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Investigation of nasal *Staphylococcus aureus* carriage by real-time PCR in patients receiving hemodialysis treatment

Songül Çetik Yıldız, Cemil Demir

Department of Medical Services and Techniques, Mardin Artuklu University, Vocational Higher School of Health Services, Mardin, Turkey

ABSTRACT

Objectives: *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), and methicillin-resistant *S. aureus* (MRSA), which are significant nosocomial pathogens, have become a growing global problem because their carriage and diseases have become resistant to many antibiotics. This study aimed to investigate and determine the rate of MRSA carriage among patients receiving hemodialysis treatment using molecular methods.

Methods: In the 254 hemodialysis patients, the nasal carriage rates, susceptibility and resistance to *S. aureus*, CoNS and MRSA were examined using culture and real-time PCR methods. Nasal samples from hemodialysis patients were examined using real-time PCR. Microscopic examination was performed using the Gram staining method, and *S. aureus* was identified using catalase and coagulase. The strains were then tested for antibiotic susceptibility. Staphylococci was isolated from 231 of the 254 patients.

Results: *S. aureus* carriage was detected in 50 patients, MRSA in 16, methicillin-susceptible *S. aureus* (MSSA) in 33, CoNS in 66, methicillin-resistant CoNS (MR-CoNS) in 38, and methicillin-susceptible CoNS (MS-CoNS) in 28. *S. aureus* and MRSA strains exhibited 100% susceptibility to nitrofurantoin, and vancomycin. MSSA strains showed the highest susceptibility to chloramphenicol, clindamycin (84.8%), and co-trimoxazole (36.4%). CoNS showed 100% susceptibility to vancomycin, and 16.7% susceptibility to ampicillin. Vancomycin was found to be the most effective antibiotic against *S. aureus*, CoNS, and MRSA pathogens identified in patients undergoing hemodialysis, whereas penicillin resistance was found.

Conclusions: It can be concluded that one of the most effective ways to prevent the formation of antibiotic resistant strains is the hygiene of the hospital and hospital staff. Real-time PCR is very important for analyzing with high sensitivity.

Keywords: *Staphylococcus aureus*, nasal carriage, antibiotic resistant, infection, real-time PCR

Staphylococci have had a significant majority among pathogens due to the diseases they have caused in humans and animals since 1881, when they were defined as infectious agents [1]. *Staphylococcus aureus*, which is resistant to methicillin-resistant *S. aureus* (MRSA), and coagulase-negative staphylococci (CoNS), is among the most common nosocomial infectious agents. It is critical that multiple drug resist-

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Address for correspondence: *Songül Çetik Yıldız, PhD., Assistant Professor, Mardin Artuklu University, Vocational Higher School of Health Services, Department of Medical Services and Techniques, Diyarbakır Yolu üzeri 5. km, Mardin Artuklu Üniversitesi Kampüsü, 47200 Artuklu, Mardin, Turkey. E-mail: songulcetik@gmail.com, Phone: +90 482 2126949 ext. 7270*

ance, particularly in MRSA infections and strains, reduces treatment options [2, 3]. Depending on the origin of the disease, MRSA can be nosocomial or community acquired. Community-acquired MRSA strains cause invasive infections, as well as skin and soft tissue infections, at an accelerating rate. Nosocomial MRSA infections can lead to severe disease and death in humans [4]. CoNS is a clinical concern because it is commonly isolated from foreign body infections, such as invasive catheters, prosthetic heart valves, and joint prostheses. Staphylococci (*S. aureus*, CoNS) inhabit the nasal mucosa and are responsible for approximately 1/3 of bloodstream infections. Approximately 80% of *S. aureus* isolated from the bloodstream were identical to those isolated from nose [5]. MRSA can spread through contaminated hands and inorganic materials after active nasal colonization, putting the carriers and others around them at risk [6]. Medical personnel and students colonized with *S. aureus* and MRSA pose risks in terms of the development and transmission of nosocomial infections [7]. Hospitalization, surgery, dialysis, indwelling catheters or percutaneous medical devices (tracheostomy tubes, gastrostomy tubes, or Foley catheters), long-term care facility residency, and positive culture for MRSA are risk factors for patients in terms of MRSA infection [8].

