandenesch et al., 2003; Voyich et al., 2005). Exoproteins have been shown as indispensable components to contribute in its pathogenic activity by enhancing the virulence of an infection. All of this exported proteome is defined as secretome (Greenbaum et al., 2001; Antelmann et al., 2001).

The secretome of *S. aureus* to be exported to the cell surface and extracellular milieu of the human host by different located secretion systems such as general secretory (Sec), twin-arginine translocation (Tat) and early secreted antigen target 6 kDa (ESAT-6) (Navaratna et al., 1998; Rice et al., 2003; Burts et al., 2008), among which the General secretory (Sec) system is the most major secretion pathway in various bacterial species (Buist et al., 2006). Generally, secretome contain an N-terminal signal peptide that is needed to target them from the ribosome to the translocation machinery in the cytoplasmic membrane. Then, the N-terminal signal peptide will be removed by signal peptidase (SPase) and exoproteins will be retained in an extracytoplasmic compartment of the cell or secreted into the extracellular milieu after its correct conformation folding (Uhlen et al., 1984; Navarre and

Schneewind, 1999; Mazmanian et al., 2001).

Since the secretome appear to have major direct roles in virulence, discovering virulence factors have become important in understanding *S. aureus* pathogenesis and their interactions with the host. Started in 2001, the unending reports of complete genome sequences for a variety of *S. aureus* have stimulated the rapid establishment in „high-throughput“ approaches for defining proteins that are differentially expressed, located or secreted to outside of the cell. For example, two-dimensional gel electrophoresis (2DE) was used with tryptic peptide mass mapping via matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (Pandey and Mann, 2000; Righetti et al., 2004) and the development of serological proteome analysis (SERPA) has facilitated the identification of vaccine candidates (Vytvytska et al., 2002).

In this context, we will discuss the *S. aureus* secretion system with prime focus on Sec pathway and role of secreted proteins. Importantly, the genomics and proteomics studies applied in the field of *S. aureus* secretome with the main target on proteomic approaches taken and the virulence factors discovered will also be highlighted.

Ways of protein secretion

Sec pathway will be one of the transport pathways and can be divided into three stages (Economou, 2002;

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Sibbald et al., 2006): (a) targeting to the membrane translocation machinery by secretion-specific or general chaperones, (b) translocation across the membrane by the Sec translocon, formed by the heterotrimeric membrane protein complex SecYEG and the peripheral ATPase SecA and (c) post-translocational folding and modification.

Targeting

In *S. aureus*, N-terminal signal peptide of the ribosome-nascent secretome can be recognized by Ffh protein (contain the M-domain that binds signal peptides of preproteins), also a component of secretion-specific chaperone called signal recognition particle (SRP) and thus targeted to the membrane with the aid of FtsY protein, act as a high affinity receptor for SRP (Bunai et al., 1999; De Leeuw et al., 2000; Tjalsma et al., 2004). Then, the nascent secretome will be directed to the translocation machinery after stimulated by negatively charged phospholipids in the membrane and Sec translocon, especially SecA protein (Sibbald et al., 2006). SecA also may act as a chaperone for preprotein targeting, by promoting the rapid folding of signal peptides (after removed by SPase), thereby excluding them from the Sec secretion process (Economou and Wickner, 1994; Eser and Ehrmann, 2003).

Transmembrane crossing

As described above, once the SRP-ribosome-nascent secretome complex is bound to its FtsY protein, the ribosome has docked on the translocation pore, thus translation force has indirectly translocate polypeptides across the membrane to the Sec translocon (SecYEG) (Powers and Walter, 1997; de Gier and Luirink, 2001). In other way, *S. aureus* also can transfer its preprotein to SecA dimer, resulting in conformational rearrangements as the ATP molecules have bind to SecA, that promote their insertion of polypeptides into the channel of SecYEG (Löfdahl et al., 1983; Veenendaal et al., 2004). Then, SecA will return to its original conformation and disassociated from the translocation channel (Sibbald et al., 2006). The polypeptides will be further translocated across the cyto-plasmic membrane via the proton motive force which is generated by binding and hydrolysis cycle of ATP molecules (Veenendaal et al., 2004). Recently, SecA1 and SecY1 also have been shown to be required for the secretion of serine-rich adhesin for platelets (SraP) (Siboo et al., 2008).

