ORIGINAL RESEARCH

Staphylococcus aureus contamination rate on environmental surfaces and hands of staff in ICU and NICU ward of Rohani hospital in Babol, Iran

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ABSTRACT

Introduction: This study surveyed the frequency of *Staphylococcus aureus* contamination on different surfaces in the ICUs in central Iran. Contamination of the environmental surfaces is a cause of nosocomial infection. The survey of *S. aureus* contamination in ICU and NICU was the purpose of this study.

Materials and Methods : Samples were taken from different surfaces of ICUs and NICU. Antibiotic resistance of isolated *Staphylococcus aureus*, SCC*mec* typing patterns, and abundance of *mecA*, *PVL*, *TSST-1* genes were investigated.

Results: 63.9% of positive cultures were detected as *Staphylococcaceae* family. *S. aureus* was identified in 6.6% of the total samples. Most of these isolates were resistant to cefazolin (93.9%), erythromycin (69.7%) and levofloxacin (63.6%). *PVL* and *mecA* genes accounted for 21.2% and 24.2%, respectively. SCC*mec* type I and HA-MRSA were seen in 87.5% of isolates, and just 22.5% of isolates had SCC*mec* IV and CA-MRSA

Conclusion : Contamination on surfaces in the ICUs belonging to non-*Staphylococcus aureus* was at a high

level. The high prevalence of SCC*mec* type I demonstrated that the origin of bacterial surface contamination in hospitals is patients infected by MRSA or MRSA carriers.

INTRODUCTION

Hospital infections under the title nosocomial infections or healthcare-associated infections occur within 42 to 72 hours of hospitalization of patients in both developing and developed countries all over the world. It causes a lot of mortality in hospitalized patients, and the economic burden of these infections is high [1,2]. Patient health status, methods of infection control, age, the function of the immune system, multiple chronic conditions, frequent exposure with health facilities, use of invasive procedures or chemotherapy and immunotherapy, and also the prevalence of different pathogens in the local communities are some of the risk factors of these kinds of infections. One of the most important factors in causing nosocomial infections is the contamination of environmental surfaces [3], especially those kinds of surfaces which are in contact with patients because a lot of pathogens can grow on the surfaces, and these pathogens can be transmitted through sneezing and cough [4] . *Staphylococcus aureus* (*S. aureus)* is one of the most important pathogens causing surface contamination. Because of its various toxins, *S. aureus* can be a reason for different infections such as Toxic Shock Syndrome (TSS) caused by the activity of *TSST-1* superantigens and can threaten human life [5]. Methicillin-resistant *S. aureus* (MRSA) is more common in the intensive care unit (ICU) and sections of the hospital where patients with MRSA infection are hospitalized. It can be transmitted by direct contacts, infected wound, or contaminated hands, which are common among health care staff. Ultimately, Healthcare-Acquired Methicillin-resistant *S. aureus* (HA-MRSA) causes blood infections, pneumonia, and urinary tract infections. On the other hand, some kinds of MRSA infections are acquired from society, termed Community-Associated Methicillin-Resistant *S. aureus* (CA-MRSA), and cause skin infections by *PVL* toxin [6]. This bacterium is resistant to beta-lactam antibiotics due to its *mecA* gene encoding the penicillin-binding protein (PBP) 2a protein that has little affinity for beta-lactam antibiotics. This gene is located on a chromosomal cluster called Staphylococcal Cassette Chromosome mec (SCC*mec*) and according to this cluster, variants 1 to 13 are identified as having 1,2,3,8 typologies in HA-MRSA and types 4 and 5 in CA-MRSA [7,9]. Determining SCC*mec* is one of the typing methods to describe the specific genetic traits of MRSA. The types of SCC*mecs* are coded based on the type of recombinase enzyme, identified by mecA complexes and ccr genes. So far, 11 different types of SCC*mec* are known, of which 5 types (SCC*mec* I-V) are predominant in Iran [29]. Another method of molecular typing of bacteria is typing of the gene encoding *S. aureus* staphylococcal protein A (spa). Spa typing is one of the differentiating factors that contain the X polymorphism region

with a short sequence, and various studies show that researchers have identified various patterns of this gene [11,12]. The aim of this study was the survey of environmental surface contamination in different ICU sections at Rohani hospital of Babol-Iran to *S.aureus* and genotyping the strains with the SCC*mec* typing method.

