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Evaluation of MRSA Colonization Epidemiology before and after Practical Training in Hospitals and Healthcare Settings among Health Profession Students in Hong Kong

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Abstract

Staphylococcus aureus has been a global threat to the medical and healthcare community as it is notorious for causing nosocomial infections which can result in serious consequences such as pneumonia and bacteremia. *S. Aureus* infections can also put heavy pressure on the economy of the society because of the extra resources needed on the preventing the widespread of the bacteria and providing medical treatments for infected individuals. This burden will be doubled should the bacteria become resistant to antibiotics such as methicillin and vancomycin. As medical and healthcare workers can be colonized by the bacteria during their duty hours in the hospital and thus carrying HA-MRSA, they can also spread the bacteria into the community after their working hours, causing CA-MRSA. Therefore, it is important to know the MRSA carriage rate of this medical and healthcare professional in order to prevent a widespread of the bacteria. Besides hospital workers, students going to hospitals for their clinical training can also be colonized by the bacteria during their time of stay. This study investigates the MRSA colonization rate in the nasal cavities of MLS students before and after their clinical training in various hospitals. A prevalence study is also conducted among MLS and nursing students to find out their nasal MRSA carriage rate. It is found that MLS students generally have a higher nasal MRSA carriage rate than that of nursing students whether they have been to clinical training or not. Moreover, the MRSA colonization rate was relatively higher among MLS students before they go to clinical practicum. Despite it is shown that association is statistically insignificant between pre-exposure and post-exposure to the clinical environment, clinical exposure is still a major factor in affecting the MRSA colonization rate and a larger sample size can be recruited when further studies are done. In order to distinguish between HA-MRSA and CA-MRSA among MRSA isolates, questionnaires can be distributed to subjects and molecular methods such as ribotyping can be employed.

Introduction

Staphylococcus aureus has been a leading cause of nosocomial infections for the past century which has known to cause infections ranging from mild skin infections to life-threatening septicemia [1]. The bacteria can be spread through direct contact with infected individuals and/or contaminated objects. It can be inhaled as droplets from coughing or sneezing as well [2]. After the discovery of the antibiotic penicillin by Sir Alexander Fleming, it had been widely used for the treatment of bacterial infections, including that caused by *Staphylococcus aureus* [1]. After acquiring resistance to penicillin in the fifties, methicillin was developed to combat *Staphylococcus aureus* infections. But in 1961, the first strain of methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated by some British scientists [1]. MRSA are strains of *Staphylococcus aureus* which are resistant to β -lactams, a group of antibiotics that includes penicillin and its derivatives such as methicillin. Its resistance to methicillin is contributed by the presence of *mec* a gene, which codes for a penicillin-binding protein, PBP2a. This altered version of protein has a lower affinity for β -lactams, making the bacteria resistant to the attack of this class of antibiotics [3]. To tackle MRSA infections, clinicians have turned to vancomycin, an antibiotic belonging to the group of the glycopeptide [4].

MRSA is a well-known endemic disease in Hong Kong hospitals. The incidence of MRSA clinical isolates has been reported as 0.5% per death and the carriage rate on intensive care unit entry as 12.1% [5]. The incidence rate of hospital-acquired MRSA infections in Hong Kong was 0.26-0.29/1000 patients from 2009 to 2011[5]. In 2005, the Department of Health was notified of 112 CA-MRSA infections. *S. aureus*

with heteroresistance to vancomycin (hetero-VRSA) was described in Hong Kong during 2000 (HKSAR). Although no isolate of VRSA was reported in Hong Kong, the prevalence of hetero-VISA is increasing worldwide. As the years go by, the bacteria start to evolve into a more resistant strain caused by practices such as inappropriate antimicrobial use and prolonged hospitalisation. Vancomycin has been regarded as the first-line drug for treatment of MRSA. Unfortunately, there has been an increase in the use of this antibiotic for other infections, such as pseudo membranous colitis due to *Clostridium difficile* and coagulase-negative staphylococcal infections in hospitalized patients [6]. In 2002, the first case of vancomycin-resistant *Staphylococcus aureus* (VRSA) infection was reported in the United States. Despite of its rare occurrence, it is still considered a threat to the community and a concern to the medical sector as there is a possibility that *Staphylococcus aureus* infections could become untreatable, as treatment of with vancomycin has been regarded as one of the few last resorts for MRSA infections [1].

Over the years, numerous researches have been conducted on hospital staff and hospitalised patients on the subject of MRSA and VRSA [3,7-9]. However, there is another group of people who are sharing the same space and the potential of acquiring infections with them as well. Every year, medical students around the world would receive their clinical training in hospitals and there is a chance for them to be exposed to various types of pathogens, including *Staphylococcus aureus*. They could be colonized by MRSA and/or VRSA during their practicum and the pathogen could be spread to the community after their training, causing infections which could become a medical and financial burden to the society.