One of the greatest challenges in the treatment of nosocomial infections is the resistance of MRSA isolates to multiple antibiotics in hospitals [9, 10]. MRSA strains are more easily transmitted than others in hospitals, and they are often difficult to eliminate once they have established themselves [11]. Because MRSA is resistant to all types of penicillin (methicillin, oxacillin, nafcillin, cloxacillin, and dicloxacillin), cephalosporins, and a wide range of antibiotics, including clindamycin, erythromycin, tetracycline, and aminoglycosides, treatment options for infections are limited and can be fatal [12]. According to previous studies, approximately 30% of the general population is colonized by SA, and approximately 3% of these strains are methicillin-resistant [13, 14]. In another study, methicillin resistance was observed in 10.9% of SA strains, and 38.8% of CoNS [15]. Colonization was found in 45% of patients admitted to intensive care units in European hospitals, with nosocomial MRSA accounting for 21% of them [16].

Although methicillin-resistant staphylococci infections are difficult to treat, they are also a leading cause of morbidity and mortality. *S. aureus*, CoNS, and MRSA, which are predominantly nosocomial, have become major global health problems around the world.

METHODS

Study Design

Using nasal swab samples taken from 254 hemodialysis patients who visited the Mardin Training and Research Hospital on a regular basis between March 2019, and May 2021, the present study aimed to assess the methicillin carriage and multiple antibiotic resistances of nasal *S. aureus*, and CoNS strains. 254 hemodialysis patients, the nasal carriage rates, susceptibility and resistance to *S. aureus*, CoNS and, MRSA were examined using culture and real-time PCR methods.

Isolation of Staphylococci and Culture-Identification

Microscopic examination was performed on one of the two samples taken from each patient's nasal cavity using the Gram staining method and *S. aureus* identification was performed using catalase and coagulase operations in order to be employed in culture processes.

The collected nasal swab samples were followed by phenotypic identification procedures. It was inoculated on commercially obtained (Oxoid) 5% sheep blood agar and incubated at 37°C for 24 hours. Growing bacteria were evaluated in terms of colony morphology and the identification of *S. aureus* suspicious colonies was made by catalase test, gram stain, coagulase test and mannitol fermentation test. The presence of coagulase was demonstrated by a commercial latex agglutination test, the Staphylase test kit (Oxoid Limited, England). Catalase and coagulase tests are positive and staphylococci that grow on Mannitol Salt agar by forming a yellow zone are interpreted as *S. aureus*.

Antibiotic Susceptibility

The antibiotic susceptibility of the *S. aureus* and CoNS strains was determined using oxacillin, penicillin, rifampicin, tetracycline, and vancomycin discs.

After 18 h of incubation at 37 °C, the inhibition zone diameters of the strains were measured. Results were interpreted using the criteria specified by the Clinical and Laboratory Standards Institute (CLSI) [4]. The control strains used in this study were ATCC 25923 (MSSA), ATCC 43300 (MRSA) and *S. aureus* ATCC 25923 (*S. aureus*). Suspensions of *S. aureus* and MRSA strains diluted in PBS were transferred to 100 µl sterile microcentrifuge tubes and heated at 95 °C, 7 min to generate DNA [17]. In line with this protocol, DNA quantity was measured using spectrophotometry at 260-280 nm.

Determination of the presence of bacteria strains using real-time PCR

To identify *S. aureus* and CoNS using real-time PCR, the femB gene-specific SA detection kit was used according to the manufacturer's instructions. The final volume of the real-time PCR procedure was 20µL with 5µL of DNA. A LightCycler 480-II instrument was used for amplification. The protocol was as follows: initial denaturation at 95°C for 2min, 50cycles of 10 s at 95°C, 1min at 60°C, with a single reading.

