THE PREVALENCE OF NASAL COLONIZATION OF *Staphylococcus aureus* AND IT'S ANTIBIOTIC SUSCEPTIBILITY PATTERNS AMONG PRE-CLINICAL AND CLINICAL STUDENTS AT UNIVERSITI MALAYSIA SABAH

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THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCES

FACULTY OF MEDICINE AND HEALTH SCIENCES
UNIVERSITI MALAYSIA SABAH
2017

DECLARATION

The materials in this thesis are original except for quotation, excerpts, equations, summaries and references, which have been duly acknowledged.

9th March 2017

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DEGREE : **MASTER OF SCIENCE (MEDICAL SCIENCE)**

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ACKNOWLEDGEMENT

In The Name of Allah, The Most Beneficent and The Most Merciful.

I would like to express my utmost gratitude and special appreciation to my supervisory team; Associate Professor Dr. Than Myint, Associate Professor Dr. Tin Sabai Aung and Dr. Su Su Hlaing for their guidances, encouragements, supports, invaluable advices, kind suggestions and constructive comments during this project. I also record my sincere appreciation to Dr. Su Su Hlaing and Professor Dr. Nor Amalina Emran (former supervisor) for their guidance, encouragement and providing financial support in this project. My sincere thanks to Medical Laboratory Technologist staff of Pathobiology and Medical Diagnostic Department, Faculty of Medicine and Health Sciences, especially for their assistance. I am deeply indebted and grateful to my family especially my mother, Mdm Junaidah bt Ameran, and my wife, Hellen Masandid and my childrens for their concern, patience and cheering me up and stood by me through the good times and bad. Last but not least, i would like to thank everyone who has helped me directly or indirectly towards completing this research project.

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9th March 2017

ABSTRACT

This study was a cross-sectional descriptive study to determine the prevalence of nasal colonization of *Staphylococcus aureus* (*S. aureus*) among pre-clinical and clinical medical students and nursing students attending Faculty of Medicine and Health Sciences at the Universiti Malaysia Sabah (UMS). From April 2013 to November 2013, nasal swabs were collected from anterior nares of 449 students. Nasal swabs were inoculated on Mannitol Salt Agar (MSA) for isolation of Staphyococcus followed by Gram stain and identification of *S. aureus* by coagulase test using Staphylase test kit and tube coagulase test. Antibiotic susceptibility test was done on all *S. aureus* isolates using Kirby-Bauer method and six antibiotics discs penicillin, oxacillin, erythromycin, clindamycin, tetracycline and trimethroprim-sulfamethoxazole were included. Cefoxitin susceptibility was also performed to detect Methicillin Resistant *Staphylococcus aureus* (MRSA). *S. aureus* isolates were also tested with Slidex ® MRSA Detection kit (bioMérieux® SA) to detect Penicillin-binding protein 2a product of MRSA. The results showed that the prevalence of nasal colonization of *S. aureus* among 449 subjects was 31.0%, all were MSSA and none of the isolates were MRSA. Antibiotic susceptibility testing revealed that all 139 *S. aureus* isolates were sensitive to oxacillin, trimethroprim-sulfamethoxazole and cefoxitin, whereas 116 (83.5%), 1 (0.7%), 3 (2.2%) and 24 (17.3%) were resistant to penicillin, erythromycin, clindamycin and tetracycline respectively. This study also found out that the potential association of *S. aureus* colonization was not significant to all factors except the university program of students (p<0.046). In conclusion, the prevalence of *S. aureus* nasal colonization among pre-clinical and clinical students at UMS was 31.0%. No MRSA was detected and the *S. aureus* isolates were highly resistant to penicillin.

ABSTRAK

KELAZIMAN KOLONISASI Staphylococcus aureus DAN CORAK KECENDERUNGANNYA TERHADAP ANTIBIOTIK DALAM KALANGAN PELAJAR PRA-KLINIKAL DAN KLINIKAL DI UNIVERSITI MALAYSIA SABAH