As both nurses and medical laboratory technicians work in hospitals and other different healthcare settings, there is a possibility of contracting hospital-acquired infections (HAI), which can also be known as nosocomial infections. HAI could range from mild infections such as urinary or skin infections to infections with serious consequences such as bacteremia and surgical site infections. It is suggested that nosocomial infection is one of the leading causes of morbidity and mortality, which means that it poses a threat not only to patients but also medical and healthcare workers [10]. It is a burden to the society as well as it increases the costs required to provide medical services such as drugs and laboratory and other diagnostic tests [11]. Micro-organisms acquired through nosocomial infection may also be spread into the community via discharged patients and medical and healthcare workers. The result would be devastating if the organism is multi-drug resistant [12,13]. Micro-organisms involved in HAI can be bacteria, virus, fungi and parasites, and the routes of transmission to hospital staff include the exogenous cross-infection, which is the transmission of the flora from a patient or member of staff to another member of staff, and the exogenous environmental infection, which is contracted when an individual comes in contact with a contaminated object [10].

Previous researches have been conducted to investigate the colonization of MRSA on medical and healthcare workers of various disciplines. Among all categories, nurses appear to be the group of hospital personnel that has the highest rate of MRSA colonization, which is then generally followed by doctors. The MRSA carriage rate of medical laboratory technicians is among the lowest of all categories [14-16]. The high carriage rate of MRSA among nurses could be due to the fact that they are constantly in direct contact with patients, making them a potential source of infection to patients and themselves alike [16]. Compared with their frontline co-workers, medical laboratory technicians tend to be involved mainly in the behind-the-scenes work of the entire medical and health care profession. The chances of laboratory personnel having close contact with patients are significantly lower than that of nurses, which explains their low carriage rate of MRSA. However, it does not mean that there is little risk in being nosocomially infected. Laboratory workers are prone to laboratory-acquired infections (LAI), which is defined by the Public Health Agency of Canada (2009) as all infections acquired through laboratory or laboratory-related activities regardless of whether they are symptomatic or asymptomatic in nature, in which infections include laboratory associated infections and autopsy related infections. Bacteria, fungi, viruses and parasites are all organisms that can cause LAI and numerous cases of LAI have been reported by healthcare organizations and researches [17,18]. Among the four major disciplines of medical laboratory science, namely hematology, clinical chemistry, clinical pathology and medical microbiology, LAI is found most prevalent in the microbiology section. It could be due to the fact that it is the duty of workers in the microbiology section to handle specimens related to microorganisms, hence a much-increased chance of acquiring. Apart from the normal work force, medical and healthcare students could be a source of MRSA infection as well as they would be exposed to different healthcare settings during their clinical training. It is mentioned in a letter to the editor of the Brazilian Journal of Infectious Diseases by Khanal [19] that a nursing student on clinical training is found to be colonized by MRSA [19]. A similar condition is reported as well in a research done by Shibabaw [20]. These findings are agreed by Zakai [20], who further adds that the colonization of MRSA in students is possibly due to their time spent in various healthcare settings, as no carriage of MRSA is detected in the control group of students who have not yet had their clinical training [20]. A research conducted by Dulon supports the theory that clinical exposure is the major cause of MRSA carriage as well [8]. Although no data related to the colonization of MRSA in nurses is found in HK, the situation cannot be ignored as nurses all around the world are exposed to patients on a daily basis.

This research is conducted to find out the nasal *S. aureus* carriage rate in MLS students before and after their clinical practicum, which could generally reveal their hygiene practice and the level of infection control measures taken by the hospitals. It would be important and beneficial to know how many students would be colonized by the bacteria as it could help prevent a high bacterial colonization rate in the students who would be going on their clinical training in the future. A prevalence study will also be conducted among MLS and nursing students to find out whether clinical practicum would increase the bacterial colonization rate in students and the difference in hygienic practice between MLS and nursing students. Help could be provided to whosoever found to be having high bacterial colonization rate.

Materials and Methods

Sample collection and processing

Students from the Tung Wah College, Hong Kong who are about to go into clinical training are recruited. They include medical laboratory science and nursing. All students were in the 19-21 age groups. About 80% are female. No antibiotics use one month before sample collection. The first batch of samples will be taken before they go into clinical training and the second batch of samples will be taken after they return from their clinical training. Nasal samples were taken by trained nurses using nasopharyngeal swabs which will be inserted into the anterior naris of one of the nostrils and rotated against the anterior nasal mucosa for 3 seconds. This process is repeated with the same swab in another nostril.