MATERIALS AND METHODS

This project was a cross-sectional study that was conducted for one year from September 2017 to March 2018. The samples were moistened by physiological saline solution swabs and were taken from 5 surfaces include overbed tables, bedside tables, beds side rails, room floors, and nurses' hands in 3 different sections (general ICU, surgery ICU, and NICU) at Rohani hospital ***(Table 1)***. Samples were cultured in mannitol-salt agar medium and then incubated overnight at 37° C.

Table 1: Number of samples taken from different sections of the hospital

Section	Samples
General ICU	190
Surgery ICU	190
NICU	114
Total	494

Identification of *S. aureus*

Bacterial tests used for identification were catalase, coagulase, & DNase tests.

Bacterial antibiotic resistance test

Antibiotic resistance test was performed by using standard disk diffusion method. For each isolate, we used distilled water to prepare bacterial suspension equivalent in density to 0.5 McFarland barium sulfate standard unit (average turbidity, 10^8 CFU/ml). Isolates were cultured on Mueller-Hinton agar using 12 different disks including clindamycin (2 µgr), vancomycin (30 gr), gentamicin (10 µgr), tetracycline (30 µgr), rifampin (5 µgr), oxacillin (1 µgr), trimethoprim and sulfamethoxazole (1.25 and 23.75 µgr), erythromycin (15 µgr), levofloxacin (5 µgr), and ceftaroline (5 µgr). After that, the plates were incubated for 24 hours at 37º C, and inhibition zones were measured to survey susceptibility and resistance [10].

PCR test

DNA of *S. aureus* isolates was extracted based on DNA extraction kit protocol, and then extracted samples were analyzed quantitatively in terms of purity and concentration on a NanoDrop spectrophotometer. Finally, the purified DNA was used for PCR.

Specific primers were used for each of these genes (*TSST-1*, *PVL*, *mecA*) [11,12]. These primer sequences were used to amplify genes on a thermocycler with the final volume of 25 µL. The primer sequences and PCR compounds used in PCR are shown in ***(Table 2)***, ***(Table 3)***, and **(*Table 4*)**.

Table 2: Primer sequence used for 3 genes (*PVL*, *TSST-1*, *mec*A)

Gene	Primer sequences	Product size (base pair)	Source
mecA	F: AAAATCGATGGTAAAGGTTGGC R: AGTTCTGCAGTACCGGATTTGC	533	Sabbagh *et al.(11)*
PVL	F:ATCATTAGGTAAAATGTCTGGACATGATCCA R: GCATCAACTGTAATTGGATAGCAAAAGC	433	Sabbagh *et al.(11)*
TSST-1	F: ACCCCTGTTCCCTTATCATC R: TTTTCAGTATTTGTAACGCC	326	Mehrotra et al.(12)

Table 3: PCR-compounds for *mec*A, *PVL*, *TSST-1* genes

PCR-compounds	*Volume*
Sterile deionized water	7.5 μL
Super Master Mix 2X	12.5 μL
Primer –F (10 Pmol)	1 μL
Primer –R (10 Pmol)	1 μL
DNA Template	3 μL
Total	25 μL

Table 4: Required temperature of three primers *TSST-1*, *PVL*, *mec*A

Gene	Initial Enzyme activation	Denaturation	Annealing	Extension	Final extension
mecA	94°C- 5 min 1 cycle	94°C- 1 min 30 cycles	50°C- 30sec	72°C- 90 sec	72°C- 5min 1 cycle
TSST-1	94°C- 5 min 1 cycle	94°C- 2 min 35 cycles	57°C- 2 min	72°C- 1 min	72°C- 7min 1 cycle
PVL	95°C- 5 min 1 cycle	94°C- 30sec 30 cycles	55°C- 30sec	72°C- 1min	72°C- 5min 1 cycle

SCC*mec* typing

For typing of strains that were *mecA*-positive, we used SCC*mec* typing with 8 primers according to Mehrotra et al. [12]. In this type of SCC*mec*, the PCRs will have different typing between 1 and 2 bands with different sizes. PCR was performed in a total volume of 50 µl ***(Table 5)***.

Statistics analysis

Data were analyzed using SPSS-V25 software and Chi-Square tests.

