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Nasal and extra-nasal methicillin resistant *Staphylococcus aureus* colonization among hemodialysis patients; is routine culturing of other body sites necessary?

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ABSTRACT

Introduction: Asymptomatic *Staphylococcus aureus* carriers have become a great concern because of being at risk of subsequent S. aureus infections. The role of nasal *S. aureus* carriages as an endogenous source for staphylococcal infections especially methicillin resistant *S. aureus* (MRSA) infections has been known that seems to be contributed to morbidity, mortality, and also the cost of end-stage renal disease management. Besides, many recent studies have demonstrated that extra-nasal sites may be important unrecognized reservoirs for resistant *S. aureus*.

Objectives: The purpose of the present research was to identify the frequency and factors associated with extra-nasal *S. aureus* colonization among maintenance hemodialysis patients.

Patients and Methods: A total of 179 hemodialysis patients were enrolled in this study. Swab cultures were obtained from anterior nares, posterior pharynx, and the inguinal area. Culture plates were analyzed for the presence of methicillin-resistant or methicillin-susceptible *S. aureus* using standard microbiological techniques for *S. aureus* and MRSA.

Results: 113 out of 179 patients (63%) were men and 66 (37%) were women with the mean age of 59.8±13.6 years. 36 out of 179 patients (20%) were colonized with *S. aureus* which 5 patients (2.7%) were colonized with MRSA. Prevalence of extra-nasal *S. aureus* colonization was 12% (22/179 patients), the prevalence of nasal *S. aureus* colonization was 10% (18/179 patients) and 2.7% of patients (5/179 patients) were colonized with *S. aureus* in more than one body site. Around 3 out of 5 MRSA colonized patients (60%) were extra-nasal carriers. There was a significant association between type of venous access for dialysis with the extra- nasal colonization ($P=0.03$) and also an association between underlying disease of diabetes mellitus type 2 with the extra-nasal colonization ($P=0.01$).

Conclusion: This study has emphasized the importance of extra-nasal evaluation along with nasal site sampling as an endogenous risk factor for staphylococcal infections among hemodialysis patients.

Implication for health policy/practice/research/medical education:
In a study on 179 hemodialysis patients, we found the importance of extra-nasal evaluation along with nasal site sampling as an endogenous risk factor for staphylococcal infections among hemodialysis patients.

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Introduction

The risk of healthcare-associated infections, in particular those caused by *Staphylococcus aureus* including methicillin-resistant *S. aureus* (MRSA) are high (1). *S. aureus* has been isolated as one of the most common pathogens causing bloodstream infections among hemodialysis patients (2). The risk of invasive *S. aureus* and MRSA infection among patients receiving maintenance hemodialysis is higher than the general population. Although the rate of annual invasive MRSA infections in the general population has been reported at 0.2-0.4 infections per 1000 persons (3), rates among patients receiving maintenance hemodialysis

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have been estimated at 37 cases per 1000 persons (4) that shows a 100 fold higher risk. The mortality rate following an invasive MRSA infection among patients on maintenance hemodialysis accounts for 17% (4).

Asymptomatic *S. aureus* carriers have become a great concern because of being at risk of subsequent *S. aureus* infections (5-7). The role of nasal *S. aureus* carriages as an endogenous source for staphylococcal infections especially MRSA infections has been known that seems to be contributed to morbidity, mortality, and also the cost of end-stage renal disease management (2). Besides, many recent studies have demonstrated that extra-nasal sites may be important unrecognized reservoirs for MRSA (8-15).

MRSA colonization in the nares, axilla, inguinal area, and rectum of admitted patients with an *S. aureus* infection was 25, 6, 11, and 13%, respectively and overall 37% of them were colonized by MRSA (16). A study of households with a history of a recent *S. aureus* skin infection demonstrated that up to 50% of household members were colonized by *S. aureus* and a nares-only survey would miss 48% of *S. aureus* colonization and 51% of MRSA colonization (17). Nasal screening alone, compared with multiple-site screening, missed approximately 27% of MRSA colonized patients at hospital admission in a French study (15). Because body colonization, especially of extra-nasal sites, is more widespread than initially believed, interventions to prevent *S. aureus* and MRSA infection may need to consider extra-nasal decolonization as well as traditional nasal decolonization with agents such as mupirocin (18).

