Staphylococcus aureus nasal carriage in young healthy adults in Brunei Darussalam

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ABSTRACT

Introduction: *Staphylococcus aureus* (*S. aureus*) is a gram-positive coccus bacterium which is normally a skin flora but may cause opportunistic infections such as skin and soft tissue infections, bacteraemia and necrotising fasciitis. Nasal carriage of *S. aureus* is a well-known factor for subsequent infections and transmission. Emergence of antibiotic-resistant strains including Methicillin-Resistant *S. aureus* (MRSA) has become a public health problem. Therefore, an understanding of *S. aureus* nasal carriage status and its transmission is important for control of *S. aureus,* formulation of treatment strategies and related health planning. **Materials and Methods:** Nasal specimens were collected from 115 undergraduate students from Universiti Brunei Darussalam who volunteered to take part in the study. *S. aureus* identification and antibiotic susceptibility testing including those of MRSA were carried out by standard laboratory protocols. A questionnaire was also administered to volunteers to help identify associated factors to *S. aureus* nasal carriage and possible transmission. **Results:** Twenty-two percent (25/115) of participants tested positive for *S. aureus* nasal carriage and none for MRSA. *S. aureus* isolates have a varying low resistance to amoxicillin/clavulanic, tetracycline and chloramphenicol. No significant differences exist between carrier and non-carrier states in relation to factors that may be associated with *S. aureus* nasal carriage. **Conclusion:** Given that this is a preliminary study, a study with a larger sample size is required to further understand *S. aureus* nasal carriage in Brunei Darussalam.

Keywords: Drug resistance, nasal carriage, risk factor, *Staphylococcus aureus*, Brunei

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a gram-positive coccus bacterium which is normally a skin flora but may cause opportunistic infections ranging from skin and soft tissue infections, mucositis, bone/joint infection, pneumonia, endocarditis, bacteraemia, to life-threatening infections of septicaemia, necrotising fasciitis, and toxic shock syndrome. [1] Currently, *S. aureus* is a leading cause of such human infections worldwide both in hospital as well as community settings. [2] *S. aureus* is also frequently found inside the nostrils, throat, intestine and vagina. [3] Nasal carriage of *S. aureus* is a well-known factor for subsequent infections and transmission. [4]

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Infections can take place when the colonising strains enter a normally sterile site due to trauma to the skin or mucosal surface. [5] Transmissions of the bacteria happen from person to person by direct hand to skin contact: through fomites and unwashed hands. [6] Treatment is complicated with emergence of antibiotic-resistant strains; Methicillin-Resistant *S. aureus* (MRSA) [7] which has become a public health concern due to its resistance to many drugs thereby resulting in the availability of limited treatments for infections caused by the organism. [8]

Given the seeming ease of possible transmission of *S. aureus* and its multi resistant antibiotic forms including MRSA, this study aimed to determine the prevalence of nasal carrier status of *S. aureus* and MRSA among healthy young adults in Brunei Darussalam, specifically students studying at the Universiti Brunei Darussalam (UBD). This study also evaluate antimicrobial resistance of isolated strains as well as identify factors that may be associated with *S. aureus* nasal carriage. This study population constitutes the most socially active group in the Sultanate. By implication therefore carriers within this group are a reservoir for active transmission of diseases of *S. aureus*. Thus understanding the prevalence of carrier status of *S. aureus* and the antibiotic resistance patterns in this group is imperative to formulating good national health planning and appropriate strategies for control and treatment of *S. aureus* infections.

MATERIALS AND METHODS

Recruitment of participants: Participants for this study were recruited from the undergraduate student population of Universiti Brunei Darussalam from January 2013 to March 2013. A total of 115 undergraduate students across the University's Academic Faculties and Institutes volunteered to take part in the study. The following exclusion criteria were used: 1) undergoing clinical attachments in the hospital or health care institutions as several studies have shown that hospital environment has a role in the development of multi resistant antibiotic *S. aureus* including MRSA [13]; 2) antibiotics usage at the time and within four weeks of sample collection as it can affect the carrier status as well as the organisms that a person carries; and 3) nasal diseases such as rhinitis because the consequent irritation and inflammation could hamper the determination of nose picking habit. [14]

Detailed explanations and participant information sheets were given to the qualifying volunteers and their questions and concerns were satisfactorily addressed. A written informed consent was received from each participant thereafter. In addition, a short questionnaire was later used in identifying and evaluating factors associated with nasal carriage of *S. aureus* was administered to each participant on the day of sample collection. Each participant's name was recorded in the database and was kept strictly confidential that it could only be accessed by the researchers. The names were linked to both questionnaire and respective nasal swabs to ensure identification of the participants in case positive samples of MRSA were detected and for individuals to be referred to a General Practitioner for treatment.

Microbiological analysis: Nasal specimens were obtained using sterile cotton swabs that had been moistened with sterile saline (to pre-

vent nasal cavity irritation) by rotating one cotton swab tip in both nares for about five seconds each and was then immediately placed in Trypticase Soy Broth (TSB) transport medium and cultured within 24 hours.

