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Nasal Carriage of *Staphylococcus aureus* in Preclinical and Clinical Dental Students of Tabriz University of Medical Sciences and Identification of Methicillin-Resistant Isolates in 2019

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Abstract

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is the cause of severe and treatment-resistant, hospital- and community-acquired infections across the world. This study aimed to identify and compare the nasal carriage of *S. aureus* and methicillin-resistant in clinical and preclinical dental students in 2019.

Materials and Methods: This study is a cross-sectional study (descriptive-analytic) which was conducted during a period of time from 2019 to 2020 at the School of Dentistry, Tabriz University of Medical Sciences. Two groups of dental students (i.e., clinical and preclinical programs) were selected. Nasal samples were taken and S. aureus was detected using the standard microbiology tests. Disc diffusion on Mueller-Hinton Agar was used to identify MRSA. *P* values of ≤0.05 were regarded as statistically significant.

Results: A total of 92 dental students were examined and S. aureus was identified in 44 (47.8%) students, but no cases of MRSA were detected among the participants.

Conclusions: The difference between clinical and preclinical groups was not significant.

Keywords: Dental students, Methicillin-resistant, Staphylococcus aureus, Nasal carriage

Introduction

Bacterial infection is a global problem. In many cases, bacterial resistance to antimicrobial agents could complicate treatments. Several strains of infectious agents have developed resistance to most of the available antibiotics and therefore, global monitoring has increased to examine antimicrobial resistances. This is particularly true for Methicillin-resistant *Staphylococcus aureus* (MRSA) which is one of the most important hospital- and community-related pathogens. Methicillin is a semisynthetic penicillin derivative produced in 1959 and is resistant to beta-lactamase resulting in significant decrease in infections related to penicillin-resistant strains. According to the definition proposed by the Center for Disease Control and Prevention, MRSA means resistance to methicillin and other common antibiotics. It has been reported to be found occasionally among people who refer to healthcare systems or have contact with MRSA patients. MRSA colonization is estimated to be around 1.5% in human population one-sixth of which is community-acquired MRSA (CA-MRSA) (1,2). Over the last 45 years, hospital-acquired MRSA (HA-MRSA) and CA-MRSA clones have spread across the world, and there is a high risk of related epidemies. Taking into account the importance of CA-MRSA and HA-MRSA strains, it is vital to identify them and determine their antibiotic resistance patterns in each region in order for helping doctors prescribe effective antibiotics (3).

Given the lack of a comprehensive research in East Azerbaijan as well as considering dental students' close contact with patients and the importance of MRSA strain, the present study aimed to identify the nasal carriage of *S. aureus* in preclinical and clinical students of dentistry at Tabriz University of Medical Sciences.

Materials and Methods

In this cross-sectional (descriptive-analytic) study, nasal samples were taken from clinical students, 2014 entry (n=54); and preclinical students, 2018 entry (n=38).

Exclusion criteria were: those taking anti-staphylococcus antibiotics over the last month; those with immunosuppressive systemic diseases probably affecting the rate of nasal bacterial colonization including immunodeficiency, diabetes, kidney failure, and cancer;

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Key Messages

- Nasal carriage of *S. aureus* is an important cause of bacterial spread.

organ transplant recipients, drug addicts, those with history of using permanent catheter, and those with recently-proven staphylococcal diseases (4).

One student from the clinical group was excluded from the study due to an underlying disease.

Samples were taken from nasal vestibule of both nostrils (1 cm anterior area) using a wet swab and sterile physiological serum, and through inoculation onto mannitol salt agar medium. The samples were immediately transferred to a laboratory for analysis. In the laboratory, plates containing mannitol salt agar media inoculated with samples were kept in the incubator at 37°C for 24 hours. After the incubation, samples that had been able to ferment mannitol and change the medium color from pink to yellow were cultured in blood agar medium for bacterial purification and formation of a single colony, and then were incubated at 37°C for 24 hours. *S. aureus*-suspicious were analyzed by means of gram staining and examining enzymatic reactions including catalase, coagulase, as well as DNase tests and hemolysis. Disc diffusion on Mueller-Hinton agar was used to identify MRSA (5).

