Arch Clin Infect Dis. 13(5):e61917. doi: 10.5812/archcid.61917.

Published online 2018 August 12. **Research Article**

High Frequency of Methicillin-Resistant *Staphylococcus aureus* in Intensive Care Unit in Karaj, Iran

Aslan Bijari [1], Masoumeh Hallaj Zade [1], Shiva Hatami [2], Enayat Kalantar [2], Mohammad Noori Sepehr [3], Kourosh Kabir [4], Ehsan Zahmatkesh [5], Mohammmad Hassan Naseri [6] and Davood Darban Sarokhalil [1, 2, *]

[1]Department of Microbiology, School of Medicine, International Branch, Iran University of Medical Sciences, Tehran, Iran
[2]Department of Microbiology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran
[3]Department of Environmental Health Engineering, School of Health, Alborz University of Medical Sciences, Karaj, Iran
[4]Department of Epidemiology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran
[5]Department of Paediatrics, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran
[6]Department of Internal Medicine, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran

[*]*Corresponding author*: Department of Microbiology, Faculty of Medicine, Alborz University of Medical Sciences, Karaj, Iran. Tel: +98-2186703183, Fax: +98-2188058649, Email: davood_darban@yahoo.com

Received 2017 September 09; **Revised** 2017 December 23; **Accepted** 2018 January 14.

Abstract

Objectives: The current study aimed at finding the frequency of MRSA infections, contamination, and colonization in the teaching hospitals of Karaj city, Iran for the first time.

Methods: The current cross sectional study was conducted in Karaj on three teaching hospitals from July 2013 to July 2014. Sample collection from personnel and surfaces was conducted twice and monthly, respectively, during the study period. Also, all *Staphylococcus aureus* species isolated from patients were included in the study. Antimicrobial susceptibility test was performed by the standard disk diffusion method. All isolates were subjected to *mupA* and *mecA*-specific polymerase chain reaction (PCR)to identify high-level mupirocin-resistant and MRSA isolates, respectively. Chi-square test was employed for data analysis.

Results: The majority of *S. aureus* species were isolated from personnel and surfaces of the hospitals. One hundred sixty-eight *S. aureus* and 49 MRSA species were isolated from Karaj teaching hospitals. The main frequency of MRSA was isolated from intensive care unit (ICU) (75%) and high rate of resistance to rifampicin (53%) was observed in MRSA isolates. Although 10 *S. aureus* species were resistant to mupirocin by disk diffusion, no *mupA* gene was detected in the isolates.

Conclusions: In conclusion, in comparison with the other studies from Iran, low frequency of MRSA was observed in the investigated hospitals. However high frequency (75%) of MRSA in ICU indicated that antibiotic policy is urgently needed to prevent the resistance development. Moreover, antibiotic susceptibility monitoring and regular screening surfaces and personnel of hospitals in terms of MRSA colonization, especially ICU, are indispensable.

Keywords: Methicillin-Resistant *Staphylococcus aureus*, MRSA, Intensive Care Unit, ICU, Iran

1. Background

Nosocomial infection rate in ICUs is one of the most common hospital-acquired infections. High mortality, increased healthcare costs, and prolonged hospital stays are the results of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) (1-3). The intensive care unit (ICU) is more frequently colonized with MRSA rather than other departments in hospitals. Patients admitted to ICUs are at high risk for MRSA infection due to following reasons: more colonization of antimicrobial-agent resistant microorganisms such as MRSA in ICU, length of stay, severity of illness, and application of intravascular devices. Moreover, patients are admitted to ICU from different wards and discharged to many other wards or hospitals and can easily transmit MRSA inter or intra-hospitals (4). Prevalence of MRSA infection in ICUs varies in different regions, from 55% in the US and Iran to about 1% in the Netherlands (3, 5).