Objectives

The purpose of the present research was to identify the frequency and factors associated with extra-nasal *S. aureus* colonization among maintenance hemodialysis patients.

Patient and Methods

Study population

This cross-sectional investigation conducted at four hemodialysis centers in Tehran, Iran from March to June 2017. All adults receiving dialysis at participating centers who had inclusion criteria were eligible for participation. Inclusion criteria were; being on maintenance hemodialysis, and not using any kind of antibiotics since two weeks before sampling. The patients gave their written informed consent prior to sampling.

Separate sterile-cotton-tripped swabs were rotated into the patients' anterior nares and pharynx as well as using sterile swabs soaked in trypticase soy broth (TSB) to be rubbed on the skin of the groins. The specimens were cultured in 5% sheep blood agar and incubated at 37°C for 24 hours. *S. aureus* isolates were identified based on their colony morphologies, biochemical activities and the coagulase test (19). Susceptibility patterns to different antibiotics, including cefoxitin (30 µg), penicillin (10 IU), clindamycin (2 µg), erythromycin (15 µg), vancomycin (30 µg), cotrimoxazole (1.25/23.75 µg), doxycycline (30 µg), and rifampin (5 µg) were determined with the guidelines of the Clinical and Laboratory Standards Institute (CLSI), using the Kirby-Bauer disk diffusion method (20). Methicillin resistance susceptibility was determined by using cefoxitin (30 µg) disks (PadtanTeb, Iran) using Muller-Hinton agar plates inoculated with a suspension (equivalent to 0.5 McFarland standards) of the *S. aureus* clinical isolates. The plates were incubated at 35°C for 24 hours and inhibition zones were measured (21,22). The CLSI 2010 criteria were used for interpretation. Cefoxitin disk diffusion tests regarding *S. aureus* ≥20 mm was considered as susceptible and ≤19 mm as resistant (23). The double disk approximation test (D-test) was applied for evaluating the inducible clindamycin resistance (ICR). Therefore, erythromycin (15 µg) and clindamycin (2 µg) disks were set in close proximity (15-20 mm) on an agar plate inoculated with a standardized suspension of the isolate. Plates were analyzed after 24 hours incubation at 37°C (24). Since the zone of inhibition around the clindamycin disk on the side facing the erythromycin disk is flattened (D shaped), the isolate was classified as having ICR (positive D-test) (25).

A questioner form was completed for each patient including the past medical history and behavioral risk factors for *S. aureus* colonization.

Ethical issues

1) The research followed the tenets of the Declaration of Helsinki. 2) Informed consent was obtained 3). This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences. This work has been extracted from the thesis of Atefeh Sadat Akhavi Mirab in School of Medicine, Shahid Beheshti University of Medical Sciences (# 81m).

Statistical analysis

Data were analyzed by using the SPSS version 16. We used descriptive statistics to describe the basic features of the data and to determine association between variables. *P* value <0.05 was considered statistically significant.

Results

A total of 179 patients were enrolled with the mean age of 59.8 ± 13.6 years (a range of 22 to 87 years). 113 (63%) patients were men and 66 (37%) were women. Table 1 shows the baseline characteristic of the patients. Table 2 indicates the distribution of hemodialysis patients based on the body site of *S. aureus* colonization. Overall, 36 out of 179 patients (20%) were colonized with *S. aureus* which 5 patients (2.7%) were colonized with MRSA. Prevalence of extra-nasal *S. aureus* colonization was 12% (22/179 patients), the prevalence of nasal *S. aureus* colonization was 10% (18/179 patients) and 2.7% of patients (5/179 patients) were colonized with *S. aureus* in more than one body site. The prevalence of MRSA colonization in nares, inguinal, and pharynx were 1.7% (3/179), 1.2% (2/179)

Table 1. Baseline characteristic associated with extra nasal *S. aureus* colonization among hemodialysis patients, Tehran, Iran, March-June 2017