Swabs were cultured on Mannitol Salt Agar (MSA) plates were and incubated at 37^0C for 24 hours. The identification of *S. aureus* was performed in accordance with standard laboratory protocols, including typical colonial morphology, Gram staining, Catalase and Coagulase tests. Isolates which were yellow or golden colonies with mannitol fermentation; Gram positive cocci; Catalase as well as Coagulase positive were identified as *S. aureus*. They were then subjected to antibiotic susceptibility testing which was performed using standardized disc diffusion method on Mueller-Hinton agar with amoxicillin/clavulanic acid (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), tetracycline (30 µg), trimethoprim/ sulphamethoxazole (25 µg), oxacillin (1 µg), and vancomycin (30 µg) (Oxoid).

S. aureus isolates were screened for methicillin resistance by disk diffusion method of the National Clinical and Laboratory Standards Institute. [9] Cultures from MSA were plated on Mueller-Hinton Agar (MHA) and a 1-µg Oxacillin disc test was placed on the inoculated plate. Zone diameters were measured and recorded after 24-hour incubation at 35^0C, the results were classified as sensitive (≥13 mm), intermediate (11–12 mm), or resistant (≤10 mm). The accuracy of the overall testing procedure was monitored by using *S. aureus* ATCC 29213 as control strain.

Potential Risk factors: Several variables based from published data in the literature based from published data in the literature were investigated as potential characteristics indicative of association with nasal carriage of *S. aureus*. These factors included sex, antibiotic usage in the past two months, prior exposure to MRSA, family members working in the hospital, hospitalisation in the past (the volunteer or any family member of the volunteer), the number of children in the household, sharing towels, razors or soap with friends or family members, nose picking, hand hygiene, keeping of animal pets, and current exposure to cigarette smoke (operationalized as either a current smoker or living in a house with a current smoker) and prevalence were then calculated. [15-19]

Ethics statement: This cross-sectional study received approval from the UBD Research and Ethics Committee before the commencement of the Project in January 2013.

Statistical analysis: IBM SPSS Statistics Software, version 17.0 was used for data analysis. Pearson Chi-square test was carried out to determine the 'p values' of factors associated with nasal carriage of *S. aureus.* P values less than 0.05 were regarded as being significant.

RESULTS

A total of 115 undergraduate students, 45 (39%) males and 70 (61%) female with an age range of 19 to 24 years participated in the study. Overall nasal carriage of *S. aureus* in the participants was 25 (22%); no MRSA case was detected. Among the *S. aureus* nasal carriers, 12 (48%) were males and 13 (52%) were females.

The antibiotic sensitivity patterns of

Table 1: Antibiotic susceptibility of *Staphylococcus aureus* isolates from volunteers.

Antibiotic	Code	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Oxacillin	OX	25(100)	0	0
Vancomycin	Va	25 (100)	0	0
Gentamicin	Cn	25 (100)	0	0
Tetracycline	Te	20 (80)	0	5 (20)
Chloramphenicol	C	23 (92)	0	2 (8)
Amoxicillin/clavulanic acid	AMC	16 (64)	0	9 (36)
Trimethoprim/sulfamethoxazole	SXT	24 (96)	1 (4)	0

25 isolates of *S. aureus* from the carriers are shown in Table 1. All the isolates were sensitive to oxacillin, vancomycin and gentamicin. Few of the isolates were resistant to commonly used antibiotics: amoxicillin/clavulanic (36%); tetracycline (20%) and chloramphenicol (8%). One isolate (4%) was intermediately resistant to trimethoprim/sulfamethoxazole.

The antibiotic sensitivity patterns showed that three isolates were resistant to tetracycline and amoxicillin/clavulanic (n=1); tetracycline and chloramphenicol (n=1); and choramphenicol and amoxicillin/clavulanic as well as intermediately resistant to trimethoprim/ sulfamethoxazole (n=1).

The potential risk factors associated with *S. aureus* nasal carriage are shown in Tables 2 and 3. Though the prevalence of *S. aureus* nasal carrier tends to be higher among nose-pickers (64%, n=16/25) when compared with non-nose pickers (49%, n= 44/90), the difference is not significant. A similar situation applies to the possession of a dog as a pet amongst the participants: *S.* (16%, n=4/25) for carriers and (10%, n=9/90) for non-carriers.

Table 2: Variables associated with *S. aureus* carriage.

Characteristics	Positive n (%)	Negative n (%)	P value
Sex			
Male	12 (48)	33 (37)	0.304
Female	13 (52)	57 (63)	
Children in household			
0	15 (60)	52 (58)	
1	3 (12)	18 (20)	
2	4 (16)	13 (14)	0.873 for trend
3	2 (8)	4 (4)	
≥4	1 (4)	2 (2)	
	0	1 (1)	
Sharing towels, razors or soap with family members or friends?			
Yes	4 (16)	24 (27)	0.272
No	21 (84)	66 (73)	
Nose picking			
Yes	16 (64)	46 (51)	0.253
No	9 (36)	44 (49)	
Washing hand regularly			
Yes	25 (100)	83 (92)	0.150
No	0	7 (8)	
Keeping of animal pets			
None	7 (28)	31 (34)	
Cat	12 (48)	42 (47)	
Dog	4 (16)	9 (10)	0.491 for trend
*Other**	2 (8)	2 (2)	
*Cat and other**	0	5 (6)	
*Cat, dog and other**	0	1 (1)	

Note: Other* denote pets such as chicken, snake, rabbit or turtle

Table 3: Variables associated with *S. aureus* nasal carriage based on clinical characteristics.