With the knowledge, the stability establishment of SecDF-YajC complex in *S. aureus* is thought to enhance translocation through SecYEG by promoting membrane cycling of SecA (Duong and Wickner, 1997; Bolhuis et al., 1998). However, either the *S. aureus* SecDF-YajC complex associates specifically with the SecA1/SecY1 tran-

slocase, or SecA2/SecY2 translocase, or both (Sibbald et al., 2006).

Maturation and release

During transmembrane crossing, the cleavage activity will take place by exposing the junction between the signal peptide and the mature part of the translocating chain to the catalytic site of SPase (van Roosmalen et al., 2004). In this review, two different signal peptides are discussed for the *S. aureus*, including secretory (Sec-type) signal peptides and lipoprotein signal peptides which contain three domains: the N, H and C domains.

The N-terminal domain will facilitate the interaction between preprotein with the secretion machinery and/or phospholipids in the membrane, whereas the H domain will facilitate membrane insertion and display the SPase recognition and cleavage site at the extracytoplasmic membrane surface. Finally, the C domain specifies the cleavage site for specific SPase, either SPase I or SPase II (van Roosmalen et al., 2004; Sibbald et al., 2006).

In *S. aureus*, SPase I (SpsA and SpsB) will cleave the proteins with Sec-type signal peptides, meanwhile proteins with a lipoprotein signal peptide will be processed by the SPase II (LspA) after the lipid modification of the lipobox Cys residue has been fully completed by phosphatidyl glycerol diacylglycerol transferase (Lgt) (Cregg et al., 1996; Bruton et al., 2003; Sibbald et al., 2006; Sankaran and Wu, 1994; Stoll et al., 2005). As described in previous reviews, Lgt will recognize the lipobox and catalyze the transfer activity of the diacylglycerol moiety of phosphatidylglycerol (PG) to the sulfhydryl group of the lipobox N-terminal Cys residue at the +1 position of the signal peptide cleavage site in prolipoprotein (Sankaran and Wu, 1994; Stoll et al., 2005).

Mostly, *S. aureus* also possess different chaperones, such as, PrsA and DsbA folding catalysts, to ensure that the Sec-dependent manner transported proteins will correctly and rapidly fold into their protease-resistant and native conformation before degraded by proteases in the cell wall or extracellular milieu (Meima and van Dijk, 2003; Sarvas et al., 2004; Dumoulin et al., 2005; Stoll et al., 2005).

Consequently, whether the released mature chain will be targeted to extracellular milieu, cytoplasmic membrane or cell wall (Matsuyama et al., 1993), is under the control by several retention signals such as, lipoprotein retention signal, non-covalently cell wall binding domain and covalently cell wall binding domain (Baba and Schneewind, 1998; Mazmanian et al., 2001; Sutcliffe and Harrington, 2002; Sibbald et al., 2006).

Secreted proteins in *S. aureus* pathogenesis

S. aureus require an arsenal of secretome which released into the host milieu or displayed at the cell surface

as their effective virulent factors. Interestingly, this contribution of virulent factors divided into several steps that begin with colonization, establishment of bacterial spread after the defense systems of human host have been corrupted and followed by development of sepsis or specific toxinoses (Fedtke et al., 2004). As a point, the primary goal of virulent factors may be to convert local host tissues into nutrients required for bacterial growth.

Colonization

Colonization of *S. aureus* is a multifactorial process with various ligands affecting initial colonization and prolonged persistence in different ways. Thus, the ability of *S. aureus* to adhere to extracellular matrix components, and/or soluble plasma proteins is thought to be essential for colonization which allows it to play a central role in host-to-host transmission and the maintenance of stable carriage of *S. aureus*. Besides that, the success of *S. aureus* colonization also considered to be important in an establishment of infections. For example, the pathogenesis of endovascular infections, including endocarditis and metastatic infections are caused by the interaction of *S. aureus* with endothelial cells (EC). Importantly, *S. aureus* have to express a range of cell wall-anchored MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), which specifically bind towards fibrinogen, fibronectin, laminin, collagen, vitronectin and thrombospondin with the aim of promoting colonization (Rivas et al., 2004; Clarke and Foster, 2006).

Several surface-exposed proteins including the fibrinogen-binding proteins clumping factor A (ClfA) and B (ClfB) (McDevitt et al., 1994; Eidhin et al., 1998); the collagen-binding protein Cna (Patti et al., 1992); protein A, which can bind Von Willebrand factor and the Fc region of immunoglobulin G (IgG) (Löfdahl et al., 1983; Uhlen et al., 1984; Hartleib et al., 2000); and two fibronectin-binding proteins, FnBPA and FnBPB (Jonsson et al., 1991; Meenan et al., 2007), play major roles during colonization of *S. aureus*. Some of the surface-exposed proteins are required to function together with wall teichoic acid (WTA) to achieve the most effective colonization, such as, broad-spectrum ligand-binding protein IsdA (potential to bind fibrinogen and fibronectin), which promote adhesion to desquamated epithelial cells (Clarke et al., 2006).

