UNIVERSITI PUTRA MALAYSIA

COMPARATIVE ANALYSIS OF EXOPROTEOME OF STAPHYLOCOCCUS AUREUS ISOLATED FROM ASYMPTOMATIC CARRIER AND DIFFERENT INFECTION TYPES

LIEW YUN KHOON

FPSK(p) 2013 17

COMPARATIVE ANALYSIS OF EXOPROTEOME OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM ASYMPTOMATIC CARRIER AND DIFFERENT INFECTION TYPES

LIEW YUN KHOON

DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

20131

COMPARATIVE ANALYSIS OF EXOPROTEOME OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM ASYMPTOMATIC CARRIER AND DIFFERENT INFECTION TYPES

By

LIEW YUN KHOON

Thesis Submitted to the School of Gradute Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

November 2013

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

COMPARATIVE ANALYSIS OF EXOPROTEOME OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM ASYMPTOMATIC CARRIER AND DIFFERENT INFECTION TYPES

By

LIEW YUN KHOON

November 2013

Chairperson: Associate Professor Vasanthakumari Neela, Ph.D

Faculty: Medicine and Health Sciences

Staphylococcus aureus (*S. aureus*) is a highly versatile pathogen that can survive under diverse *in vitro* and *in vivo* environmental conditions. The success of *S. aureus* is mainly driven by the extracellular proteins (exoproteins). Understanding the exoproteome of *S. aureus* isolates from different host and clinical manifestations as well as the host humoral and inflammatory responses is important in identifying potential virulence and diagnostic markers, and vaccine candidates.

Firstly, silver staining technique was optimized prior to proteomic study to obtain a clear resolution of proteome. The modified silver staining helped to visualize the lower molecular mass and low abundant protein spots. Besides that, enhanced-resolution images of co-migrating spots with variable abundance intensities were also achieved. The modified silver staining allowed the detection of proteins loaded at

extremely low concentrations, ranging from 0.0048 to 0.0480 µg/µL. Therefore, all further investigations were carried out with modified silver staining method.

Analysis of the exoproteome of pig-associated *S. aureus* strain (sequence type 9 (ST9)) isolated from human and pig showed similar protein patterns, however variation in the protein spot intensity was observed. The protein spots intensities were on average higher in *S. aureus* ST9 strain isolated from pigs than pig handlers. Variation in the spot positional correlation between the isolates from two different hosts was found to be less. From the comparative exoproteome, IsaA was found to be dominantly expressed in *S. aureus*, irrespective of their source.

A comprehensive analysis of the exoproteome (p*I* 4-7) of *S. aureus* of similar or distinct genetic backgrounds (based on sequence type) isolated from healthy carriers (n = 6) and different clinical manifestations such as SSTIs (n = 6) and bacteremia (n = 6) was performed. These included ST8, ST30, ST1963 and ST1964 from carriers, ST30, ST239, and ST1 from SSTIs and ST1, ST80, ST1179 and ST1899 from bactermia patients. There was considerable heterogeneity in the exoproteomes even of clonally closely related *S. aureus* isolates. Generally, spot patterns of *S. aureus* isolates within each group were more similar to each other than those of strains obtained from different groups. However, considering the pronounced overall heterogeneity in the exoproteomes of *S. aureus*, the identification of infection-related protein signatures will be challenging.

In two-dimensional gel electrophoresis (2-DGE), twelve exoproteins spots were found to be selectively expressed *in vitro* by bacteremia isolates. These signature proteins were identified as DnaK, Pgk, GroEL, Anae109_2543, PanB, cysteine synthase A, N-acetyltransferase and EF-Tu. In two dimensional-immunoblot (2D-IB), surprisingly, none of them were immunogenic. However, this could not be really concluded that these proteins are not expressed *in vivo* as 2D-IB overlook the elicited antibodies against native proteins as it only detects the antibodies against denatured proteins.