Characteristics	Positive n (%)	Negative n (%)	p value
Use of antibiotic in past 2 months			
Yes	3 (12)	7 (8)	0.507
No	22 (88)	83 (92)	
Prior exposure to MRSA infection			
Yes	1 (4)	3 (3)	0.630
No	24 (96)	87 (97)	
Any family member working in hospital?			
Yes	8 (32)	32 (36)	0.741
No	17 (68)	58 (64)	
Any family member being hospitalised currently or in the past for more than one year?			
Yes	11 (44)	50 (56)	0.738
No	14 (56)	40 (44)	
Current smoker			
Yes	0	6 (7)	0.185
No	25 (100)	84 (93)	
Any family member who is/are currently smoking?			
Yes	9 (36)	40 (44)	0.450
No	(64)	50 (56)	

No apparent differences exist in relation to the other factors that were examined.

DISCUSSION

In this study, 22% (25/115) of the participants tested positive for *S. aureus* nasal carriage. This is slightly lower than the reported global incidence of between 25% and 30% [20]; and suggests that 22% of the participants in the study may run a risk of acquiring nosocomial infections. [10] They are also a reservoir for the transmission of *S. aureus* to people that they interact with. [4] However, none of the participants tested positive for MRSA which suggests that the studied population does not carry this drug-resistant strain. This is not surprising as life-threatening MRSA infections typically occur more amongst patients in healthcare settings, and in general less than 2% of carriers are found in the community. [20]

The treatment of staphylococcal infections is generally carried out with a group of antibiotics called beta-lactams which include methicillin, oxacillin, penicillin, and amoxicillin; MRSA is however generally resistant to these antibiotics.

In the present study, all the *S. aureus* isolates were sensitive to oxacillin, vancomycin and gentamicin. This suggests that should any of the isolates cause infections in the participants, individuals concerned could be effectively treated with any of these antibiotics. In general, these isolates show lower rates of resistance to amoxicillin/clavulanic acid (36%); tetracycline (20%) and chloramphenicol (8%) in comparison with two previous studies [2, 11] which were conducted in Nigeria. For example, Ajoke *et al.*, reported a high rate of resistance to tetracycline (72.2%) and amoxicillin/clavulanic acid (68.4%), while Onanuga and Temedie observed 47.5% resistance to chloramphenicol. The differences in antibiotic resistance patterns among the *S. aureus* isolates in these studies may be due to differences in the availability and/or relative

ease of access to antibiotics in the two countries where the studies were carried out. For example, in Brunei Darussalam antibiotics are dispensed only with doctors' prescriptions which consequently leads to fewer misuse of antibiotics. On the contrary, in Nigeria, antibiotics are readily available over the counter [12] and this may have result in more misuse, inappropriate and/or incomplete use of antibiotic dosages resulting in bacteria mutation and antibiotic resistance. Another plausible reason is the differences in the study populations with respect to their age ranges, education levels and occupations. Why was the present study focused on students with age ranging from 19 to 24 years old? Ajoke *et al.*, randomly selected people with age ranging from 18 to 40 years old and Onanuga and Temedie had studied both students and villagers with an age range of 15 to 35 years old. Older population especially villagers commonly have less knowledge on misues of antibiotics compared to students, and this may be the reason why antibiotic resistance of *S. aureus* isolated from students tends to be lower. [2]

In this study, the prevalence of *S. aureus* nasal carriage tends to be higher among nose pickers (64%) than non-nose pickers (49%). However, there is no significant difference observed between them ($p>0.05$) which suggests that nose picking habit may not be a notable factor in *S. aureus* nasal carriage in this population. With respect to the keeping of a dog as pets, there is no difference in prevalence observed between nasal carriers and non-carriers ($p>0.05$) which suggests that keeping a dog as a pet may also not be a notable factor in *S. aureus* nasal carriage in this study population. The aspect of keeping a dog as a pet and its effect on *S. aureus* nasal carriage in Brunei Darussalam becomes more pertinent and therefore certainly deserves more studies. This is because about 66% of the population of Brunei are Muslims and Islam forbids the keeping of dogs as pets or even playing with them. Given that this is a preliminary study with a relatively much smaller sample size than desired, these observations may not be replicable in a larger sample size. This is especially so since proportionately there are no similarities in the prevalence for each of factor.

In conclusion, approximately 22% of the participants of this study are found to be nasal carriers of *S. aureus* but no MRSA carriage is detected. A low rate of antibiotic resistance to at least three antibiotics (amoxicillin/clavulanic acid, tetracycline and chloramphenicol) is observed amongst the isolated *S. aureus* strains. A more elaborate study with a much larger sample size and a wider age range is therefore being considered.

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