	All patients (n=179)	Extra-nasal colonization (n=22)	No extra-nasal colonization (n=157)	OR	95% CI	*P* value
Age (y) Mean+SD	59.8±13.9	58.5±11.95	59.98±14.18	_	_	_
Male gender	113(63.12%)	15 (68.2%)	98 (62.42%)	1.3	0.497, 3.34	0.64
History of a skin or soft-tissue infection	6(3.35%)	1(4.5%)	5(3.18%)	1.45	0.16, 12.9	0.55
Hospitalization in the previous 3 months	50(27.93%)	5(22.7%)	45(28.6%)	0.73	0.25, 2.1	0.56
History of surgery in the previous 3 months	20(11.17%)	1(4.5%)	19(12%)	0.34	0.04, 27	0.47
History of antibiotics in the previous 3 months	54(30.16%)	5(22.7%)	49(31.2%)	0.65	0.22, 1.85	0.41
Type of vascular access						
Venous catheter	60(34.5%)	3(13.6%)	58(36.9%)			
Arteriovenous graft or fistula	119(66.5%)	19(86%)	99(63%)	0.27	0.07, 0.95	
Duration of dialysis more than 1 year	146(81.6%)	19(86%)	127(80.8%)	0.67	0.18, 2.4	0.77
Diabetic mellitus	71(39.7%)	14(63.6%)	57(36.3%)	3.07	1.2, 7.7	0.01
Coronary disease	94(52.5%)	14(63.6%)	80(50.9%)	1.68	0.67, 4.2	0.26
Polycystic disease	7(3.9%)	1(4.5%)	6(3.8%)	1.12	0.13, 10.4	1

and 0.5% (1/179) respectively. Three out of five MRSA colonized patients (60%) were extra-nasal carriers.

There was a significant association between type of venous access for dialysis with the extra-nasal colonization (95% CI: 0.07-0.95, $P=0.03$) and also an association between underlying disease of diabetes mellitus type 2 with the extra-nasal colonization (95% CI: 1.2-7.7, $P=0.01$). There were associations between extra-nasal *S. aureus* colonization with a dialysis length of more than one year and also with a history of skin and soft tissue infections even though these associations were not statistically significant.

Four out of 18 patients with nasal colonization (22%) had been detected to be colonized by *S. aureus* in their extra-nasal sites, whereas 18 out of 161(11%) non-nasal colonized patients were colonized by *S. aureus* in their extra-nasal sites.

All MRSA isolates were susceptible to vancomycin and rifampin. The resistant pattern of MSSA and MRSA isolates has shown in Tables 3 and 4 respectively.

Discussion

This study showed a 20% prevalence of *S. aureus* colonization following a survey on their anterior nares, pharynx and inguinal regions with 2.7% of MRSA colonization that is very lower than the study by Eells et al with a prevalence of 42% and 6% for *S. aureus* and MRSA (26). This difference might be related to the techniques. In this survey, anterior nares of patients were the site of *S. aureus* colonization in 10% (18 patients) which is less than other research findings that reported at 33%, 46% and 43% (27-29). This might be explained by the number of enrolled patients in their studies. Extra-nasal colonization prevalence in our research was 12% which is very lower than the study of Eells et al (26) that could be due to their higher colonization rates overall.

Four out of 18 patients (22%) in the present study with nasal *S. aureus* colonization also had extra-nasal colonization but the results of the study by Baker and et al showed that 56% patients with nasal *S. aureus* colonization had extra-nasal colonization (30). This difference may be due to obtaining samples from more sites of body (oropharynx, axilla, hand, perirectal, wound, and catheter insertion site) in their study in comparison with our research that was done from inguinal region and oropharynx as extra nasal carrier investigations.

Eighteen out of 161(11%) non-nasal colonized patients in our study were colonized by *S. aureus* in their extra-nasal sites which means extra-nasal surveillance resulted in 11% additionally identification of *S. aureus* carriers. This finding is less than the results of other studies which reported up to 33% additional detection (26,31). However, all reports have emphasized the benefit of extra-nasal investigations in addition to nasal surveys.

The prevalence of MRSA colonization in the present study was 2.7% which is similar to Lu et al with a prevalence of 2.36% (32) and less than 5.6%-12% reported by others (26,33,34). The MRSA isolates showed a sensitivity of a 100% to vancomycin and rifampin, 50% to clindamycin and erythromycin and 83% to cotrimoxazole and doxycycline. In the study by Mohajeri et al (35), sensitivity to cotrimoxazole and doxycycline was 29% and 50% respectively and in the study of Tashakori et al was 25% (36). In the study of Wu et al susceptibility to vancomycin, tigecycline, rifampin and clindamycin was 100%, 100%, 95.7%, 2.1% respectively (37).