A programmed surveillance on screening of personnel, surfaces of departments of hospitals and patients in terms of nosocomial microorganisms such as MRSA is one the strategies to control infection (4). The first step to control the MRSA infection is having suitable and enough knowledge about the rate of infections, contamination, and colonization of the bacteria in the healthcare systems (4). Al-

though several studies are conducted in different regions of Iran (5), there are no data regarding the MRSA rate in the teaching hospitals of Karaj, one the most populated cities in Iran. Moreover, there are limited data on nosocomial infections in ICU in Iran. To the best of authors' knowledge, this is the first report on MRSA frequency in Karaj teaching hospitals. Then the current study aimed at finding the prevalence of MRSA contamination and colonization in the hospitals of Karaj, Iran.

2. Methods

2.1. Collection and Identification of Bacterial Isolates

The current cross sectional study was conducted in Karaj on three teaching hospitals from July 2013 to July 2014. The majority of *S. aureus* isolates were isolated from personnel and surfaces of hospitals. Sample collection from personnel was performed twice during the study. Specimens were collected by swabbing both nostrils of the personnel and subsequently culturing on the brain-heart infusion (BHI) agar. Sample collection from the surfaces of the three hospitals was performed monthly and cultured on the same media. The following hospital departments were included in the study: hospital No.1, emergency, burns, ICU, and internal medicine; hospital No. 2, females' surgery, neonates, children, emergency, and ICU; and hospital No. 3, dialysis, angiosis, ICU, emergency, and males' internal medicine. The isolates were cultured on the sheep blood agar and mannitol salt agar, and identified by conventional biochemical tests including catalase, tube coagulase, mannitol fermentation, and DNase (6). All *S. aureus* species isolated from patients were included in the study.

2.2. Antimicrobial Susceptibility Testing

The clinical and laboratory standards institute (CLSI) guideline was used for antimicrobial susceptibility tests by the standard disk diffusion method. The tested antibiotics included cefotaxime (30 μg), tigecycline (15 μg), vancomycin (30 μg), linezolid (30μg), synercid (quinupristin/dalfopristin) (15 μg), mupirocin (20 μg), teichoplanin (30 μg), and rifampicin (5 μg) (Mast, UK). *S. aureus* ATCC 25923 was used as the control strain.

2.3. DNA Extraction and Identification of High-Level Mupirocin Resistant and MRSA Isolates

DNA was extracted from *S. aureus* colonies by boiling method described previously (7). The polymerase chain reaction (PCR) was the amplification of *S. aureus*-specific nuclease (*nucA*) gene as described previously (8). Moreover, all isolates were subjected to *mupA* and *mecA*-specific PCR to identify high-level mupirocin resistant and MRSA isolates, respectively (7, 9).

2.4. Statistical Analysis

SPSS version 16 was used to analyze data (SPSS, Chicago, IL, USA). Differences of *S. aureus* and MRSA isolates frequencies among hospitals and departments were assessed using chi-square test. A P-value of < 0.05 was considered statistically significant.

3. Results

One hundred sixty-eight *S. aureus* and 49 MRSA species were isolated from Karaj teaching hospitals. The number and frequency of the isolates are shown in Table 1. More *S. aureus* and MRSA species were isolated from hospital No.1 and the P-value was significant (< 0.05). The number and frequency of *S. aureus* and MRSA species isolated from surfaces of different units of hospitals are shown in Table 2. Almost 75% of MRSA species were isolated from ICU departments and the P-value was significant. Table 3 shows the No. and frequency of *S. aureus* and MRSA isolates in personnel. Finally, antibiotic susceptibility pattern of MRSA and methicillin-sensitive *S. aureus* (MSSA) isolates is shown in Table 4. Although 10 *S. aureus* isolates were resistant to mupirocin based on disk diffusion findings; *mupA* was not observed in the isolates and accordingly it is concluded that the isolates had low resistance to mupirocin.