Table 5: PCR thermal cycle program for SCC*mec* typing

Gene	Initial Enzyme activation	Denaturation	Annealing	Extension	Final extension
SCC*mec*	94°C- 5 min 1 cycle	94°C- 30sec 30 cycles	55°C- 30sec	72°C- 1 min	72°C- 4min 1 cycle

RESULTS

Among the 494 samples taken, 316 samples (63.9%) were diagnosed as *Staphylococcaceae* family (*S. aureus* and non-*Staphylococcus aureus*). *S. aureus* accounted for 6.6% (33 samples), while non-*S. aureus* accounted for 52% (283 samples).

In the general ICU, the highest rate of contamination belonged to nurses' hands, with a rate of 79%. The lowest rate of contamination (65.8%) was seen in samples that were taken from side rails of the bed. 89.5% of *Staphylococcus* isolates from surgery ICU were taken from the floor, and the lowest contamination rate was related to overbed desks at 58%. Most of the contamination seen in the NICU related to the floor (68.5%), and the lowest rate of *Staphylococcus spp*. contamination was 52.5%, belonging to staff hands ***(Figure 1)***.

According to variance analysis results conducted for the survey of contamination on different surfaces, there were significant differences between the floor and the bedside rails, the floor with the bedside table, and also the floor with hands of staff (p-value <0.01).

The highest frequency of isolated *Staphylococcus spp*. among different surfaces related to the floor of surgery ICUs with 31 isolates (16.3%), and the lowest frequency belonged to the staff hands in NICU with 14 isolates (12.2%). Based on the Chi-square test, the frequencies of *Staphylococcus spp*. at different surfaces were statistically significant (p-value <0.01). The most frequent of *Staphylococcus spp*. contamination was seen in general ICU (62.1%), while this frequency in NICU was lower than other sections (48.2%). Overall, *S. aureus* was confirmed in 33 (6.6%) samples taken. Fourteen of these samples were taken from the general ICU, 13 samples were obtained from surgery ICU, and 6 others belonged to NICU. Based on the Chi-square test, the

frequencies of *Staphylococcus spp*. and *S.aureus* at different surfaces were statistically significant (p-value <0.01 and <0.05, respectively). In general, ICU as a section with the majority of contamination, the highest and lowest contamination rates were observed in samples taken from the floor (2.6%) and beside tables (0.5%), respectively. The results of the antibiotic susceptibility test demonstrated that most of the *S. aureus* isolates were resistant to ceftaroline (93.9%), while all of them were sensitive to vancomycin. These results are shown in ***(Figure 2)***.