Certain studies showed that initial colonization would be important in formation of biofilm (extracellular material called slime) (Gotz, 2002; O'Gara, 2007). Extracellular matrix protein-binding protein (Emp) recently showed essential for biofilm formation under low-iron growth conditions (Johnson et al., 2008). As a result, device-related infection has emerged as a major problem to the long-term use of medical devices in treating various diseases and abnormalities due to the stable form of *S. aureus* biofilm (Willcox et al., 2008).

Human defense system evasion

To our knowledge, *S. aureus* is well known to evade host defenses, to adapt to different environmental conditions for intracellular or extracellular survival, invade or destroy host cells and spread within the tissues after initial colonization.

Following an initial colonization, *S. aureus* have to address the variable moisture condition between hyper- and hypo-osmotic condition by producing Ehb protein to avoid plasmolysis under hyper-osmotic condition (Kuroda et al., 2008). Surprisingly, *S. aureus* also secretes carotenoid pigment and catalase that has an important role for enhanced oxidant and neutrophil resistance (inactivates the toxic hydrogen peroxide and free radicals) (Gresham et al., 2000; Mayer-Scholl et al., 2004; Voyich et al., 2005) and increased its survivability in phagocytes. To become lysozyme resistant in phagocytes, *S. aureus* has to encode an integral membrane protein via *oatA* gene (Bera et al., 2005).

Generally, adhesins or fibronectin-binding proteins are utilized to facilitate the host colonization, but in certain case they are involved with evasion of human immune response activity. For example, formation of a fibronectin bridge to the fibronectin-binding integrin $\alpha 5 \beta 1$ expressed on the host cell surface also could be observed, and then FnBPs trigger bacterial invasion to a variety of non-professional phagocytic cells (Sinha et al., 1999; Fowler et al., 2000). Subsequently, *S. aureus* succeed to evade host defenses and resist antibiotic killing. *S. aureus* that escapes into the cytoplasm will kill the host cell by multiple virulence factors, such as α -toxin (Novick, 2003). Besides that, ClfA could protect *S. aureus* far away from macrophage phagocytosis and enhances immunostimulatory activity, also act as mediator of *S. aureus*-induced platelet aggregation. According to the reports, the induction of localized joint inflammation and erosive lesions of cartilage and bone are significantly caused by Clfs (Palmqvist et al., 2005).

Furthermore, *S. aureus* also secrete the cell wall-anchored protein A, Spa, to inhibit the phagocytic engulfment and cause the immunological disguise and modulation. Spa is the best characterized protein for its capacity to bind the Fc region of IgG (Forsgren and Sjoquist, 1966). In addition, *S. aureus* also produce the zymogen staphylokinase that cleaves human plasminogen into active plasmin, which in turn cleaves IgG (Rooijackers et al., 2005b). Consequently, Fc-receptor mediated phagocytosis and also complement activation via C1q pathways are inhibited. Spa also acts as a B-cell superantigen through interactions with the heavy-chain

variable (V_H clan III-encoded B-cell receptor) part of Fab fragments and sequesters immunoglobulins by forming large insoluble immune complexes with human IgG (Forsgren and Sjoquist, 1966). Notably, superantigen-triggered B-cell responses do not favor the development of Spa-specific memory B-cells (Kozlowski et al., 1998;

Graille et al., 2000). Recently, studies show that SpA also recognizes the TNF-receptor 1, a receptor for tumor-necrosis factor- (TNF-) and cause the staphylococcal pneumonia (Gomez et al., 2006).

To escape the powerful complement fixation, *S. aureus* also produce the Sbi protein (Sbi-E) that consist of four major globular domains (I, II, III and IV) which binds host complement components Factor H (major fluid-phase complement regulator that controls alternative pathway activation at the level of C3) and C3 as well as IgG and 2-glycoprotein I (plasma component) and interferes with innate immune recognition by blocking the alternative complement pathway (Haupt et al., 2008).

