259-270

CMJ Original Research

September 2020, Volume: 42, Number: 3

Cumhuriyet Tıp Dergisi (Cumhuriyet Medical Journal)

http://dx.doi.org/10.7197/cmj.vi.645617

Nasal Carriage of *Staphylococcus aureus* by medical students: assessment of antibiotic susceptibility and risk factors

Tıp öğrencilerinde Nasal Staphylococcus aureus taşıyıcılığı: Antibiyotik duyarlılığı ve risk faktörlerinin değerlendirilmesi

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Received/Accepted: November 12, 2019 / August 18, 2020

Conflict of interest: There is not a conflict of interest.

SUMMARY

Objective: *Staphylococcus aureus* (*S. aureus*) is the leading vector for both hospital-sourced and population-sourced infections globally and nasal carriage may be responsible for these serious infections. The aim of this study was to assess the nasal *S. aureus* carriage rates, antibiotic susceptibility and risk factors among preclinical period medical students.

Method: A total of 183 preclinical period students from Ahi Evran University Faculty of Medicine voluntarily participated in our study. They were requested to complete a survey form containing questions about demographic information, hygiene habits and medical history and possible risk factors were recorded. *S. aureus* isolates proliferating from nasal swab culture samples were studied with antibiotic susceptibility tests.

Results: Of the total of 183 students, 39 (21.4%) had *S. aureus* proliferation identified from nasal swab samples. None of these isolates were methicillinresistant *S. aureus* (MRSA). Antibiotic susceptibility tests found highest resistance against penicillin (85%). There were negative correlations between nasal spray use and allergy history with nasal *S. aureus* carriage.

Conclusions: Medical students may each be mobile sources of bacteria in hospitals, especially as MRSA carriers. According to our results, it is necessary to note the importance of training and standard infection control precautions to prevent the increase in MRSA carriage rates in the clinical period as none of our preclinical period students carried MRSA. Advanced studies are important to monitor carriage rates.

Keywords: Staphylococcus aureus, nasal carriage, medical students.

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ÖZET

Amaç: *Staphylococcus aureus* (*S. aureus*), dünya çapında hem hastane kaynaklı hem de toplum kaynaklı enfeksiyonların önde gelen etkenlerinden olmakla birlikte burun taşıyıcılığı bu ciddi enfeksiyonların sorumlusu olabilir. Bu çalışmanın amacı, preklinik dönem tıp öğrencilerinde nasal *S. aureus* taşıma oranını, antibiyotik duyarlılığı ve risk faktörlerini değerlendirmektir.

Yöntem: Ahi Evran Üniversitesi Tıp fakültesi öğrencilerinden 183 preklinik dönemde olan öğrenci gönüllü olarak çalışmamıza katılmıştır. Demografik bilgilerin, hijyen alışkanlıklarının ve tıbbi öykünün sorgulandığı anket doldurmaları istenmiştir ve olası risk faktörleri kaydedilmiştir. Burun sürüntü kültür örneklerinde üreyen *S. aureus* izolatlarına antibiyotik duyarlılık testi çalışılmıştır.

Bulgular: Toplam 183 öğrencinin burun sürüntü örneğinin 39'unda (%21,4'ünde) *S. aureus* üremesi saptanmıştır. Bu izolatların hiçbiri metisilin dirençli *S. aureus* (MRSA) değildi. Antibiyotik duyarlılık testinde en yüksek direnç penisiline (%85) karşı tespit edilmiştir. Nasal sprey kullanımı ve alerji öyküsü ile nasal *S. aureus* taşıyıcılığı arasında negatif korelasyon saptanmıştır.

Sonuç: Tıp öğrencileri özellikle MRSA taşıyıcısı olarak hastanelerde hareketli birer bakteri kaynağı olabilirler. Sonuçlarımıza göre MRSA taşıyan preklinik dönem öğrencimiz olmamasından dolayı kliniğe geçildiği dönemde MRSA taşıyıcılık oranlarının artmaması için eğitimin ve standart enfeksiyon kontrol önlemlerinin önemine dikkat çekmek gerekmektedir. İleri çalışmalarla taşyıcılık oranlarının takibi önemlidir.

