UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION AND CHARACTERIZATION OF BIOFILM-PRODUCING CLINICAL ISOLATES OF Staphylococcus aureus

SALMAN SAHAB ATSHAN

FPSK(p) 2013 8
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DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

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IDENTIFICATION AND CHARACTERIZATION OF BIOFILM-PRODUCING
CLINICAL ISOLATES OF Staphylococcus aureus

By

SALMAN SAHAB ATSHAN

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in fulfilment of the requirements for the degree of Doctor of Philosophy

June 2013
DEDICATION

To my parents, daughter, son and my wife for invaluable support and extraordinary courage
IDENTIFICATION AND CHARACTERIZATION OF BIOFILM-PRODUCING CLINICAL ISOLATES OF Staphylococcus aureus

By

SALMAN SAHAB ATSHAN

June 2013

Chairman: Prof. Mariana Nor Shamsudin, PhD

Faculty: Medicine and Health Sciences

Staphylococcus aureus is considered the major etiological agent of human infections. It is a biofilm-forming bacterium, which embedded itself in a matrix of extracellular polysaccharide (slime), and facilitates the adherence of these microorganisms to biomedical surfaces causing many persistent infections. The main issue with biofilm has become a global public health problem that is impacted by the insufficient management of patients infected with biofilm growth as extremely adaptable to antibiotic pressure. The ability of S. aureus to form biofilm is a long-known fact but the problem involving the issue of biofilm identification has remained since the availability of the phenotypic approach of growth on highly selective or differential media can provide identification of biofilm formation but with a high margin of error through many false negative outcomes. In line with these shortcomings, the present study embarked on
several strategies to overcome the issue of inaccurate biofilm identification through the development of an improved method that can provide positive identification. In this study, it was found that our modified-Congo red agar was significantly different from published-CRA (P <0.05). The modified agar constituents provided not only stable 100% formation of black, also showed very high correlation with standard methods and with the presence of icaADBC biofilm genes. In the second part of the work, the ability to adhere and produce biofilms of genotypically different clones of S. aureus was characterised. The study found the isolates that belonging to similar spa, SCCmec and ST types have similar abilities to produce biofilms. Moreover, isolates that have different spa types showed high variation in their ability to produce biofilms. The results indicate that differences in biofilm production capacities are caused by the differences in surface protein A (spa) type and are not due to differences in MLST and SCCmec types. In the third part of the work, the prevalence and distribution of microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and biofilms genes in different clones of S. aureus were determined. The study found icaADBC, fnbA, eno, ebpS, clfA and clfB genes to be present with a high prevalence and were equally distributed between the various clone types of 60 MSSA and MRSA clinical isolates, while the prevalence of other MSCRAMM genes were found to be variable. In the fourth part of the work, the transcriptional profiles of specific staphylococcal genes encoding MSCRAMMS and icaADBC were determined during gradual changes in complexity of the biofilm production under different growth phases. The results indicate that the relative expression of MSCRAMMS and icaADBC genes in comparison with the phenotypic biofilm morphology can be utilized as a model to study the up- and down- regulation of such genes. Delayed expression of certain genes during stationary phase biofilms grown at significantly higher
levels are considered important for biofilm development and for the survival of composing cells in a nutrient-scarce niche. In the fifth part of this work, the extracellular 2DE protein profiles among genotypically different clone types and under different time-points of biofilm developed growth of *S. aureus* were characterized. The main results of 2DE studies showed a high degree of extracellular protein heterogeneity among the various clone types and under different time-points growth, indicating that different regulation modes of growth processes are operating under different clone types and under altered time conditions. In the sixth part of this work, the antimicrobial susceptibility patterns (glycopeptide, β-lactam, lipopeptide, oxazolidinones and glycylicycline) of different *S. aureus* clone types were determined. The results revealed that MICs and the bactericidal activities of these agents’ classes within the different *spa* types were largely different. However, the MIC and MBC among clones within the same *spa* and MLST type were slightly different. Moreover, the minimum biofilm reduction concentrations (MBRCs) of these agents in the prevention of biofilm formation *in vitro* were overall greater than the CLSI-defined planktonic MIC breakpoint for resistance and quite variable among different clone types. The diversity in the antibiotic susceptibilities of isolates within the various clone types emphasises the need for continuous monitoring for the clones and clinicians should consider a correct antibiotic rather than empirical treatment. In the last part of this study, the effect of sub-inhibitory concentrations of vancomycin and tigecycline on the steady-state mRNA transcription levels of MSCRAMM and the *icaADBC* target gene, as well as on secretion of exoproteins of different clone types of *S. aureus* isolates were studied. The results indicate that the effects of these antibiotics generally affecting all virulence factors of selected target genes and the secretion of exoproteins. Thus might enhance
the virulence of this bacterium, therefore using these antibiotics to treat *S. aureus* infections may contribute to unpredictable results.