We found a significant association between types of venous access for dialysis with the extra-nasal colonization which is different with the result of Eells et al. We also determined a significant association between underlying disease of diabetes mellitus type 2 with the extra-nasal colonization which is similar to the study by Lederer et al (33). In the study of Lederer et al, *S. aureus* nasal colonization had a relationship with old aging, diabetes mellitus and previous hospitalization (33). Our research indicated a non-significant association between extra-

Table 2. The distribution of hemodialysis patients based on the body site of S. aureus colonization (n=179)

	Percentage of colonized patients, No. (%)
Anybody site colonization	
S. aureus	36 (20)
MSSA	31 (86)
MRSA	5 (14)
Nasal colonization	
S. aureus	18 (10)
MSSA	15 (83)
MRSA	3 (17)
Throat colonization	
S. aureus	15 (8)
MSSA	14 (93.4)
MRSA	1 (6.6)
Inguinal-region colonization	
S. aureus	8 (4.5)
MSSA	6 (75)
MRSA	2 (25)
Extra-nasal colonization	
S. aureus	22 (12)
MSSA	19 (86.3)
MRSA	3 (13.7)
Colonization at >1body site	
S. aureus	5 (2.7)
MSSA	4 (80)
MRSA	1 (20)
Extra-nasal colonization only	
S. aureus	18 (10)
MSSA	15 (83.3)
MRSA	3 (6.7)
Nasal colonization only	
S. aureus	14 (7.8)
MSSA	12 (85.7)
MRSA	2 (14.2)
Throat colonization only	
S. aureus	12 (6.7)
MSSA	11 (91.6)
MRSA	1 (8.3)
Inguinal-region colonization only	
S. aureus	5 (2.7)
MSSA	4 (80)
MRSA	1 (20)

nasal *S. aureus* colonization with a history of skin and soft tissue infections. The findings of Eells et al are similar to our results (26).

Conclusion

In summary, this study has emphasized that extra-nasal investigations for *S. aureus* detect additional *S. aureus* carries and also detect more MRSA colonized hemodialysis people. Moreover, extra-nasal investigations might be helpful to recognize the original site of staphylococcal infections in cases of recurrent infections. Besides, it might be a strategy to decrease colonization pressure if necessary.

Limitations of the study

In this research, we did not evaluate all possible body sites for *S. aureus* colonization affecting the number of carriage. Besides, the sample size of our study was not ideal due to our limited resources which could have increased the chance of missing colonized people.

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Authors' contribution

NSZ; study design and manuscript drafting. ZA; study design, manuscript reviewing. ASAM; data collection and statistical analysis. LG; statistical analysis. AA; do laboratory work.

Conflicts of interest

The authors declare no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Table 3. Resistant pattern of MSSA, hemodialysis patients, Tehran, Iran, March-June 2017 (n=35)

Susceptibility results	Antibiotics						
	Clindamycin	Penicillin	Vancomycin	Doxycycline	Erythromycin	Cotrimoxazole	Rifampin
Resistance	8 (23%)	11 (31.5%)	4 (11.5%)	7 (20%)	15 (43%)	6 (17%)	3 (8.5%)
Intermediate	-	-	-	-	1 (3%)	-	-
Sensitive	27 (77%)	24 (68.5%)	31 (88.5%)	28 (80%)	19 (54%)	29 (83%)	32 (91.5%)

Table 4. Resistant pattern of MRSA, hemodialysis patients, Tehran, Iran, March-June 2017 (n=6)

Susceptibility results	Antibiotics						
	Clindamycin	Rifampin	Cotrimoxazole	Erythromycin	Doxycycline	Vancomycin	Penicillin
Resistance	3(50%)	_	1(17%)	3(50%)	1(17%)	_	2(33%)
Intermediate	-	-	-	-	-	-	-
Sensitive	3 (50%)	6(100%)	5(83%)	3(50%)	5(83%)	6(100%)	4(67%)

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