Kajian rentas ini dijalankan adalah untuk mengesan kelaziman kolonisasi Staphylococcus aureus (S. aureus) dalam kalangan pelajar pra-klinikal dan klinikal pelajar perubatan dan kejururawatan di Fakulti Perubatan dan Sains Kesihatan, Universiti Malaysia Sabah (UMS). Dari April hingga November 2013, sampel palitan hidung telah diambil di bahagian hadapan hidung 449 orang pelajar. Sampel kemudiannya disuntik ke atas 'Mannitol Salt Agar (MSA)' untuk pemencilan Staphylococcus, pewarnaan Gram dan S. aureus dikenalpasti menggunakan Kit Ujian Staphylase dan koagulasi tiub. Ujian Kecenderungan antibiotik telah dijalankan pada semua pencilan S. aureus menggunakan kaedah Kirby-Bauer dan enam antibiotik iaitu penicillin, oxacillin, erythromycin, clindamycin, tetracycline dan trimethroprim-sulfamethoxazole telah digunakan. Kecenderungan pada cefoxitin juga turut diuji untuk mengesan methicillin-rintang S. aureus (MRSA). Pencilan S. aureus turut diuji menggunakan 'Slidex®MRSA Detection kit (bioMérieux® SA)' untuk mengesan produk protein iaitu Penicillin-binding protein 2a yang dihasilkan oleh MRSA. Hasil kajian mendapati kelaziman kolonizasi S. aureus di kalangan 449 subjek adalah 31.0%, kesemuanya adalah MSSA dan tiada MRSA dikesan. Ujian kecenderungan antibiotik mendapati bahawa kesemua pencilan S. aureus adalah sensitif pada oxacillin, trimethroprim-sulfamethoxazole dan cefoxitin, sementara 116 (83.5%), 1 (0.7%), 3 (2.2%) dan 24 pencilan (17.3%) adalah rintang terhadap penicillin, erythromycin, clindamycin dan tetracycline. Kajian ini juga mendapati bahawa hubungan kolonisasi S. aureus adalah tidak signifikan pada semua faktor berpotensi kecuali program universiti yang diikuti oleh pelajar-pelajar ($p<0.046$). Sebagai kesimpulan, kelaziman kolonisasi S. aureus dalam kalangan pelajar pre-klinikal dan klinikal di UMS adalah 31.0%. Tiada pencilan MRSA dikesan dan semua pencilan S. aureus adalah sangat rintang terhadap penicillin.

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LIST OF ABBREVIATIONS

α	-	Alpha
β	-	Beta
δ	-	Delta
γ	-	Gamma
ATCC	-	American Type Culture Collection
CA-MRSA	-	Community Associated MRSA
Clf	-	Clumping factor
Cna	-	Collagen-binding protein
CoNS	-	Coagulase Negative Staphylococci
CoPS	-	Coagulase Positive Staphylococci
CPs	-	Capsular Polysaccharides
DA	-	Clindamycin
DD	-	Disc Diffusion
DNA	-	Deoxyribonucleic acid
E	-	Erythromycin
Efb	-	Extracellular fibrinogen binding protein
Fnb	-	Fibronectin-binding protein
FOX	-	Cefoxitin
HA-MRSA	-	Hospital Associated MRSA
IsdA	-	Iron regulated surface determinant A
LTA	-	Lipoteichoic acid
MLST	-	Multi-locus Sequencing Typing
MRSA	-	Methicillin Resistant *Staphylococcus aureus*

MSA	-	Mannitol Salt Agar
MSCRAMM	-	Microbial Surface Components Recognizing Adhesive Matrix Molecule
MSSA	-	Methicillin Susceptible *Staphyloocccus aureus*
OX	-	Oxacillin
P	-	Penicillin
PBP2a	-	Penicillin-binding Protein 2a
PCR	-	Polymerase Chain Reaction
PFGE	-	Pulsed-field Gel Electrophoresis
PVL	-	Panton-valentine Leukocidin
RFLP	-	Restriction Fragment Length Polymorphism
SaPI	-	*Staphylococcus aureus* Pathogenicity Island
SaSG	-	*Staphylococcus aureus* surface protein G
SCC	-	Staphylococcal cassettes chromosome
SE	-	Staphylococcal Enterotoxin
spa	-	Staphylococcal Protein A
SSI	-	Surgical Site Infection
SSTI	-	Skin and Soft Tissue Infection
SXT	-	Staphyloxantin
TE	-	Tetracycline
TMP/SMX	-	Trimetroprim/Sulfamethaxazole
TSB	-	Tryptone Soya Borth
TSST-1	-	Toxic Shock Syndrome Toxin-1