Figure 1: Frequency of *Staphylococcus spp.* surface contamination in ICUs

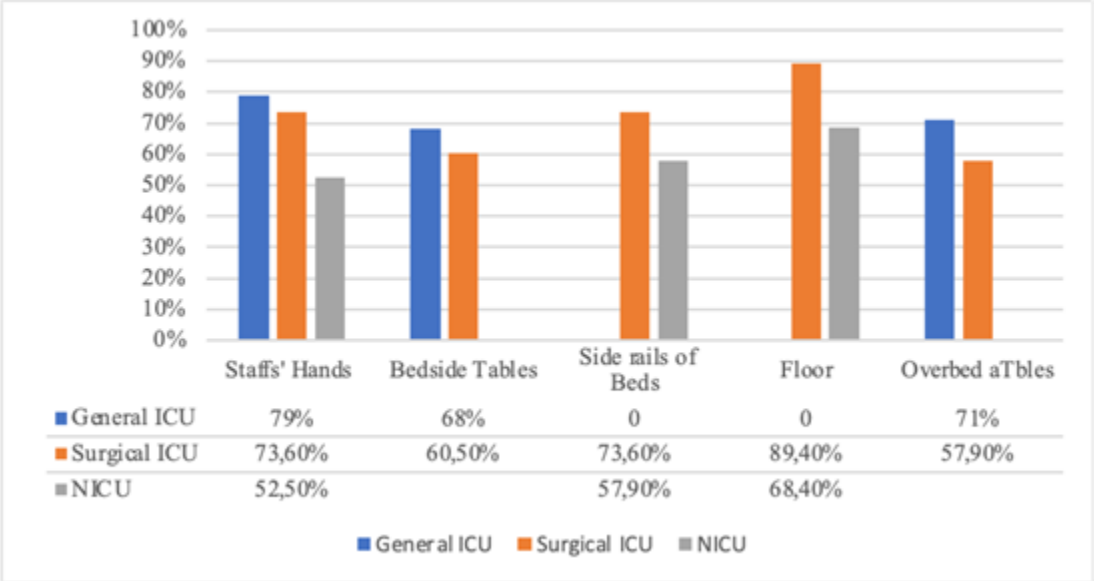
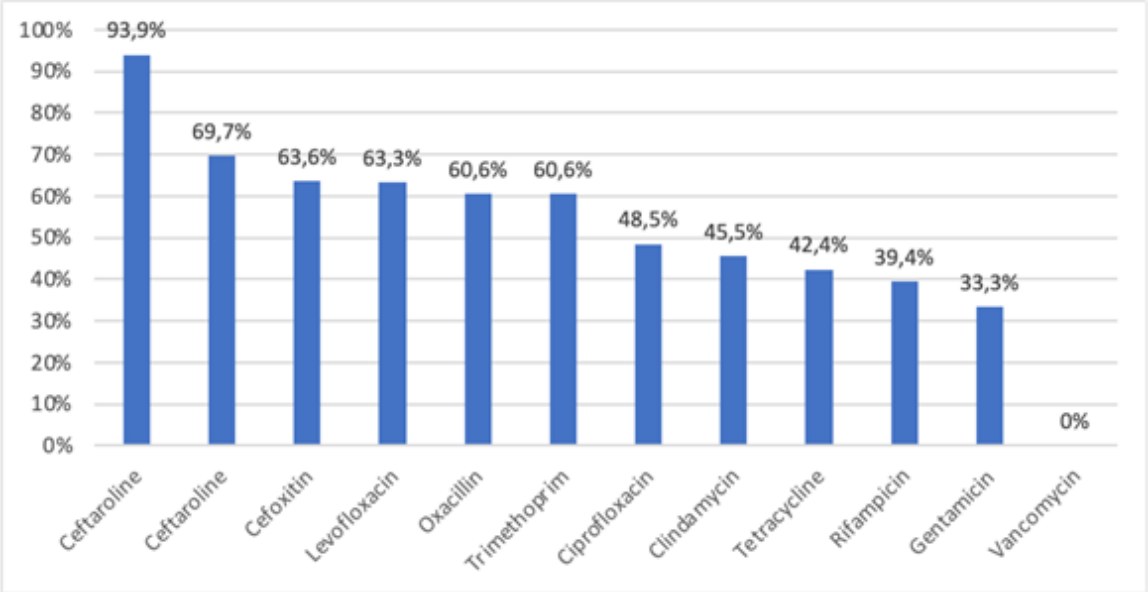


Figure 2: Percentage of antibiotic-resistance in *S. aureus* isolates



Of all 33 *S. aureus* isolates, *mecA* gene was detected in 8 (24.2%). Two out of these 8 (14.3%) belonged to general ICU (SCC*mec* type 1) isolated from the overbed table and floor. In addition, 4 strains in surgery ICU (30.7%) also had this gene, and all of them were SCCmec type 1 (isolated from the floor, overbed table, side rail of beds, staff's hand). In the NICU, we detected 2 *mecA* positive isolates (33.3%), and both of them were isolated from the floor. One of them was SCC*mec* type I and the other was SCC*mec* type IV ***(Figure 3)***.

Seven strains (21.2%) had *PVL* gene. These strains isolated from the side rail of beds and floor in general ICU (21.4%),2 of them belonged to bedside table and overbed tables in surgery ICU (15.3%), and 2 strains were isolated from the floor of NICU (33.3%) ***(Figure 4)***.

Figure 3: The result of PCR for *mecA*. C+: Positive control, C-: Negative control, Right line: 100bp DNA ladder, Line 3: Positive isolates (533bp).