Anahtar sözcükler: Staphylococcus aureus, nasal taşıyıcılık, tıp öğrencileri

INTRODUCTION

Staphylococcus aureus (S. aureus) is encountered as a pathogenic bacteria in many significant infections including skin infections like scalded skin syndrome, cellulitis, folliculitis, and abscess, pneumonia, sepsis, meningitis, osteomyelitis, surgical area infections and toxic shock syndrome.¹The main colonization area for S. aureus is the nose, but it may be isolated from other regions of the body. Nasal colonization especially forms a risk for many hospital- and communityacquired S. aureus infections.² In recent years, increased resistance rates to antibiotics among S. isolates have been encountered. aureus Methicillin-resistant S. aureus (MRSA) especially and reduced susceptibility rates to vancomycin cause problems with treatment.3,4 Previously MRSA isolates were mostly isolated in hospitalacquired infections, but recently communityacquired MRSA isolates have been determined.^{5,6} As a result, studies to identify nasal S. aureus carriage which may cause these infections and may spread within community are important.

While medical students in the first three-year preclinical period reflect community, when they enter clinics they act as mobile elements carrying bacteria between patients. It is known that nasal colonization may increase among health workers due to MRSA found in the hospital environment.⁷ In Turkey in spite of increasing presence of *S. aureus* in hospital environments, there are few studies about potential risk factors for occurrence or colonization of *S. aureus* among medical students. This study was performed to assess the nasal *S. aureus* carriage rates, antimicrobial susceptibility profiles and related risk factors

among medical students before exposure to clinical internships and additionally to attract attention to the topic among students before they work in clinics.

MATERIAL AND METHODS

Study design

Our study was performed with a Medical Microbiology student research group from Ahi Evran University Faculty of Medicine from February 2019 to March 2019. Of the total of 218 students in Ahi Evran University Faculty of Medicine, 183 participated. Students accepting participation provided an informed consent form and were requested to complete a written survey about demographic information and medical history. Variables included on the survey were age, gender, dormitory residence, pet ownership, previous infections, allergies other noninfectious pathologies, smoking habits, hygiene habits, antibiotic use in the previous 3 months and surgery or hospital stay in the previous 6 months. Our study received permission from Ahi Evran University Faculty of Medicine Clinical Research Ethics Committee (Decision No: 2019-01/03).

Exclusion Criteria

Participants receiving any antistaphylococcal antibiotic within the previous 2 weeks, and students with active upper respiratory tract infection during the study were excluded from the study.

Collection of Samples and Bacteriologic Identification

Nasal swab samples were taken by inserting a sterile swab 2 cm into the anterior region of the

nose and slowly turning it 3-4 times. Culture samples in Stuart's transport medium (Or-bak, Turkey) at room temperature reached the laboratory within 24 hours. Samples were seeded on mannitol salt agar and incubated at 37 °C for 24-48 hours. Positive cultures were investigated for colony morphology, gram staining and other conventional tests. Yellow colonies on mannitol salt agar were passaged for 5% sheep's blood agar. Bacteria positive for beta hemolytic gold color pigment, gram positive cocci morphology, catalase and coagulase tests were identified as *S. aureus*.

Antibiotic Susceptibility Tests

The antibiotic susceptibility tests for S. aureus isolates were studied in line with recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) using Mueller Hinton agar medium with the Kirby-Bauer disc diffusion method.⁸ Methicillin-resistant cefoxitin (30 µg) discs were screened. Additionally, susceptibility tests were performed using penicillin, erythromycin, clindamycin, tetracvcline. linezolid. trimethoprimsulfamethoxazole, rifampicin, gentamicin, ciprofloxacin and fucidic acid discs (Bioanalyse[®], Turkey). Vancomycin susceptibility was studied with E-test (bioMérieux®, France) using the gradient diffusion method. For identification of inducible clindamycin resistance, erythromycin and clindamycin discs were placed as 15 mm distance on an MHA plate. Situations where the inhibition zone around the clindamycin disk had a straight line at the edge closest to the erythromycin disc (D-shape) were accepted as positive for inducible clindamycin resistance. In our study, (MSSA) S. aureus ATCC 29213 and (MRSA) ATCC 33591 strains were used as quality control strains.

