COMPARATIVE GENOMICS ANALYSIS AND MOLECULAR CHARACTERIZATION OF COMMUNITY AND HOSPITAL-ASSOCIATED METHICILLIN-RESISTANT

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FPSK(p) 2020 12

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By

ZARIZAL BIN SUHAILI

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

December 2019

DEDICATION

This thesis is dedicated to my parents, my wife and children

With love, respect and a bunch of memories

Indeed, we belong to Allah and indeed to Him we will return.

So which of the favors of your Lord would you deny?

Ar-Rahman [Verse:16].

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

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December 2019

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Published genomics data regarding CA-MRSA and HA-MRSA isolates from Malaysia are scarce. Therefore, the aim of this study was to characterise the Malaysian CA-MRSA and HA-MRSA isolates in depth using molecular typing, as well as whole-genome sequencing. CA-*S. aureus* isolates were obtained from the nasal swabs of undergraduate students whereas HA-*S. aureus* isolates were archived samples obtained from hospital laboratories. Out of 168 *S. aureus* nasal carriage isolates obtained, the occurrence of MRSA was 8.3% and 15% among undergraduate students of the agriculture biotechnology and health sciences programmes, respectively, with multidrug resistance were observed in 15% (26/168) of the isolates. Among the 146 hospital archived samples of *S. aureus*, 28% (41/146) were MRSA, out of which 63% (i.e. 26/41) were categorised as multidrug-resistant (MDR; resistant to three or more classes of antimicrobial compounds).

The most predominant *SCCmec* type among both CA- and HA-MRSA was *SCCmec*-III, with the highest occurrences observed among HA-MRSA (68%; 28/41) compared to CA-MRSA (41%; 9/22) isolates. Other *SCCmec* types that were found in CA-MRSA were *SCCmec*-IV (32%), *SCCmec*-I (23%), and *SCCmec*-II (4%), while in HA-MRSA were *SCCmec*-IV (22%), *SCCmec*-V (7%), and *SCCmec*-I (2%). *spa* type t037 was the most predominantly found among CA-MRSA (50%) and HA-MRSA (61%). Higher occurrence of the *pvl* gene (which encodes the pore-forming Panton-Valentine leucocidin) at 27% (11/41) among HA-MRSA compared to CA-MRSA at 23% (5/22). Another virulence factor, the staphylococcal surface protein gene (*sasX*), was more prevalent in HA-MRSA at 61% (25/41) compared with CA-MRSA (50%; 11/22).

Nine *S. aureus* isolates representing HA-MRSA (SAZ_1, SAZ_10, SAZ_16, SAZ_31, and KT/Y21), hospital-associated methicillin-susceptible *S. aureus* (HA-MSSA, M314250), and CA-MRSA (ZS_Z30, ZS_Z37 and ZS_Z46) were subjected to whole-genome sequence analysis. Genomic content of all isolates were diverse with the presence of various mobile genetic elements (MGEs) such as insertional sequence (IS), transposons, plasmids, genomic islands (νSAα and νSAβ), phages, Staphylococcal pathogenicity islands (SaPIs: SaPI1, SaPI2, SaPI3, and SaPI5) as well as both virulence and resistance determinants. In-silico, MLST and *spa* type of nine representative isolates revealed five sequence types (ST)-*spa* types combinations, i.e. ST1-t127 (M314250), ST30-t122 (SAZ_31), ST239-t037 (SAZ_10, ZS_Z30, ZS_Z37, and ZS_Z46), ST239-t421 (SAZ_1), and ST772-t657 (SAZ_16 and KT/Y21). Interestingly, one of the HA-MRSA isolates, SAZ_10, harboured a novel 35 kb conjugative plasmid designated pSAZ10A that carried multiple antimicrobial resistance (AMR) determinants (*aadD*, *ileS*, and *tcaA*) as well as complete conjugative transfer (*tra*) genes.

Genome analyses showed the presence of multidrug efflux pumps and AMR genes, which were likely to contribute to the MDR phenotypes in these isolates. Moreover, various virulence factors were revealed among both CA and HA-MRSA isolates, which were likely to play essential roles in their pathogenesis. Pan-genome analysis of all representative isolates and other 49 global reference strains revealed an open genome with a large accessory genome allowing acquisition of exogenous DNA and facilitating successful adaptation to the selective hospital and community environment.

Thus, this analysis provided us an insight into the characteristics and adaptability related to both virulence and resistance of MRSA and MSSA of Malaysian isolates, which will assist in the surveillance, prevention and control of pathogenic *S. aureus* particularly in community- and hospital-associated environment.

