

Staphylococcus aureus Nasal Carriage in Medical Students of Universiti Teknologi MARA

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ABSTRACT

Introduction: To determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriage and detection of *S. aureus* leukotoxins among medical students of Universiti Teknologi MARA. **Methods:** Both sides of the anterior nares of 136 volunteers, comprising 68 preclinical and 68 clinical medical students, were swabbed and immediately cultured onto mannitol salt agar for growth of *S. aureus*. Standard microbiological techniques were conducted to identify and confirm the *S. aureus* colonies and susceptibility test against oxacillin were conducted by using Kirby-Bauer method to determine their resistance to methicillin. Polymerase chain reaction was performed for detection of leukotoxins, i.e., Pantone-Valentine Leukocidin (PVL) and γ -haemolysin genes. **Results:** Nineteen students (14%) consisting of 10 preclinical (14.7%) and 9 clinical (13.2%) were nasal carriers of *S. aureus*. However, none of the *S. aureus* isolates were MRSA. No PVL gene was detected but eight of them were positive for γ -haemolysin gene. **Conclusion:** There were no MRSA nasal carriers among the medical students, but a low prevalence of *S. aureus* nasal carriers was detected. These carriers do not pose as high risk because none of the strains of *S. aureus* possess both the γ -haemolysin toxin and the PVL toxin that are associated with tissue necrosis.

KEYWORDS: *Staphylococcus aureus*, MRSA, nasal carriage, medical students, PVL, γ -haemolysin.

INTRODUCTION

Staphylococcus aureus is a normal flora that primarily resides in the nose and throat of healthy individuals. It was estimated that between 20-30% of healthy population colonise *S. aureus* in vestibulum nasi of their anterior nares [1, 2, 3]. In hospital settings, there are many reports that associate healthcare workers being the source of cross-transmission of *S. aureus* between patients [4, 5, 6]. *S. aureus* has been associated with surgical wound infections [7], hospital-associated pneumonia, catheter-associated infections [8] and bacteraemia [9]. Although asymptomatic nasal colonisation with *S. aureus* is common amongst healthcare workers, transmission of methicillin-resistant *S. aureus* (MRSA) to critically-ill patients could result in debilitating outcomes and complications to treatment [10]. Nosocomial infections by MRSA result in morbidity of hospitalised patients, prolonged duration of hospitalization and increasing the cost of healthcare [11, 12].

Strategies for an effective preventive measure are needed to eliminate the source of the infections and/or reduce transmission of MRSA among healthcare workers. Medical students who will be future healthcare workers play a role in the intervention strategy of *S. aureus* transmission in the hospital settings. They need to be aware of their potential risk as *S. aureus* nasal carriage and be prepared with sufficient knowledge in infection control. This study aims to determine the prevalence of nasal carriage of MRSA among medical students of UiTM and to detect the presence of leukotoxins that are associated with lysis of leukocytes and erythrocytes. These two toxins, i.e. Pantone-Valentine Leukocidin (PVL) and γ -haemolysin, are in the family of synergohymenotropic. Presence of both toxins will be able to destroy the cell membrane of host's defense cells and erythrocytes by synergistic action [13].

METHODS

Study Design

A total of 136 volunteers, consisting of medical students from the Faculty of Medicine, UiTM Sungai Buloh were enrolled in this study. The number of participants involved in this study was calculated based on the formula to achieve statistical significance [14]. They comprised 68 preclinical (Years 1 and 2) and 68 clinical-year (Years 3 and 4) students. Students in preclinical years are basically not exposed to hospital environment as opposed to students in clinical years who have already some early exposure in hospitals during their clinical postings. Oral and written informed consents were obtained from the students prior to collection of samples. A self-administered questionnaire on their demographic profile (name, age and posting), past medical history (history of chronic illnesses or fever) and history of antibiotic usage for the past two weeks, were distributed to each of them.