Statistical Analysis

Descriptive statistics are given as frequency and percentage for categoric variables and as median, minimum and maximum values for numeric variables. Group comparisons used the chi-square test and the Mann-Whitney U test. To be able to determine factors affecting *S. aureus* carriage, univariate and multivariate logistic regression analysis was used. According to univariate group comparison results, p values smaller than 0.20 (p<0.20) were included in the multivariate logistic regression model. For selection of regression analysis variables, the Backward Wald method was used. Odd's ratio and 95% confidence intervals for Odd's ratio are given. To calculate the risk of S. aureus carriage among study participants, a mathematical model was created with the aid of multivariate logistic regression with ROC curve analysis applied to determine the differentiative power of the model. All statistical analyses accepted p<0.05 as statistical significance. All analyses were performed with SPSS 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

RESULTS

A total of 183 medical faculty students participated in our research; all were in the preclinical periods with 90, 54 and 39 students voluntarily participating from period I, II and III, respectively. Of the students participating 39.3% were male and 60.7% were female with mean age 19.857 ± 1.266 years. The mean age for female students was 19.748 ± 1.099 , while the mean age for male students was 20.028 ±1.483 years. Of the 183 students, 39 (21.4%) were identified to have S. aureus proliferation. S. aureus was isolated from nasal swab samples from 18 (20%) period I students, 15 (27%) period II students and 6 (15%) period III students. All of the proliferating bacteria were methicillin-susceptible S. aureus (MSSA). The highest resistance was 85% against penicillin. The resistance rates to other antibiotics are given in the table (Table 1). According to the survey results, 80% of students did not smoke, 57.3% lived in dormitories, 80% did not have a family member working in a hospital, 95% regularly washed their hands, 74% regularly washed their nasal cavities, 31.7% had frequent nose touching habits and 80% regularly washed their hands after touching their nose. There were no significant correlations found between variables like age, gender, period, smoking, antibiotic use, etc. with S. aureus carriage (p>0.05).

Antibiotics	Resistance (%) n=39
Penicillin	33 (85)
Methicillin	-
Fusidic Acid	5 (13)
Erythromycin	9 (23)
Clindamycin	4 (10)
Tetracycline	5 (13)
Linezolid	-
Rifampicin	-
Trimethoprim-sulfamethoxazole	-
Gentamicin	20 (51)
Ciprofloxacin	1 (2.5)
Vancomycin	-
Inducible Clindamycin resistance	3 (7.5)

Table 1: Antibiotic resistance rates in S. aureus isolates

There were correlations found between nasal spray use and allergy history with *S. aureus* carriage (p<0.05). Of the 39 students with bacterial proliferation, 6 (15.4%) had allergy history while of the 143 students without bacterial proliferation, 48 (33.6%) had allergy history (p<0.05). Of the 39 students with bacterial proliferation, 10 (25.6%) did not use nasal spray while of the 143 students without bacterial proliferation, 65 (45.5%) did not use nasal spray (p<0.05) (Table 2).

Table 2: Group comparisons

Variable	Proliferation		p-value
	Absent	Present	
Gender			
Male	51 (35.7)	20 (51.3)	0.96
Female	92 (64.3)	19 (48.7)	
Period			
Ι	69 (48.3)	18 (46.2)	0.401
II	41 (28.7)	15 (38.5)	
III	33 (23.1)	6 (15.4)	
Smoking			
Yes	22 (15.4)	4 (10.3)	0.675
No	112 (78.3)	33 (84.6)	
Used to-quit	9 (6.3)	2 (5.1)	
Do you live in a dormitory?			

