UNIVERSITI PUTRA MALAYSIA

PHENOTYPIC AND MOLECULAR ANALYSIS OF STAPHYLOCOCCUS AUREUS FOR CARRIAGE AND TRANSMISSION IN A STUDENT POPULATION IN A MALAYSIAN PUBLIC UNIVERSITY

NORHIDAYAH MAT AZIS

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NORHIDAYAH BINTI MAT AZIS

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Chairman : Assoc. Prof. Mohd. Nasir bin Mohd. Desa, PhD
Faculty : Medicine and Health Sciences

Staphylococcus aureus is a human commensal bacteria that colonize the skin and mucosal surfaces of healthy individuals. This opportunistic pathogen is the most common nosocomial pathogen responsible for life-threatening diseases in humans worldwide. The anterior nares are the most frequent colonization site for *S. aureus* and nasal carriage is a major risk factor for infections and a source of transmission of this pathogen. The incidence of *S. aureus* and MRSA are growing at an alarming rate not only in the healthcare settings but also in the community. In this regards, this study aims to assess the *S. aureus* carrier rate and persistence, risk factors for nasal carriage, antimicrobial resistance and epidemiological molecular characteristics among the university student population. A set of self-administered questionnaires on socio-demographics, hygienic practices, medical and medication history together with a consent form were distributed prior to nasal swab collection. The collection was done twice in a one month interval during October and November 2013 from 192 and 180 health sciences students, respectively, at the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. Identification of bacteria isolated was done based on basic phenotypic methods. All *S. aureus* isolates were subjected to antibiotic susceptibility test (AST) by Kirby-Bauer disc diffusion method against eight antibiotics and screened for MRSA by PCR detecting the *mecA* gene. All *mecA* positive isolates were subjected to staphylococcal cassette chromosome (SCC) *mec* typing, multilocus sequence typing (MLST) and eBURST analysis. All isolates were further characterized by *spa* typing, screening of PVL genes and enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR). In this study, the colonization rates of *S. aureus* was 31.3% (60/192) and 33.3% (60/180) of the student population during the first and second sampling respectively. Thirty-nine (65%) students were detected for *S. aureus* at both isolation events and referred as persistent carriers. There is no significant correlation between the carriage and the tested risk factors except for the habit of touching the nose and chronic illnesses ($P<0.05$). All 120 *S. aureus* isolates from both isolation events were susceptible towards vancomycin, ciprofloxacin and gentamycin. The highest frequency of resistance was observed for penicillin at both isolations (70% and 65% respectively). This was followed by tetracycline with a similar resistance rate (11.67%) in both isolation events. Low level of resistance was observed against erythromycin at both events. This indicates the persistence of the antimicrobial resistance pattern in the

population over the short study period. As for methicillin resistance, out of the 120 isolates of *S. aureus*, 10 (8.33%) were positive for the *mecA* gene with four and six isolates from first and second isolation events respectively; four isolates were from two individuals. However, among the *mecA* positive isolates, only eight isolates showed resistance towards cefoxitin (four isolates from each isolation event) while the other two *mecA* positive isolates (from second event) were cefoxitin-susceptible by both dics and Etest methods. The *mecA*-positive isolates belonged to SCC*mec* types I (n=9) and V (n=1). MLST analysis of MRSA isolates revealed three STs: ST508 (n=1), ST88 (n=1) and ST96 (n=1) while other seven of MRSA isolates showed non typeable sequences type. This indicates the tendency of MRSA to persist, although at a low rate with limited genotypes. eBURST analysis showed that MRSA isolates found in this study were potentially related to those MRSA found in Asian countries and might be disseminated regionally. Based on ERIC analysis, the majority of isolates were largely genetically distinct. As for the persistent *S. aureus* carriers, it was found that for 19 (48.72%) of them, respective individual carried *S. aureus* of a similar *spa* type during both isolation events. This indicates the persistence of certain *spa* types in the respective individuals over the short term period. Although the prevalence of *S. aureus* carriage and MRSA in this study cannot be generalized to entire population due to limitations of the study, but this indicates the need of periodic screening to monitor *S. aureus* and MRSA status among community.