5. Discussion

Antibiotic pressure and indiscriminate administration of broad-spectrum antibiotics lead to high prevalence of resistant *S. aureus* especially MRSA in the world (1, 10). The contamination of hospital environments, especially ICUs, with MRSA is the main concern in recent years. Nevertheless, there are limited reports on the incidence rates of MRSA infection in ICUs in Iran (11-13). Furthermore, ICU-acquired infections are reported with high morbidity and mortality in Iran (14, 15). In the current study, the prevalence of MRSA varied greatly between investigated hospitals, from 16% to 40% (Table 1). As compared with the recent studies findings, the data showed that the rate of MRSA was lower than those of the other studies in Iran (52.7% - 93.3%), India (78%), Oman (52%), Australia (30.3%), and Turkey (35% - 43%) (1, 5, 16-18). According to Table 1, the frequency rate of MRSA in hospital No. 1 was higher than the other two hospitals. Some important reasons for the higher rate in this hospital included the high number of admitted patients, poor hygiene, and lack of infection control strategies. Despite low rate of MRSA colonization in Karaj hospitals, out

Table 1. Number and Frequency of *Staphylococcus aureus* and MRSA Species Isolated From Karaj Teaching Hospitals

Hospital	Surface		Personnel		Patient		Total	
	S. aureus	MRSA (%)	*S. aureus*	MRSA (%)	*S. aureus*	MRSA (%)	*S. aureus*	MRSA (%)
No. 1	51	23 (45)	35	10 (28.5)	4	3 (75)	90	36 (40)
No. 2	25	3 (12)	11	4 (36)	6	0	42	6 (16.5)
No. 3	22	2 (9)	6	0	8	4 (50)	36	7 (16.5)
Total	98	28 (28.5)	52	13 (25)	18	7 (39)	168	49 (29)

Table 2. Number and Frequency of *Staphylococcus aureus* Species Isolated From Surfaces of Karaj Teaching Hospitals

Department	ICU	Emergency	Internal Medicine	Surgery	Neonates	Burns	Angiography	Dialysis	Total
No. of *S. aureus* (%)	43 (44.5)	19 (19)	18 (18.5)	7 (7)	4 (4)	3 (3)	2 (2)	2 (2)	98 (100)
No. of MRSA (%)	21 (75)	2 (7.25)	2 (7.25)	1 (3.5)	1 (3.5)	1 (3.5)	0	0	28 (100)

Table 3. Number and Frequency of *Staphylococcus aureus* and MRSA Species isolated From Personnel of Karaj Teaching Hospitals

Hospital	No. 1	No. 2	No. 3	Total (%)
No. of samples (each nostril)	98	98	23	219
No. and frequency of *S. aureus* isolates (%)	25 (25.5)	7 (7)	4 (17)	36 (16.5)
No. and frequency of personnel carrying MRSA (%)	6 (6)	2 (2)	0	8 (3.5)

Table 4. Antibiotic Susceptibility Pattern of MRSA and MSSA Isolates

Antibiotic (Disk)	MRSA (N = 49)	MSSA (N = 119)	Total (N = 168)
	No. of Resistant Isolates (%)	No. of Resistant Isolates (%)	No. of Resistant Isolates (%)
Rifampicin	26 (53)	1 (0.8)	27 (16)
Mupirocin	4 (8)	6 (5)	10 (6)
Teicoplanin	0	0	0
Vancomycin	0	0	0
Synercid	0	0	0
Linezolide	0	0	0
Tigecyclin	0	0	0

of 98 and 28 *S. aureus* and MRSA isolates, 43 (44.5%) and 21 (75%) species were isolated from ICU surfaces, respectively (Table 2) which showed an alarm in the hospitals. High frequency of MRSA in ICU was in agreement with those of previous studies (19). Also in the studies conducted in Tehran, *S. aureus* were the most frequent bacteria isolated from ICU surfaces (13, 20). Factors such as prolonged hospital stay, infection sites, invasive procedures, underlying disease conditions, and exposure to multidrug-resistant bacteria mostly cause higher rates of infection among patients in ICUs (11).

