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Staphylococcus Aureus Antibiotic Resistant Patterns Altered in Student Nurses after Clinical Experience

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Abstract

Background: *Staphylococcus aureus* (*S. aureus*) carriage is a risk factor for nosocomial infections in patients and healthcare workers and may include nursing students undergoing clinical experience.

Aim: To investigate a group of nursing students undergoing their first clinical posting for *S. aureus* carriage in the nose and/or throat; determine the antibiotic resistant patterns of individual *S. aureus* isolate as well as screen the participants for methicillin resistant *S. aureus* (MRSA).

Methods: Nose and throat swab samples from 17 first year diploma students of nursing, four male with a mean age of 21 years and thirteen female with a mean age of 19.3 years who were undergoing their first clinical posting were investigated. Pre- and post- a four weeks clinical experience at a tertiary level hospital swab samples were processed by standard methods for the isolation, identification and antibiotic susceptibility testing of *S. aureus* including MRSA.

Findings: *S. aureus* was isolated from both nose and throat of four of the students; four for each of nose and throat in the pre-clinical experience samples. In post clinical experience sampling, four students had *S. aureus* in their nose and throat and four and six from nose and throat respectively. No MRSA was isolated from any of the samples. Several of the students' post clinical experience *S. aureus* isolates show higher antibiotic resistance.

Conclusion: Nursing students on clinical posting most likely to be at an increased risk of *S. aureus* carriage and antibiotic resistance.

Keywords: Antibiotic resistance; Carrier status; *Staphylococcus aureus.*

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a normal flora of the human body and can be found in anatomical locales such as on the skin and in the oropharynx.[1] However, it may be opportunistically pathogenic being capable of causing various infections of the skin and soft tissues, bacteraemia, endocarditis, meningitis, osteomyelitis and pneumonia in both healthy individuals and those with underlying illness.[2]

S. aureus has the capability of acquiring resistance against all classes of antibiotics by either horizontal transfer of a resistance gene from other bacterium or mutation of an existing bacterial gene.[3] Clinically, *S. aureus* has been known to have acquired the most important resistance trait which is being methicillin-resistant.[3]

Approximately 60% and 20% of the population are intermittent or occasional and persistent carriers of *S. aureus* respectively while the remainder of the population are non carriers.[4] *S. aureus* is carried by approximately 30% of the healthy population [5] although the rate of carrier status for the throat is a variable frequency ranging from 4% to 64%. [6]

S. aureus being the most potent pathogen causing nosocomial infections[7] exposes the patients as well as healthcare workers to a higher risk of such infections. This may extend to healthcare students undergoing their clinical postings due to their exposure to the hospital environment and the frequency of contact with the patients. The nurses are likely to be at the most risk as they are the largest group of hospital workers and in very close contact with the patients who they directly handle.[8] Hence, it could be argued that they run a higher risk of being colonised by different pathogens including *S. aureus* which they may subsequently transmit and could indeed be of public health significance.

The first year students of diploma in nursing at the Institute of Health Sciences, Universiti Brunei Darussalam had not been exposed to professional hospital environment, therefore, making them a suitable candidate for this study.

The aim of this study was to investigate the students' carrier status with respect to *S. aureus* in the nose and/or throat as well as the relative acquisition of the carrier status of *S. aureus* during their clinical posting, compare the antibiotic susceptibility patterns in pre- and post-clinical posting nasal and/or throat swabs, and specifically screen the participants for the presence of MRSA before and after the clinical posting.

METHODS

This study was approved by Research and Ethics Committee of Universiti Brunei Darussalam, Brunei Darussalam. Seventeen students were recruited from among first year students of the Diploma in Nursing programme of the Institute of Health Sciences, Universiti Brunei Darussalam: they had not been exposed to the hospital environment in their professional capacities. Swab samples were collected from the anterior nasal mucosa and oropharynx by the use of sterile cotton swab moistened with sterile saline to prevent any irritation to the participants and facilitate the ease of bacterial collection unto the swab. [9] Samples from the anterior nasal mucosa were obtained by rotating one cotton swab tip in both nares for about 5 seconds each; and from the oropharynx by rotating another cotton swab tip on left and right palatoglossal arch. The swab specimens were transported in modified peptone water within 1 hr of collection to the Institute of Health Science's Research Laboratory for analysis. Swab samples were collected on the day before the commencement of the four weeks clinical posting at the Raja Isteri Pengiran Anak Saleha Hospital (RIPAS Hospital) the premier tertiary hospital in Brunei Darussalam and the day after its completion.

Modified peptone water (MPW) as previously used by Isibor and Amadi, was inoculated with the swab samples immediately after collection from the study participants. [10] Briefly, after an overnight incubation at 37°C, the broth culture was sub-cultured in Mannitol Salt Agar (Oxoid) (MSA) and incubated overnight at 37°C. Colony morphology of the growth on the MSA was further analyzed for suspected colonies of *S. aureus*.