The sample will be inoculated onto chromogenic agar (BBL™ CHROMagar™ Staph aureus) for the isolation of *Staphylococcus aureus*. Catalase tests will be performed to aid the selection of bacteria of *Staphylococcus spp.* for further tests and coagulase tests will be performed using Staphaurex and plasma and serum in test tubes to confirm that the bacteria isolated are indeed *Staphylococcus aureus*. 2-3 colonies will be picked from chromogenic agar for sub-culturing on 5% sheep blood agar (BioMérieux, Marcy l'Etoile, France) in order to obtain a pure culture of the bacteria.

Identification of MRSA

Two sets of tests will be conducted respectively for the detection of MRSA. In the conventional antibiotic susceptibility testing, the minimum inhibitory concentration is determined by the disk diffusion method for MRSA using inoculum of 0.5 McFarland Standard. In the disk diffusion method, discs of antibiotics, i.e. cefoxitin, will be placed on top on the inoculated the surface of Mueller-Hinton agar. Previous researchers are seen to have used oxacillin as the antibiotic of choice. However, cefoxitin seems to be a better choice of antimicrobial agent in this investigation as it is a better inducer of the *mecA* gene, and hence will give a more reproducible and accurate result. In the broth microdilution method, the 0.5 McFarland turbidity standard inoculum will be standardized using Muller-Hinton broth and vancomycin of two-fold serial-diluted concentrations will be added to the wells with the standardized inoculum. The mixtures will be incubated overnight for 24 hours at 37°C before results are read. Interpretation will be done according to the CLSI guidelines.

Molecular testing and confirmation

DNA will be extracted from *S. aureus* isolates using the Qiagen DNA Purification kit as per manufacturer's instructions (QIAGEN, Dutch). The molecular method is employed as well in detecting the presence of MRSA and VRSA. Quantitative PCR is used to detect the presence of *mecA* (primers (MR1: GTGGAATTGGCCAATACAGG and MR2: TAGGTCTGCAGTACCGGAT), which are responsible for the resistance of bacteria to methicillin. The other pair of

primer for (*nuc*) detection as a confirmatory gene for *S. aureus* (primer (TN1: GACTATTATTGGTTGATCCACCTG and TN2: GCCTTGACGAATAAAGCTTTCG). In order to select only for *Staphylococcus aureus*, the *nuc* gene, which encodes for the thermostable nuclease of *Staphylococcus aureus*, is replicated as well. Therefore, the presence of MRSA is shown by the presence of both *mec A* and *nuc* genes while that of VRSA is demonstrated by the presence of both *nuc* and *mec A* genes. The bacterial suspension will be prepared by suspending a tiny amount of bacterial isolate from a pure culture into double-distilled water (ddH₂O). With the addition of the working Master mix solution, the mixture will be ready for the PCR, in which the lysis of the bacterial cells, and thus the extraction of bacterial DNA, will occur during the process of denaturation. A standard curve will be constructed using a serial dilution of the positive control. A mixture with the absence of the target DNA will serve as the contamination control to make sure that the reaction mixture is not contaminated while another mixture that contains a DNA which lacks the target sequence will serve as the negative template control to ensure that the primers are not annealed to unintended sequences. An amplification control is included as well to ensure that the reaction is indeed working even if the target is not being amplified. Spa typing will be performed on each of the MRSA isolates as described. A *spa* type is the composition of the VNTRs in the 3' end of the staphylococcal protein a gene (*spa*).

Statistical analysis

Statistical analyses were conducted using Graph Pad Prism 6.0 software (GraphPad Software, La Jolla, CA, USA). Study population characteristics were defined by descriptive analysis. Wilcoxon matched pairs test was used to determine whether the chance of being colonized by *Staphylococcus aureus* is related to clinical practicum of medical students. Differences for which - $P < 0.05$ were considered statistically significant.

Ethics Statement

Informed consent was obtained from all participants with approval from the Committee on the Use of Human and Animal Subjects in Teaching and Research of Tung Wah College. Participants were informed of their nasal swab results individually in writing if they had provided their address. MRSA carriers were referred to the participant information sheet and advised to inform their medical practitioners of the swab results.

Results

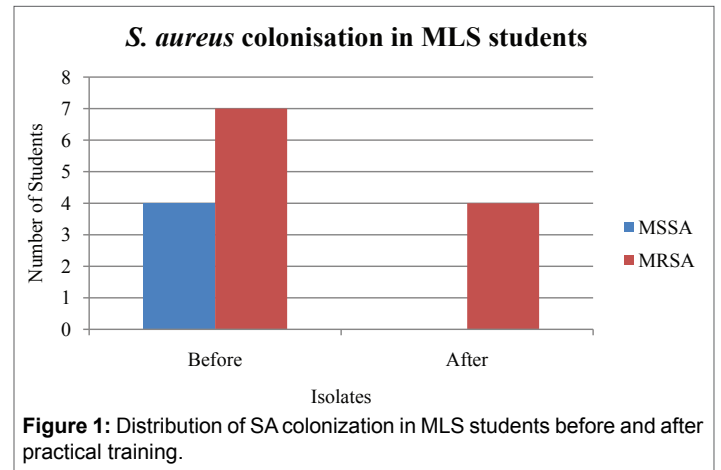
A total of 92 samples were collected from the targeted subjects, 30 of which were from medical laboratory science students while the remaining 62 were from nursing students. After collecting the isolates from the agar plate both conventional biochemical tests and PCR were done to verify the identity of the isolated colonies.