In order to evade the complete innate immune system efficiently, especially complement attack, *S. aureus* has to excrete five additional secretome such as staphylococcal complement inhibitor (SCIN) (Rooijackers et al., 2005a) and chemotaxis inhibitory protein (CHIPS), by which both are genetically clustered on SaPI5, a novel pathogenicity island that is carried by bacteriophages (Haas et al., 2004; Rooijackers et al., 2005a); extracellular fibrinogen-binding protein (Efb) (Lee et al., 2004), the Efb homologous protein (Ehp) (Hammel et al., 2007), and the extracellular complement-binding protein (Ecb) (Jongerijs et al., 2007). In 2005, studies identified that SCIN acts on surface-bound C3 convertases, C3bBb and C4b2a by stabilizing these complexes, thereby reducing the enzymatic activity and inhibit the reaction of complement towards *S. aureus* (Rooijackers et al., 2005a). On the other hand, CHIPS that are produced by *S. aureus* will block the function of C5a and formylated peptide receptors required for chemotaxis of neutrophils (Haas et al., 2004; Rooijackers et al., 2005a). Meanwhile, Efb, Ehp and Ecb also have been found to bind C3 and C3d that prevent further activation of C3b by blocking the activity of C3b-containing convertases (Lee et al., 2004; Hammel et al., 2007).

S. aureus also produce four types of haemolysins known as α -, β -, γ -, and δ -toxin with one type of leukocidin, Pantan-Valentine leukocidin (PVL), to affect large numbers of epithelial, immune and red blood cells. According to Jarry and Cheung, *S. aureus* can escape from the phagolysosome after being internalized by a cystic fibrosis epithelial cell line, CFT-1, with the help from secreted Hla protein (Jarry and Cheung, 2006). Most recently, δ -toxin showed the potential to kill the proliferation of human T-lymphocytes in order to evade the host immune system (Doery et al., 1963; Huseby et al., 2007). Other human cells which are susceptible to δ -toxin are polymorphonuclear leukocytes, resting lymphocytes and monocytes. In 2009, δ -toxin has been shown to induce neutrophil-mediated lung injury through both its sphingomyelinase activity and syndecan-1 (Hayashida et al., 2009). Furthermore, *S. aureus* also secrete two bicomponent toxins, α -toxin (Hlg) and PVL act as two synergistically acting proteins, one S component (HlgA, HlgC or LukS-PV) and one F component (HlgB or LukF-

PV). Hlg is strongly haemolytic with weak leukocytes, whereas PVL may lyse polymorphonuclear neutrophils and macrophages with its heterooligomeric pore-forming exotoxin (Prevost et al., 1995; Genestier et al., 2005). Consequently, *S. aureus* may escape from the immune defense system and spread through the blood to other body areas, causing a variety of systemic infections.

Development of sepsis or specific toxinoses

The final and perhaps most important aspect of *S. aureus* infections we shall consider here, is a remarkable observation that virtually all of *S. aureus* exotoxins are associated with specific toxinoses and sepsis. These exotoxins usually will cause disease in toxic shock syndrome, TSS (including menstrual TSS and non-menstrual TSS), food poisoning and „scalded skin“ syndrome. So far, there are three clinically important secretome: staphylococcal enterotoxins (SEs), toxic shock syndrome toxin (TSST) and exfoliatin toxins (ETs).

The SEs (types A to R) are mostly associated with the food poisoning and frequently happens in the United States and around the world (Wieneke et al., 1993; Dinges et al., 2000; Loir et al., 2003). Actually, SEs and TSST-1 are under pyrogenic toxin superantigens (PTSAGs) family that stimulates proliferation of T-lymphocytes regardless of the antigen specificity of these cells which results in elevated levels of pro-inflammatory cytokines. As super-antigens, these PTSAGs bind directly to outside of conventional peptide-binding groove of major histocompatibility class II molecules (MHC class II) via N terminus of PTSAGs and then presented to T cells without internalization or “proteolytic processing” by host antigen-presenting cells (APC) (Hurley et al., 1995; Kum et al., 1996).

Subsequently, PTSAGs will be recognized by the T cell receptor (TCR) that is strictly dependent on the variable region of a chain (V) from the TCR and not requires recognition by all five variable elements (V , D , J , V and J) of the TCR, like conventional antigens (Davis and Bjorkman, 1988). Then, it will swiftly result in cell-signaling cascades and leading to elevated expression of pro-inflammatory cytokines (Chatila et al., 1988; Scholl et al., 1992; Andersen et al., 1999). PTSAGs also capable of activating the transcriptional factors NF- κ B and AP-1, which subsequently elicit production of pro-inflammatory cytokines such as, interferon gamma (IFN γ), interleukin 2 (IL2), IL6 and tumour necrosis factor beta (TNF β) may be released from T cells; meanwhile IL1 and TNF may be produced by macrophages (Gjertsson et al., 2001). Thus, massive released proinflammatory cytokines are believed to be responsible for many of the clinical features of toxic shock syndrome.

