J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 47(1): 277-283, 2023
Doi: 10.33483/jfpau.1211002

ORIGINAL ARTICLE / ÖZGÜN MAKALE

NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS* IN PHARMACIST AND PHARMACY PERSONNEL

ECZACI VE ECZANE PERSONELİNDE STAPHYLOCOCCUS AUREUS BURUN TAŞIYICILIĞI

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ABSTRACT

Objective: *The aim of this study was to determine the Staphylococcus aureus (S. aureus) nasal carriage rates and risk factors in pharmacist and pharmacy personnel.*
Material and Method: *300 nasal swabs were collected from volunteers (pharmacist and pharmacy personnel) working in pharmacies in Ankara, Turkey. Samples were identified as S. aureus by phenotypic methods. Methicillin resistance of the strains was determined in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) by the disk diffusion method and the presence of the mecA gene was investigated by Polymerase Chain Reaction (PCR). Volunteers were asked to answer some questions (age, sex etc.) and risk factors for nasal S. aureus carriage were investigated.*
Result and Discussion: *S. aureus was detected in 64 (21.3%) of 300 samples and of which 4 (1.3%) were identified as Methicillin Resistance Staphylococcus aureus (MRSA). S. aureus carriage rates were found to be 25.7% in pharmacist and 20% in pharmacy personnel. There was no significant difference between these two groups ($p>0.05$). A significant difference was found between some risk factors (smoking, diabetes, and outpatient treatment in hospital within the past year) and nasal S. aureus carriage ($p<0.05$). We think that compliance with hand hygiene and effective infection control policies can reduce the rates of S. aureus and MRSA carriage.*
Keywords: *mecA, methicillin resistance Staphylococcus aureus, polymerase chain reaction, Staphylococcus aureus*

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Submitted / Gönderilme : 01.12.2022
Accepted / Kabul : 28.12.2022
Published / Yayınlanma : 20.01.2023

ÖZ

Amaç: *Bu çalışmanın amacı eczacı ve eczane personelindeki Staphylococcus aureus (S. aureus) burun taşıyıcılık oranlarının ve risk faktörlerinin belirlenmesidir.*

Gereç ve Yöntem: *Ankara'daki eczanelerde çalışan eczacı ve eczane personelinden oluşan gönüllü bireylerden 300 nazal sürüntü örneği toplandı. Örnekler fenotipik yöntemler ile S. aureus olarak tanımlandı. Suşların metisilin direnci CLSI önerileri doğrultusunda disk difüzyon yöntemi ile belirlendi ve mecA geninin varlığı polimeraz zincir reaksiyonu (PZR) ile araştırıldı. Gönüllülerden bazı sorulara (yaş, cinsiyet vb.) cevap vermesi istenerek nazal S. aureus taşıyıcılığı için risk faktörleri araştırıldı.*

Sonuç ve Tartışma: *300 örneğin 64'ü (21.3%) S. aureus ve 4'ü (1.3%) metisilin dirençli Staphylococcus aureus (MRSA) olarak tespit edildi. Nazal S. aureus taşıyıcılık oranları eczacılarda % 25.7, eczane personelinde % 20 olarak belirlendi. Bu iki grup arasında anlamlı bir fark bulunmadı (p>0.05). Bazı risk faktörleri (sigara içmek, şeker hastası olmak ve son bir yıl içerisinde hastanede ayakta tedavi görmek) ile nazal S. aureus taşıyıcılığı arasında anlamlı bir fark bulundu (p<0.05). El hijyenine ve enfeksiyon kontrol politikalarına uyumun S. aureus ve MRSA taşıyıcılık oranlarını azaltabileceğini düşünmekteyiz.*

Anahtar Kelimeler: *mecA, metisilin dirençli Staphylococcus aureus, polimeraz zincir reaksiyonu, Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a bacterium that causes community and hospital-acquired infections [1]. Although *S. aureus* is colonized in various parts of the body, its main ecological niche is the anterior nares [2]. *S. aureus* is permanently colonized in the anterior nares of approximately 30% of people [3]. Endogenous nasal colonization is a common source of infection and a strong risk factor for subsequent colonization [4,5]. *S. aureus* nasal carriage rates are higher in health care workers (HCWs) than in the community [6]. HCWs who interact with hospital and community can cause cross contamination of hospital-acquired and community-acquired MRSA [7]. It is difficult to treat infections caused by MRSA [8]. MRSA carriers can cause major problems for critically ill patients in the hospital [9]. The hands of HCWs can cause the transmission of an infectious microorganism to the patient [10]. It is recommended that healthcare workers be screened for MRSA in order to reduce the spread of MRSA in the hospital [11]. It is important to know the rates of *S. aureus* and MRSA carriage in healthcare workers for appropriate antibiotic treatment [12]. Hand hygiene and environmental decontamination are measures that can be taken to control and prevent the spread of MRSA [13].

The aim of this study was to determine the rates of *S. aureus* nasal carriage and risk factors in pharmacist and pharmacy personnel who were in contact with patients and their relatives.

