

**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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**FACULTY OF MEDICINE AND HEALTH  
SCIENCES  
UNIVERSITI MALAYSIA SABAH  
2017**

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**UTMS**

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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.

9<sup>th</sup> March 2017

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OF *Staphylococcus aureus* AND IT'S ANTIBIOTIC  
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AND CLINICAL STUDENTS AT  
UNIVERSIT MALAYSIA SABAH**

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

VIVA DATE : **9<sup>TH</sup> MARCH 2017**



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Norfarid Irfan bin Mohd Subri

9<sup>th</sup> 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 *Staphylococcus* 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 trimethoprim-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, trimethoprim-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 trimethoprim-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 kolonisasi *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, trimethoprim-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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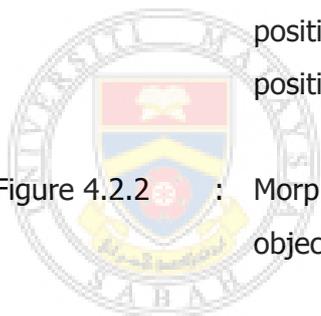
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## LIST OF ABBREVIATIONS

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

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