Collection of Samples, Culture Procedure and Antibiotic Susceptibility Test

Nasal swabs were collected from both nares by using sterile cotton swab which were immediately cultured on mannitol salt agar (Oxoid). The agar plates were incubated at 37°C for 24 hours. Yellow colonies suspected of *S. aureus* on the mannitol salt agar were identified by performing Gram staining, catalase test, and coagulase test. Subsequently, the colonies were subcultured onto brilliance MRSA (BMRSA) agar and blood agar. Blue colonies that grew on the BMRSA after 24 hours incubation at 37°C indicate presence of MRSA colonies. The colonies on blood agar that produced β -haemolysis were tested for their susceptibility to oxacillin (Oxoid, UK; 1 μ g) by disk diffusion method on Muller Hinton agar, following the method recommended by the Clinical Laboratory Standard Institute [15]. A colony which produced a zone of inhibition (ZOI) 10 mm diameter or less is considered as MRSA and ZOI of 13 mm diameter or more is considered as methicillin-susceptible *S. aureus* (MSSA) strains. The breakpoint interpretation was based on CLSI guidelines. For positive control, *S. aureus* ATCC49775 was used.

Detection of PVL and γ -haemolysin genes

Polymerase chain reaction (PCR) was performed to detect PVL and γ -haemolysin (Hlg) genes present in *S. aureus*, using primers *luk-PV1* and *luk PV2* for PVL genes, and *hlg-1* and *hlg2* for γ -haemolysin genes, following a published method [16], with a modification on the preparation of DNA template. Instead of extracting DNA using an extraction kit, heat-boiling method was used because of the ease of the method and economical. The heated culture suspension was briefly spun by rapid centrifugation and the supernatant was used in the reaction. The reference strain was concurrently run in the reactions as a positive control. PCR products were resolved by electrophoresis through 1.5% agarose gel and observed using GelDoc (Biorad, US). Amplicons of 433-bp and 937-bp were indicative of the presence of PVL and γ -haemolysin genes, respectively.

Statistical Analysis

The categorical variables were analysed and compared by using chi-square test of SPSS version 22, where two-tailed p-value less than 0.05 was considered as significant.

RESULTS

Out of 136 medical students, 19 (14%) of them were *S. aureus* nasal carriers; 10 (14.7%) and 9 (13.2%) were preclinical and clinical students, respectively. Table 1 summarizes the demographic information of the students and the results of isolation and PCR. The number of *S. aureus* isolated from each group of students showed no significant difference ($p < 0.05$) between preclinical and clinical students, irrespective of their previous exposure to hospitals' environment. It was also found that *S. aureus* carrier status was also not associated with gender, past medical history and antibiotic used.

The results of the antimicrobial susceptibility test on the isolated *S. aureus* showed that all of the isolates produced ZOI more than 13 mm diameter, indicating that all the *S. aureus* were MSSA. None of these strains showed the presence of PVL genes but γ -haemolysin gene was detected in eight of the strains.

Table 1 Socio-demographic of medical students and results of *S. aureus* detection.

	Preclinical Students (n=68)	Clinical Students (n= 68)	P-value
Gender			0.389
Male	20 (29.4%)	20 (29.4%)	
Female	48 (70.6%)	48 (70.6%)	
History of cold and fever in past 2 weeks	11 (16.2%)	13 (19.1%)	0.653
Antibiotic used in the past 2 weeks	1 (1.5%)	3 (4.4%)	0.310
<i>S. aureus</i>			
Isolated	10 (14.7%)	9 (13.2%)	0.805
MRSA	0	0	
PVL detected	0	0	
γ -haemolysin detected	5	3	

DISCUSSION

Many reports have associated healthcare workers as the causal for transmission of *S. aureus* infections in hospital settings. As a proactive awareness for UiTM medical students before they are fully engaged as housemen in hospitals, this study has screened approximately 11.9% of the total population (n=1142) of medical students in UiTM. We have found that prevalence of 14% of *S. aureus* nasal carriers is relatively low compared to the prevalence in some population of medical students in universities in other countries. In medical schools in Spain and Thailand, 39.3% and 29.7% their students, respectively, were *S. aureus* nasal carriers [17, 18]. Although the percentage obtained in our study may not reflect the true prevalence of nasal *S. aureus* carriage among our medical students, it may benefit students who have enrolled in this research of their carriage status. The students who are nasal *S. aureus* carriers are aware and could prepare themselves with knowledge on the preventive methods to eliminate or reduce transmission to their patients. Being *S. aureus* nasal carriers, these students have a higher risk of acquiring exogenous *S. aureus* than non-carriers. This assumption is based on a previous report that hospitalised patients who were previously *S. aureus* nasal carriers have higher risks to acquire *S. aureus*

during their stay in the hospital compared to those who were non-carriers [19].