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

ANALISIS PERBANDINGAN GENOMIK DAN PERINCIAN MOLEKULAR TERHADAP *Staphylococcus aureus* RINTANG-METISILIN BERKAITAN KOMUNITI DAN HOSPITAL

Oleh

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Penerbitan data genomik berkaitan pencilan CA-MRSA and HA-MRSA di Malaysia adalah terhad. Oleh itu, matlamat utama kajian ini adalah untuk mencirikan CA-MRSA dan HA-MRSA Malaysia secara mendalam menggunakan penentuan molekular dan juga penjujukan keseluruhan genom. Pencilan CA-*S. aureus* diperolehi dari calitan rongga hidung pelajar sarjana muda, manakala pencilan HA-*S. aureus* diperolehi dari sampel arkib makmal hospital. Daripada 168 pencilan bawaan rongga hidung yang diperolehi, kehadiran MRSA di kalangan pelajar sarjana muda bioteknologi pertanian dan sains kesihatan masing-masing adalah pada 8.3% dan 15%. Ciri rintang pelbagai ubatan diperhatikan dalam 15% (26/168) dari pencilan terbabit. Daripada 146 sampel arkib hospital *S. aureus*, 28% (41/146) adalah MRSA, di mana 63% (i.e. 26/41) dikategorikan sebagai rintang pelbagai ubatan (MDR; rintang terhadap tiga atau lebih kelas sebatian antimikrob).

Jenis *SCCmec* yang utama di kalangan kedua-dua CA- dan HA-MRSA adalah *SCCmec*-III, dengan peratusan yang paling tinggi diperhatikan dalam kalangan HA-MRSA (68%; 28/41) berbanding pencilan CA-MRSA (41%; 9/22). Jenis *SCCmec* lain yang didapati dari CA-MRSA adalah *SCCmec*-IV (32%), *SCCmec*-I (23%), dan *SCCmec*-II (4%), manakala dari HA-MRSA adalah *SCCmec*-IV (22%), *SCCmec*-V (7%), dan *SCCmec*-I (2%). Tambahan pula, 8 dan 10 jenis *spa* berbeza telah diperolehi dari kalangan pencilan CA- dan HA-MRSA, dengan jenis *spa* t037 yang paling utama (CA-MRSA; 50%, HA-MRSA; 61%). Pencilan HA-MRSA memperlihatkan peratusan tertinggi gen *pvl* (mengkodkan pembentuk-liang Panton-Valentine leucocidin) pada 27% (11/41) berbanding CA-MRSA pada 23% (5/22). Gen protein permukaan staphylococcal (*sasX*) merupakan sejenis faktor virulen yang lebih lazim terdapat pada HA-MRSA dengan 61% (25/41) berbanding CA-MRSA (50%; 11/22).

Sembilan pencilan mewakili HA-MRSA (SAZ_1, SAZ_10, SAZ_16, SAZ_31, dan KT/Y21), *S. aureus* rentan-meticilin berkaitan-hospital (HA-MSSA, M314250) dan CA-MRSA (ZS_Z30, ZS_Z37, dan ZS_Z46) telah dipilih untuk analisis penjujukan keseluruhan genom. Kandungan genomik kesemua pencilan adalah berbeza dengan kehadiran pelbagai unsur genetik mudah alih (MGEs) seperti jujukan sisipan (IS), transposon, plasmid, pulau genom (νSAα dan νSAβ), faj, pulau patogenisiti Staphylococcal (SaPIs: SaPI1, SaPI2, SaPI3, and SaPI5) di samping kedua-dua penentu virulen dan ketahanan. *In-silico*, MLST dan jenis *spa* dari sembilan wakil pencilan menunjukkan lima jenis kombinasi jujukan (ST)-*spa* iaitu ST1-t127 (M314250), ST30-t122 (SAZ_31), ST239-t037 (SAZ_10, ZS_Z30, ZS_Z37, dan ZS_Z46), ST239-t421 (SAZ_1), dan ST772-t657 (SAZ_16 dan KT/Y21). Menariknya, salah satu pencilan HA-MRSA, SAZ_10, mengandungi suatu plasmid konjugatif baru 35 kb dikenali sebagai pSAZ10A yang membawa pelbagai penentu (*aadD, ileS*, dan *tcaA*) rintangan antimikrob (AMR) di samping gen pemindahan konjugatif (*tra*) yang lengkap.

Analisa genom menunjukkan kehadiran pam efluks pelbagai ubatan dan gen AMR, yang menyumbang kepada fenotip MDR dalam pencilan terbabit. Tambahan pula, pelbagai faktor virulen dipamerkan oleh kedua-dua pencilan CA dan HA-MRSA, yang memainkan peranan penting dalam pathogenesis. Analisa pan-genom terhadap kesemua wakil pencilan dan 49 strain rujukan global mempamerkan genom terbuka dengan kehadiran aksesori genom yang besar bagi memperolehi DNA eksogenus dan memudahkan penyesuaian terhadap persekitaran hospital terpilih dan komuniti.