Colonization of SA in MLS students before and after practicum

In the investigation of MRSA colonization in MLS students before and after practicum, it is shown in table 1 that eleven *S. aureus* isolates were found among the fifteen individuals before they go into clinical training, in which four (36%) of them are sensitive to cefoxitin and contains only *nuc* gene and seven (64%) of them are resistant to cefoxitin and contains both *nuc* and *mec A* genes. In the samples after they return from practical training, four of the subjects were found to be colonized by *S. aureus* and all of them are resistant to cefoxitin and contains both *nuc* and *mec A* genes. The Wilcoxon matched pairs was employed in finding out the association between the colonization of MSSA and MRSA in MLS students before and after practicum. With a Wilcoxon matched pairs test, the p-value is 0.456, which is greater than 0.05, indicating that the association is statistically insignificant. The distribution of the MRSA colonization is shown in Figure 1.

Table 1: MRSA colonization in MLS students before and after practicum and prevalence of colonization in nursing students.

	No. of samples	SA isolates		MSSA isolates		MRSA isolates	
		n	%	n	%	n	%
MLS	Before	15	73	4	27	7	47
	After	15	27	0	0	4	27
Nursing	62	9	15	6	10	3	5
Total	92	24	26	10	11	14	15



Prevalence of SA in MLS and nursing students

It is found in the prevalence study conducted in all subjects (both MLS and nursing students) that MLS students have a higher MRSA carriage rate than first year nursing students, both before and after their practical training. Among the sixty-two nursing students sampled, nine of them were found to be colonized with *S. aureus*, in which six (10%) of them were MSSA as they are sensitive to cefoxitin and only *nuc* gene was amplified. Three (5%) of them were MRSA as they are resistant to cefoxitin and both *nuc* and *mec A* genes were amplified. Fifteen MLS students were sampled before and after their practical training. Eleven out of fifteen subjects were found to be carrying *S. aureus* before practicum, in which four of them were MSSA carrier and seven of them were MRSA carrier, accounting for 27% and 47% respectively (Table 1). *S. aureus* was isolated from four out of fifteen isolates after practicum, and all of them were found to be MRSA as they were are resistant to cefoxitin and both *nuc* and *mec A* genes were amplified, accounting for 27% of the MLS students. The results of the molecular test could be seen in the four gel electrophoresis images below (Figures 2-5), with lane 1 to 18 representing the genes isolated from nursing students, 19 to 26 from MLS students after practicum and 31 to 52 from MLS students before their practical training, and the band for *mec A* gene above that of the *nuc* gene. Lane 27, 28, 53, and 54 are the positive controls while lane 29, 30, 55, and 56 are negative controls. Table 1 and Figures 6 and 7 below illustrate the prevalence of *S. aureus* colonization in MLS and nursing students, with figure 6 in actual numbers and figure 7 in percentages.

Situations before and after clinical training in MLS students

Table 2 illustrates the situations of the nasal cavities of MLS students before and after their practical training in various hospitals. The situations can be divided into four categories, in which: 1) MRSA was present in the nostrils both before and after practicum, 2) MSSA isolated before practicum had turned into MRSA after practicum, 3) MSSA had disappeared after practicum and 4) MRSA had vanished after practicum. Three out of the eleven (27%) subjects belong to the first category. Only one (9%) of them was colonized with MSSA before training but was

Table 2: Situations concerning the colonization of MSSA and MRSA in MLS students before and after practical training.

	MLS	
	n	%
MRSA present before and after practicum	3	27
MSSA before Practicum has turned into MRSA after practicum	1	9
MSSA disappeared after practicum	3	27
MRSA disappeared after practicum	4	37
Total	11	100

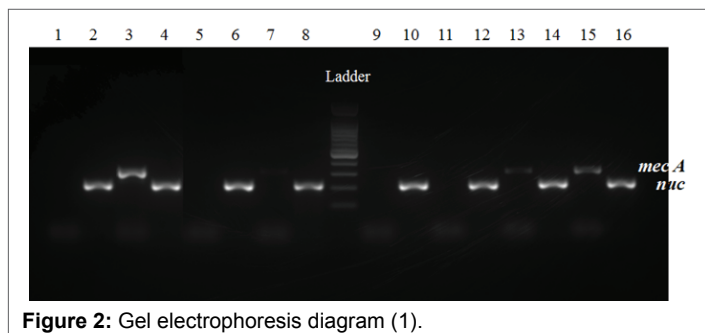


Figure 2: Gel electrophoresis diagram (1).

