



***Staphylococcus aureus* Infection Risk in a Population of Health Sciences Students at a Public University**

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Abstract

Background: This study was undertaken to evaluate *Staphylococcus aureus* carriage and persistence in health sciences students at the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

Methods: Consent form and self-administered questionnaires on socio-demographics, hygienic practices, medical and medication history were distributed followed by nasal swab collection; sampling was done twice in one month interval in October and November 2013. Bacterial identification followed the standard phenotypic methods. Antibiotic susceptibility of isolates against oxacillin and cefoxitin was tested by disc diffusion method. Methicillin resistance determinant gene (*mecA*) was detected through polymerase chain reaction-assay.

Results: *S. aureus* was isolated from 31.3% (60/192) and 33.3% (60/180) of the student population during the first and second sampling respectively. Among the *S. aureus*-positive participants, about 65% of them were persistent carrier (*S. aureus* was detected during both sampling exercises). Six methicillin-resistant *S. aureus* (MRSA) were detected (four and two isolates in the two sampling events respectively) as inferred by decreased susceptibility to oxacillin and cefoxitin and presence of *mecA* gene; two of the strains were from a single individual. Fisher's exact test showed no significant correlation between carriage and the tested risk factors except for the habit of touching nose and chronic illnesses ($P < 0.05$), with a higher incidence of *S. aureus* among those associated with the two risk factors.

Conclusions: As far as the limited sampling period is concerned, these findings indicate that a proportion of the student population may be at infection risk. Avoiding frequent nose-touching could be one of the preventive measures.

Keywords: *Staphylococcus aureus*, Infection risk, Nasal carriage, MRSA

Introduction

Staphylococcus aureus is part of human normal flora colonizing the anterior nares, skin and throat (1).

In a population, up to 20% may be the persistent carriers of *S. aureus* while the rest either intermit-

tently or rarely carry the organism (2). *S. aureus* is an opportunistic pathogen and thus, under favourable conditions, colonization might impose threats in healthy individuals particularly in those with persistent *S. aureus* presence (3). Worldwide, it is one of the most common causes of skin, soft tissue and nosocomial infections with high morbidity and mortality (4). Nowadays, *S. aureus* becomes a serious health-care concern due to the emergence of drug resistant *S. aureus* particularly the methicillin-resistant *S. aureus* (MRSA) (5).

MRSA has become reputable not only in clinical setting but also in the community. For the latter, the risk factors for *S. aureus* acquisition and infection could be linked to recent hospitalization, recent antibiotic exposure, skin cut and underlying chronic illnesses (2). Social factor such as living in crowded environment, close contact with persons who are heavily colonized by the organism and poor self-hygiene may further facilitate *S. aureus* dissemination (6). MRSA could have been transmitted from hospital and proliferated in the community posing threat largely of population especially those with impaired immune system. Nevertheless, MRSA could have also emerged in the community itself (7). MRSA has been associated with multidrug resistance that complicates treatment due to limited antimicrobial choices (1-5).

In view of the increasing incidence of MRSA and its associated clinical complications, periodic screening for *S. aureus* carriage and methicillin resistance in community is crucial for surveillance purposes. Population living in proximity is of interest due to ease of transmission of the organism.

For this reason, we conducted a study in a population of health sciences students who lived and interacted within a close locality for *S. aureus* carriage over a period and the presence of MRSA. Potential risk factors were measured and compared for prospective associations with *S. aureus* carriage.

Materials and Methods

Study population and isolation of S. aureus

This cross-sectional study was conducted in Faculty of Medicine and Health Sciences, Universiti

Putra Malaysia involving first and second year health sciences students who agreed to participate by signing the written consent form. Samples were obtained twice in about one month interval in October and November 2013 for monitoring the short-term persistence pattern of the carriage. A total of 192 and 180 samples were collected from the first and second samplings respectively. Each participant was given a set of self-administered questionnaire to collect information about socio-demographics, hygienic practices, medical and medication history prior to first isolation (5, 8).

Samples were collected from both nares by inserting a sterile nasal swab into nostril about 1.5 cm in depth and rotating against the interior surface of nostril. The swabs were then cultured on Mannitol Salt Agar (MSA) (Merck, Germany) and incubated at 37°C for 24 h. The yellow colonies on MSA indicated the presence of *S. aureus* due to mannitol fermentation. Further isolation of yellow colonies for pure culture was carried out. Colonies, which showed consistent result of positive reaction for gram stain, coagulase and catalase test, were confirmed as *S. aureus*. All isolates were stored in Luria-Bertani broth with 20% glycerol at -80°C.

Antibiotic susceptibility test

The Kirby-Bauer disc diffusion method was used to determine the antibiotic susceptibility of *S. aureus* isolates. In screening for methicillin resistance, 1 µg of oxacillin and 30 µg of cefoxitin discs (Oxoid, UK) were used with reference strains *S. aureus* ATCC 700699 and ATCC 25923 as positive and negative controls respectively. Guidelines from the Clinical and Laboratory Standard Institute (CLSI) (9) were followed to interpret the diameter of the inhibition zones produced.

MecA gene screening

All *S. aureus* isolates that showed resistant towards oxacillin and cefoxitin were subjected to polymerase chain reaction (PCR)-assay for detection of *mecA* gene. The primers used were *mecA*-F (5'-AAA ACT AGG TGT TGG TGA AGA TAT ACC-3') and *mecA*-R (5'-GAA AGG ATC TGT ACT GGG TTA ATC AG-3') (10). The PCR conditions included an initial denaturation step at

95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 40 s, annealing at 56°C for 40 s and extension at 72°C for 50 s, and a final extension at 72°C for 5 min (10). *S. aureus* ATCC 700699 and *S. aureus* ATCC 25923 were used as positive and negative controls respectively. Upon gel electrophoresis, samples with band fragment of 147 bp were reported as positive for *mecA* gene.