In Malaysia, medical students of Universiti Putra Malaysia (UPM) [20], *S. aureus* nasal carrier was relatively low, i.e., 10% of 209 students, thus, comparable to our results. Similarly, none of their *S. aureus* were MRSA. However, health science students from UPM revealed a higher number of *S. aureus* nasal carriage at two samplings carried out within one-month interval, i.e. 31.3 and 33.3% from 372 of their students [21], with low prevalence (1.6%) of MRSA carriers.

Exposure to hospital environment has shown to increase the prevalence of *S. aureus* and MRSA nasal colonisation in medical students of Louisiana Medical University [22]. Similarly in Saudi Arabia, the prevalence of MRSA nasal carrier was higher in housemen than medical students of a university in Jeddah, who had less exposure to hospital environment [23]. Our study, however, revealed that exposure of clinical postings in hospitals did not seem to affect the *S. aureus* nasal carriage rate among the students. It is possible that the duration of students' exposure in hospitals in Malaysia may differ than those in Louisiana and Saudi Arabia. Our finding is similar to a study in Australia, where *S. aureus* nasal carrier rates in their medical students did not vary (35.2 to 42.6%) with the duration of hospital exposure [24]. Interestingly, they found that there was an increase in the number of resistant strains among medical students in their clinical years compared to those who were in pre-clinical years. Unfortunately, we are not able to corroborate their observation as antimicrobial susceptibility pattern of our isolates was not determined to our study.

It is estimated that ~20% of healthy individuals are persistent carriers, ~60% are intermittent carriers and ~20% are persistent non-carriers [1]. From our study, it is not possible to identify persistent carriers, intermittent carriers, and persistent non-carriers, as only a one-time screening was carried out. One report has hypothesized that host genetic factors, such as host adhesions, immune response, and secretion of antimicrobial peptide (AMP) β -defensin could influence the pattern of nasal carriage [25]. Apart from host factors, bacterial factors

such as the phenotype of the strain (e.g., presence of a capsule, biofilm or expression of surface adhesins) and transmissibility of the strain also, have some role. Recently, it was demonstrated that host innate inflammatory factors such as chemokines, growth factors, cytokines IL-22 as well as staphylococcal protein A, determine the duration of *S. aureus* nasal carriage [26, 27].

PVL is cytolytic that destroys leukocytes and causes tissue necrosis, whereas γ -haemolysin is leukotoxic and haemolytic [28]. PVL is usually found in < 5% of strains of *S. aureus*, in contrast to γ -haemolysin, which is being produced by >99% of *S. aureus* clinical strains. Fortunately, none of our students carry MRSA in their nares. Although 19 students carry MSSA strains, none harbours PVL. Eight of these students, however, had γ -haemolysin gene. This finding thus, illustrates that PVL toxin is rarely encountered whilst γ -haemolysin is constitutive in most *S. aureus* strains. Strains of *S. aureus* that do not produce synergohymenotropic toxin are considered to be less pathogenic [28].

For those who are *S. aureus* nasal carriers, intranasal mupirocin is one of the treatments that are usually advised as it was found effective for short-term eradication of *S. aureus* colonisation [29]. A meta-analysis on its usage showed its effectiveness for the decolonisation of *S. aureus* where it can reduce *S. aureus* colonisation by 56% [30]. Besides nasal application of a non-antibiotic, alcohol-based antiseptic was also effective in reducing *S. aureus* and total bacterial carriage [31]. It was able to reduce *S. aureus* colony-forming units from baseline by 99%. Furthermore, it was also found to be safe and convenient. Apart from above measures to prevent spread of *S. aureus*, healthcare workers should always practice personal hygiene, in particular, hand hygiene.

One limitation of this study is that, Year Five students were not included as subjects, as at the time of this study, they were sitting for their final examination. This cohort of students should be the most appropriate target groups since they will soon-to-be housemen in health-care settings. Their awareness on their *S. aureus* nasal carriage status could help them to take proactive measures to help curb the transmission of the bacteria to their patients. A longitudinal study

involving final year students and when they are housemen, could perhaps provide information on the impact of longer exposure to patients on the status of *S. aureus* nasal carriage.

CONCLUSIONS

There is low prevalence of *S. aureus* nasal carriers among medical students of UiTM, but none are MRSA carriers. The colonised MSSA however, are less pathogenic because none of the *S. aureus* isolates had both the PVL and γ -haemolysin toxins. Screening for *S. aureus* nasal carriers in medical students is important to create their awareness of being potential source of transmission of *S. aureus* and the importance of an infection control in hospital settings.

Conflicts of Interest

Authors declare none.

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