When the immunogenicity of the exoproteins was analyzed, healthy carrier did not elicit strong IgG response to numerous exoproteins when compared to infected groups. However, IsaA was commonly recognized by almost all individual human sera. Signature immunogen spots for different microbe-host interaction outcomes (bacteremia, SSTIs, or healthy carrier) were successfully revealed. Surprisingly, antibody against iron-regulated surface determinant system (Isd) proteins which was previously targeted for vaccine was not expressed throughout different *S. aureus* infection types. Hence, the current result hypothesizes that Isd system proteins may not be good targets for vaccine against *S. aureus* infections.

When inflammatory responses against different infection types and in healthy carriers were investigated through cytometric bead array (CBA), the pattern of cytokines and chemokines production varied among different infection types over the course of infection. Interleukin-6 (IL-6) was significantly higher in most of the *S. aureus* infected patients when compared to healthy carriers ($p < 0.035$). IL-17A in SSTIs patients was observed to be statistically higher than bacteremia patients. Interestingly, bacteremia patients elicited higher titers of monokine induced by interferon-γ (MIG)

than SSTIs patients and healthy carriers during their acute phase and convalescent phase. Further studies on testing the reliability, specificity and sensitivity of the chemokine MIG is recommended to evaluate its potential to be used as biomarker for early diagnosis of bacteremia infection.

In conclusion, the exoproteome of clonally related strains also varies, resulting in different infection types and clinical outcomes. A comprehensive understanding of the exoproteome, as well as the cytokine and chemokine responses during different host-pathogen interaction outcomes has identified potential marker for early diagnosis for bacteremia. Vaccine preparation using Isd proteins need to be re-evaluated for its coverage against array of staphylococcal infections.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PERBANDINGAN ANALISIS EKSOPROTEOM *STAPHYLOCOCCUS AUREUS* YANG DIASINGKAN DARIPADA PEMBAWA ASIMPTOMATIK DAN JANGKITAN PELBAGAI JENIS

Oleh

LIEW YUN KHOON

November 2013

Pengerusi: Profesor Madya Vasanthakumari Neela, PhD

Fakulti: Perubatan dan Sains Kesihatan

Staphylococcus aureus (*S. aureus*) merupakan satu patogen serba boleh yang mampu hidup secara *in vitro* dan *in vivo* dalam pelbagai keaadaan persekitaran. Semua ini disebabkan oleh eksoprotin yang dirembeskan oleh *S. aureus*. Kefahaman tentang eksoproteom *S. aureus* dan tindak balas humoral serta inflamotori adalah penting untuk mengenalpasti keupayaan virulen, penentu diagnosis dan calon vaksin.

Teknik pewarnaan perak dioptimasi sebelum kajian proteomik dimulakan. Pewarnaan perak yang diubahsuai membolehkan titik protin yang mempunyai jisim molekul yang rendah dan titik protin yang kecil digambarkan. Selain daripada itu, peningkatan resolusi untuk titik protin juga dicapai. Pewarnaan perak yang diubahsuai mamperbaiki had pengesanan untuk titik protin di 2-DGE, dari kepekatan protin 0.0048 ke 0.0480 µg/µL.

Corak protin yang serupa tetapi berbeza dari segi intensiti titik protin telah dicerap untuk pencilan *S. aureus* (ST9) daripada khinzir dan pengendali khinzir. Intensiti titik protin daripada pencilan berkaitan khinzir adalah lebih kuat. Variasi titik protin posisi kaitan antara khinzir dan pengendali khinzir adalah sikit. Ini menunjukkan virulen faktor yang diperolehi dari alam sekitar khinzir oleh *S. aureus* mempunyai peluang untuk dihasilkan dalam manusia. IsaA protin adalah protin yang dihasilkan secara dominan oleh semua pencilan *S. aureus* tanpa berkait dengan sumber.

