Iranian Journal of Microbiology
Volume 6 Number 2 (April 2014) 79-83

Staphylococcus aureus nasal carriage and patterns of antibiotic resistance in bacterial isolates from patients and staff in a dialysis center of southeast Iran

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Received: December 2012, **Accepted:** December 2013.

ABSTRACT

Background and Objective: *Staphylococcus aureus* is an important infection in hemodialysis patients. We studied the prevalence of nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) and its antibiotic resistance pattern in patients receiving hemodialysis as well as in dialysis unit staff.

Materials and Methods: From June to September 2012, we evaluated 74 cases including 61 patients on hemodialysis and 13 dialysis unit staff. Nasal swabs were taken from all cases and were cultured on a blood medium agar. We identified *S. aureus* based on conventional laboratory methods. For antimicrobial resistance patterns, we used disk diffusion method. Oxacillin MIC, oxacillin and cefoxcitin disk diffusion methods were used for detection of MRSA. Disk approximation test (D-test) was applied for the frequency of erythromycin induced clindamycin resistance.

Results: *S. aureus* carrier state was determined in 12 of the 61 patients on hemodialysis (19.67%) and 5 of the 13 dialysis unit staffs (38.46%). In hemodialyzed patients, MRSA and MSSA carrier of *S. aureus* were 6.56% and 13.11%, respectively. All nasal carriage states in studied staffs were MSSA. All isolated *S. aureus* were found to be sensitive to vancomycin, teicoplanin, and rifampin. However, reduced sensitivity of MRSA isolates to other antibiotics was noted. Resistance frequencies to tested antibiotic was as follows: cefteriaxone and penicillin (100%), tetracycline and doxycilin (75%), gentamicin, cloxacillin, and cefazolin (50%), ciprofloxacin, trimethoprim-sulfamethoxazol, erythromycin, and clindamycin (25%). The resistance rate of isolated MSSA against tested antibiotics was lower than isolated MRSA. Inducible clindamycin resistance was shown in 25% of identified MRSA strains.

Conclusion: *S. aureus* nasal carrier state was lower than former reports from other parts of Iran. The antibiotic resistance patterns also differed, perhaps due to different pattern of administering antibiotics at our hospital. Screening of these patients should be noted as a health priority and microbial sensitivity tests should be considered in order to optimize treatment options.

Keywords: Nasal carriers, MRSA, *Staphylococcus aureus*, Hemodialyzed patients, Iran

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INTRODUCTION

Infection has become a major cause of morbidity and is the second most common cause of death in patients receiving hemodialysis (1, 2). *Staphylococcus aureus* is currently the most common isolated

pathogen causing infection in such patients. Several studies have found that patients colonized with *S. aureus* are at risk to develop invasive infections compared to noncolonized patients (3-5). Although *S. aureus* can be isolated from multiple sites of the carriers but the nose is regarded as the major site of *S. aureus* carriage from where the organism can spread to other parts of the body (6). Hemodilayzed patients are prone to staphylococcal infections due to their immune compromised state, skin colonization and multiple needle punctures. The carrier states in patients receiving hemodialysis is important not only for predisposing to subsequent infections, but also as a potential of transmission among dialysis unit staff and community (7). Different methods of treatment are introduced for eradication of nasal carriage which results significantly lower infection rate (8-9). Therefore to avoid the spread of MRSA (methicillin-resistant *S. aureus*) and to obtain proper infection control procedures, the occurrence of nasal carriage of *S. aureus* must be noticed in patients admitted in hemodialysis units.

Different studies determined the *S. aureus* nasal carriers states ranged from 36.9% to 45.8% in different dialysis centers in Iran (10-13). The aim of the present study was to determine the prevalence of MRSA nasal carriage among hemodialyzed patients referred to Ali Ebne Abitaleb Hospital, Rafsanjan, Iran and to investigate the sensitivity pattern of these strains against various antibiotics.

MATERIALS AND METHODS

This cross-sectional study was conducted over a period of three months (June-September 2012) at the hemodialysis center of Ali Ebne Abitaleb hospital, Rafsanjan, Iran (southeast Iran). Sixty-one patients undergoing regular hemodialysis who had not received antibiotics during the last week and had no recent hospitalization for any reasons other than hemodialysis were entered into the study. Also, 13 subjects who were working at the dialysis unit were included in this investigation.