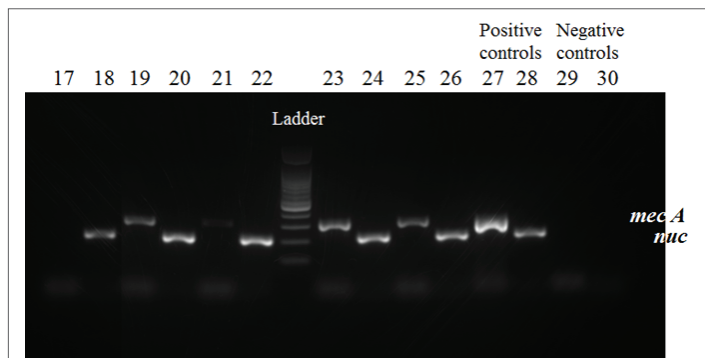


Figure 3: Gel electrophoresis diagram (2).

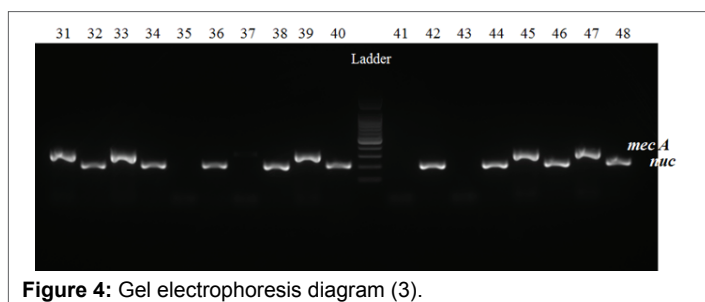


Figure 4: Gel electrophoresis diagram (3).

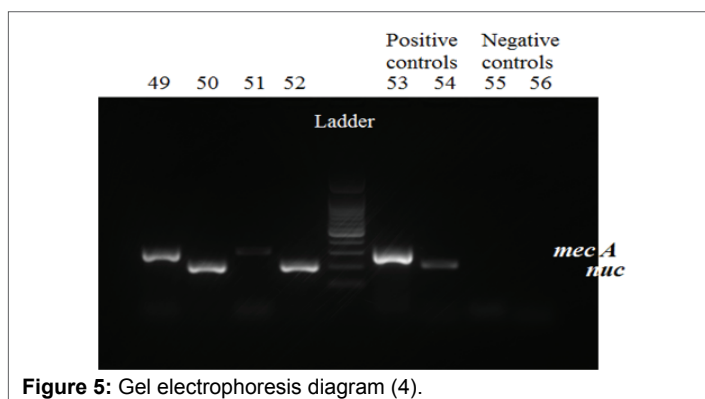


Figure 5: Gel electrophoresis diagram (4).

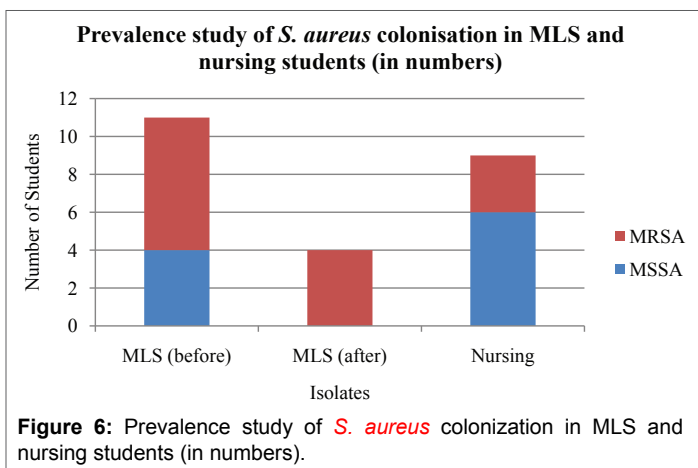


Figure 6: Prevalence study of *S. aureus* colonization in MLS and nursing students (in numbers).