Perbandingan *S. aureus* eksoproteom (p*I* 4-7) dengan latar belakang genetik yang sama atau berbeza juga dijalankan. Ini termasuk strain-strain ST8, ST30, ST1963 dan ST1964 daripada pembawa; ST30, ST239, dan ST1 daripada jangkitan kulit dan tisu lembut; ST1, ST80, ST1179 dan ST1899 daripada bakteremia. Heterogenisiti dalam eksoproteom yang diperolehi daripada strain-strain yang berkait rapat juga boleh dicerap. Variasi ini lagi jelas untuk strain-strain berkait rapat yang diperolehi dari kumpulan pesakit yang berlainan jangkitan. Kelperbagaian corak eksoproteome mencabar kita untuk mengesan unik exoprotin yang berkaitan dengan jenis jangkitan tertentu.

Hanya dua belas unik titik eksoprotin yang dihasilkan secara *in vitro* oleh pencilan bakteremia sahaja boleh dikesan dengan teknik gel elektroforesis dua dimensi (2-DGE). Unik protin ini dikenal sebagai DnaK, Pgk, GroEL, Anae109_2543, PanB, “cysteine synthase A”, N-acetyltransferase dan EF-Tu. Protin-protin itu tidak menunjukkan cirri-ciri immunogenik. Akan tetapi, kita tidak boleh membuat kesimpulan bahawa dua belas protin ini tidak dihasilkan secara *in vivo*. Ini adalah

kerana antibodi yang bertindak balas terhadap struktur protin yang natif mungkin telah terlepas perhatian oleh immunoblot dua dimensi (2D-IB).

Walau bagaimanapun, kita nampak tindak balas IgG terhadap eksoprotin lebih kuat di kumpulan pesakit jika berbanding dengan pembawa yang sihat. Antibodi bertindak ke atas IsaA protin dapat dijumpai untuk semua kumpulan yang dikaji. Unik titik immunogen daripada hasil interaksi hos dan mikrob tertentu berjaya ditemui. Ganjilnya kita nampak antibodi untuk Isd protin tidak selalu dihasilkan semasa jangkitan *S. aureus*. Isd protin pernah dicalonkan sebagai vaksin tetapi tidak berjaya di manusia baru-baru ini. Jadi, Isd protin kemungkinan besar Isd protin tidak selalu dihasilkan semasa jangkitan dan vaksin yang diperbuatkan berdasar Isd protin bukan sasaran yang sesuai.

Corak penghasilan sitokin dan kemokin adalah berbeza antara satu sama lain dalam pelbagai jenis *S. aureus* jangkitan. IL-6 dihasilkan oleh pesakit secara signifikan tinggi jika berbanding dengan pembawa yang sihat ($p < 0.035$). Pesakit SSTIs kebanyakan mempunyai titer IL-17A yang tinggi. Yang penting, pesakit bakterimia memperolehi titer MIG yang signifikan tinggi semasa awal jangkitan dan semasa pemulihan. Dengan itu, MIG dicalonkan sebagai diagnosis untuk bakterimia sekiranya kajian terhadap kebolehpercayaan, kespesifikan dan kepekaan dijalani.

Keseluruhannya, data kita menyimpulkan eksoproteom adalah kelperbagaian untuk strain-strain berasal dari latar belakang genetopik yang sama. Kefahaman secara terperinci atas eksoproteom, tindak balas sitokin dan kemokin untuk hasil interaksi hos dan microb membolehkan kita temui sasaran yang berpotensi untuk diagnosis

bakterimia. Kita juga mencadangkan vaksin terhadap Isd protin perlu dikaji semula untuk melindungi pelbagai jenis jangkitan dari strain-strain *S. aureus*.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my heartfelt gratitude to the chairperson of supervisory committee, Assoc. Prof. Dr. Vasanthakumari Neela, who gave me the opportunity to do this research. She showed her genuine support and guidance throughout this study. She also let me to be independent make decision in research. A huge appreciates to her for her trust in me. She has a very good patience with gracious attitude and put a lot of effort when editing my thesis as well as manuscripts for publication. All of these have indirectly encouraged me to become good personalities in my life. In addition, I deeply thank again Assoc. Prof. Dr. Vasanthakumari Neela for her help in arranging my trip to French conference (ISSSI 2012). I gained valuable experiences during that oversea conference so I thank you with all my heart.