Yes	82 (57.3)	23 (59)	0.999
No	61 (42.7)	16 (41)	
Do you have pets?		. ,	
Yes	20 (14)	3 (7.7)	0.417
No	123 (86)	36 (92.3)	
Do you have chronic disease?		. ,	
Yes	13 (9.1)	1 (2.6)	0.308
No	130 (90.9)	38 (97.4)	
Do you have allergies?		. ,	
Yes	48 (33.6)	6 (15.4)	0.03
No	95 (66.4)	33 (84.6)	
Do any of your family members work in hospitals?			
Yes	112 (78.9)	33 (86.8)	0.358
No	30 (21.1)	5 (13.2)	
Hand-washing frequency			
Regular	136 (95.1)	38 (97.4)	0.999
Irregular	7 (4.9)	1 (2.6)	
Nasal cavity washing			
Irregular-rare	39 (27.3)	7 (17.9)	0.3
Regular-frequent	104(72.7)	32 (82.1)	
Nasal touching			
Rare	101 (70.6)	23 (59)	0.179
Frequent	42 (29.4)	16 (41)	
Hand washing after nasal touching			
Regular	115 (81)	30 (76.9)	0.651
Irregular	27 (19)	9 (23.1)	
Have you had a surgical operation?			
Yes	6 (4.2)	5 (12.8)	0.06
No	137 (95.8)	34 (87.2)	
Have you stayed in hospital?			
Yes	10 (7)	6 (15.4)	0.115
No	133 (93)	33 (84.6)	
Have you used antibiotics?			
Yes	59 (41.3)	18 (46.2)	0.589
No	84 (58.7)	21 (53.8)	
Have you used nasal spray?			
Yes	65 (45.5)	10 (25.6)	0.028
No	78 (54.5)	29 (74.4)	
Have you used inhaler steroids?			
Yes	3 (2.1)	1 (2.6)	0.999
No	139 (97.9)	38 (97.4)	
Have you had skin infection?			
Yes	11 (7.7)	4 (10.3)	0.742
No	132 (92.3)	35 (89.7)	
Variable	Proliferation		p-value
	Absent	Present	

Age	20 [18 - 26]	20 [18 - 23]	0.771
How many people do you share a room with?	3 [0 - 4]	5 [2 - 9]	0.329
How many people in your family?	5 [2 - 9]	5 [3 - 7]	0.625

Generally according to the univariate logistic regression analysis results, students with chronic disease, with pets, with family member working in the hospital, and regular hand washing after nasal touching had lower risk of bacterial proliferation (p>0.05). Those with skin infections, who used inhaler steroids, had stayed in hospital, used antibiotics, frequently touched their noses, frequently washed their nasal cavities, regularly and frequently washed their hands, stayed in dormitories and smoked or had quit smoking had higher risk of bacterial proliferation (p>0.05) (Table 3).

According to multivariate logistic regression analysis, students with allergies had 68.2% less risk of bacterial proliferation compared to students without allergies (Odd's ratio: 0.318, 95% CI: 0.109-0.930). Students who used nasal spray had 56.1% less risk of bacterial proliferation compared to students who did not use nasal spray (Odd's ratio: 0.439, 95% CI: 0.187-1.033). Additionally, those who had surgery were identified to have 7 times increased risk of bacterial proliferation (Odd's ratio: 7.136, 95% CI: 1.678-30.356) (Table 4).

Variable	Univariate		
	Odds Ratio (95% Confidence interval)	p-value	
Age	1.032 (0.782 - 1.362)	0.822	
Gender			
Male	1	-	
Female	0.527 (0.258 - 1.077)	0.079	
Period			
Ι	1	-	
II	1.402 (0.639 - 3.08)	0.399	
III	0.697 (0.253 - 1.919)	0.485	
Smoking			
Yes	1	-	
No	1.621 (0.521 - 5.036)	0.404	
Used to-quit	1.222 (0.189 - 7.9)	0.833	
Do you live in a dormitory?			
No	1	-	
Yes	1.069 (0.521 - 2.195)	0.855	
Do you have pets?			
No	1	-	
Yes	0.513 (0.144 - 1.823)	0.302	
Do you have chronic disease?			
No	1	-	
Yes	0.263 (0.033 - 2.077)	0.205	
Do you have allergies?			
No	1	-	

