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Comparative Exoproteomics and Host Inflammatory Response in *Staphylococcus aureus* Skin and Soft Tissue Infections, Bacteremia, and Subclinical Colonization

Yun Khoon Liew,[a,b] Rukman Awang Hamat,[a] Alex van Belkum,[c] Pei Pei Chong,[d] Vasanthakumari Neela[a]

Department of Medical Microbiology and Parasitology, Universiti Putra Malaysia, Serdang, Malaysia[a]; School of Pharmacy, International Medical University, Kuala Lumpur, Malaysia[b]; bioMérieux, La Balme Microbiology Unit, La Balme les Grottes, France[c]; Department of Biomedical Sciences, Universiti Putra Malaysia, Serdang, Malaysia[d]

The exoproteome of *Staphylococcus aureus* contains enzymes and virulence factors that are important for host adaptation. We investigated the exoprotein profiles and cytokine/chemokine responses obtained in three different *S. aureus*-host interaction scenarios by using two-dimensional gel electrophoresis (2-DGE) and two-dimensional immunoblotting (2D-IB) combined with tandem mass spectrometry (MS/MS) and cytometric bead array techniques. The scenarios included *S. aureus* bacteremia, skin and soft tissue infections (SSTIs), and healthy carriage. By the 2-DGE approach, 12 exoproteins (the chaperone protein DnaK, a phosphoglycerate kinase [Pgk], the chaperone GroEL, a multisensor hybrid histidine kinase, a 3-methyl-2-oxobutanoate hydroxymethyltransferase [PanB], cysteine synthase A, an *N*-acetyltransferase, four isoforms of elongation factor Tu [EF-Tu], and one signature protein spot that could not be reliably identified by MS/MS) were found to be consistently present in more than 50% of the bacteremia isolates, while none of the SSTI or healthy-carrier isolates showed any of these proteins. By the 2D-IB approach, we also identified five antigens (methionine aminopeptidase [MetAPs], exotoxin 15 [Set15], a peptidoglycan hydrolase [LytM], an alkyl hydroperoxide reductase [AhpC], and a haptoglobin-binding heme uptake protein [HarA]) specific for SSTI cases. Cytokine and chemokine production varied during the course of different infection types and carriage. Monokine induced by gamma interferon (MIG) was more highly stimulated in bacteremia patients than in SSTI patients and healthy carriers, especially during the acute phase of infection. MIG could therefore be further explored as a potential biomarker of bacteremia. In conclusion, 12 exoproteins from bacteremia isolates, MIG production, and five antigenic proteins identified during SSTIs should be further investigated for potential use as diagnostic markers.

Staphylococcus aureus is capable of causing a wide range of infections, including skin and soft tissue infections (SSTIs), bacteremia, osteomyelitis, and more. However, certain sequence types (STs) of *S. aureus* may better colonize and infect patients. For instance, necrotizing pneumonia or sepsis is commonly associated with ST1 of clonal cluster 1 (CC1) (1). In addition, the burden of multidrug-resistant (MDR) *S. aureus* renders infection control challenging in hospital settings. Furthermore, non-MDR strains may also cause severe staphylococcal infections (2–4).

In *S. aureus*, exoproteins play a major role in virulence, particularly during invasion and host tissue damage. *S. aureus* produces exogenous phenol-soluble modulins that exhibit strong cytolytic activity against human neutrophils, erythrocytes, and monocytes (5). The exoprotein LukGH was recently reported to exhibit synergistic effects with Panton-Valentine leukocidin on human neutrophil lysis (6). Similarly, the exoprotein SasX facilitates intercellular aggregation and promotes biofilm formation (7). A continuous search for new *S. aureus* virulence factors is ongoing, and comparative exoproteomics of strains isolated from different infection types may help in the identification of additional virulence factors.

Several studies have reported heterogeneous virulence gene expression in strains from different infection types and different clones (8, 9). These studies also reported exoproteome heterogeneity likely due to genetic regulation, posttranslational modification, or targeted protein degradation or stabilization. Such heterogeneity complicates the identification of potential biomarkers or vaccine candidates for *S. aureus*.

It is well known that some exoproteins are antigenic. This antigenicity has been shown in both *S. aureus*-infected patients and healthy carriers (10, 11). The exoproteins Hla, IsdB, IsaA, and ClfA elicit significantly higher levels of antibodies in patients than in healthy carriers. Such immunogens play key roles in host protection by enhancing opsonic activity and inducing cytokine and chemokine production to promote immune cell recruitment (12, 13). Unfortunately, the efficacy of these protective actions has not been transformed into a licensed vaccine, underscoring the need for a better understanding of host responses to the different types of infection caused by *S. aureus*. A comprehensive analysis of exoproteome variations and immunoproteomics may allow correlations between different infection types and host immune profiling to be discovered. In turn, this knowledge may help to identify new disease-specific protein markers.

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Address correspondence to Vasanthakumari Neela, vasantha@upm.edu.my.

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