Although health care workers are the main reservoirs for MRSA, they may be the victims in the health-care settings. The nasal carriage rate of *S. aureus* and MRSA in the personnel in the current study was 16.5% and 3.5%, respectively, which was lower than those of other studies conducted in other regions of Iran (1, 21, 22). However, another study from Ghana reported low nasal carriage among inpatients and health staff (23). Some factors such as quality and size of samples, application of different techniques, and different interpretation guidelines may affect the prevalence of nasal carriage of *S. aureus* strains (21). Similar to high contamination with *S. aureus* and MRSA in hospital No. 1, Table 3 also shows the highest colonization of personnel with those strains in this hospital. These data also approved the probable poor hygiene and lack of infection control strategies in the mentioned hospital. However, the number of studied personnel in hospital No. 3 was less than those of other hospitals and it was a limitation in the current study.

The ICUs are the regions with remarkable drug usage and high frequency of drug-resistant pathogenic bacte-

ria. It is well known that antibiotic-resistant organisms are becoming progressively widespread in the medical center surroundings as a result of the wide consumption of antibiotics (4, 11). In the current study, the MRSA isolates were frequently resistant to rifampicin compared to MSSA isolates (Table 4). Although some studies did not observe significant correlations between MRSA and resistance to rifampicin, other studies reported high rates of resistance to the rifampicin among the MRSA isolates (11, 13, 21). As reported before (1, 11), there is a relationship between methicillin-resistance and resistance to other antibiotics such as rifampicin (24). Fortunately, no resistance to the effective drugs such as tigecycline, linezolid, synercid (quinupristin/dalfopristin), teichoplanin, and vancomycin -extensively used in the region to treat patients with MRSA infection- was observed in the current study.

Mupirocin is one of the most effective antibiotics currently used to eradicate MRSA. In contrast with a previous study (1) there was no simultaneous resistance to methicillin and mupirocin in the current study *S. aureus* isolates. In spite of low resistance to mupirocin in the isolates, high susceptibility to mupirocin was not observed in the current study isolates. Since mupirocin was not used to eradicate MRSA in the personnel of the mentioned hospitals, in agreement with other studies, previous exposure to mupirocin can be the cause of such high resistance in *S. aureus* isolates. Moreover, low rate of resistance to mupirocin was more common among MRSA than MSSA isolates (8% versus 5%). The prevalence of mupirocin-resistant MRSA was different in studies from Jordan (2.6%), Greek (1.6%), Korea (5%), China (6.6%), and Iran (70%) (1). In conclusion, in comparison with the other studies from Iran, there was low frequency of MRSA in the investigated hospitals. However, high frequency of MRSA (75%) in ICU indicated that an antibiotic policy is urgently needed to prevent the resistance development. Moreover, antibiotic susceptibility monitoring and regular screening of the hospital surfaces and personnel in terms of MRSA colonization, especially ICUs, are indispensable.

Acknowledgments

The current study was part of the project 2168344 supported by Alborz University of Medical Sciences, Karaj, Iran.