**Conclusion:** We conclude that a considerable difference exists among similar and various clone types of *S. aureus*. This variation could have contributed to the degree of virulence even within the same clone and enhanced heterogeneity in the infection potential. Thus, new genetic diversity suggests that the development of a rapid and precise identification profile for each clone type in human infections is very important to prescribe appropriate antibiotics and reduce the empirical treatment.
Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGENALPASTIAN DAN PENCIRIAN Staphylococcus aureus PENGHASIL BIOFILEM DARI ISOLAT KLINIKAL

Oleh

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Staphylococcus aureus dianggap ejen etiological utama jangkitan manusia. Ia adalah bakteria biofilm-membentuk, yang tertanam sendiri dalam matriks polisakarida extracellular (lendir), dan memudahkan pematuhan ini mikroorganisma pada permukaan bioperubatan menyebabkan banyak jangkitan berterusan. Isu utama dengan lapisan yang telah menjadi satu masalah kesihatan awam global yang dipengaruhi oleh pengurusan yang tidak mencukupi pesakit yang dijangkiti dengan pertumbuhan biofilm sebagai sangat cepat menyesuaikan diri kepada tekanan antibiotik. Kebolehan S. aureus untuk menghasilkan biofilem telah lama diketahui namun masalah yang melibatkan penentuan biofilem masih wujud disebabkan kaedah penentuan berdasarkan fenotipik memerlukan medium yang sangat spesifik dan berbeza. Kaedah ini berupaya menentukan biofilem namun kadar kesalahan adalah tinggi disebabkan keputusan
yang salah-benar. Justeru, sejajar dengan keperluan yang sangat tinggi, kajian ini mengemukakan beberapa strategibagi mengatasi isu pengenalan biofilm tepat melalui pembangunan kaedah yang lebih baik yang boleh memberikan pengenalan positif. Keputusan yang didapati daripada CRA yang telah diubahsuai dalam kajian ini adalah berbeza secara signifikan daripada CRA yang telah diterbitkan (P<0.05). Kandungan agar yang diubahsuai bukan sekadar menghasilkan 100% pigmen hitam yang stabil malah menunjukkan hubungkait yang sangat tinggi dengan kaedah piawai dan kehadiran gen biofilem icaADBC. Bahagian kedua kajian, ini melibatkan pencirian kebolehan pelbagai klon S. aureus yang berbeza secara genotipik untuk melekat dan menghasilkan biofilm. Kajian ini telah menunjukkan bahawa pencilalan yang mempunyai jenis spa, SCCmec dan MLST yang sama mempunyai kebolehan yang sama untuk menghasilkan biofilm. Tambahan pula pencilalan yang mempunyai jenis spa yang berbeza menunjukkan variasi yang tinggi dalam kebolehan mereka untuk menghasilkan biofilm. Keputusan ini menunjukkan bahawa perbezaan kapasiti dalam penghasilan biofilm adalah disebabkan oleh perbezaan jenis protein permukaanA (spa) dan bukan disebabkan oleh perbezaan jenis MLST dan SCCmec. Pada bahagian ketiga kajian, kelaziman dan taburan molekul-molekul komponen matriks lekit permukaan mikrob (MSCRAMMs) dan gen-gen pada klon-klon berbeza S. aureus telah ditentukan. Kajian mendapat bahawa icaADBC, fnbA, eno, ebps, gen-gen clfA and clfB hadir dengan kelaziman yang tinggi dan sekata di antara berbagai-bagai klon jenis 60 MSSA and MRSA dari pencilalan klinikal, Kelaziman gen-gen MSCRAMM pula adalah pelbagai. Pada bahagian keempat kajian, profil transkripsi gen-gen khusus staphylococcal yang mengekod MSCRAMMS and icaADBC telah ditentukan semasa perubahan kompleksiti penghasilan biofilm yang konsisten pada masa pertumbuhan yang berbeza. Keputusan menunjukkan bahawa ekspresi relatif gen-gen MSCRAMMS dan icaADBC

**kesimpulan:** Kami menyimpulkan bahawa perbezaan yang besar wujud di kalangan jenis klon yang serupa dan pelbagai S. aureus. Perubahan ini boleh menyumbang kepada tahap kejahatan walaupun dalam genotip yang sama klon dan kepelbagaian dipertingkatkan dalam potensi jangkitan. Oleh itu kepelbagaian genetik baru menunjukkan bahawa pembangunan profil pengenalan pesat dan tepat bagi setiap jenis klon dalam jangkitan manusia adalah sangat penting untuk menetapkan antibiotik yang betul dan mengurangkan rawatan empirikal.
ACKNOWLEDGEMENTS

In the Name of Allah the Compassionate the Merciful

First of all I am thankful to Allah (S.W.T), the Allah Mighty, who blessed me strength and courage to complete this work and make this day possible.

I would like to thank my supervisor, Professor. Dr. Mariana Nor Shamsudin, for her guidance, advice and support throughout my work. She has helped me a lot from the beginning of my program at Universiti Putra Malaysia, I am really unable to find apt words of appreciation for the help she did, and I don’t intend to exaggerate with words. All I want to say is sincere and straight from my heart: ‘thank you’

I am also very grateful to my co-supervisor, Professor Dr. Zamberi Sekawi, Associate Professor Dr. Rukman Awang Hamat, Dr. Leslie Than Thian Lung and Associate Professor Chong Pei Pei for their help and constructive and criticism during my work.

I would like to thank all lecturers for their advice and their patience. Deep thanks to Encik Zainan Ahmed Ariffin, Encik yousef, and Encik Zainal for their guidance and help and also I am extremely grateful and appreciative of all staff members and postgraduate students in the Department of Medical Microbiology.

I would like to express my gratitude to my wife Salwa A. Abduljaleel for her affection and constant support. Deep thanks to my brother Dr. Saddam for his understanding, encouragement and open- mindedness.

Last but not least, my deep apologizes for my mistakes during this time period and deep apology to all the peoples that a name didn’t mentioned here.
I certify that a Thesis Examination Committee has met on 28.6.2013 to conduct the final examination of Salman Sahab Atshan on his thesis entitled “identification and characterization of biofilm-producing clinical isolates of Staphylococcus aureus” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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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.

SALMAN SAHAB ATSHAN

Date: 28 June 2013
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