Statistical analysis

The data collected from questionnaires was analyzed using SPSS version 21.0 (IBM, USA). Fisher's exact test was used to analyze the categorical variables in relation to *S. aureus* carriers and non-carriers. The results were considered as statistically significant if P values ≤ 0.05 .

Results

A total 192 and 180 health sciences students participated during first and second sample collections respectively. Among the participants, *S. aureus* nasal colonization was found in 60 (31.3%)

students during the first sampling and 60 (33.3%) students during the second sampling. Among the 60 students positive for *S. aureus* in both sampling exercises, 39 (65%) were the same individuals (persistent carrier) and the rest carried in either one of the two sampling periods (intermittent carrier). Based on CLSI-interpretive criteria, six MRSA were tentatively detected during both sample collections as inferred by decreased susceptibility to oxacilin and cefoxitin. PCR further confirmed presence of the methicillin resistance-determinant gene (*mecA*) in these isolates based on the successful 147 bp-amplicon size. Among the persistent carriers, only one individual carried MRSA in both sampling exercises.

The demographic, medical and hygienic features of the students are indicated in Table 1. There are no significant associations between carriage and the tested risk factors except for the underlying chronic illnesses (asthma; $n = 9$) and habit of touching nose ($P < 0.05$). All six MRSA were isolated from healthy students and five of them were carried by those with habit of touching nose.

Table 1: Distribution of *S. aureus* in relation to risk factors

Risk factor	Health sciences students*		<i>P</i> values
	Positive for <i>S. aureus</i> <i>n</i> = 60 (31.3%)	Negative for <i>S. aureus</i> <i>n</i> =132 (68.7%)	
Gender			
Male	12 (20)	22 (17)	0.68
Female	48 (80)	110 (83)	
Race			
Malay	40 (67)	97 (73)	0.39
Others	20 (33)	35 (27)	
Presence of chronic illnesses	7 (12)	4 (3)	0.04
History of cold and fever in past 2 weeks	14 (23)	32 (24)	1.00
Antibiotics in past 2 weeks	3 (5)	9 (7)	0.76
Presence of unhealed wound	0	2 (2)	1.00
Use of intravascular device	1 (2)	1 (1)	0.53
Often touch nose	54 (90)	102 (77)	0.04
Often wash hand after touching nose	33 (55)	86 (65)	0.20

Significant values ($P < 0.05$)/ *Questionnaires were analyzed based on *S. aureus* isolation from the first sampling only.

Discussion

Results from this study demonstrated that the prevalence of *S. aureus* in both sampling periods

were about similar to a previous study by Neela et al. (11) (31.5%) among healthy students at the same locality. The observed prevalence rate is also within the range as reported in other studies (20-

35%) (8, 12). In this study, a significant association was observed between the carrier and the habit of touching nose and chronic diseases. The present analysis found that 90% of the student positive for *S. aureus* had frequently touched their noses. This could partly explain the reason of being carrier among the students. *S. aureus* are easily transmitted via hands and thus habit of touching nose may facilitate transmission and colonization in the nares. As for presence of chronic illnesses ($n = 11$), the associated students with such risk factor were low and mostly asthmatic, but a majority of them (64%) were *S. aureus* carriers. Those with chronic underlying diseases may have a compromised immune system to allow a more successful bacterial colonization. Thus, this group is at a higher risk of *S. aureus* infection.

The demographic factors such as gender and ethnicity may also have contributing roles in *S. aureus* nasal carriage (2, 13). However, as far as the studied population in this study was concerned, such association was not observed. The medical factors such as recent history of cold or fever, and consumption of antibiotic also showed no association with *S. aureus* carriage, while the presence of unhealed wound and use of intravascular device involved a very few respondents and neglected. Amin Nordin et al. (8) and Choi et al. (12) conducted a similar study on a specific Malaysian population and found no correlation. Although these medical factors were reported by others as the potential risk factors for *S. aureus* carriage (4), differential outcomes are expected due to the socioeconomic, health status and lifestyle of the studied population which may vary in different regions with different climates to affect the colonization and transmission of *S. aureus* (14).

The sample collections in this study were conducted twice within one-month interval, thus allowing us to identify short transient pattern. More than half of the respondents positive for *S. aureus* (65%) were found to be persistent carriers. This may reflect a prolonged colonization of *S. aureus* at a certain extent, which may increase the risk of infection in part of the population. The presence of MRSA was low but should be of concern particularly the incidence of MRSA that was

detected in a single individual in both sampling periods. The MRSA could be of community origin and may potentially disseminate at a larger extent if no precautionary measures are being taken.

Conclusions

As far as the limited sampling period is concerned, findings in this study indicate that a proportion of the student population may be at infection risk. Implementation of preventive measures in several aspects, such as hand-hygiene practice in particular, is warranted although the other factors included in this study may be the playing roles as well. A larger sample size involving more participants from various localities is needed to invigilate the status of *S. aureus* colonization and persistence pattern, and the dissemination of MRSA in community.

Ethical considerations

This study was approved by the Ethics Committee for Research involving Human Subjects (JKEUPM), Universiti Putra Malaysia with reference number UPM/TNCPI/RMC/1.4.18.1 (JKEUPM)/F1.

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