References

1. Abbasi-Montazeri E, Khosravi AD, Feizabadi MM, Goodarzi H, Khoramrooz SS, Mirzaii M, et al. The prevalence of methicillin resistant Staphylococcus aureus (MRSA) isolates with high-level mupirocin resistance from patients and personnel in a burn center. *Burns*. 2013;**39**(4):650–4. doi: 10.1016/j.burns.2013.02.005. [PubMed: 23499497].
2. Radhakrishna M, D'Souza M, Subbannayya K, Vishwas SK, Shashidar KM. Prevalence of methicillin resistant Staphylococcus aureus carriage amongst health care workers of critical care units in Kasturba Medical College Hospital, Mangalore, India. *J Clin Diagn Res*. 2013:2697–700.
3. Wassenberg M, Kluytmans J, Erdkamp S, Bosboom R, Buiting A, van Elzakker E, et al. Costs and benefits of rapid screening of methicillin-resistant Staphylococcus aureus carriage in intensive care units: a prospective multicenter study. *Crit Care*. 2012;**16**(1):R22. doi: 10.1186/cc11184. [PubMed: 22314204]. [PubMed Central: PMC3396263].
4. Thompson DS. Methicillin-resistant Staphylococcus aureus in a general intensive care unit. *J R Soc Med*. 2004;**97**(11):521–6. doi: 10.1258/jrsm.97.11.521. [PubMed: 15520145]. [PubMed Central: PMC1079644].
5. Askari E, Soleymani F, Arianpoor A, Tabatabai SM, Amini A, Naderinasab M. Epidemiology of mecA-methicillin resistant Staphylococcus aureus (MRSA) in Iran: A systematic review and meta-analysis. *Iran J Basic Med Sci*. 2012;**15**(5):1010–9. [PubMed: 23493646]. [PubMed Central: PMC3586924].
6. Mahon CR, Lehman DC, Manuselis G. *Textbook of diagnostic microbiology*. 3, illustrated ed. USA: Saunders Elsevier; 2007.
7. Perez-Roth E, Claverie-Martin F, Villar J, Mendez-Alvarez S. Multiplex PCR for simultaneous identification of Staphylococcus aureus and detection of methicillin and mupirocin resistance. *J Clin Microbiol*. 2001;**39**(11):4037–41. doi: 10.1128/JCM.39.11.4037-4041.2001. [PubMed: 11682527]. [PubMed Central: PMC88484].
8. Sahebekhtiari N, Nochi Z, Eslampour MA, Dabiri H, Bolfion M, Taherikalani M, et al. Characterization of Staphylococcus aureus strains isolated from raw milk of bovine subclinical mastitis in Tehran and Mashhad. *Acta Microbiol Immunol Hung*. 2011;**58**(2):113–21. doi: 10.1556/AMicr.58.2011.2.4. [PubMed: 21715281].
9. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant Staphylococcus aureus. *J Clin Microbiol*. 2005;**43**(10):5026–33. doi: 10.1128/JCM.43.10.5026-5033.2005. [PubMed: 16207957]. [PubMed Central: PMC1248471].
10. Hasani A, Sheikhalizadeh V, Hasani A, Naghili B, Valizadeh V, Nikoonijad AR. Methicillin resistant and susceptible Staphylococcus aureus: Appraising therapeutic approaches in the Northwest of Iran. *Iran J Microbiol*. 2013;**5**(1):56–62. [PubMed: 23467268]. [PubMed Central: PMC3577566].
11. Hassanzadeh P, Motamedifar M, Hadi N. Prevalent bacterial infections in intensive care units of Shiraz University of medical sciences teaching hospitals, Shiraz, Iran. *Jpn J Infect Dis*. 2009;**62**(4):249–53. [PubMed: 19628899].
12. Mirzaii M, Emaneini M, Jabalameli F, Halimi S, Taherikalani M. Molecular investigation of Staphylococcus aureus isolated from the patients, personnel, air and environment of an ICU in a hospital in Tehran. *J Infect Public Health*. 2015;**8**(2):202–6. doi: 10.1016/j.jiph.2014.09.002. [PubMed: 25458916].
13. Mirzaii M, Emaneini M, Maleknejad P, Jonaidi N, Fooladi AA, Aligholi M, et al. Distribution of bacterial contamination in a teaching hospital in Tehran - a special focus on Staphylococcus aureus. *Acta Microbiol Immunol Hung*. 2012;**59**(1):1–11. doi: 10.1556/AMicr.59.2012.1.1. [PubMed: 22510282].
14. Hashemian M, Talaie H, Akbarpour S, Mahdavinejad A, Mozafari N. Central nervous system depressants poisoning and ventilator associated pneumonia: an underrated risk factor at the toxicological intensive care unit. *Iran Red Crescent Med J*. 2016;**18**(1). e30989. doi: 10.5812/ircmj.30989. [PubMed: 26889400]. [PubMed Central: PMC4753022].
15. Salimi A, Talaie H, Rezaie Hemami M, Mahdavinejad A, Barari BB, Razi P, et al. Teicoplanin as an anti-methicillin resistant Staphylococcus aureus agent in infections of severely poisoned intensive care

