ELUCIDATION OF GENETIC DIVERSITY IN METHICILLIN-RESISTANT
Staphylococcus aureus ISOLATED FROM CANCER
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Dedicated to

Prophet Mohammad
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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

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February 2013

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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

iii
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

PERUNGAIAN 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

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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). Mayoriti 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...

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 ekspresi 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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Alreshidi Mateq Ali
Serdang, February 2013
I certify that a Thesis Examination Committee has met on 15 February 2013 to conduct the final examination of Alreshedi Mateq Ali A on his thesis entitled "Elucidation of Genetic Diversity in Meticillin-resistant Staphylococcus aureus Isolated from Cancer and Non-cancer Patients in Malaysia and Saudi Arabia" in accordance with the Universities and University College 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 or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

ARESHIDI MATEQ ALI A

Date: 15 February 2013
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>x</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>xii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xxi</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxiv</td>
</tr>
</tbody>
</table>

CHAPTER

1 INTRODUCTION

2 LITERATURE REVIEW

2.1 The genus Staphylococcus

2.2 Morphological and Biochemical Characteristics

2.3 Emergence of MRSA

2.4 Genome

2.4.1 Core Genome

2.4.2 Accessory genome

2.5 Resistance of MRSA to Antimicrobial agents

2.5.1 Resistance to antibiotics

2.5.2 Resistance to antiseptics

2.6 Prevalence and rise of incidence of MRSA infection

2.7 Virulence factors of MRSA

2.7.1 Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs)

2.7.2 Toxins

2.7.3 Accessory gene regulator (agr)

2.7.4 Biofilm associated proteins

2.8 Typing

2.8.1 SCCmec Typing

2.8.2 Multi-Locus Sequence Typing (MLST)

2.8.3 Spa Typing

2.9 Worldwide distribution of MRSA clones and status in Malaysia and Saudi Arabia

xv
2.10 Gene Expression
2.10.1 Quantitative real-time Polymerase Chain Reaction (qPCR) 40
2.11 MRSA and Cancer 45

3 MATERIALS AND METHOD 48
3.1 Bacterial isolates 48
3.2 Confirmation of isolates by conventional techniques 49
  3.2.1 Gram Stain 49
  3.2.2 Growth on Mannitol-salt Agar 50
  3.2.3 Catalase test 50
  3.2.4 Coagulase test 51
3.3 Antimicrobial susceptibility tests 51
  3.3.1 Disk diffusion test 51
  3.3.2 D-Zone test 53
  3.3.3 Minimum inhibitory concentration (MIC) test for vancomycin 54
  and daptomycin
3.4 Molecular identification of MRSA isolates 55
  3.4.1 DNA extraction 55
  3.4.2 DNA quality and quantity determination 56
  3.4.3 Detection of mecA and pvl genes gene 56
  3.4.4 Electrophoresis 57
3.5 Study I: Genotyping of MRSA 58
  3.5.1 SCCmec typing 58
  3.5.2 Staphylococcus aureus protein A (spa) typing 59
  3.5.3 Multi-Locus Sequence Typing (MLST) 62
  3.5.4 Agr typing 64
3.6 Study II: To investigate the virulence gene profiles of MRSA in 65
  cancer patients from Malaysia and Saudi Arabia
  3.6.1 Detection of adhesion (MSCRAMM) genes 65
  3.6.2 Detection of toxin genes 66
3.7 Study III: To determine the prevalence of antiseptic resistance genes 68
  and examine the efficacy of the selected antiseptic agents against
  MRSA
  3.7.1 Detection of antibiotic and antiseptic resistance genes 68
  3.7.2 Determination of MIC for antiseptic agents 69
3.8 Study IV: To establish a quantitative Real-Time PCR assay and 70
  investigate the quantitative gene expression in selected genes within
  strains isolated from cancer and non-cancer patients
  3.8.1 RNA extraction 70
  3.8.2 DNase treatment 71
3.8.3 Measurement of RNA concentration purity and integrity 71
3.8.4 cDNA Synthesis 72
3.8.5 Primer design 73
3.8.6 PCR efficiency 73
3.8.7 Determination of reference gene expression stability 73
3.8.8 Quantitative real-time Polymerase Chain Reaction (qPCR) 74
3.9 Statistical analysis 76

4 RESULTS 77
4.1 Description of samples and patient demographics 77
4.1.1 MRSA isolates from Malaysia 77
4.1.2 MRSA isolates from Saudi Arabia 78
4.2 Confirmation of isolates by conventional techniques 79
4.2.1 Culture media 79
4.2.2 Biochemical reaction 79
4.2.3 Oxacillin and Cefoxitin Susceptibility Test for MRSA Isolates by disc diffusion method 80
4.3 Antibiotic susceptibility test 81
4.3.1 Antibiotic resistance Profile 81
4.3.2 Detection of inducible clindamycin resistance (D-test) 84
4.3.3 Minimum inhibitory concentrations for vancomycin and daptomycin 87
4.4 Molecular identification of MRSA isolates 88
4.4.1 Total genomic DNA extraction 88
4.4.2 Detection of mecA and pvl genes 89
4.5 Study I: Genotyping of MRSA 90
4.5.1 SCCmec typing 90
4.5.2 Spa typing 92
4.5.3 Multi-locus sequence typing (MLST) 99
4.5.4 Determination of agr types 101
4.6 Study II: To investigate the virulence gene profiles of MRSA from two countries 103
4.6.1 Adhesion genes 103
4.6.2 Toxins genes 113
4.7 Association of virulence genes and clones 125
4.8 Study III: To determine the prevalence of antiseptic resistance genes and examine the efficacy of the selected antiseptic agents against MRSA isolates 128
4.8.1 Detection of antiseptic resistance qacA/B and smr genes 128
4.8.2 Minimum inhibitory concentrations for antiseptic agents 129
4.9 Study IV: To investigate the quantitative gene expression in selected genes among strains isolated from cancer and non-cancer patients

4.9.1 RNA concentration, purity and integrity

4.9.2 Validation of reaction specificity and efficiency

4.9.3 Identification of optimal reference genes

4.9.4 Relative expression of MRSA virulence genes by qRT-PCR

5 DISCUSSION

6 CONCLUSIONS

REFERENCES

APPENDICES

BIODATA OF STUDENT

LIST OF PUBLICATIONS