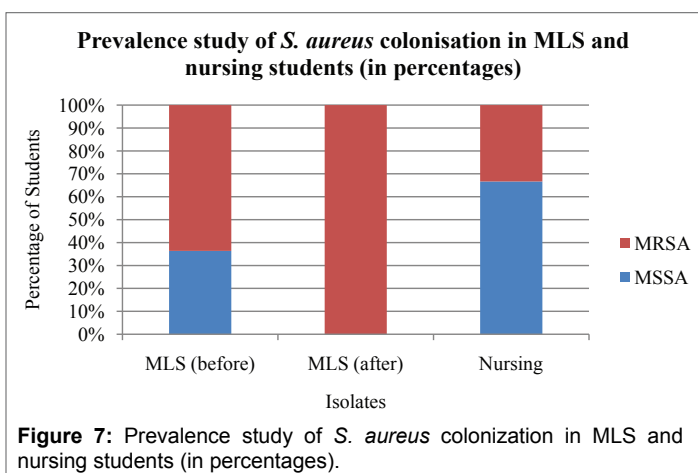


Figure 7: Prevalence study of *S. aureus* colonization in MLS and nursing students (in percentages).

discovered with MRSA after practicum. MSSA found in three of the subjects (27%) before training had disappeared after practicum. The same happened to MRSA that was present in four of the eleven (37%) subjects before clinical training.

Discussion

It is shown in table 1 that among the fifteen samples collected from the MLS students before their practical training eleven of them were found to be colonized with *S. aureus* in their nasal cavities, in which seven of them were MRSA, accounting for 64% of all isolates and 47% of samples collected. After their practical training in various hospitals, four *S. aureus* isolates were found among fifteen students and all of which turned out to be MRSA. This indicates that spending time in hospitals could not be a direct reason for the surge in MRSA colonization in the nasal cavities of the subjects, especially when medical laboratory technicians work with the patient samples which are potential sources of the bacteria.

MRSA present before and after practicum

The MRSA found in the nostrils of the subjects before practical training could either be community-acquired or hospital-acquired. The subjects could acquire strains of CA-MRSA from interaction with their friends and/or family members who had been to hospital settings within the past year where they had acquired strains of MRSA. The presence of MRSA after their clinical training could be due to the persistent presence of the strains of MRSA which had colonized their nostrils before their practicum and/or the acquisition of HA-MRSA from their time in the hospital. To find out the real cause of the presence of MRSA in their nasal cavities, questionnaires could be distributed to the subjects, including questions

about whether the subjects and/or their family members had been hospitalised or visiting clinics around the period of sample collection, and the duration and reason for hospitalisation and/or clinic visits. Molecular methods such as ribotyping could also be employed to distinguish between HA-MRSA and CA-MRSA by cleaving with different restriction enzymes. The characteristic pattern unique to each species or strains among species would be compared to the reference database for identification [21-23]. It is considered the most applied method in determining bacterial taxonomy as the results are discriminatory and reproducible. Other molecular methods of genotyping such as Multilocus sequence typing (MLST) or Pulsed Field Gel Electrophoresis (PFGE) can also be used [22,23].

Acquiring CA-MRSA through family and/or friends

Students could have acquired MRSA before practicum from their friends and/or family. These people could have had hospital stays recently and acquired MRSA during their hospitalization. When they were discharged, they carried home the bacteria which were hospital-acquired. Another reason for this phenomenon could be that their friends and family members are medical and healthcare workers who had been colonized by HA-MRSA at work, hence carrying around the bacteria in the community [8,24]. The students could have been colonized by MRSA during meetings with their friends and family members, who might have sneezed in their direction, hence the students being colonized through the airborne route [25]. The subjects might also have been in contact with objects being contaminated by MRSA from their friends and family, e.g. objects which have been sneezed on, thus being colonized through indirect contact. Since the subjects had not been exposed to hospitals and healthcare settings before, they were acquiring CA-MRSA from their close relations [8]. This spread of bacteria could be dangerous to the society as there is a possibility of CA-MRSA becoming endemic. Precautions should be taken to prevent a widespread of CA-MRSA, e.g. wash hands frequently and wearing masks if one feels ill.

Acquisition of HA-MRSA from hospitals

The MRSA found in the nasal cavities of MLS students could be acquired in both of the above-mentioned ways. If the student was colonized by MRSA before having practical training without suffering from infections, he would bring the MRSA into the hospital settings and not only cause endogenous self-infection but also spread the infection to co-workers. During the time when students spent in the laboratory for practical training, there are numerous occasions that they would be exposed to infectious agents (MRSA in this case), such as inhaling the bacteria in the air through the airborne pathway and touching objects which are contaminated by the bacteria [26]. The risk of being colonized by MRSA could be higher if the individual works in the microbiology section as he would be exposed to the source of the bacteria. As they come across the infectious agents and/or objects contaminated by the infectious agents, they would be colonized by MRSA and infections might develop due to cross contamination [8].