Oleh itu, analisis ini memberi gambaran tentang ciri-ciri dan kebolehsesuaian yang berkaitan dengan kedua-dua pencilan virulen dan rintangan MRSA dan MSSA dari Malaysia yang dapat membantu dalam pengawasan, pencegahan dan kawalan patogenik *S. aureus* terutamanya dalam persekitaran berkaitan komuniti dan hospital.

ACKNOWLEDGEMENTS

With the name of Allah, the Al-mighty, most compassionate and merciful with His blessing giving me the strength and passion, to finish the research until this thesis completed. I also would like to take this opportunity to thanks to number of peoples that have helped made this journey worth.

To my supervisor, Prof. Madya Dr. Mohd Nasir Mohd Desa. Many thanks for giving these valuable opportunities to explore more on the current state-of-the arts of research. Being humble and tolerable supervisor not forget the guidance as well as highly motivational advices, truly courage and support in finishing the learning process. Appreciation also goes to my committee members, Prof. Madya Dr Syafinaz Amin Nordin, Prof. Dr. Yeo Chew Chieng and Dr Faizal Ghazali for all the unconditional technical supports and valuable guidance.

To the members of Dr Nasir's Laboratory, Mr Sabri. All the prostgraduates survivors Dr Abdul Rahman, Diana Drazaly, Abdulrahman Mansoor, Nurul Hana Zainal Baharin, Nor Asmara Tasrip and Norhidayah Mat Azis.

I would like to express my deepest gratitude and thanks to my beloved Abah Suhaili bin Sani and Mak Tubiah Binti Ahmad and all family members for understanding, love and supports.

Not forgetting my soulmates, Nor Maizalina Binti Inan and two lovely daughters, Zara Afiqah and Zara Aimy, love till the end.

Alhamdulillah, akhirnya sempurna.

This thesis was 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:

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphosim
agr	Accessory gene regulator typing
AST	Antimicrobial susceptibility test
ATCC	American Type Culture Collection
AMR	Anti-microbial Resistant
BSAC	British Society for Antmicrobial Chemotheraphy
BPGA	Bacterial Pangenome Analysis Pipeline
CA-MRSA	Community-associated Methicillin-Resistant *Staphylococcus aureus*
CARD	Comprehensive Antimicrobial Resistance Database
CC	Clonal complex
CDS	Coding sequence
CLSI	Clinical and Laboratory Standards Institutes
$cMLS_B$	Constitutive Macrolides-Lincosamides-Streptogramin B
COG	Clusters of orthologous groups
CoNS	Coagulase negative staphylococci
CoPS	Coagulase positive staphylococci
CSAR	Contigs Scaffolding using Algebraic Rearrangement
ddH_2O	double-distilled water
ddNTPs	dideoxynucleotides triphosphates
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EUCAST	European Committee on Antimicroial Susceptibility Testing
HA-MRSA	Hospital-associated Methicillin-Resistant *Staphylococcus aureus*
HGT	Horizontal genome
HUSM	Hospital Universiti Sains Malaysia
HCW	Health care workers
$iMLS_B$	inducible Macrolides-Lincosamides-Streptogramin B
KEGG	Kyoto encyclopedia of genes and genome
LA-MRSA	Livestock-associated Methicillin-Resistant *Staphylococcus aureus*
Mbp	Megabase pairs
MGE	Mobile genetic elements

MIC	Minimal inhibitory concntration
MLEE	Multilocus enzyme electrophoresis
MLS_B	Macrolides-Lincosamides-Streptogramin B
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSA	Mannitol Salt Agar
MSSA	Methicillin-Susceptible *Staphylococcus aureus*
NaCl	Sodium hydrochloride
NCCLS	National Committee for Clinical Laboratory Standards
NGS	Next Generation Sequencing
NMRR	National Medical Research Registration
NT	Non-typeable
OD	Optical density
PBP-2A	Penicillin binding protein 2A
PCR	Polymerase Chain Reaction
PFGE	Pulse Field Gel Electrophoresis
PHASTER	Phage search tool enhanced released
PVL	Panton-Velentine Leukocidin
RGI	Resistance Gene Identifier
RNA	Ribonucleic Acid
SCCmec	Staphylococcal cassette chromosome *mec*
SMRT	Single-molecules, real-time
SLST	Single locus sequence typing
SNP	Single nucleotide polymorphism
spa	Staphylococcal protein A typing
SSTI	Skin and Soft Tissue Infection
TSA	Trypticase Soy Agar
TSST	Toxic shock syndrome toxin
VISA	Vancomycin-intermediate *Staphylococcus aureus*
VRSA	Vancommycin-resistant *Staphylococcus aureus*
VFDB	Virulence Factor Database
WGS	Whole-genomes sequencing