I would also like to thank my members of the supervisory committee, especially grateful to Dr. Rukman Awang Hamat who has motivated me once I was in depression. His encouragement has stimulated me to be a positive thinking person. He also sincerely helped me when I was in emergency. I truly thank Assoc. Prof. Dr. Chong Pei Pei, for her collaboration and providing comfortable facilities for proteomic work before my laboratory received the proteomic equipments.

Furthermore, I would like to thank Dato' DR. Chang Kian Meng, Dato' DR. Ardi bin Haji Awang, Profesor DR. Azhar Md. Zain, DR. Muhaimi Bt. Othman and DR. Basir bin Towil who have approved this current study design.

Thank you to Dr. Syafinaz Amin Nordin for giving me a lot of useful assistance. She had kindly helped me to discuss with the hospital authorities for clinical samples collection. I would also like to thank Mr. Mohd. Zafrul provided me some of the pig associated *S. aureus* isolates. At the same time, I am thankful to Catherine and Fatimah, who kindly supplied me the samples of fish mucus and human plasma protein, respectively. I wish to express my gratitude to Assoc. Prof. Dr. Maha Abdullah for allowing me to use the flow cytometer (Fortessa flow cytometer) in immunology lab. I also wish to thank Assoc. Prof. Dr. Cheah Yoke Kqueen, who helped me on BioNumerics software. Thank you Julia Kolata and Syazwan, I could not have done the 2-dimensional immunoblotting without you both. I want to thank Ms. See Hui Shien, technician of Biomarketing Sevices Sdn. Bhd. for helping me with optimization of flow cytometer setting. In addition, I have to thank Mr. Jason, staff of Chemoscience Sdn. Bhd., who provided the fundamental knowledge on PDQuest software to me.

A special thanks Professor Alex van Belkum, Dr. Willem J. B. van Wamel and Professor Richard V. Goering, who has visited us for their brilliant comments and advice on my study. Furthermore, their outstanding enthusiasms in research and intellectual maturity have inspired me to become a diligent and successful researcher.

I would like to acknowledge Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysis for supporting me in the beginning and offering adequate preparation for the study. I also thank to all my labmates, who has welcomed me into their hearts. You have all put a smile on my face in my long exhausting hours in lab. Thank you very much.

Thank you God who must be helping me. My deep appreciation also goes to GRF and Yayasan Biasiswa Sarawak Tunku Abdul Rahman that sponsored the scholarship to me. Thank you to Pn. Alina for her cooperation in processing my Yayasan Sarawak scholarship payment. On the other hand, my research work was financially supported by Universiti Putra Malaysia through RUGS grant (04-01-09- 0795RU).

Last but not least, I heartfuly thank you to my family, beloved Chuan Loo, Chu Chu, Fei Fei and Bo Bo for their love throughout my life. You all mean the world to me.

I certify that a Thesis Examination Committee has met on 14th November 2013 to conduct the final examination of Liew Yun Khoon on his Doctor of Philosophy thesis entitled "Comparative Analysis of Exoproteome of *Staphylococcus aureus* Isolated from Asymptomatic Carrier and Different Infection Types" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysis [P.U.(A)106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were a follws:

Rajesh a/l Ramasamy, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Zamberi bin Sekawi, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Cheah Yoke Kqueen, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Barbara M. Broker, PhD
Professor
Institute of Immunology and Transfusion Medicine
University Medicine Greifswald
Germany
(External Examiner)

NORITAH OMAR, PhD
AssociateProfessor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 19 December 2013

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Vasanthakumari Neela, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Rukman Awang Hamat, MD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Chong Pei Pei, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

_____
BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

LIEW YUN KHOON

Date: 14th November 2013

TABLE OF CONTENTS