MATERIAL AND METHOD

Our study was carried out between June and September 2014 with 300 volunteers consisting of pharmacist and pharmacy personnel working in pharmacies in Ankara, Turkey.

Isolation and Identification of Bacteria

Swab samples from both nostrils of the volunteers were transferred to stuart transport medium (Oxoid, England). The samples were passaged on 5% sheep blood agar medium (Blood Agar Base Merck, Germany) and incubated at 37°C for 24 hours. After incubation, isolates were confirmed as *S. aureus* by conventional methods [14].

Antibiotic Susceptibility Testing

The phenotypic determination of methicillin resistance of strains was carried out by disk diffusion method using oxacillin (1µg, Oxoid, Basingstoke, UK) and cefoxitin disks (30 µg, Oxoid, Basingstoke, UK). The results were evaluated according to Clinical and Laboratory Standards Institute recommendations [15].

DNA Isolation and *mecA* Gene Amplification

The presence of the *mecA* gene was investigated to determine the methicillin resistance of the strains by molecular method. DNA isolation was performed from *S. aureus* strains in accordance with the recommendations of Okamoto et al. [16]. After DNA isolation, the samples were stored at -80°C until they were studied. For the amplification of the *mecA* gene, the primers *mecA*-1-F (5 GTAGAAATGACTGAACGTCCGATAA-3), *mecA*-2-R (5 CCAATTCCACATTGTTTCGGTCTAA-3) were used. As a result of PCR performed with these primers, amplicons with a size of 310 bases were produced [17]. PCR buffer (added $MgCl_2$), dNTPs, Taq DNA polymerase (GeneDirex Inc., USA), primers (Iontek, Turkey), ddH2O, template DNA were used in the PCR reaction. (Table 1). PCR reactions were performed in a thermal cycler (GeneAmp PCR System 9700 PE Applied Biosystems, Norwalk, CT, USA). (Table 2). The amplicons were evaluated in 2% agarose gel electrophoresis (RunVIEW, Cleaver Scientific, UK) containing 0.5 ug/ml ethidium bromide (Thermo Fisher Scientific, St. Leon-Rot, Germany) and photographed on the imaging device (Vılbert Lourmat Photodocumentation and Imaging Systems, France). Amplicons were size confirmed with a DNA Ladder (Thermo Scientific™ O'RangeRuler™ 50 bp DNA Ladder, Lithuania).

Table 1. PCR Reaction Mix

Components	Volume	Stock Concentration	Final Concentration
PCR Buffer (added $MgCl_2$)	5 µl	10X	1X
Taq Polymerase	0.25 µl	5 U/µl	1.25 U
Forward Primer	2 µl	10 µM	0.4 µM
Reverse Primer	2 µl	10 µM	0.4 µM
dNTPs	0.4 µl	25mM	0.2 mM
ddH_2O	35.35 µl		
Template DNA	5 µl		
Final Volume	50 µl		

Table 2. PCR Protocol

Step	Cycles	Temperature	Time
Initial denaturation	1	94°C	5 min.
Denaturation	40	94°C	30 s.
Annealing	40	55°C	30 s.
Extension	40	72°C	1 min.
Final Extension	1	72°C	1 min.

Statistical Analysis

The data were analyzed in SPSS 20.0 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). Results were compared using the Independent Sample t test. $p<0.05$ was considered statistically significant.

RESULT AND DISCUSSION

In this study, 70 (23.3%) of the volunteers were pharmacists, 41 (13.7%) of the pharmacists were males and 29 (9.7%) of them were females. Of the 230 (76.7%) pharmacy personnel, 162 (54%) were males and 68 (22.7%) were females. Volunteers were asked to answer some questions (age, sex, outpatient treatment in hospital within the past year, using antibiotics in the last month, smoking, diabetes). *S. aureus* ATCC 25923, *S. aureus* ATCC 43300 were used as control strains in this study.

S. aureus was detected in 64 (21.3%) of 300 nasal swabs were collected from pharmacist and pharmacy personnel. MRSA was found in 4 (1.3%) of the strains, of which 3 (1%) were resistant to cefoxitin and 1 (0.3%) was resistant to both oxacillin and cefoxitin. The presence of *mecA* gene of

MRSA strains was investigated by PCR and it was determined that all of them produce amplicons with a size of 310 bases. Thus, methicillin resistance was genotypically confirmed in the strains.

18 (25.7%) *S. aureus* strains were isolated from pharmacists, of which 11 (15.7%) were males and 7 (10%) were females. 46 (20%) *S. aureus* strains were isolated from pharmacy personnel, of which 33 (14.3%) were males and 13 (5.7%) were females. *S. aureus* carriage rates were found to be 25.7% in pharmacists and 20% in pharmacy personnel. There was no significant difference between these two groups ($p>0.05$) (Table 3). Nasal carriage rates of *S. aureus* have been reported to range from 22.7% to 48% among different countries and categories of healthcare professionals [18-20]. The *S. aureus* carriage findings in our study were similar to the findings in other studies conducted in Turkey 20% [21], Egypt 22.9% [22], China %21.6, [23] and Spain %24.3, [24]. In some countries such as Germany 33.8% [25], France 38.8% [1], America 43.8% [26] the rates of *S. aureus* carriage in studies were higher than our findings.