MSSA before practicum has turned into MRSA after practicum

MRSA was isolated from one of the subjects who previously had only MSSA isolated from his nasal cavity. This aggravated condition could be due to the fact that the individual had unhygienic personal practices and/or because of the poor infection control measures were taken by the hospital he stayed in during practicum. Mutations in the genome of *S. aureus* can also cause the conversion of MSSA to MRSA [27].

Unhygienic personal practices

Unhygienic personal practices could cause the colonization of MRSA on an individual. Therefore, it is important for medical and health care

workers to adhere to the infection prevention and control protocol during their duty hours. There are some standard and essential procedures which could prevent the colonization of bacteria: frequent hand hygiene, wearing protective equipment and preventing injuries by sharps.

Hand washing is probably the easiest way to prevent bacterial colonization. Hands should be washed after handling specimens and performing laboratory tests, even if gloves are worn. Studies have shown that some healthcare workers may not have washed their hands after the removal of gloves [8]. Hand hygiene could be performed using soap and water and other anti-septic agents. Disposable gloves should be worn during work and discarded after single use. Other protective gears such as gowns, masks and face shields could be worn when necessary for personal protection against infectious agents. Precautions should be taken as well while using needles or sharp instruments to prevent blood-borne pathogens and wounds should be properly dressed to prevent any contact with the bacteria. In this case of being colonized by MRSA after practical training, the student might not have complied with these regulations during his time at the hospital. As mentioned before, *S. aureus* transmits mainly through the air and contact, in both direct and indirect way. It could be speculated one might not have performed proper hand hygiene at the appropriate times, e.g. after performing tests, before meals and prior to have direct contact with bodily parts. The bacteria could adhere to the skin and nasal cavities when the individual has contact with himself after direct contact with the bacteria and/or indirect contact with objects contaminated with the bacteria [28]. Infrequent changing of laboratory gowns also poses a threat of bacterial colonization. As *S. aureus* could be airborne [26], the bacteria might be lodged onto the individual when the person is unaware that he is breathing in the bacteria.

Inadequate infectious control measures were taken by the hospital

Poor infectious prevention and control measures practiced by hospitals were shown to have detrimental effects, and one of which is the increased resistance of microorganisms towards antibiotics which is demonstrated by one of the MLS students who had come back from his practical training with MRSA in his nasal cavities. Hospitals and healthcare settings should have a clear and strict set of infection prevention and control protocol which could be followed by medical and healthcare workers. In the laboratory, cleansing and disinfection are of utmost importance as it could prevent the spreading and acquiring of bacteria by employees. Bench tops should be carefully cleaned and disinfected before and after work. Careless and superficial cleaning proves to be not only useless but dangerous (Hospital hygiene and infection control) as there is a possibility of spreading the organism over a larger surface area and increases the opportunity of contaminating other objects and probably people. Cleaning with soap and water removes visible dirt. Disinfection afterward will kill bacteria and other micro-organisms. Proper sterilization is required for the preparation of bacteria-free instruments: autoclaving growth media at 121°C for 15-20 minutes and incinerating forceps after use. Medical wastes should also be sterilized before disposal in order to prevent the spreading of infections, e.g. autoclaved. The linen department in the healthcare settings should handle and wash the gowns of the hospital staff properly so no micro-organisms should be adhering to the gowns after laundry [29].

Mutation from MSSA to MRSA

Although the resistance to methicillin in *Staphylococcus aureus* could be mediated by either the production of beta-lactamase by the bacteria or the acquisition of the *mec A* gene which would cause the production of an abnormal copy of penicillin-binding protein PBP2a, the latter appears to be the major cause [30]. The acquisition of gene

mec A from other *Staphylococcus* species such as *Staphylococcus sciuri* leads to the production of an additional but abnormal PBP2a which has a lower affinity for penicillin and a higher rate of releasing the bound drug compared to the normal version of PBP [31]. Mutations in these regulatory genes would enhance the level of synthesis of PBP2a which was originally kept at minimum level. As these mutations could be caused by factors such as antibiotic and salt concentration, pH, temperature and other environmental factors [30], students could be exposed to mutation inducers during their time in the hospital. They could also be ill at the time and take antibiotics on their own. These could lead to the isolation of MRSA in their nasal cavity after their clinical training.

MSSA and MRSA disappeared after practicum

A total of 7 subjects have lost the MSSA or MRSA they had previously after their practicum. This phenomenon seems unusual but it could be achieved by good infection control measures in the healthcare settings and good hygiene practice by the individual himself. According to the CDC, such measures include hand hygiene, gloving, gowning, mouth, nose, eyes protection, appropriate handling of laundry and environmental measures. These are all the things that can be done in a hospital setting which could prevent healthcare and medical workers from being colonized by MRSA. Hand washing has been a basic procedure suggested by CDC for many decades which includes washing hands with anti-microbial or non-antimicrobial soap and alcohol-based solutions [32]. Wearing protective equipment such as gloves and masks during duty hours could also protect workers from being colonized by MRSA.