Specimens were obtained from both nostrils of the subjects. The swabs were immediately placed in the transport medium and transported to the Microbiology Laboratory of the hospital. The specimens were cultured in 5% sheep blood agar and incubated at 37°C for 48 hours. *S. aureus* isolates were identified based on colony morphology, biochemical activities and coagulase test (14). Susceptibility patterns to different antibiotics**,** including penicillin (10IU), cefazolin (30 µg), cloxacillin (1 µg), cefteriaxone (30 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazol (1.25/23.75 µ), doxycilin (30 µg), clindamycin(2 µg), erythromycin (15 µg), tetracycline (30 µg), gentamicin (10 µg), rifampin (5 µg), vancomycin (30 µg), and teicoplanin (30 µg), were determined with the guidelines of the CLSI, using the Kirby-Bauer disk diffusion method (15). Methicillin resistance susceptibility were tested with oxacillin (1 µg) and cefoxcitin (30 µg) disks (Mast Diagnostic Group, UK) 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 (16, 17). Interpretive criteria (in mm) for oxacillin disk diffusion tests regarding *S. aureus* were 13 mm as susceptible, 11-12 mm as intermediate, and 10 mm as resistant. Interpretive criteria (in mm) for *S. aureus* using cefoxcitin disk were $\geq$ 20 mm as susceptible and $\leq$ 19 mm as resistant (18). Minimum inhibitory concentrations (MICs) of two antibiotics (methicillin and vancomycin) were determined by MIC strip using Muller-Hinton agar plates supplemented with 2% NACL. The Clinical Laboratory Standards Institute (CLSI) 2010 criteria were used for interpretation. Approximation test was carried out for evaluating the inducible clindamycin resistance (ICR). In this respect, erythromycin (15 µ) and clindamycin (2 µ) disks were set in close proximity (15-20 mm) on an agar plate inoculated with standardized suspension of the isolate. Plates were analyzed after 24 hrs incubation at 37°C (19). If 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 inducible clindamycin resistance (positive D-test) (20). *S. aureus* ATCC 25923 was used as control strain.

Data was analyzed by using the SPSS software (statistical Package for the Social Science, version 16).

RESULTS

Sixty-one patients on hemodialysis, with a male to female ratio of 1.03 and 13 dialysis unit staff with a male to female ratio of 1.6 participated in this study. Demographic data for the patients on hemodialysis are outlined in Table 1.

S. aureus carrier state was determined in 12 of the

61 patients on hemodialysis (19.67%) and 5 of the 13 dialysis unit staff (38.46%). Methicillin resistance rate carrier was 6.56% among the studied cases. The MIC valve of oxacillin was 0.5 or lower in 13 (12.90%) isolated *S. aureus* and defined as MSSA (methicillin-sensitive *S. aureu*s) according to the CLSI criteria. The frequency of the MSSA and MRSA carriage also varied among patients on hemodialysis and the staff of dialysis center. In hemodialyzed patients, MRSA and MSSA carrier of *S. aureus* were 6.56% and 13.11% respectively. All isolated strains of *S. aureus* in the studied staff were of MSSA and no MRSA was detected. The results of the analyses of potential risk factors for nasal carriage of *S. aureus* on hemodialysis patients showed no significant difference regarding sex (P = 0.749), age (P = 0.636), diabetic or non-diabetic state (P = 0.076), and hemodialysis duration (P = 0.670) in patients on hemodialysis with *S. aureus* and non-carriers. The sensitivity of *S. aureus* isolates (MRSA and MSSA) to the tested antibiotics is shown in Table 2. In hemodialyzed patients, the highest resistance rates were observed for ceftriaxone (100%), tetracycline, doxycilin, ciprofloxacin, and trimethoprim-sulfamethoxazol (75%), cefazolin, cloxacillin (50%) among the MRSA strains, while the lowest resistance rate was to erythromycin and clindamycin (25%). None of MRSA strains were resistant to vancomycin, teicoplanin and rifampin. The resistant rate of isolated MSSA among hemodialyzed patients and dialysis unit staff was lower than isolated MRSA in both groups. Results of two different oxacillin and cefoxcitin disc diffusion techniques for identification of MRSA isolates were compatible with MIC oxacillin method. Among all isolated *S. aureus* strains, only 25% were resistant to erythromycin, and D-test was positive in 25% of them.