Suspected colonies of *S. aureus* emanating from the morphological appearance were further investigated following standard microbial identification protocols consisting of Gram staining, Catalase test and, Coagulase test using Prolex™ Staph Latex Kit (Pro-Lab Diagnostics, Canada). *S. aureus* ATCC 29213 was used as positive control.[11, 12]

Antibiotic susceptibility testing

All confirmed *S. aureus* isolates were tested for susceptibility to the following antibiotics using standardized disc diffusion method on Mueller-Hinton Agar (MHA): amoxicillin/clavulanic acid (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), tetracycline (30 µg), trimethoprim/sulphametoxazole (25 µg), oxacillin (1 µg), and vancomycin (30 µg) (Oxoid). In addition *S. aureus* isolates were tested with oxacillin discs to determine their MRSA status. Susceptibility testing of the *S. aureus* isolates to the antibiotics was carried out according to the CLSI publication M100-21 (2011). [13]

RESULTS

Of the 17 participants, 13 (76.5%) were female while the remaining 4 (23.5%) were male. In preclinical posting swab samples, *S. aureus* was isolated from both nose and throat of four of the participants and four

participants each for only nose and throat; making a total of 12 (70.6%) *S. aureus* carriers. Five (29.4%) of the participants did not carry *S. aureus* in their nose and/or throat at the preclinical experience sampling. In the post-clinical experience swab samples, *S. aureus* was isolated in both nose and throat of four of the participants, and, four and six in only nose or throat respectively; making a total of 14 (82.4%). The four carriers of *S. aureus* in both nose and throat in pre-clinical experience samples were all female. However, in the post-clinical experience samples one male and three female participants were carriers in both nose and throat: the three female were among the four carriers identified in the preclinical experience sampling. The male who has become a carrier in both nose and throat in the post-clinical experience sampling only carried *S. aureus* in the throat prior to going on clinical posting. Interestingly, the fourth female carrier in both nose and throat during pre-clinical experience sampling has become a carrier only in the throat post clinical experience. Three of the four participants who carried *S. aureus* only in their nose during preclinical sampling persisted in the post clinical sampling with the fourth person becoming a non-carrier in neither the nose nor throat. In summary, in post-clinical posting sampling, four (28.6%) were nasal carriers, six (42.9%) were throat carriers, and four (28.6%) were both nasal and throat carriers of *S. aureus* (Table 1).

Isolation Site of *S.aureus*	Pre-Clinical Posting	Post- Clinical Posting	Total
Nose	4	4	17
Throat	4	6	
Nose & Throat	4	4	
Non – carriers	5	3	

Table 1: *S. aureus* isolation in the anterior nares and throat of the diploma in nursing and midwifery students, pre- and post- clinical posting

Antibiotic susceptibility testing revealed that all isolates of *S. aureus* were sensitive to gentamicin, trimethoprim/sulphamethoxazole, chloramphenicol, vancomycin and oxacillin in both pre- and post-clinical samples. None of the *S. aureus* isolates was methicillin resistant. With respect to the preclinical posting samples, only one sample was resistant to amoxicillin and another four were resistant to tetracycline. As for post-clinical samples, all were sensitive to amoxicillin and four were resistant to tetracycline. Table 2 summarizes the result of antibiotic susceptibility testing for the *S. aureus* isolates in the nose and throat for pre- and post- clinical experience.

Antibiotics	Status	Pre-Clinical Posting	Post-Clinical Posting
AMC	S	11	14
	R	1	0
TE	S	8	9
	R	4	4
C	S	12	14
	R	0	0
VA	S	12	14
	R	0	0
SXT	S	12	14
	R	0	0
CN	S	12	14
	R	0	0
OX	S	12	14
	R	0	0

Table 2: Results of antibiotic susceptibility testing for S. aureus isolates in the nose and throat for pre- and post-clinical posting
AMC: amoxicillin/clavulanic acid, TE: tetracycline, C: chloramphenicol, VA: vancomycin, SXT: trimethoprim/sulphamethoxazole, CN: gentamicin, OX: oxacillin. S: sensitive, R: resistant

Eleven of the participants were persistent carriers of *S. aureus* over the period of sample collection. Five of them showed different antibiotic susceptibility patterns in pre- and post clinical experience samples. Three of the *S. aureus* isolates from the throat were resistant to tetracycline in both pre- and post- clinical experience analyses; one isolate from the throat became resistant to tetracycline in the post clinical experience while another isolate from the throat became susceptible to tetracycline. One isolate from the nose became susceptible to amoxicillin/clavulanic acid post-clinical posting (Table 3).