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ANALISIS FENOTIP DAN MOLEKULAR *STAPHYLOCOCCUS AUREUS* BAGI PEMBAWA DAN PENYEBARAN DALAM POPULASI PELAJAR DI UNIVERSITI AWAM MALAYSIA

Oleh

NORHIDAYAH BINTI MAT AZIS

November 2015

Pengerusi : Prof. Madya Mohd. Nasir bin Mohd. Desa, PhD
Fakulti : Perubatan dan Sains Kesihatan

Staphylococcus aureus ialah bakteria yang biasanya hidup di kulit dan permukaan mukosa individu yang sihat. Patogen oportunis ini ialah patogen utama bagi jangkitan mikrob di hospital yang bertanggungjawab menyebabkan jangkitan yang mengancam nyawa kepada manusia di seluruh dunia. Lubang hidung ialah kawasan yang paling kerap dikolonisasi oleh *S. aureus* dan pembawa *S. aureus* berisiko besar untuk mendapat jangkitan dan juga merupakan sumber utama kepada penyebaran patogen ini. Insiden *S. aureus* and MRSA telah berkembang pada kadar yang membimbangkan bukan sahaja di pusat kesihatan malah dalam kalangan komuniti. Sehubungan itu, kajian ini bertujuan untuk mengkaji kadar pembawa *S. aureus*, faktor-faktor risiko pembawa, ketahanan antimikrob dan ciri-ciri epidemiologi molekul dalam tempoh masa yang pendek dalam kalangan populasi pelajar. Satu set soal selidik yang mengandungi soalan mengenai sosio-demografik, amalan kebersihan, perubatan dan sejarah ubat bersama borang persetujuan diedarkan sebelum pengambilan sampel swab hidung. Pengambilan sampel dilakukan dua kali dalam tempoh sebulan pada bulan Oktober dan November 2013 dari masing-masing 192 dan 180 pelajar di Fakulti Perubatan dan Sains Kesihatan, Universiti Putra Malaysia. Pengenalan bakteria dilakukan berdasarkan ujian asas fenotipik. Semua isolat *S. aureus* dikenakan ujian kepekaan antibiotik terhadap lapan jenis antibiotik oleh ujian Kirby-Bauer dan penyaringan MRSA oleh PCR yang mengesan gen *mecA*. Isolat yang positif terhadap gen *mecA* telah menjalani ujian pengelasan staphylococcal kromosom kaset *mec* (SCC*mec*), pengelasan jujukan multilokus (MLST) dan analisis eBURST. Semua isolat telah dicirikan oleh pengelasan *spa*, penyaringan gen PVL dan ujian ERIC. Dalam ujian ini, kadar kolonisasi *S. aureus* ialah 31.3% (60/192) pada pengumpulan sampel pertama dan 33.3% (60/180) pada pengumpulan sampel kedua. Seramai 39 pelajar (65%) telah dikesan sebagai pembawa *S. aureus* pada kedua-dua aktiviti persampelan dan dirujuk sebagai pembawa berterusan. Tiada hubungan yang signifikan antara pembawa dan kesemua faktor yang diuji kecuali amalan menyentuh hidung dan penyakit kronik ($P < 0.05$). Kesemua 120 isolat dari kedua-dua aktiviti persampelan sensitif terhadap antibiotik vancomisin, ciprofloxasin dan gentamisin. Frekuensi ketahanan tertinggi terhadap antibiotik penisilin dapat dilihat dalam kedua-dua aktiviti persampelan (70% pada persampelan pertama dan 65% pada persampelan kedua). Ini diikuti oleh ketahanan terhadap antibiotik tetrasiklin pada kadar 11.67% dalam kedua-dua persampelan. Ketahanan yang rendah terhadap antibiotik eritromisin juga dapat dilihat

dalam kedua-dua persampelan. Ini menunjukkan kadar berterusan corak ketahanan antimikrob dalam populasi pelajar sepanjang tempoh masa kajian yang pendek. Bagi isolat yang rintang terhadap metisilin, 10 isolat (8.33%) dari 120 isolat adalah positif terhadap gen *mecA* (empat isolat dari persampelan pertama dan enam isolat dari persampelan kedua). Empat isolat adalah dari dua individu yang sama. Walaubagaimanapun, dalam kalangan isolat yang positif terhadap gen *mecA*, hanya lapan isolat menunjukkan ketahanan terhadap antibiotik sefoksitin (empat isolat dari setiap persampelan) manakala dua lagi isolat yang positif terhadap gen *mecA* (dari persampelan kedua) adalah sensitif terhadap antibiotik sefoksitin melalui kaedah Kirby-Bauer dan Etest. Isolat yang positif gen *mecA* dikelaskan kepada SCC*mec* jenis 1 (n=9) dan V (n=1). Analisis MLST terhadap isolat MRSA menunjukkan tiga STs: ST508 (n=1), ST88 (n=1) dan ST96 (n=1) manakala tujuh isolat MRSA menunjukkan jenis urutan yang tidak lengkap. Ini menunjukkan kecenderungan berterusan MRSA walaupun pada kadar yang rendah dengan genotip terhad. Analisis eBURST menunjukkan bahawa isolat MRSA yang ditemui dalam kajian ini berpotensi mempunyai kaitan dengan isolat MRSA yang ditemui di negara-negara Asia dan berkemungkinan disebarkan serantau. Berdasarkan analisis ERIC, majoriti isolat adalah isolat berbeza dari segi genetik. Bagi pembawa berterusan *S. aureus*, ditemui 19 (48.72%) daripada mereka membawa *S. aureus* yang sama pengelasan *spa* dalam kedua-dua persampelan. Ini menunjukkan penerusan jenis *spa* tertentu yang dibawa oleh pembawa *S. aureus* dalam tempoh masa yang pendek. Walaupun kadar prevalens pembawa *S. aureus* dan MRSA dalam kajian ini tidak boleh diumumkan kepada seluruh populasi disebabkan oleh batasan kajian, tetapi ini menunjukkan keperluan untuk melakukan pemeriksaan berkala bagi memantau status *S. aureus* dan MRSA dalam kalangan komuniti.