In this study, MRSA was detected in 4 (1.3%) volunteers and 3 (1%) of them were isolated from males pharmacy personnel and 1 (0.3%) from female pharmacy personnel. MRSA carriage ranges from 0.37 % to 13 % in some studies [11,12,21,27]. The MRSA rates in our study were similar to the studies in China 1% [23] and Spain 1.3% [24].

The difference in *S. aureus* and MRSA carriage rates depend on many factors such as culture techniques, sample size, inadequate hand hygiene, limited infrastructure, lack of personnel protective equipment, insufficient information about transmission routes [28]. In our study, which examined some risk factors for *S. aureus* carriage, the difference between the smoker and non-smoker group was found to be significant ($p<0.05$). Smoking decreases IgA production, increases mucus production, impairs epithelial elastic properties and affects phagocyte activities. As a result, bacterial colonization is facilitated, the inflammatory response is exacerbated, host immunity is further impaired, bacterial colonization is promoted in the respiratory tract [29]. Our study was consistent with studies in which smoking was found to be a risk factor of *S. aureus* carriage [21,29,30]. In many studies, it has been reported that diabetes increases the colonization of *S. aureus* [32-36]. Anafo et al [31] found that there was a significantly associated between *S. aureus* carriage and diabetes. Our study was in line with this study ($p<0.05$) (Table 3). In this study, there was no significant association between nasal *S. aureus* carriage and other risk factors (age, gender, antibiotic use in the last month ($p>0.05$) (Table 3). However, a significant difference was observed between the volunteers who received outpatient treatment at the hospital in the last year and the *S. aureus* carriage ($p<0.05$) (Table 3).

Pharmacist and pharmacy personnel are healthcare workers who are in contact with the patients. Therefore, we think that our results were similar to hospital-acquired nasal *S. aureus* and MRSA carriage rates. HCWs are the source of transmission of *S. aureus* and MRSA in the hospital and environmental setting. Compliance with hand hygiene is the most effective way to prevent and control the transmission of *S. aureus* and MRSA. Additionally, we predict that *S. aureus* and MRSA carriage rates can be reduced with rapid detection and treatment of *S. aureus* and MRSA carriers, Judicious Use of Antibiotics and effective infection control policies.

Table 3. *S. aureus* carriage rates and risk factors

***S.aureus* Culture Results**			
Age	**Total**	**Positive**	**Negative**
18-30	156 (52)	33 (21)	123 (79)
31-40	107 (35.7)	26 (24)	81 (76)
41-57	37 (12.3)	5 (13.5)	32 (86.5)
	N:300 (n%)	64 (21.3)	236 (78.7)
Sex/Pharmacist			
Male	41 (58.6)	11 (15.7)	30 (42.9)
Female	29 (41.4)	7 (10)	22 (31.4)
	N:70 (n%)	18 (25.7)	52 (74.3)

Table 3 (*continue*). *S. aureus* carriage rates and risk factors

Sex/Pharmacy Personnel	Total	Positive	Negative
Male	162 (70.4)	33 (14.3)	129 (56.1)
Female	68 (29.6)	13 (5.7)	55 (23.9)
	N: 230 (n%)	46 (20)	184 (80)
Diabetes			
Yes	8 (2.7)	4 (50)	4 (50)
No	292 (97.3)	60 (20.55)	232 (79.45)
	N: 300 (n%)	64 (21.3)	236 (79.7)
Antibiotic use in last one month			
Yes	96 (32)	18 (18.75)	78 (81.25)
No	204 (68)	46 (22.55)	158 (77.45)
	N: 300 (n%)	64 (21.3)	236 (79.3)
Smoking			
Yes	64 (21.3)	23 (35.94)	41 (64.06)
No	236 (78.7)	41 (17.37)	195 (82.63)
	N: 300 (n%)	64 (21.3)	236 (79.7)
Outpatient treatment in hospital within the past year			
Yes	76 (25.33)	46 (60.52)	30 (39.48)
No	224 (74.67)	18 (8.03)	206 (91.97)
	N: 300 (n%)	64 (21.3)	236 (79.7)

ACKNOWLEDGEMENTS

The authors thank Sivas Cumhuriyet University and Sivas Cumhuriyet University Scientific Research Projects for their financial support to this study (Project No: ECZ-010). We thank the volunteers who participated in our study.

AUTHOR CONTRIBUTIONS

Concept: H.B., S.Y.; Design: H.B.; Control: S.Y.; Sources: H.B.; Materials: H.B., S.Y.; Data Collection and/or Processing: H.B., S.Y.; Analysis and/or Interpretation: H.B., S.Y.; Literature Review: H.B., S.Y.; Manuscript Writing: H.B., S.Y.; Critical Review: H.B., S.Y.; Other: H.B., S.Y.

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

Ankara University Faculty of Medicine Clinical Research Ethics Committee. No: 04-177-14. 10.3.2014.

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