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ELUCIDATION OF GENETIC DIVERSITY IN METHICILLIN-RESISTANT *Staphylococcus aureus* ISOLATED FROM CANCER AND NON-CANCER PATIENTS IN MALAYSIA AND SAUDI ARABIA

ALRESHIDI, MATEQ ALI A

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By

ALRESHIDI, MATEQ ALI A

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Dedicated to

Prophet Mohammad

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ALRESHIDI, MATEQ ALI A

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Chairman: Professor Mariana Nor Shamsudin, PhD

Faculty: Medicine and Health Sciences

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a versatile pathogen capable of causing a wide range of human diseases and became a leading cause of nosocomial infections worldwide. Cancer patients are unique cohort with multiple risk factors for MRSA infection. Since a little is known about the characteristics of MRSA strains among the hospitalized cancer patients in Malaysia and Saudi Arabia, it is important to elucidate the phenotypic and genotypic characteristics of local MRSA clones for the efficient management of infection in cancer patients. In the current study, a total of 240 non-consecutive MRSA isolates were obtained from cancer and non-cancer patients in Malaysia and Saudi Arabia (60 each). The majority of MRSA isolates were multiresistant to more than four classes of antibiotics. Five and three antibiotic susceptibility profiles were observed among the MRSA isolates from cancer and non-cancer patients in

Malaysia. For the isolates from Saudi Arabia, five and 14 antibiotic susceptibility profiles were observed among the MRSA isolates from cancer and non-cancer patients, respectively. Three isolates were vancomycin-intermediate (VISA) however, all of them were susceptible to daptomycin. Although there was no statistical significance between the susceptibility of isolates from cancer and non-cancer patients, the high level of multiple drug resistance among MRSA isolated from cancer patients in both countries was observed. In addition, the susceptibility of all MRSA isolates against three antiseptics agents; benzalkonium chloride (BAC), benzethonium chloride (BZT) and chlorhexidine digluconate (CHG) were determined. All isolates were susceptible to all tested antiseptics with MIC ranging from 0.5-2 µg/ml. Antiseptic resistance gene *qacA/B* was detected in 98.3% and 83.3% of the isolates from cancer and non-cancer in Malaysia respectively. For the isolates from Saudi Arabia, *qacA/B* was detected in 46% and 35% from cancer and non-cancer, respectively. *Smr* gene was detected in one isolate each from cancer and non-cancer patients in Malaysia. The carriage of *qacA/B* highly correlated with reduced susceptibility to CHG and BAC.

Spa typing revealed four different *spa* types in the isolates from Malaysia. Eleven and 25 *spa* types were detected among isolates from cancer and non-cancer patients in Saudi Arabia, respectively including four new *spa* types identified in this study. All isolates from Malaysia belonged to ST239 whereas six and nine STs were detected among isolates from cancer and non-cancer patients in Saudi Arabia, respectively. Three *agr* types were detected in this study; the majority of MRSA isolates belonged to *agr* I. *Agr* III was detected in 25 and 17 isolates from cancer and non-cancer patients, respectively,

whereas *agr* II was detected in five isolates from non-cancer patients in Saudi Arabia. No *agr* type IV was detected in this study. Virulence genes profiling showed that all strains were commonly positive for adhesion genes except *ebps* and *bbp* genes which were not detected in any isolate. Although the presence of adhesion genes slightly varied among MRSA isolates from cancer and non-cancer patients, these variations were not found to be statistically significant. In contrast, the presence of toxin genes *seb*, *sec*, *seg* and *sei* was found to be significant between cancer and non-cancer patients, these significances were not consistent between isolates from cancer and non-cancer in both countries.

Relative quantitative real-time reverse transcriptase polymerase chain reaction (qPCR) assay was designed and applied in order to study the expression levels of selected genes encoding the adherence and toxins virulent factors. Relative quantification qPCR showed a significant higher expression level of common genes tested among strains isolated from cancer patients not only within the clone but also among different lineages.

In conclusion, this study demonstrated that although all MRSA strains studied from cancer and non-cancer patients possessed several virulence determinants, the isolates from cancer patients were more multiresistance to antibiotics with low susceptibility towards antiseptic agents and the expression rather than carriage of virulence determinants may mediate higher pathogenicity potential. These data will aid in developing more effective infection control strategy to improve the management of MRSA infection in cancer patients.

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

PERUNGKAIAN KEPELBAGAIAN GENETIC *Staphylococcus aureus* TAHAN METHICILLIN YANG DIPENCILKAN DI KALANGAN PESAKIT-PESAKIT KANSER DARI MALAYSIA DAN ARAB SAUDI

Oleh

ALRESHIDI, MATEQ ALI A

Februari 2013

Pengerusi: Profesor Mariana Nor Shamsudin, PhD

Fakulti: Perubatan dan Sains Kesihatan

Staphylococcus aureus tahan methicillin (MRSA) merupakan satu patogen serba boleh yang mampu menyebabkan pelbagai penyakit di kalangan manusia dan menjadi punca utama jangkitan nosokomial di seluruh dunia. Pesakit kanser adalah satu kohort yang unik dan mempunyai pelbagai faktor-faktor risiko bagi jangkitan MRSA. Oleh kerana sedikit sahaja yang diketahui tentang ciri-ciri strain MRSA di kalangan pesakit-pesakit kanser di Malaysia dan Arab Saudi, satu kajian perlu dijalankan bagi menjelaskan ciri-ciri fenotip dan genotip MRSA klon-klon tempatan supaya pengurusan jangkitan MRSA pada pesakit kanser dapat dibuat dengan lebih berkesan. Di dalam kajian ini, sejumlah 240 pencilan MRSA telah diperolehi secara tidak berturutan daripada pesakit-pesakit kanser dan bukan kanser dari Malaysia dan Arab Saudi (60 setiap satu). Majoriti pencilan