Several freshly cultured *S. aureus* colonies (24 h culture) were selected and transferred to a test tube containing 3 mL of sterile physiological serum, and the resulting turbidity was compared to the turbidity of half-McFarland suspension ($1\text{-}2 \times 10^8$ CFU/mL) and was adjusted accordingly. After a 15-minute interval, a sterile cotton swab was dipped into the bacterial suspension and rolled several times. The swab tip was then pressed against the upper tube wall to remove excessive solution from the cotton tip. The surface of the plate containing Mueller-Hinton agar medium was lawn cultured with the swab. The swab was then rolled over the medium. After 3 to 5 minutes (this process should not exceed 15 minutes), a sterile forceps was used to place antibiotic discs on the culture medium at proper distances. In 30 minutes, the plates were reversely incubated at 35-37°C for 24 hours. To measure growth inhibition zone diameter accurately, the plate was held a few centimeters above a black reflectionless background, and the diameter around each antibiotic disc was measured with a ruler. The results were then compared with the accompanying antibiotic disc table and reported as sensitive (S), resistant (R), and intermediate (I). Standard *S. aureus* strain (ATCC 25923) was used to ensure quality control of the antibiotic discs. Images of the bacterial detection steps in the medium and antibiotic resistance test are provided .

Statistical analysis was performed using SPSS software version 17. The *P*-value<0.05 was statistically regarded as being significant.

Results

Clinical dental students (2014 entry) and preclinical dental students (2018 entry) were selected for the present study. The age, sex, nasal isolation of *S. aureus*, as well as the resistance of the isolated *S. aureus* to methicillin were assessed for all students. The clinical group included 30 male students and 24 female ones; the preclinical group, on the other hand, included 27 male students and 11 female ones (Table 1). In the clinical group, nine students were married and 45 ones were single; whereas in the preclinical group, only one student was married and 37 ones were single. In the clinical group, 29 students were non-dormitory Tabriz residents and 25 students were dormitory residents. In the preclinical group, 20 students were non-dormitory Tabriz residents and 18 students were dormitory residents (Table 1). Five students were smokers and 49 students were nonsmokers in the clinical group, while 12 students were smokers and 26 were nonsmokers in the preclinical group.

The mean age of the preclinical group including students aged 19-30 years was 20.89 ± 2.34; and the mean age of clinical group including students aged 23-38 years was 24.57 ± 3.82.

The Frequency of *S. aureus* nasal carriage in the clinical and preclinical groups are shown in Figures 1 and 2.

Comparison of the study results showed that there was no statistically significant difference between two groups in nasal carriage of *S. aureus* ($P = 0.833$).

Among 44 positive cultured samples obtained from 92 samples, no single case was found resistant in disc

Table 1. Demographic Information of the Students

Variable		Pre-clinical Group		Clinical Group	
		Percent	No.	Percent	No.
Gender	Male	71.1	27	55.6	30
	Female	28.9	11	44.4	24
Smoking	Smoker	31.58	12	9.26	5
	Non-smoker	68.42	26	90.74	49
Habitation	Dormitory	47.37	18	46.29	29
	Non-dormitory	52.63	20	53.71	25

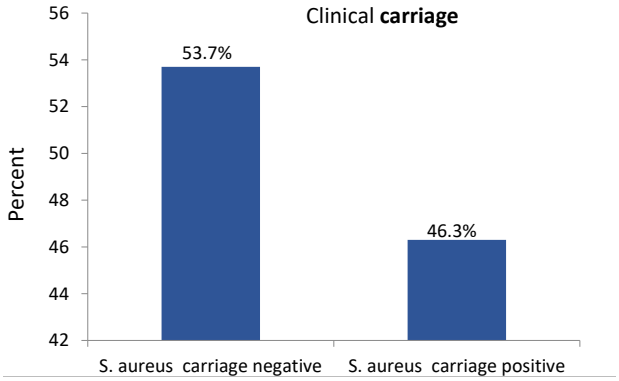

Figure 1. Frequency of *S. Aureus* Nasal Carriage in the Clinical Group Students.

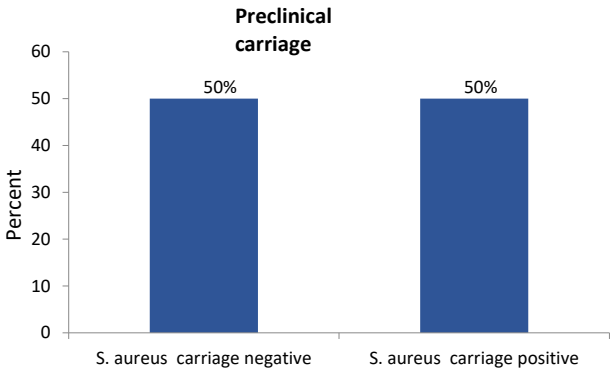

Figure 2. Frequency of *S. Aureus* Nasal Carriage in the Preclinical Group Students.

diffusion oxacillin test. In other words, no cases of MRSA were detected.

Discussion

This study reported a on dental students of preclinical and clinical programs at Tabriz University of Medical Sciences. The carriage rates of *S. aureus* were 50% and 46.3% in students of the preclinical and clinical groups, respectively. No cases of MRSA were detected in two groups.

Most of the studies in this area have targeted medical students and there has been little research on dental students and dentists.