Table 3: Univariate logistic regression analysis results

Yes	0.36 (0.141 - 0.918)	0.032
Do any of your family members work in hospitals?		
No	1	-
Yes	0.566 (0.203 - 1.574)	0.275
Hand-washing frequency		
Irregular	1	-
Regular	1.956 (0.233 - 16.392)	0.536
Nasal cavity washing		
Irregular-rare	1	-
Regular-frequent	1.714 (0.699 - 4.203)	0.239
Nasal touching		
Rare	1	-
Frequent	1.673 (0.804 - 3.48)	0.169
Hand washing after nasal touching		
Irregular	1	-
Regular	0.783 (0.333 - 1.84)	0.574
Have you had a surgical operation?		
No	1	-
Yes	3.358 (0.967 - 11.659)	0.056
Have you stayed in hospital?		
No	1	-
Yes	2.418 (0.82 - 7.132)	0.110
Have you used antibiotics?		
No	1	-
Yes	1.22 (0.599 - 2.488)	0.584
Have you used nasal spray?		
No	1	-
Yes	0.414 (0.188 - 0.912)	0.029
Have you used inhaler steroids?		
No	1	-
Yes	1.219 (0.123 - 12.058)	0.865
Have you had skin infection?		
No	1	-
Yes	1.371 (0.412 - 4.569)	0.607

Table 4: Multivariate logistic regression analysis results

Variable	Multivariate		
	Odds Ratio (95% Confidence interval)	p-value	
Do you have allergies?			
Yes	0.318 (0.109 - 0.93)	0.036	
No	1	-	
Have you had a surgical operation?			
Yes	7.136 (1.678 - 30.356)	0.008	
No	1	-	
Have you used nasal spray?			
Yes	0.439 (0.187 - 1.033)	0.059	
No	1	-	

The model obtained with the aid of variables like having allergies or not, using nasal spray and surgical operations was found to have differentiating power for individuals with and without bacterial proliferation of AUC = 0.666 (95% CI: 0.578-0.754) according to ROC curve analysis (p<0.05). Accordingly, the differentiating power of the model was identified to be at moderate levels (Figure 1).



Diagonal segments are produced by ties.

Figure 1: The differentiating power of the model by ROC Curve analysis.

DISCUSSION

There are very few publications about the nasal *S. aureus* carriage and risk factors among medical students in Turkey. Generally, health workers in hospitals (like doctors, nurses, assisting personnel) have been assessed for MRSA colonization. In our study, 21.4% of medical students were identified to have nasal *S. aureus* carriage and none had MRSA. A study by Güçlü et al. in 2006 in Turkey found 27.9% of medical students had nasal *S. aureus* carriage and 10% MRSA was identified.⁹ Another study by Türkdağı et al. in 2015 found 17.3% nasal

S. aureus carriage and reported they identified 2.9% MRSA.¹⁰ International studies have found nasal *S. aureus* carriage and MRSA rates as 19.3% and 2.4% in Jordan in 2018, 24.7% and 0.3% in China in 2017, and 30.8% and 6.6% in Ireland, respectively.¹¹⁻¹³ A 2013 study in Thailand found nasal *S. aureus* carriage was 20.3%, while they did not identify any MRSA.¹⁴ Similarly, a 2012 study in Malaysia found 10% carriage rates; however, they stated they did not find any MRSA isolates.¹⁵ The comparison of nasal *S. aureus* carriage and MRSA rates of medical students with other studies is presented in Table 5.

Table 5: Comparison of nasal S. aureus carriage and MRSA rates by medical students in the literature.

Publication	Nasal S.aureus %	MRSA %	
Bischoff et al., 2004 USA ¹⁹	29	2	
Güçlü et al., 2006 Bolu, Turkey ⁹	27.9	10	
Bettin et al., 2012 Colombia ¹⁶	25	1.6	
Gualdoni et al., 2012, Austria ²³	25.3	-	
Cirkovic et al., 2012 Serbia ²⁴	*	0.37	
Chen et al., 2012 Taiwan ²⁰	19.3	2.2	
Nordin et al., 2012 Malaysia ¹⁵	10	-	
Treesirichod et al., 2013 Thailand ¹⁴	20.3	-	
Türk Dağı et al., 2015 Konya, Turkey ¹⁰	17.3	2.9	
Ansari et al., 2016 Nepal ¹⁷	15	4	
Okamo et al., 2016 Tanzania ²⁵	21	0.3	
Sarkar et al., 2016 Saudi Arabia ²⁶	43	0.4	
Orlin et al., 2017 Israel ²⁷	33	12	
Abroo et al., 2017 Iran ¹⁸	19.6	13.14	
Budri et al., 2017 Ireland ¹³	30.8	6.6	
Chen et al., 2017 China ¹²	24.7	0.3	
Al-Tamimi et al., 2018 Jordan ¹¹	19.3	2.4	

*Only MRSA isolation performed.