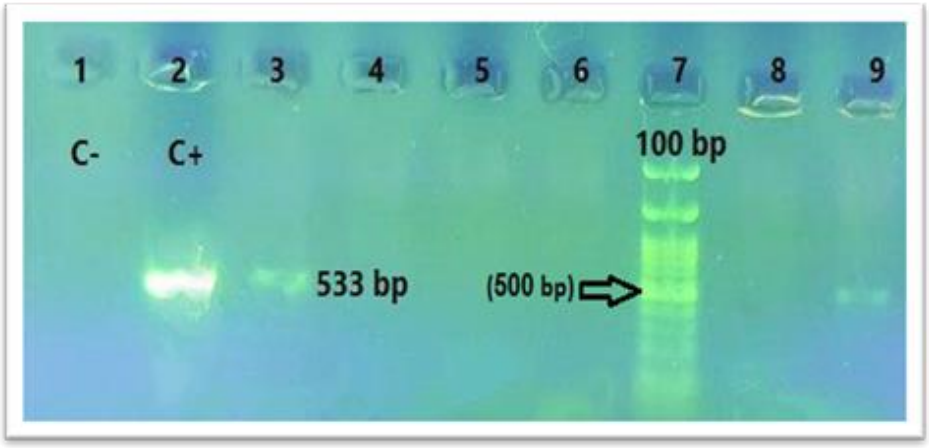
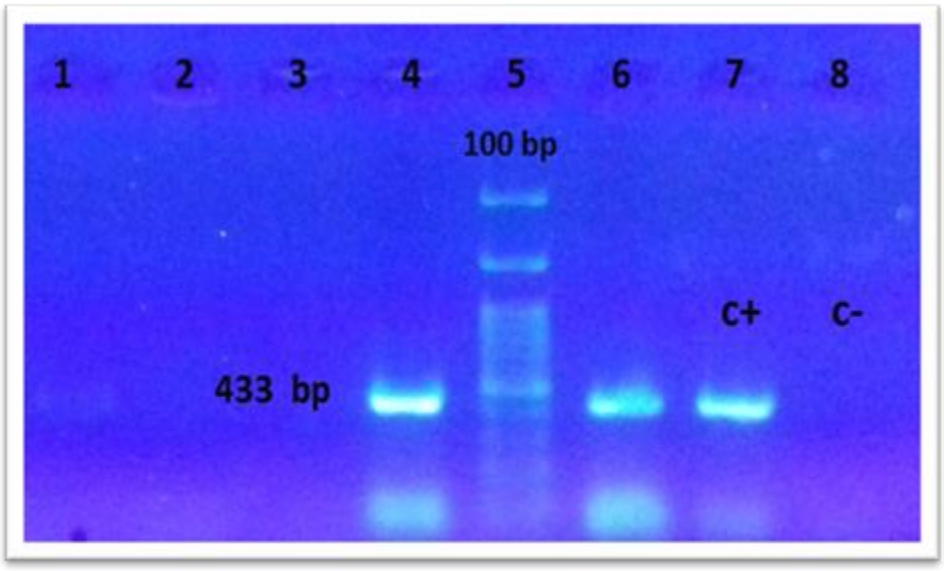


Figure 4: The result of PCR for *PVL*. C +: Positive control, C -: Negative control, line 5: 100bp DNA ladder, Line 4 and 6: Positive isolates (433bp).



Four strains (12.1%) had both *mecA* and *PVL* genes. It is worth mentioning that *TSST-1* gene was not identified in any of these samples.

DISCUSSION

Microbial populations constantly inhabit the community and hospital environment. Hospital surfaces are often contaminated with microbes from patients, visitors, and medical staff and act as potential reservoirs for the spread of microbial agents. Study of the bacteriological profile of common sites of hand contact would help to locate the possible reservoirs of bacteria and to apply suitable disinfection techniques [13]. The ability of *S. aureus* and MRSA to form biofilms on inanimate objects prolongs their survival and spread [14]. Identification of the more frequently contaminated sites and the most commonly identified potential pathogen is important for infection control practices and promotion of new interventions [15]. Increasing drug resistance among MRSA isolates and the emergence of vancomycin-resistant *S. aureus* (VRSA) isolates have further exacerbated the problem. Identification of sites colonized by MRSA and other potential nosocomial pathogens would minimize the transmission among patients and thus help reduce the incidence of nosocomial infections in the ICU [16].

In this study, the prevalence of bacterial contamination was 68.6% of samples taken from different surfaces of hospital sections. 63.9% of these isolated bacteria were *Staphylococcus spp*. and 33 isolates (6.6%) detected as *S. aureus*. Of the 33 *S. aureus* isolates, 8 (24.2%) had the *mecA* gene.