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

Mohd. Nasir Bin Mohd Desa, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Syafinaz Binti Amin Nordin, MPath
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

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

Date:

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Signature: _____

Name of Chairman of Supervisory Committee: Associate Professor Mohd. Nasir Bin Mohd Desa

Signature: _____

Name of Member of Supervisory Committee: Associate Professor Syafinaz Binti Amin Nordin, MPath

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
CLSI	Clinical And Laboratory Standards Institute
MDR	Multidrug Resistant
mg	milligram
MHA	Mueller-Hinton Agar
MIC	Minimum Inhibitory Concentration
min	minute
mL	millilitre
mM	millimol
mm	millimeter
MSA	Mannitol Salt Agar
RPM	Revolutions per minute
sec	second
^{O}C	Degree celcius
µL	microliter

CHAPTER 1

INTRODUCTION

Staphylococcus aureus is a commensal microorganism that colonizes the skin and mucosal surfaces of healthy individuals (Khorvash *et al*., 2012). This bacteria has been reported to colonize about 20% of the human population (Syafinaz *et al*., 2012). Unfortunately, *S. aureus* is an opportunistic human pathogen that is responsible for community and hospital-acquired infections. It may cause infections under favourable conditions and can easily transmit through direct contact (Syafinaz *et al*., 2012).

The anterior nares are the primary reservoirs of *S. aureus* with the carrier rate ranging from 20-30% in a healthy population (Shibabaw *et al*., 2014). Colonization of nares play a significant role in the epidemiology and pathogenesis of *S. aureus*. It may lead to subsequent infections of the skin, blood, heart, lung, and soft tissues. *S. aureus* infections are growing problem worldwide with increasing morbidity in hospitals and the community with mortality rates of between 6% to 40%, thus placing a burden to the healthcare systems worldwide (Khorvash *et al*., 2012).

Bacterial, environmental and host factors play significant roles as the leading mechanism for *S. aureus* nasal carriage. Bacterial factors such as staphylococcal toxins helps these bacteria to resist and invade host defense (Weidenmaier *et al*., 2012). Environmental factors such as living in crowded environments, hospitalization, close contact with persons who are heavily colonized by the organism and poor self-hygiene may further facilitate *S. aureus* dissemination (Gorwitz *et al.*, 2008). Host factors such as gender, age, ethnic groups, recent antibiotic exposure, skin cut and underlying chronic illness have been identified as earlier determinants of *S. aureus* nasal carriage in previous case-control or cross-sectional studies involving the community (Wertheim *et al*., 2005).

There are three major patterns of *S. aureus* colonization which are persistent carriage, intermittent carriage and noncarriage (Wertheim *et al*., 2005). These three patterns can be distinguished over time. According to Wertheim *et al.* (2005), based on a longitudinal study in a human population, about 20-30% of individuals are colonized persistently by *S. aureus* while 30% are colonized intermittently. There are about 50% of individuals who are never or rarely colonized by *S. aureus*. Comparing to intermittent carriage and noncarriage, persistent carriage appear to have higher *S. aureus* loads, higher risks of developing subsequent infection and lower levels of immunoglobulins to staphylococcal antigens (Verkaik *et al*., 2009). The definition of persistent carriage varies from one study to another since there is no general agreement on the number of positive cultures to be defined as persistence (Wertheim *et al*., 2005).