As for the part of the hospital, proper measures should be taken to protect staff from MRSA colonisation, such as disinfecting facilities and equipment regularly and ensure the laundry department is handling the protective equipment properly to avoid the wide spreading of the bacteria [12,29].

Colonization of MRSA in Nursing Students

In the prevalence study done among the sixty-two first year nursing students, nine samples were found to be containing *S. aureus*, in which three of them were methicillin resistant and accounting for 33 % of all *S. aureus* isolates. This number is significantly lower than that found in the MLS students, both before and after training.

Compared to MLS students before their clinical practicum

47% of MLS students were found to be colonized with MRSA before training, which is higher than that among nursing students and higher than the percentage after the MLS students had returned from their clinical training. One of the reasons for such a high nasal MRSA carriage rate could be due to the colonization through microbiology practical lessons. Before clinical practicum, MLS students were having microbiology laboratory sessions on a weekly basis for more than three months, and *S. aureus* is a commonly encountered bacterium during the laboratory sessions. Students might have been colonized through careless direct contact of the bacterium and/or through indirect contact with MSSA or MRSA contaminated objects in the laboratory, in which MSSA may mutate into MRSA under suitable conditions. Nursing students, however, were not having microbiology laboratory classes. So there was less chance for them to be in contact with the bacteria and be colonized. Another important reason for the lower MRSA carriage rate in nursing students is that great emphasis has been put on hand hygiene in their course. Nursing students are trained in their course to deal hands-on with patients, which include procedures that involve direct contact with patients such as performing catheterization. More stress might have been put on the importance of disinfection and hand washing before and after

handling patients. Numerous rules and protocols have also been suggested for the benefits and importance of hand hygiene (CDC, 2002). As the concept of hand hygiene is repeated continuously over the course, this perception will linger in the nurse students' brain and this could become part of their daily life practice. MLS students, on the other hand, might not have disinfected their hands so often as the nursing students. This is not because of their lack of training, but due to the fact that they are not required to have direct contact with patients most of their time, and one tends to be less perceptive to the concept of hand hygiene. They would be spreading the bacteria in numerous circumstances to different people: to themselves when they touch parts of their faces with contaminated hands (hence the higher percentage of MRSA colonisation in their nasal cavities) and to others when they cough or sneeze and come in contact with public facilities [8].

Compared to MLS students after their clinical practicum

27% of MLS students were found being colonized with MRSA after training, which is higher than that among the nursing students but not as much as that before going for their practical training. As MLS students, had been exposed to the clinical environment for months while nursing students had not, clinical exposure is a highly possible reason for the high MRSA colonization rate, especially for the fact that MLS students had spent one-fourth of their time in the microbiology laboratory, which is the potential source of the bacteria. Moreover, the lessons on hand hygiene had equipped the nursing students against bacterial colonization more effectively than that in MLS students. However, the situation among MLS students had improved among MLS students as the MRSA carriage rate after the practicum is not as high as that before the clinical training. As mentioned in the previous paragraphs, MRSA could be transmitted through airborne and/or direct and indirect contact. Taking good personal hygiene measures, e.g. wearing protective equipment and frequent hand washing, could effectively cause the decreased MRSA carriage rate, and this practice might have been employed by the MLS students during their time spent in the hospitals [25,26]. They might also have learned new and practical ways of protecting themselves from HAI from their seniors in the hospitals. The hygiene conditions in the hospitals might also have been kept at a maximum level in order to prevent nosocomial infections in patients and medical and healthcare workers [28].

Conclusion

It is well known that *Staphylococcus aureus* infections could cause serious problems to the society economically and medically as it increases the medical costs and risk of infections. The situation could be worse if the bacteria become resistant to antibiotics such as methicillin and vancomycin. It is found in the study that clinical exposure is an important reason for acquiring MRSA as the percentage of nasal MRSA carriage is higher in MLS students who had been to clinical training in hospitals than that of the first-year nursing students who had not been exposed to the clinical environment. Personal measures could be taken to prevent MRSA colonization such as wearing protective equipment and proper hand hygiene. Hospital and healthcare settings should tighten their infection prevention and control regulations to minimize nosocomial infections (HA-MRSA in this case) in medical and healthcare workers and patients. These measures could also decrease the chance to the spreading of CA-MRSA in the community. Despite the finding that clinical exposure increases the chance of MRSA colonization in MLS students, the association does not appear to be statistically significant between pre-exposure and post-exposure to the clinical environment. To further prove the point that clinical exposure could increase the chance of MRSA colonization, a larger sample size could be used. Questionnaires and molecular typing methods could be employed as well to distinguish the potential source of the MRSA isolated in the samples.

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