A study was conducted by Yusuf *et al.* in 2015, investigating the rate of multiple drug resistance (MDR) infection on different surfaces in two sections of adult and neonatal ICUs. It showed that 113 sample cultures were 62.8% positive in adult ICUs and 59.2% positive in neonatal ICUs, which is almost consistent with our study. However, it was different in the rate of *S.aureus* (27%) with a recent study (6.6%) [17]. Zazouli *et al.* in 2015 in Sari-Iran observed the extent of multidrug-resistant bacteria contamination on surfaces and type of equipment of the different sections, (ICU, ear-nose-throat [ENT], surgery room, burn wards, and recovery section). *S. aureus* accounted for 20.8% (80% in ENT section, 53% in men burn ward, 40% in NICU and ICU) [18]. In another study in Nigeria, the rate of contamination with *S. aureus* on different surfaces was 50.8%. In addition, the highest rate of contamination with *S.aureus* was observed in samples taken from brancards (100%), staff hands (100%), and handles (50%) [19].

Bhatta *et al.* reported that the prevalence of *S. aureus* and MRSA was 54.4% (49/90) isolated from surfaces of intensive care units [16]. Nkuwi *et al.* reported that the prevalence of *S. aureus* and MRSA was 20.5% and 19.9%, respectively, in general sections, ICU, and surgery room [20]. Another result

reported from the USA demonstrated that *S. aureus* and MRSA frequency on the surfaces of ICU was 2% and 10%, respectively [21] . The prevalence of *S. aureus* in the current investigation was 6.6% of detected bacterial contamination, which is higher than results of the studies conducted in the USA and lower than those found in other studies as 50.8%, 20%, and 20.83% from studies conducted by Hummual *et al.*, Nkuwi *et al.*, and Zazouli, respectively.

The cause of the difference in bacterial contamination, especially MRSA, in different hospitals can be due to the use of appropriate disinfectants, regular disinfection of surfaces, and hand hygiene principles of staff when caring for patients. It can also be due to different sample sizes. The number of patients, staff, and visitors in each section also contributes to the rate of contamination [22,23]. French *et al.* reported that hospital surface contamination seems to be higher in the rooms of patients infected by MRSA (70% and 60% before and after disinfecti on, respectively) [24].

According to the Clinical & Laboratory Standards Institute (CLSI) guidelines, methicillin resistance in MRSA is due to the expression of *mecA* gene or other mechanisms such as changes in the affinity of PBPs. So, in this study, the cefoxitin-resistant MRSA strains were tested for the presence of *mecA* gene. Eight of 21 samples were *mecA* positive. Whereas in a study conducted by Nkuwi *et al.***,** MRSA strains were identified just by using cefoxitin disk (19.9%). It is likely that this frequency of MRSA could be lower if *mecA* gene presence was the factor for diagnosing MRSA strains [20,25]. A study was conducted in 2008 to investigate the molecular characteristics and typing of SCC*mec* gene chromosomal cast complex in MRSA strains isolated from clinical samples of Shahid Beheshti Hospital in Kashan. This study showed that more than half of *S. aureus* strains have the *mecA* gene, and more than 60% of them were isolated from the CA-MRSA community. Also, SCC*mec* type II was one of the most abundant genotypes isolated from the samples [22]. In the Thapaliya *et al.* study in Cambodia, the prevalence of contamination with staphylococcus and MRSA on 152 environmental surfaces of university buildings (hospital and educational) were reported as 22.4% and 5.9%, respectively, and the isolates were analyzed by PCR for *PVL* gene presence.

One of these isolates was *PVL* positive and the antibiotic resistance with disk diffusion assays showed resistance to erythromycin, oxacillin, clindamycin, tetracycline, and ciprofloxacin (35.3%, 26.5%, 14.7%, 5.9%, and 8.8%, respectively) [26]. These results differ from the findings presented here. In our study, *PVL* positive strains accounted for 21.2%, and resistance to erythromycin 70%, oxacillin 61%, clindamycin 42%, ciprofloxacin 46%, tetracycline 41%, which are higher than in the Thapaliya study. Possible explanations for these differences can be related to geographic area, the hospital's environmental condition, or indiscriminate antibiotic consumption.