unit patients/ Tehran- Iran. *Acta Biomed*. 2014;**84**(3):189–95. [PubMed: 24458163].
16. Parhizgari N, Khoramrooz SS, Malek Hosseini SA, Marashifard M, Yazdanpanah M, Emaneini M, et al. High frequency of multidrug-resistant Staphylococcus aureus with SCCmec type III and Spa types t037 and t631 isolated from burn patients in southwest of Iran. *APMIS*. 2016;**124**(3):221–8. doi: 10.1111/apm.12493. [PubMed: 26709106].
17. Goudarzi M, Goudarzi H, Sa Figueiredo AM, Udo EE, Fazeli M, Asadzadeh M, et al. Molecular characterization of methicillin resistant Staphylococcus aureus strains isolated from intensive care units in Iran: ST22-SCCmec IV/t790 emerges as the major clone. *PLoS One*. 2016;**11**(5). e0155529. doi: 10.1371/journal.pone.0155529. [PubMed: 27171373]. [PubMed Central: PMC4865093].
18. Coombs GW, Pearson JC, Nimmo GR, Collignon PJ, Bell JM, McLaws ML, et al. Antimicrobial susceptibility of Staphylococcus aureus and molecular epidemiology of meticillin-resistant S. aureus isolated from Australian hospital inpatients: Report from the Australian Group on Antimicrobial Resistance 2011 Staphylococcus aureus Surveillance Programme. *J Glob Antimicrob Resist*. 2013;**1**(3):149–56. doi: 10.1016/j.jgar.2013.04.005. [PubMed: 27873625].
19. Dibah S, Arzanlou M, Jannati E, Shapouri R. Prevalence and antimicrobial resistance pattern of methicillin resistant Staphylococcus aureus (MRSA) strains isolated from clinical specimens in Ardabil, Iran. *Iran J Microbiol*. 2014;**6**(3):163–8. [PubMed: 25870749]. [PubMed Central: PMC4393492].
20. Tajeddin E, Rashidan M, Razaghi M, Javadi SS, Sherafat SJ, Alebouyeh M, et al. The role of the intensive care unit environment and healthcare workers in the transmission of bacteria associated with hospital acquired infections. *J Infect Public Health*. 2016;**9**(1):13–23. doi: 10.1016/j.jiph.2015.05.010. [PubMed: 26117707].
21. Jannati E, Arzanlou M, Habibzadeh S, Mohammadi S, Ahadi P, Mohammadi-Ghalehbin B, et al. Nasal colonization of mecA-positive, oxacillin-susceptible, methicillin-resistant Staphylococcus aureus isolates among nursing staff in an Iranian teaching hospital. *Am J Infect Control*. 2013;**41**(11):1122–4. doi: 10.1016/j.ajic.2013.02.012. [PubMed: 23706805].
22. Askarian M, Zeinalzadeh A, Japoni A, Alborzi A, Memish ZA. Prevalence of nasal carriage of methicillin-resistant Staphylococcus aureus and its antibiotic susceptibility pattern in healthcare workers at Namazi Hospital, Shiraz, Iran. *Int J Infect Dis*. 2009;**13**(5):e241–7. doi: 10.1016/j.ijid.2008.11.026. [PubMed: 19269873].
23. Egyir B, Guardabassi L, Nielsen SS, Larsen J, Addo KK, Newman MJ, et al. Prevalence of nasal carriage and diversity of Staphylococcus aureus among inpatients and hospital staff at Korle Bu Teaching Hospital, Ghana. *J Glob Antimicrob Resist*. 2013;**1**(4):189–93. doi: 10.1016/j.jgar.2013.05.006. [PubMed: 27873611].
24. Montazeri EA, Khosravi AD, Jolodar A, Ghaderpanah M, Azarpira S. Identification of methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from burn patients by multiplex PCR. *Burns*. 2015;**41**(3):590–4. doi: 10.1016/j.burns.2014.08.018. [PubMed: 25441547].