The observations of increasing rates of antibiotic resistance among *S. aureus* isolates is a significant problem throughout the world. Medical students in the preclinical period simultaneously reflect community. Most studies investigating antibiotic susceptibility of *S. aureus* isolates from the noses of medical students found similar results to our study. For example, a study from Colombia in 2012 found 2.97% clindamycin resistance, 14.85% erythromycin resistance, inducible clindamycin resistance in one isolate and no gentamicin, trimethoprim-sulfamethoxazole, vancomycin or

rifampin resistance in any isolate.¹⁶ A study from Nepal published in 2016 found high resistance to penicillin G (73.0%) among 30 isolates tested, with 2nd highest frequency for ciprofloxacin resistance (36.7%) and stated that inducible clindamycin resistance was present in 4 isolates (13.3%) with the D-region test. The same study emphasized that no vancomycin or teicoplanin resistant isolates were found.¹⁷ A study of medical students from Iran in 2017 identified 93.4% penicillin resistance, 27% erythromycin resistance, 19% clindamycin resistance, 12.4% trimethoprim-sulfamethoxazole resistance, 6.6% ciprofloxacin resistance, 2.2% gentamicin resistance and 4.4% mupirocin resistance.¹⁸

When the socio-demographic, potential and habitual risk factors are investigated according to student responses, there was no significant correlations with nasal S. aureus carriage apart from negative correlations with nasal spray use and allergy history. When S. aureus colonizes the nose, it is a bacteria with high capacity to form biofilms. In the stage of S. aureus entering the nasal anterior region, we think the use of nasal spray and allergy history may mechanically prevent the infection linked to secretions and sneezing. Additionally, according to logistic regression analysis, surgical operation history increases the risk of S. aureus carriage by 7 times. This shows colonization linked to hospital stay. If we examine the research, Bischoff et al. in a study researching the nasal S. aureus carriage of medical students found significantly high rates of carriage with increasing age and with male gender.¹⁹ Research in Jordan in 2018 identified significant correlations between students with chronic disease and male gender with S. aureus carriage.¹¹ In Taiwan, Chen et al. found correlations between hospital stay in the previous 1-year, antibiotic use and male gender with S. aureus carriage.²⁰ A 2017 study from China identified correlations with increased age and male gender and a significant difference with regular nasal cleaning.¹² Some studies have not identified significant correlations between nasal S. aureus carriage and risk factors.14,16,17

We think a limitation of our study is that we only took nasal swab samples from students one time as nasal *S. aureus* carriage may be temporary or permanent. Permanent carriage may be identified by nasal *S. aureus* isolation with repeated sampling at certain intervals. There is a need to support our results with advanced studies about whether permanent carriage is present or not.

As medical students have close contact with patients in hospitals, they are important elements in the spread of pathogens to patients and the general population. Topical antibiotics may be used to resolve *S. aureus* colonization. However, some studies have emphasized that this is not a permanent solution with relapse observed and have not recommended it.^{21,22} Additionally, we may encounter increased resistance rates to antibiotics due to excess use. Instead, providing information to *S. aureus* carriers and providing training explaining standard precautions and especially the importance of hand hygiene to reduce bacterial spread will create awareness.

CONCLUSION

S. aureus and MRSA carriage is an important pathogen playing a role in hospital-associated infections along with population-sourced infections. The medical faculty in our university is a newly-opened faculty, with no medical students in clinic yet. Medical students in the preclinical period in our university are S. aureus carriers; however, it is a positive result that no MRSA carriage was found. Additionally, in order for there to be no MRSA carriers among our students when they enter the hospital, we shared our results with the students and organized awareness training. With continuous training, it is necessary to support and develop strategies to reduce transmission from hospital environment to students to keep nasal bacterial colonization low in the clinical period and to monitor rates with advanced studies.

Financial Disclosure: This work was supported by Ahi Evran University Scientific Research Project Unit with TIP.A4.19.008 project number.

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