MecA1(5′-GCA ATC GCT AAA GAA CTA AG-3′) and MecA2(5′-GGG ACC AAC ATA ACC TAA TA-3′), which are mecA gene-specific primers, were utilized to identify MRSA using real-time PCR [18]. The final volume for real-time PCR was set to 20µL, which contained 2µL of DNA. Real-time PCR was performed on a LightCycler 480-II using the LightCycler FastStart DNA Master SYBR Green-I kit according to the manufacturer's instructions. With a single readout, real-time PCR reaction was set to 35cycles of 10min at 95°C for initial denaturation, 0s at 95°C, 5s at 55°C, and 8 s at 72°C. Following this, The single-cycle melting curve program consisted of heating at 95°C with a hold time of 0s, heating at 58°C, 60s, and heating at 95°C, 0s.

Statistical Analysis

Statistical analyzes were performed using the SPSS 24.00 program. Categorical data are presented as numbers and percentages. Pearson Chi-Square test was used in categorical data analysis. Statistical significance was accepted as $p < 0.05$.

RESULTS

The study included 254 patients of whom 145 (57%) were male and 109 (43%) were female. In terms of age, 72 (28%) participants were 50 years or older, 124 (49%) were 30-49 years old, and 58 (23%) were under the age of 29. Of the patients, 159 (63%) were admitted for less than a year, whereas 95 (37%) were admitted for more than a year (Table 1).

The presence of staphylococci in nasal samples from 231 of 254 participants was evaluated. Nasal *S. aureus* carriage was discovered in 50 (22%) of the pa-

Table 1. Demographic features of 254 hemodialysis patients

Variables	n	%
Gender		
Male	145	57
Female	109	43
Age		
29 and under	58	23
30-49	124	49
50 and over	72	28
Duration of hemodialysis admittance		
Less than 1 year	159	63
More than 1 year	95	37

Table 2. Distribution of the bacteria strains isolated from cultures

Variables	n	%
Gender		
Male	145	5
Female	109	4
Age		
29 and under	58	2
30-49	124	4
50 and over	72	2
Duration of hemodialysis admittance		
Less than 1 year	159	6
More than 1 year	95	3

tients, MRSA 16(7%), MSSA 33 (14%), CoNS 66 (29%), MR-CoNS 38 (16%), MS-CoNS 28 (12%) (Table 2).

Antibiotic susceptibilities of the *S. aureus* strains shown in Table 3. The highest susceptibility antibiotics was nitrofurantoin, and vancomycin at a rate of 50 (100%) followed by 47 (94%) to gentamicin, 45(90%) to rifampicin, and 16 (32%) to co-trimoxazole, and erythromycin (indicating that they had developed strong resistance to these antibiotics). The lowest susceptibility 4(8%) and so highest resistance were to penicillin (Table 3).

Antibiotic susceptibilities of the CoNS strains shown in Table 4. The strains demonstrated the highest resistance to vancomycin at a rate of 100% and did not gain further resistance. CoNS strains exhibited high susceptibility and low resistance to rifampicin 54(81.8%), nitrofurantoin 51 (77.3%), ciprofloxacin and fusidic acid 48 (72.7%). The lowest susceptibility was observed for ampicillin with 11 (16.7%). The strains showed low susceptibility/high resistance 14 (21.2%) to penicillin (Table 4).

In Table 5, it was observed that MSSA (n = 33) strains showed the highest susceptibility and the least resistance. The highest susceptibility antibiotics was 28 (84.8%) to chloramphenicol and clindamycin followed by 25 (75.8%) to cefotaxime and ciprofloxacin, 18 (54.5%) toerythromycin and 12 (36.4%) to co-trimoxazole. The MSSA strains showed the lowest susceptibility and the highest resistance to ampicillin with five strains (15.2%) (Table 5).