CHAPTER 1

INTRODUCTION

Staphylococcus aureus is known as a leading cause of bacterial infection and increasingly found to resist almost all antibiotics therapy. The emergence of broad-spectrum antibiotic-resistant bacteria is a global phenomenon, which poses an imminent threat to established treatment protocols for infectious diseases agents. Moreover, methicillin-resistant *Staphylococcus aureus* (MRSA) has been known to rapidly develop and acquire multidrug-resistance properties towards a wide range of structurally-related and unrelated antimicrobial agents due to the uncontrolled antimicrobial usage in hospitals throughout the world, including Asia (Chen & Huang, 2014). Currently, the prevalence of MRSA is of growing concern, particularly due to the more recent increased frequency hospital-associated to community-associated MRSA in the population. Due to the awareness of these pathogens, more efforts and related research were carried on community-associated environments (Gesualdo et al., 2013; Mohamed et al., 2016).

The emergence of MRSA in Malaysia was reported since 1970 by Lim and Zulkifli, (1987). The prevalence of MRSA is also showing an increment from 17% in 1986 (Rohani et al., 1999) to 61.4% in 2004 as reported by the Ministry of Health Malaysia (2004) in the National Surveillance Antibiotic Resistance reports. The plummeting number of prevalent isolated MRSA were also observed from 31% in 2005 to 17.7 % in 2013, as reported by the Ministry of Health Malaysia (Ministry of Health Malaysia, 2013). Additionally, the recent prevalence rates of Malaysian MRSA from clinical isolates recorded for the years 2016 and 2017 were at 18% and 19.8%, respectively (Ministry of Health Malaysia, 2016, 2017). The most dangerous threat is currently represented by MRSA strains that generally harbour additional novel mobile genetics element composing the antibiotic resistance determinant, thus warranting the utilisation of the last resort glycopeptides antibiotic. The emergence of MRSA with reduced susceptibility towards vancomycin has initiated a particularly serious concern (Hiramatsu et al., 2002). Various categories of MRSA based on epidemiologic character are commonly recognised including hospital-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and livestock-associated MRSA (LA-MRSA). HA-MRSA infections are most commonly reported among immunocompromised people who have spent time in hospitals or healthcare centres, while CA-MRSA infections occur among otherwise healthy adults and children in the community. Meanwhile, livestock-associated MRSA (LA-MRSA) refers to strains of MRSA in which animals, particularly production animals, serve as the main reservoir of infections to humans. While the prevalence and molecular diversity of both MSSA and MRSA within the hospital is very well documented, population-based studies on *S. aureus* on nasal colonisation, particularly in Malaysia are scarce. Furthermore, previous studies were often limited to the west coast of Malaysia, best known as Klang Valley, focusing on medical-related population (medical students) samples (Azis et al., 2014, 2017).

1.1 Problem Statements

Generally, limited genome sequences of *S. aureus* have been studied, and most of the studies reported the draft genome sequences of specific Malaysian strain. Moreover, limited information on the molecular and genomic comparison of multidrug resistance of both HA-MRSA and CA-MRSA involve those of the east coast of Malaysia. Most of the studies were focused on the antimicrobial susceptibility and resistance rates, as well as the molecular epidemiology using conventional typing methods such as polymerase chain reaction (PCR)-based genes profiling of the strains within the same locality, which did not dissect the details of the genomic make-up of the strain (Lim et al., 2012; Noordin, et al., 2016; Suhaili et al., 2009). Thus, the understanding of molecular epidemiology of both MSSA and MRSA strains will assist in controlling the spread and emergence of multidrug resistance clones. Therefore, this study examined a set of strains isolated sporadically from samples including clinical and community nasal swab to determine the dynamicity, evolutionary relatedness, and presence of different resistance, as well as virulence genes among both MSSA and MRSA strains.

1.2 General Objective

To characterise molecular and genomic make-up of CA- and HA-MRSA using conventional and whole-genome approach.

1.3 Specific Objectives

1. To determine the antimicrobial susceptibility patterns profiles of both MSSA and MRSA isolated from community and hospital associated samples.
2. To identify the prevalence of virulence and resistance genes determinant underlying among CA and HA- MRSA isolates.
3. To characterise the genetic relatedness of MRSA by accessory gene regulator (*agr*) typing, *spa* typing, *SCCmec* typing and whole-genome MLST.
4. To compare and analyse the genetic content and various genes responsible for virulence factors and multi-drug resistance of both CA-MRSA and HA-MRSA from WGS data.

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