MRSA adalah multiresistan terhadap lebih daripada empat kelas antibiotik. Lima dan tiga profil kerentanan antibiotik telah diperhatikan di kalangan pencilan MRSA daripada pesakit-pesakit kanser dan bukan kanser di Malaysia. Bagi pencilan dari Arab Saudi, masing-masing lima dan 14 profil kerentanan antibiotik telah diperhatikan di antara strain MRSA daripada pesakit-pesakit kanser dan bukan kanser. Tiga pencilan menunjukkan rintangan-pertengahan terhadap vancomycin (VISA), walau bagaimanapun, kesemua mereka adalah rentan terhadap daptomycin. Walaupun secara statistiknya tidak ada sebarang perbezaan terhadap kerentanan pencilan MRSA di antara pesakit kanser dan bukan kanser, tahap kerintangan yang tinggi terhadap pelbagai-bagai jenis antimikrob telah diperhatikan di kalangan pencilan MRSA dari kedua-dua buah negara.

Di samping itu, kerentanan pencilan MRSA terhadap tiga ejen antiseptik; benzalkonium klorida (BAC), klorida benzethonium (BZT) dan chlorhexidine digluconate (CHG) telah dapat ditentukan. Semua pencilan adalah rentan kepada semua antiseptik yang diuji dengan MIC antara 0,5-2 μg / ml. Gen rintangan antiseptik qacA/B telah dikesan sebanyak 98.3% dan 83.3% pencilan masing-masing daripada pesakit-pesakit kanser dan bukan kanser di Malaysia. Bagi pencilan dari Arab Saudi, gen qacA/B telah dikesan, masing-masing sebanyak 46% dan 35% daripada pesakit-pesakit kanser dan bukan kanser. Pembawaan qacA/B adalah sangat berkait rapat dengan pengurangan tahap kerentanan terhadap CHG dan BAC.

Pengetipan spa telah dapat mengasingkan empat jenis spa yang berlainan daripada pencilan dari Malaysia. Sebelas dan 25 jenis spa telah juga dikesan masing-masing di kalangan pesakit-pesakit kanser dan bukan kanser di Arab Saudi, termasuk juga empat

spa jenis baharu yang dikenal pasti di dalam kajian ini. Semua pencilan dari Malaysia tergolong di dalam ST239 manakala enam dan sembilan jenis STs telah dikesan masing-masing di kalangan pesakit-pesakit kanser dan bukan kanser di Arab Saudi. Tiga jenis kumpulan agr telah juga dapat dikesan di dalam kajian ini; secara majoritinya pencilan MRSA tergolong di dalam jenis kumpulan agr I. Jenis kumpulan agr III telah dikesan masing-masing sebanyak 25 dan 17 pencilan daripada pesakit-pesakit kanser dan bukan kanser, manakala kumpulan agr II telah dikesan pada lima pencilan daripada pesakit-pesakit bukan kanser dari Arab Saudi. Tiada jenis kumpulan agr IV dikesan di dalam kajian ini. Profil kevirulenan gen menunjukkan bahawa kesemua pencilan mempunyai gen-gen lekatan kecuali gen ebps dan bbp. Walaupun penemuan gen lekatan berbeza sedikit di dalam pencilan MRSA di kalangan pesakit-pesakit kanser dan bukan kanser, perbezaan ini adalah tidak signifikan secara statistiknya. Berbeza sekali, penemuan gen-gen toxin seb, sec, seg dan sei di antara pesakit-pesakit kanser dan bukan kanser adalah signifikan tetapi signifikasi tersebut tidak konsisten di antara pencilan-pencilan daripada pesakit kanser dan bukan kanser dari kedua-dua buah negara.

Relatif kuantitatif tindakbalas bersilang real-time (qPCR) asai telah direka dan digunakan untuk mengkaji tahap ekspresi gen-gen pengkodan yang dipilih seperti gen pelekatan dan toksin. Relatif kuantifikasi qPCR telah menunjukkan terdapat signifikasi tentang tahap ekpresi gen yang tinggi di kalangan strain-strain yang diperolehi daripada pesakit kanser bukan sahaja didapati di dalam klon tunggal tetapi juga di kalangan garis keturunan yang berbeza.

Kesimpulannya, kajian ini menunjukkan bahawa walaupun kesemua strain MRSA dikaji daripada pesakit kanser dan bukan kanser mempunyai beberapa penentu kevirulenan dengan latar belakang molekul yang sama, pencilan daripada pesakit kanser mempunyai lebih multi kerintangan terhadap antibiotik dan berkecenderungan mempunyai kerentanan yang rendah terhadap agen antiseptik dan ekpresi gen bukannya pembawaan penentu kevirulenan yang berkemungkinan menyebabkan potensi kepatogenan yang lebih tinggi. Data-data ini dijangka dapat membantu di dalam merangka strategi kawalan jangkitan yang lebih berkesan bagi memperbaiki mutu pengurusan jangkitan MRSA pada pesakit-pesakit kanser.

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

Mariana Nor Shamsudin, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Ali Abdullah Alsalamah, PhD
Professor
Faculty of Science
King Saud University
(Member)

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

Rukman Awang Hamat, Medical Doctor
Lecturer Medicine
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 or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

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Date: 15 February 2013

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