S. aureus became a serious healthcare concern due to the emergence of drug resistant *S. aureus* particularly the Methicillin-resistant *S. aureus* (MRSA). MRSA has become notorius not only in clinical settings but also in the community. In Malaysia, a previous

study reported that the occurrence of MRSA in selected healthcare settings, had continuously increased from years 1998 to 2000 (Hui Sang *et al*., 2011). Hospital-acquired MRSA (HA-MRSA) originates in the hospital and are carried in a person who had frequent and recent contact with healthcare centers or facilities within the past year, have recently undergo an invasive medical procedure or are immunocompromised (*Staphylococcus aureus* in Healthcare Settings., 2011). Meanwhile, community-associated MRSA (CA-MRSA) originates outside the hospital and acquired by persons who had not been recently hospitalized within the past year or had a medical procedure. MRSA could have been transmitted from hospital and proliferated in the community posing threat to a larger extent of the population, especially those with impaired immune system (Evans, 2008). The incidence of MRSA continues to grow at an alarming rate. Therefore, surveillance of *S. aureus* has become crucial due to emergence of the worldwide dissemination of this pathogen. Classical epidemiology data such as structured questionnaires together with typing data are important tools in understanding the epidemiology of *S. aureus* infection. Typing has become an important tool in outbreak investigations, to trace the strain origin and to study the dissemination of the pathogen's clones. Various molecular methods have been developed for the epidemiological characterization of *S. aureus*. Among the molecular typing method for *S. aureus*, pulsed-field gel electrophoresis (PFGE) have been considered as the 'gold standard' due to its excellent discriminatory power. However, this technique is technically demanding and time consuming (Shopsin *et al*., 1999). Other typing methods such as Enterobacterial Repetitive Intergenic Consensus (ERIC) and multilocus sequence typing (MLST) has proven their ability to characterize *S. aureus* strains. In addition, DNA sequencing of the polymorphic X region of the protein A gene (*spa*) has become an alternative technique for typing of *S. aureus*. Nowadays, detection of *mecA* methicillin resistance gene and SCC*mec* typing has been considered as important tools for typing MRSA strains (Shopsin *et al*., 1999).

In view of the increasing incidence of MRSA and its associated clinical complications, periodic screening for *S. aureus* carriage and methicillin resistance in the community is crucial for monitoring purposes. This study attempts to study *S. aureus* carriage over a period of time and the presence of MRSA in a population of health sciences students who lived and interacted within a close locality. Investigation of potential risk factors, supported by data at the molecular level, is necessary so as to see any strain relatedness or clonal dissemination over a short span of time. The result of this study may provide the status of *S. aureus* carriage among a healthy student population. Increased awareness of MRSA colonization may help in controlling the spread of MRSA in the community and lessen the risk of staphylococcal diseases.

1.1 Problem statement

Nasal colonization of *S. aureus* is common in communities worldwide. The rate of *S. aureus* nasal carriage rate varies according to specific group in populations (Chatterjee *et al.,* 2009; Choi *et al.,* 2006). In Malaysia, previous studies reported that the prevalence of nasal carriage of *S. aureus* is within a range of 20–25% among healthy adults. However,a recent study by Vasanthakumari *et al.* (2009) showed the increasing rate of *S. aureus* carriage (31.5%). Even though the rate of *S. aureus* carriage is increasing in the community, there are still a limited number of studies on the carriage

pattern of *S. aureus* (Vasanthakumari *et al*., 2009). The emergence of MRSA, especially CA-MRSA in the community had increased the burden in association with infection. In a nasal culture survey among healthy Malaysian population, there were 3% incidences of putative CA-MRSA reported (Shamsudin *et al*., 2008). This indicates the presence of CA-MRSA among healthy Malaysian carriers without risk factors predisposing them for acquisition. Since the presence of CA-MRSA are continuously emerging and disseminating, it is important to continue monitoring the distribution and carriage pattern of *S. aureus* in the community, targeting the active group (young and healthy adult). Study on *S. aureus* carriage pattern within a short period time helps to determine the dissemination of MRSA clones. In addition knowing the infection risk factors of *S. aureus* and its antibiotics susceptibility can reduce the spread of infections.

1.2 Hypothesis

The rate of nasal carriage of *S.aureus* is expected to be around 20% - 30% from the healthy students tested based on the previous studies at the same locality (Choi *et al*., 2006; Shamsudin *et al*., 2008). With the current trend in the emergence and dissemination of *S. aureus* and antibiotic resistance strains, the isolation of MRSA or drug resistant isolates in this study is likely.

1.3 Objectives

1.3.1 General objective

To assess the nasal carriage and molecular characteristics of *S. aureus* in a population of healthy students at Universiti Putra Malaysia.

1.3.2 Specific objectives

a) To determine the short-term (one month interval in October and November 2013) nasal carriage of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin-Susceptible *Staphylococcus aureus* (MSSA) among healthy students.
b) To determine the potential risk factors for nasal *S. aureus* and MRSA colonization.
c) To determine antibiotics susceptibility patterns of the *S. aureus* isolates.
d) To determine the genotypic status of Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates for PVL gene, *mecA* gene and SCC*mec* type.
e) To determine the epidemiological characteristics between two consecutives of *S. aureus* isolates within one month interval by *spa* typing, ERIC and MLST.

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