	TE		AMC	
Isolation Site of *S. aureus*	**Pre-**	**Post-**	**Pre-**	**Post-**
Nose	3R5S	2R3S	1R4S	0R5S
Throat	1R4S	2R3S	0R5S	0R5S

Table 3: Antibiotic susceptibility pattern of *S. aureus* isolates to tetracycline and amoxicillin/clavulanic acid

DISCUSSION

The incidence of *S. aureus* carriage in both the anterior nares and the throat in comparison to either of the sites, seen in pre- and post- clinical experience were comparable, with the post clinical posting being 12 percent higher. Overall, given that only four of the 17 participants (24 percent) were male, it is our opinion that a comparison of the incidence between the genders will be skewed and therefore misleading. Furthermore, a total number of 17 participants would

seem inadequate and unrepresentative of the study population. However, a recent study by Abdul Malik et al that investigated the *S. aureus* carriage in the same sample population reported similar incidence between the genders. [14]

The *S. aureus* carrier status of some participants changed over the four weeks clinical experience. For example, three of the five individuals who were non-carriers in the pre-clinical experience sampling have become *S. aureus* carriers post clinical experience. Furthermore, others have acquired *S. aureus* at sites where they were not present initially and one has become a non-carrier even though no antibiotic medication had been taken. While it is possible the observed changes in carriage status to be influenced by the clinical posting, it could in the main suggest that a number of the individuals could be intermittent or occasional carriers. This is in agreement with Kluytmans *et al.,* who observed that different *S. aureus* carriage status occurs over time in certain individuals even without the use of antibiotics. [4] Indeed, other workers have reported that varying composition of the local bacterial flora could concomitantly influence the nasal colonization by *S. aureus*. [15] Furthermore, the overall immune status of the individuals might improve and overcome *S. aureus* carriage as the host immune defence has an important role against nasal colonization.[16] Thus, it is highly likely that either or both happened in the case of the individual who became a non-carrier of *S. aureus* in the post clinical experience sampling as they may have undergone changes in ecological milieu of the nose and/or the throat.

We determined the *S. aureus* carrier status by investigating swab samples from both nose and throat of the participants. If the throat had been excluded from the study, 50% and 55.6% of the total *S. aureus* carriers in pre- and post-clinical experience samples respectively would have been missed. In addition, the two participants who became *S. aureus* carriers in the throat after the clinical experience would not have been detected. This observation is in agreement with a previous report by Mertz *et al.* [5, 6] Our results therefore confirm that screening only the nose as has been carried out by some workers is not sufficient in the investigation of *S. aureus* carriage. [17, 18] This is because colonization of *S. aureus* could be missed if the screening is limited to the anterior nares as the throat can be selectively colonized by *S. aureus.* We therefore argue for the combined use of the throat and anterior nares in screening for *S. aureus* carriage.

Antibiotic susceptibility testing on the *S. aureus* isolates revealed that overall, post-clinical experience isolates demonstrated more multidrug resistance. This is in agreement with an observation by Stubbs *et al* who reported a more prevalent multi-drug resistant *S. aureus* among medical students on clinical postings. [19] This suggests that increased exposure to the clinical environment might lead to multi-drug resistance in due course. It is also likely that the resistance to the antibiotic could be caused by mutation of the organisms as the plasticity of *S. aureus* genome may indeed increase its ability to adapt to selective pressure in the environment.[20]

Susceptibility patterns among some of the *S. aureus* isolates are inconsistent. For example, after their clinical experience, a carrier of *S. aureus* in both the nose and the throat became susceptible to amoxicillin/clavulanic acid in the nose while the *S. aureus* in the throat remains resistant to tetracycline. This is in contrast to another carrier of *S. aureus* in the throat which was previously resistant to tetracycline became susceptible in post-clinical sampling. The inconsistency of the susceptibility patterns for *S. aureus* isolates between the nose and the throat of the same carrier may suggest that the isolates from the nose and the throat are of different strains. This study has not carried out *S. aureus* stain typing. However, others have reported the existence of different strains of *S. aureus* carried in the nose and the throat. [21, 22] In addition, the colonizing strain in the nares can be replaced by another *S. aureus* strain [23] which, therefore, could possibly be the case for these two post- clinical experience isolates.

There were no MRSA carriers detected among the participants. This suggests none of the participants has carries the organism in detectable proportion. This is not surprising especially in the post clinical experience sampling as the students were attached to the general wards and none in the Intensive Care Units, which according to Witte *et al*, are more affected by MRSA than other clinical settings. [24] Furthermore, there is relatively low but importantly decreasing levels of MRSA at RIPAS Hospital**.** [25] Finally, it could also suggest that the participants in this study practice good level of hand hygiene, which has been proven to play an important role in reducing the of MRSA and other nosocomial infections in general.[26]

We intend to progress this study by characterising the various isolates of *S. aureus*, and relating this to their sites of colonization and antibiotic resistance.

In conclusion, we have observed that a group of diploma in nursing students undergoing their first clinical training experienced altered *S. aureus* carriage status in their nose and/or throat. The alterations were observed in the sites of *S. aureus* carriage and antibiotic susceptibility patterns.

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