A study by Nordin et al showed that 111 Malaysian medical students, 14 students (12.6%) of the preclinical program, and 7 students (7.1%) of the clinical program carried nasal staphylococcus aureus. No sample was found to be resistant in disc diffusion oxacillin test and, therefore, MRSA was not proved to be present (6). Similar results were found in the present study.

A research by Burgmann et al on 86 medical students in Vienna found that 20 students (25.3%) carried nasal *S. aureus* with zero cases of MRSA (7), which was consistent with the results from our study.

In a study by Del Pozo et al on 102 Spanish preclinical medical students, 33 students (32.4%) were determined to be nasal carrier of *S. aureus*. Two students (1.9%) out of the given population showed methicillin-resistance. Their study on 149 clinical medical students revealed that 57 students (38.3%) were nasal carrier of *S. aureus* with no cases of MRSA (8), which was in line with the findings from our study.

Seni et al carried out a research on 166 preclinical medical students in Tanzania and reported 33 cases (19.9%) of nasal carriage of *S. aureus*. They demonstrated that 33 (22.3%) out of 148 preclinical students were nasal carriers of *S. aureus*; only one student (0.3%) in the preclinical group was determined as MRSA positive among the total of 314 students. No significant difference was found between two groups (9), which was in agreement with the result from this study.

The reasons for non-significant differences between preclinical and clinical groups, the lack of increase in incidence of *S. aureus* carriage, and MRSA negative cases could have been due to the use of personal protective equipment such as mask, shield, gloves, gown, as well as washing hands (10).

Ansari et al collected the samples from 200 preclinical medical students in Nepal and reported 30 cases (29.9%) of nasal *S. aureus*. Eight students (4%) were also determined as MRSA positives (11).

In an Iranian study by Sharifi et al on 350 medical students, 63 (46%) were determined as nasal carriers of *S. aureus* where 7 students (2%) were reported to be methicillin-resistant (12).

Another Indian study by Hema et al in 2017 examined samples from 200 PhD and 200 BSc dental students and reported that 74 (18.5%) of the students carried nasal *S. aureus*. Their study results further showed that 25 students (12.5%) of the BSc program were MRSA positives, while the number of PhD dental students with MRSA positive was 49 (24.5%), suggesting a significant difference between two groups of students ($P = 0.02$) (13).

In a study by Bhatta et al in Nepal, samples from 100 preclinical medical students were collected and examined; and it was concluded that 25 students (25%) were nasal carriers of *S. aureus*. From among 100 preclinical medical students, 10 (10%) students were discovered to carry nasal *S. aureus*. The number of MRSA positive cases was 10 (0.1%) and 29 (0.29%) in the preclinical and clinical group, respectively (14).

Szymanek-Majchrzak et al performed a research in Poland and took nasal samples from 955 third-year medical students (preclinical program) between 2014 and 2016. They reported that 245 students (25.7%) were nasal carriers of *S. aureus*, while only one student (4%) was MRSA positive (15).

The differences between the results from these studies could have been associated with various factors such as the sample size, the number of encounters, the colonization

rate of *S. aureus* in the community, the use of personal protective equipment (gloves, mask, and gown), hand hygiene (i.e., washing hands with water and soap), and sterilizing equipment of hospitals and related healthcare divisions (16-20). Further researches are needed, however, to meticulously investigate the causes of these differences.

Conclusions

The findings from this study showed the prevalence of nasal carriage of *S. aureus* among the pre-clinical and clinical dental students in our healthcare center. No significant difference was found between the pre-clinical and clinical groups. According to our study results, furthermore, MRSA carriage was not detected in dental students.

It is recommended that further researches be conducted to investigate both preclinical and clinical dental students when the COVID-19 pandemic ends in order for examining the role of hygienic measures such as using personal protective equipment (mask, gloves, etc.), regular hand washing with water and soap, and equipment disinfection in frequency of *S. aureus* carriers and prevalence of MRSA.

Limitations of the Study

The inclusion criteria in this study were educational level (clinical and pre-clinical) and satisfaction with sampling. The exclusion criteria were allergies, recent infections, antibiotic usage in the previous three months, hospitalizations in the earlier six months, dissatisfaction with sampling, and negative cultures for *S. aureus* in samples.

Authors' Contributions

SAO and ATZ conceptualized and conducted the study**.** YAO and FYS contributed to the collection of samples and microbial testing, MAR contributed to designing, collecting and analyzing the data. SAO and ATZ organized the first draft of the paper**.** All authors read the draft version and approved the final manuscript**.**

Conflict of Interests

Authors have no conflict of interests.

Ethical Issues

The study was conducted after obtaining the approval of the committee for ethics at Tabriz University of Medical Sciences (IR.TBZMED,REC.1398.153).

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