When the distribution of the antibiotic susceptibilities of MRSA strains in Table 6 was examined, it was observed that the highest susceptibility antibiotics was 16 (100%) to nitrofurantoin and vancomycin (they did not exhibit resistance) followed by 11 (68.8%) to rifampicin, and 9 (56.3%) to clindamycin. The MRSA strains showed the lowest susceptibility 1 (6.3%) to ampicillin, and cefotaxime, indicating that they gained strong resistance to these antibiotics. Additionally, they demonstrated low susceptibility and so high resistance to erythromycin, and fusidic acid in 2 strains (12.5%) (Table 6).

Table 3. Distribution of the antibiotic susceptibilities of *Staphylococcus aureus* strains (n = 50)

Staphylococcus aureus	**n (%)**	
	S	I
Ampicillin	7 (14)	-
Cefotaxime	26 (52)	5 (10)
Chloramphenicol	36 (72)	5 (10)
Ciprofloxacin	39 (78)	1 (2)
Clindamycin	39 (78)	1 (2)
Co-trimoxazole	16 (32)	4 (8)
Erythromycin	16 (32)	10 (20)
Fusidic acid	26 (52)	-
Gentamicin	47 (94)	-
Nitrofurantoin	50 (100)	-
Oxacillin	33 (66)	-
Penicillin	4 (8)	-
Rifampicin	45 (90)	1 (2%)
Tetracycline	12 (24)	-
Vancomycin	50 (100)	-

S = Susceptible, I = Intermediate

Table 4. Distribution of the antibiotic susceptibilities of CoNS strains

CoNS (n = 66)	**n (%)**	
	S	**I**
Ampicillin	11 (16.7)	-
Cefotaxime	46 (69.7)	-
Chloramphenicol	30 (45.5)	2 (3)
Ciprofloxacin	48 (72.7)	-
Clindamycin	42 (63.6)	2 (3)
Co-trimoxazole	52 (78.8)	1 (1.5)
Erythromycin	36 (54.5)	-
Fusidic acid	48 (72.7)	-
Gentamicin	38 (57.6)	-
Nitrofurantoin	51 (77.3)	-
Oxacillin	26 (39.4)	-
Penicillin	14 (21.2)	-
Rifampicin	54 (81.8)	-
Tetracycline	39 (59.1)	-
Vancomycin	66 (100)	-

S = Susceptible, I = Intermediate

DISCUSSION

Real-time PCR, which is a quick and reliable procedure, was used. The real-time PCR method has been favored in previous studies because it detects MRSA, an important pathogen, faster and more precisely than the traditional culture and latex agglutination methods [19, 20].

Males comprised 57% of the 254 hemodialysis patients in the present study, while females accounted for 43%. 28% of the patients were over the age of 50, 49% were between 30-49 years, and 23% were under the age of 29. 63% of patients were admitted for one year or less, and 37% of the patients were admitted for more than one year. There was no correlation between MRSA nasal carriage and age or sex in this study (Table 1). In a similar study, nasal carriage of MRSA was not significantly related to gender or age group [21].