Early studies reported that exfoliative toxins (Ets) are superantigens that non-specifically stimulate certain V T lymphocyte clones via MHC class II molecule. Most re-

ports have shown that nanogram quantities of either Eta or Etb are sufficient to induce a substantial proliferation of T cells in human PBMC cultures (Ladhani et al., 1999). However, some studies suggest that the previous super-antigen activity of ETs was probably due to contamination with other mitogenic exotoxins (Ladhani et al., 1999; Monday et al., 1999). Absolutely, ETs are recognized as the cause of staphylococcal scalded skin syndrome (SSSS), a disease characterized by separation of the epidermis at the desmosomes, leading to a positive Nikolsky sign, which occurs predominantly in the very young before the development of protective antibodies. Interestingly, SSSS has been described relatively few times in the elderly to who have immunocompromised or have renal insufficiency, but has a high mortality up to 40 - 60% (Franken et al., 2008). Recently, three isoform of Ets have been identified as Eta, Etb and Etd, which are glutamate-specific serine proteases that specifically cleave a single peptide bond within the calcium-binding site in the extracellular region of human and mouse desmoglein 1 (Dsg1), a desmosomal cadherin-type cell-cell adhesion molecule (Amagai et al., 2002; Nishifuji et al., 2008).

Consequently, secretome of *S. aureus* play crucial and important roles in the colonization and subversion of the human host, which involves the excretion of a variety of secretome to the cell surface and extracellular milieu. To date, the secretome of *S. aureus* has been initially defined by two-dimensional electrophoresis (2-DE) and mass spectrometry (MS) as focus in this review.

Proteomics-future direction

With the aim to study the secretome of pathogenic *S. aureus* and to investigate the expression level of virulence factors based on various condition, proteins extracted from cell culture media or cell pellets can be analyzed and identified by the use of proteomic approaches (Bernardo et al., 2002; Nakano et al., 2002; Nandakumar et al., 2005; Sibbald et al., 2006; Pocsfalvi et al., 2008).

This provides a basis for future studies on the development of vaccines and diagnostic tools. Proteomic chosen as an essential tool in secretome research is because:

1. We have realized that information of complete genome sequences is not enough to derive biological function.
2. Proteomics is more focused on the gene products that are useful for drug development.
3. mRNA levels do not always correlate with protein expression (Gygi et al., 1999).
4. Prediction of genes and verification of a gene product can be achieved through exploration and information on proteomics (Teufel et al., 2006).
5. Protein modifications and protein localization are able

to be detected using proteomics (Burlak et al., 2007).

6. Protein regulation system cannot be determined only at DNA level.

Proteomics and other omics based approaches do not just depend on a hypothesis but relies in the fact and generate new theory. For example, in a recent review, proteomic approach was applied to analyze the secretome of enterotoxigenic *S. aureus* strains revealed the presence of different known enterotoxins and other virulence factors along with a number of core exoproteins. This can give a comprehensive picture of the expression both of core exoproteins and virulence factors under a given condition, where by, production of SEIL and SEIP was demonstrated for the first time at the protein level (Pocsfalvi et al., 2008).

Consequently, a greater understanding of cell wall/membrane-associated proteins in pathogenicity and antibiotic resistance mechanisms will offers the chance to identify additional antigens for their capacity to elicit a protective immune response and can aid in the discovery of vaccine and therapeutic targets (Cordwell et al., 2001). However, proteome analysis of *S. aureus* membrane and cell surface proteins is complex due to their intrinsic hydrophobic nature, alkaline pI and the number of trans-membrane spanning regions, meanwhile the high contamination of abundant cellular components are frequently observed in peptidoglycan and membrane fractions. Therefore, different techniques such as application of low- or high-percentage gels, zoom gels or chromatographic prefractionation techniques have been used to overcome these weakness by increasing the overall proteome coverage (Cordwell et al., 2000; Washburn et al., 2001).