DISCUSSION

Nasal carriage of *S. aureus* has been shown to be more common in patients receiving long-term hemodialysis than in the general population (21). The relationship between nasal and skin colonization with *S. aureus* and subsequent infection in patients has been well established by different investigations (22-23). In this study, the nasal carriage rate was 19.67% in patients on hemodialysis and 38.46% on dialysis unit staff. However, in different studies from Iran the reported rates of nasal carriage in hemodialyzed patients were higher than what we detected here. Ghazvini and Hekmat reported a rate of 40.5% in hemodialyzed patients in Mashhad, Iran (10). Ghasemian *et al*., demonstrated the frequency of 36.9% nasal carriers of *S. aureus* among hemodialyzed patients at teaching hospitals of Mazandaran University of Medical Sciences, Iran (11). Aminzadeh and colleagues reported the nasal carrier state in 45.5% of their patients on hemodialysis in Tehran, Iran (12). Keramat *et al*., study revealed the presence of 40.3% nasal carriage of *S. aureus* in patients on hemodialysis in Hamedan, Iran (13). Differences in the prevalence of nasal carriage of *S. aureus* strains may be due to the use of different techniques and different interpretation guidelines.

Data obtained in our investigation also demonstrated low rate of MRSA colonization among the studied patients compared to published previous studies from Iran (10-13). Ghasemian *et al*., reported the MRSA nasal carrier state as 74.2% in hemodialysis patients at teaching hospitals of Mazandaran in north of Iran (11). Aminzadeh and colleagues investigation revealed MRSA isolate in 100% of studied patients in Tehran dialysis center. This difference can be explained by limitation of our study due to small sample size.

In our investigation, no significant correlation was found regarding the nasal carriage of *S. aureus* and diabetic mellitus in patients on hemodialysis. Furthermore our findings indicate that age, gender and hemodialysis duration were not important risk factors in our cases. Similary, Aminzadeh and colleagues did not find any difference between genders. In contrast, Saxena *et al*., and Keramat *et al*., found a significant correlation between age and nasal carriage (13-20). In contrast to our finding, Ghasemian *et al.,* investigation revealed the length of admission to hemodialysis is an important risk factor for *S. aureus* carriage (11).

Our data about nasal carriage of *S. aureus* in dialysis unit staff demonstrated the rate of 38.46%. This report was consistent with Askarian *et al*., investigation that revealed the prevalence of nasal carriage as 31% among healthcare workers at Namazi Hospital, Shiraz, Iran (24).

The resistance profiles of isolated MRSA in this study demonstrated multidrug resistance with increasing resistance to SXT, doxycilin and tetracycline (75%), gentamicin and cloxacillin (50%). This finding supports the relationship between methicillin resistance and resistance to other antibiotics that reported by previous studies, which is a major problem in the treatment of *S. aureus*

infections as reported by previous studies (25-26). Therefore, in view of the high resistance rates to the above antibiotics, treatment of MRSA infections at our hospital may not be effective. All the *S. aureus* strains recovered from nasal carriers, both MRSA and MSSA, were susceptible to vancomycin, teicoplanin and rifampin possibly because of the use of these antibiotics in our hospital is limited.

In conclusion, This study revealed the prevalence of nasal carriage of *S. aureus* strains among patients receiving hemodialysis to be lower than that reported previously from other parts of Iran. The antibiotic resistance patterns also differed, perhaps as a different use of antibiotic in our hospital. Due to sensitivity patterns of all isolated *S. aureus* strains to vancomycin, this antibiotic is still the drug of choice for treatment of MRSA. Regular monitoring of vancomycin sensivity should be carried out and also we recommend administering this antibiotic correctly in order to avoid construction of the resistant strains. Meanwhile, patients subjected to hemodialysis are more susceptible to *S. aureus* infection thus screening of these patients should be noted as a health priority and microbial sensitivity tests should be considered in order to optimize treatment options.

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