The determination and prevention of nasal carriage in the development of nosocomial *Staphylococcus* are of great importance in terms of infection control. The nose is the primary site of *S. aureus* colonization, but it can also be isolated from other regions of the body. The present study investigated the presence of *S. aureus*, MRSA, MSSA, CoNS, MR-CoNS, and MS-CoNS in nasal swab samples from 231 of 254 individuals. *S. aureus* carriage was found in 50 (22%) patients, MRSA in 16 (7%), MSSA in 33 (14%), CoNS in 66 (29%), MR-CoNS in 38 (16%), and MS-CoNS carriage in 28 (12%) patients, according to the data. This led to the conclusion that dialysis patients are exposed to these strains throughout the dialysis process, which increases the frequency of resistant strains (Table 2). In a previous study of catheter-related bloodstream infections [22] discovered that MR-CoNS developed in 23 (21.1%) of 109 patients, MS-CoNS in 1(0.9%), MRSA in 15 (13.8%), and MSSA in 23 (21.1%). *S. aureus* was found in 39.6% (345/871) of the samples taken from clinical samples in a study conducted by [23]. MRSA was detected in 18.1%, and MSSA in 21.5%. In another study [24], 42 of 100 staphylococcal strains isolated from diverse clinical specimens were *S. aureus* strains, and 58 were CoNS strains. In another study, 54 of 139 strains isolated from diverse clinical specimens were *S. aureus* strains, while 94 were CoNS strains [25]. In certain studies, nasal *S. aureus* carriage and MRSA rates were reported as 19.3% and 2.4% in Jordan [26], 24.7% and 0.3% in China [27], 30.8% and 6.6% in Ireland [28], respectively. According to another study, over 30% of the US population carries *S. aureus* in their anterior nostrils and 1-2% carries MRSA in their nostrils [8]. When the findings of previous studies conducted on a

Table 5. Distribution of the antibiotic susceptibilities of MSSA strains (n = 33)

MSSA	n (%)	
	S	I
Ampicillin	5 (15.2)	-
Cefotaxime	25 (75.8)	5 (15.2)
Chloramphenicol	28 (84.8)	4 (12.1)
Ciprofloxacin	25 (75.8)	1 (3.0)
Clindamycin	28 (84.8)	-
Co-trimoxazole	12 (36.4)	3 (9.1)
Erythromycin	18 (54.5)	10 (30.3)

S = Susceptible, I = Intermediate

Table 6. Distribution of the antibiotic susceptibilities of MRSA strains

MRSA (n = 16)	n (%)	
	S	I
Ampicillin	1 (6.3)	-
Cefotaxime	1 (6.3)	-
Chloramphenicol	6 (37.5)	1 (6.3)
Ciprofloxacin	2 (12.5)	-
Clindamycin	9 (56.3)	1 (6.3)
Co-trimoxazole	4 (25)	1 (6.3)
Erythromycin	2 (12.5)	-
Fusidic acid	2 (12.5)	-
Gentamicin	14 (87.5)	-
Nitrofurantoin	16 (100)	-
Oxacillin	-	-
Penicillin	-	-
Rifampicin	11 (68.8)	-
Tetracycline	5 (31.3)	-
Vancomycin	16 (100)	-

S = Susceptible, I = Intermediate

global scale were compared, it was clear that the transmission rates of *S. aureus*, MRSA, and CoNS varied by region, population, and hospital. The carriage that occurs as a result of colonization of healthcare workers' skin and nasal mucosa is significant because it is a possible source of infection and plays a vital role in nosocomial epidemics.

S. aureus strains were found to have the highest susceptibility to nitrofurantoin and vancomycin, with a 100% rate of susceptibility, indicating that they did not develop any resistance to these two antibiotics. They showed a high susceptibility to gentamicin, and rifampicin (94% and 90%, respectively). They were also susceptible to clindamycin and ciprofloxacin in 78% of the cases, and chloramphenicol in 72%. They demonstrated 16% susceptibility to co-trimoxazole and erythromycin, indicating that they developed significant resistance to these medicines, according to the findings. Although the strains were 14% susceptible to ampicillin, they were only 8% susceptible to penicillin, indicating that they had the highest antibiotic resistance to penicillin. (Table 3) Penicillin resistance was found to be most common (85%) in a similar study, as well [29].