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CHAPTER 1

INTRODUCTION

1.1 Background

Staphylococcus aureus (*S. aureus*) is a Gram positive-cocci bacterium, known as a pathogen in many hospitals worldwide including Malaysia. According to the National Surveillance of Antibiotic Resistance report in 2014, 18.0% of *S. aureus* was isolated from the blood culture, which the isolates shown resistance at many antibiotics tested including erythromycin, gentamycin, co-trimoxazole, rifampicin, fusidic acid, clindamycin, ciprofloxacin and linezolid (Ministry of Health Malaysia, 2014). It was also reported that *S. aureus* as the second common bacteria isolated in blood cultures after Coagulase-negative staphylococci (CoNS) found in a multidisciplinary teaching hospital in Kuala Lumpur (Karunakaran *et al.*, 2007). The incidence of *S. aureus* infections in hospitals has been reported causing many diseases including bloodstream infections, endocarditis, surgical site infections, skin infections, lung infections, urinary tract infections and catheter-associated devices infections. Among most of the above diseases, bloodstream infections are the most common infections and reported as one of the cause of mortality in Malaysian Hospitals (Ministry of Health Malaysia, 2008a). The natural habitat of staphylococcus is widespread in nature which are the normal flora that living on the skin and mucous membrane of mammals and birds (Bannerman and Peacock, 2009). It has been accepted that the nostrils are the area where *S. aureus* can be cultured most frequently (Williams, 1963). It was due to an ability of *S. aureus* to adhere to the nasal mucosal cells of the nasal vestibules (Aly *et al.*, 1977). The colonization of *S. aureus* in the nose was also mediated by MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) family such as Fibronectin-binding protein (fnb), clumping factor B (ClfB), Iron regulated surface determinant A (IsdA), Serine-aspartic acid repeat

proteins (Sdr), Haemoglobin, *S. aureus* surface protein G (SasG). Recently, it was reported that triclosan can promote nasal colonization (Syed *et al.*, 2014).

S. aureus can transmit to other people such as from mother to their baby (Jimenez-Truque *et al.*, 2012), those people living in the crowded area such in a jail (Maree *et al.*, 2010), having sports activity such as wrestling or football that may lead to skin lesion, and sharing personal items such as soap, towels, deodorant and hair clippers (Archibald *et al.*, 2008). The transmission of *S. aureus* especially methicillin resistant *Staphylococcus aureus* (MRSA) in hospital environments was usually mediated by the hands especially among healthcare workers to hospitalized patients (Berthelot *et al.*, 2003). So far, the exact role of nasal carriage among healthcare workers for spreading *S. aureus* infection in hospitals outbreaks remain unclear although there are several reports on MRSA outbreaks in which hospital staff were responsible. (Danzmann *et al.*, 2013). In addition, *S. aureus* also can transmit from the surfaces such as clothing, towels, scrub suits, lab coat and privacy drapes (Neely and Maley, 2000), or from doctors necktie (Koh *et al.*, 2009), or those people having picking nose habits (Wertheim *et al.*, 2006). In hospitalized patients, transmission of *S. aureus* is also at risk to the patients in the wards especially, those having hospitalization history and antibiotics use history in past (Hidron *et al.*, 2005), using central venous catheter insertion, staying in the same ward with colonized patients in the same ward at the same time (Oztoprak *et al.*, 2006) and staying in the room previously occupied by colonized patients (Huang *et al.*, 2006). Besides that, infection can be acquired through airborne in hospitals (Mortimer *et al.*, 1966), however the route of airborne was relatively not important and the role of airborne transmission of *S. aureus* in hospitals is difficult to evaluate (Solberg, 2000).

The emergence of antibiotic resistant strains or known as methicillin resistant *Staphylococcus aureus* (MRSA) was shown as a challenge for the clinicians to treat the infected patients. This was because the strains were resistant to all β-lactam antibiotics including penicillin, nafcillin, oxacillin, methicillin, cephalosporins and carbapenems (Bush, 2009). It is well known that the incidence or prevalence of MRSA infection has been reported worldwide

including Malaysia. To date, the prevalence of antibiotic resistance to methicillin in Malaysia was at 17.3% among *S. aureus* isolated from Malaysian general hospitals including three university hospitals in 2014 (Ministry of Health Malaysia, 2014).

Various techniques to identify methicillin resistant *Staphylococcus aureus* (MRSA) isolates including phenotypic and genotypic methods have been documented worldwide. However, the use of antibiotic in Kirby-Bauer disc diffusion method are commonly performed in the routine microbiology laboratories in Hospitals. According to National Antibiotic Guidelines in 2008, twelve antibiotics have been listed to be used *S. aureus* isolates in the laboratory (Ministry of Health Malaysia, 2008b). For genotypic methods, the molecular characteristics and epidemiological studies of MRSA isolates have been reported from various institutions in Malaysia. These genotypic methods include phage typing (Hanifah, 1991), SCCmec typing (Mustafa *et al.*, 2012), spa typing (Neela *et al.*, 2010), dru typing (Ehsanollah *et al.*, 2011), and multi-locus sequences typing (King *et al.*, 2012). However, in the laboratory which has limited access of molecular facilities, a disc diffusion method was an adequate to detect methicillin resistant isolates. In addition, molecular methods usually are more expensive, laborious and technically demanding.