In the past four years, gel-free analysis of *S. aureus* proteins using 2D LC-MS/MS has been performed for the alkaline or hydrophobic proteins (Kohler et al., 2005). Recently, the utilization of one/two-dimensional gel-LC and a membrane shaving approach together with tandem-MS/MS analyses have extremely facilitated the detection of hydrophobic integral membrane proteins. According to the studies, 271 of integral and 86 of peripheral membrane proteins from exponentially growing cells had been identified (Wolff et al., 2008). Beside that, shotgun proteomics approach was utilized to address the most recently major problem of hospital- and community-acquired pneumonia. Studies have shown that 513 host proteins were associated with *S. aureus*, suggesting that *S. aureus* was rapidly internalized by phagocytes in the airway and significant host cell lysis occurred during early infection. Furthermore, extracellular matrix and secreted proteins, including fibronectin, antimicrobial peptides and complement components, were associated with *S. aureus* at both time points (Ventura et al., 2008).

Thus, proteomics, genomic and genome-based technologies applied to *S. aureus* offers a big opening for finding novel diagnostics, therapeutics and vaccines. As a result,

the combination of proteomic and genomic could be an advanced tool for a faster analysis of pathogenic factors in clinical isolates (Bernardo et al., 2002). Surprisingly, there are highly conserved proteome profiles between antibiotic sensitive and resistant strains (Cordwell et al., 2002). It seems likely due to undissolve hydrophobic protein within the cell wall, which may hide some of the secrets to resistance in those strains. Hence development of new approach for the micro-characterization of highly hydrophobic proteins in proteomics continues and play an important step as described earlier (Kohler et al., 2005; Wolff et al., 2008). Further-more, the comprehensive study of *in vivo* immunogenic secretome by serological proteomic approach is still not yet satisfied. For example, only 15 potential vaccine candidates could be identified by using patient's sera blotting to the secretome that express *in vitro* in the synthetic culture medium instead of *in vivo* in the host system (Vytvytska et al., 2002).

Obviously, there is a need for combination or modification of proteomics approach with other available approaches in identifying the *S. aureus* secretome, often with the aim of developing new vaccine candidates and diagnostic tools.

CONCLUSIONS

Experimental and bioinformatics studies of secreted proteins in *S. aureus* have largely been limited to studies that build on the legacy of the pre-genomic era. Relatively few researchers have taken up the challenge of describing and investigating the unexplored areas of the *S. aureus* secretome based on immunoproteomic approach.

In this paper we have tried to bridge this knowledge gap by providing an overview of the secretion systems of the *S. aureus* and secretome that are involved in *S. aureus* pathogenicity to ensure that the basic understanding can be implant to the researchers who wish to construct a detailed profile of a *S. aureus* secretome. It is very likely that our increasing knowledge of the biology of pathogen- host interactions will in turn be used to identify additional secreted proteins. With the knowledge, we can apply the secretome as therapeutics to alter secretome profiles for disease treatment. On the other hand, we have highlighted a few of many unanswered questions regarding secretion system function, unknown function of the novel proteins and additional secretome produced *in vivo*. One important research area for the future is an increased understanding of how secretion system is controlled in strains that secrete multiple proteins in an organized manner. Besides that, correlation between the secretomes or secretomes with its own secretion system should be investigated. As showed by Labandeira-Rey et al. (2007), the expression of the *luk-PV* genes will interfere with global regulatory networks, which may also enhance virulence by increasing the expression of *Spa* gene (Labandeira-Rey et al., 2007). Hence recently, Hla

has been shown to be indirectly involved in colonization²⁺ function by accelerating pump-driven extrusion of Ca²⁺ ions resulting in attenuation of calcium-mediated cellular defense functions and facilitation of bacterial adherence to the bronchial epithelium (Eichstaedt et al., 2009).

That is why, secretion system and enhanced virulence of *S. aureus* as discussed in this review made the proteomics, genomic and genome-based technologies continue to become the main role in system biology of *S. aureus*, as it can identify and quantify the molecular protein, and also can show the networks of their physical interactions among each other, including information on protein modification, protein degradation, protein localization and targeting. We believe that combination of proteomics with molecular genetics, biochemistry or biophysics can show tremendous potential for making vaccines or diagnostic tools that once might have been impossible to design, although there are some failures we meet before, Veronate is produced by Inhibitex Pharmaceuticals and an antibody-inducing polysaccharide conjugate vaccine Staph Vax, is made by Nabi Biopharmaceuticals.

Consequently, we need a better understanding of *S. aureus* secretomes together with the antibodies immunocompromised patients to make such therapies work.

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