CoNS is one of the leading foreign body infections and nosocomial bacteremia [30]. CoNS strains were found to have the highest susceptibility to vancomycin at a rate of 100% and did not acquire any resistance. CoNS strains showed high susceptibility and low resistance to rifampicin (81.8%), nitrofurantoin (77.3%), ciprofloxacin, fusidic acid (72.7%). The lowest susceptibility was observed for ampicillin (16.7%. The strains exhibited low susceptibility and high resistance to penicillin, at a rate of 21.2% (Table 4). CoNS strains were found to be more antibiotic-resistant than *S. aureus* strains [15]. In another study, MR-CoNS strains were more resistant to antibiotics than MS-CoNS, while all of them were susceptible to vancomycin and teicoplanin [24]. In a previous study, 84.2% of 172 CoNS strains were found to be resistant to penicillin, 38.8% to methicillin, 54.8% to erythromycin, 44.4% to clindamycin, 42.2% to co-trimoxazole, 25% to ciprofloxacin, 28.1% to fusidic acid; however, no vancomycin resistance was reported [15].

Table 5 showed that MSSA strains had the highest susceptibility to chloramphenicol and clindamycin (84.8%). The strains exhibited susceptibility at a rate of 75.8% to cefotaxime, 54.5% to erythromycin, and 36.4% to co-trimoxazole. The MSSA strains showed the lowest susceptibility and highest resistance to ampicillin at a rate of 15.2% (Table 5). When the distribution of antibiotic susceptibility of MRSA strains was examined, it was determined that they were the most susceptible to nitrofurantoin and vancomycin at a rate of 100%, indicating that they did not demonstrate any resistance. Susceptibilities to gentamicin, rifampicin, and clindamycin were 87.5%, 68.8%, and 56.3%, respectively. MRSA strains showed low susceptibility and high resistance to erythromycin and fusidic acid, at a rate of 12.5%. MRSAs showed the lowest sensitivity to ampicillin and cefotaxime antibiotics at a rate of 6.3%, indicating that they gained high resistance to these antibiotics (Table 6). In a previous study, the MRSA resistance rates were reported to be 29.9% for trimethoprim-sulfamethoxazole, 60.8% for clindamycin, 71.8% for erythromycin, 7.7% for teicoplanin, 90.1% for gentamicin, 88.8% for ofloxacin, 88.1% for norfloxacin and 100% for penicillin. Additionally, all the isolates were reported to be susceptible to vancomycin [23].

Consistent with the findings, the most efficient antibiotic against *S. aureus,* CoNS, MRSA was vancomycin, which had a 100% susceptibility rate. Vancomycin and nitrofurantoin susceptibilities were 100% for the SA and MRSA strains. The MRSA strains were resistant to penicillin and showed no signs of susceptibility. With the exception of vancomycin, CoNS showed no susceptibility to other antibiotics and was confirmed to be resistant.

CONCLUSION

In conclusion, multidrug resistance in *S. aureus*, CoNS, and MRSA, all of which are highly significant nosocomial infections, has become a major global health issue. Furthermore, this condition results in significant issues such as non-responsiveness and an increase in treatment costs. As treating MRSA is difficult and proper safeguards are not taken today, these bacteria may create major health concerns in the future. As a result of their close contact with healthcare professionals and members of the general public, sick, and resistant bacteria can spread quickly to community members and hospitalized hemodialysis patients. Therefore, it is suggested that patient circulation in

hospitals should be limited, to ensure hygiene and carrier rates should be identified and monitored.

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki, and the ethics committee approval for the study was obtained from the Ethics Committee of Gazi Yasargil Training and Research Hospital on 2022/59. In addition, in order to carry out the study, Permission was obtained from the TR Ministry of Health / Mardin Provincial Health Directorate.

Authors' Contribution

Study Conception: SNK; Study Design: SNK, SU, AD; Supervision: SNK, SU; Funding: N/A; Materials: SU; Data Collection and/or Processing: AD, MB, SU; Statistical Analysis and/or Data Interpretation: MB, AD; Literature Review: SNK, MB, AD; Manuscript Preparation: SNK, AD and Critical Review: SNK, MB, SU.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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