A study in the north of Iran studied the types of *Staphylococci mec* chromosomes among methicillin-resistant *Staphylococcus aureus*, and the most common type was SCC*mec* type III with a frequency rate of 76%. After that, types IV, I, and V have been confirmed with frequency rates of 11.2%, 4.8%, and 3.2%, respectively. Like other geographical regions of Iran, SCC*mec* type III MRSA strain was the most common strain isolated from patients in Gorgan. SCCmec type III has shown lower virulence factors compared to other types of SCC*mec* [23].

Another study performed in 2015 by Mirzaii *et al.* demonstrated that the prevalence of *S. aureus* was 13.8%, of which MRSA strains made up 94%. Eight samples were SCC*mec* type III (22.8%), 4 samples were SCC*mec* type I (10%), and 3 samples were SCC*mec* type IV (8%) [27]. Contaminated blood in the laboratory may cause bacterial infections with such bacteria as *Staphylococcus aureus* and *Staphylococcus epidermidis* [32]. The frequency of virulence genes in *S. aureus* strains isolated from Al-Najaf Al-Ashraf Teaching Hospital has been studied Alehawy *et al.* Among the strains, *hla* gene was present with 91% frequency, showing the highest prevalence among pathogenic genes. *Sea, mecA, clfB, femA, fnbB, TSST,* and *hlb* genes were the next most prevalent at 88%, 65%, 54%, 45%, 39%, 27%, and 13%, respectively [33].

In the present study, most of the *S. aureus* contamination belonged to general ICU (7.3%), which is lower than those observed by Mirzaiee *et al.* According to a study conducted by Abbasi in Ilam, Iran, the prevalence of *S. Aureus* and MRSA strains was 62.8% and 40.9%, respectively. Furthermore, 100% of those isolates were sensitive to vancomycin, which is in agreement with our result. The highest rate of resistance was observed with gentamicin (83.3%). SCC*mec* type I (25%) and II (30.5%) were also reported [27]. Consequently, many of the MRSA strains have also developed resistance to a range of antibiotics that include erythropoietin, aminoglycosides, tetracyclines, rifampicin, clindamycin, trimethoprim sulphate, and fluoroquinolones. Vancomycin and teicoplanin are generally the only antimicrobials that can be used in severely infected patients with MRSA. The only genuine sources of *S. aureus* in hospitals are, essentially, septic lesions and transfer sites for patients and staff [34]. The PCR method has been used to measure gene frequency. The highest levels of resistance to aminoglycosides were observed in kanamycin (47.8%), gentamicin (46.9%), and tobramycin (46.9%). Doxycycline and ciprofloxacin (50.4% and 49.5%, respectively) were the non-aminoglycoside antibiotics to which the highest levels of resistance were exhibited. The frequency of the aac (6′)-le-aph (2′′) gene was 39.1% [35].

A study in 2022 in Imam Reza Hospital, Birjand, Iran investigated the frequency of multidrug-resistant SCC*mec* type IV and SCC*mec* type I among MRSA isolates. The prevalence of MRSA isolates was 39.4%. Vancomycin and ceftaroline were effective drugs against

MRSA isolates. SCC*mec* types I, III, and IV were identified in 27.9%, 23.3%, and 37.2% of MRSA isolates, respectively. SCC*mec* types I and IV were identified as the most common types of SCC*mec* in HA-MRSA isolates, while SCC*mec* type IV (66.7%) was the most common type of SCC*mec* associated with CA-MRSA isolates [30].

Ceftaroline is used as rescue therapy for various MRSA infections. Ceftaroline should be used with caution as ceftaroline-resistant MRSA is beginning to emerge [31].

A recent study carried out by Carvalho *et al.* in Brazil in 2017 investigated the contamination of *S. aureus* and MRSA on the equipment and environment of two public hospitals in Brazil. That survey demonstrated that *S. aureus* and MRSA frequencies were 83.7% and 25.64%, respectively. This outcome was contrary to ours. Of 30 samples, 19.28% were HA-MRSA with 37% SCC*mec* type I. Among CA-MRSA strains, type IV SCC*mec* prevalence was 73%. Additionally, among HA-MRSA strains, 21% were *TSST-1* positive, while in our study, the *TSST-1* gene was not found in any isolates [28]. In the present study of 33 strains, 8 strains (24.2%) identified MRSA, of which 7 strains (87.5%) were type I SCC*mec* and HA-MRSA. One strain was type IV SCC*mec*, and one strain was CA-MRSA.

Our study findings are important to generate awareness among infection control teams and healthcare professionals regarding contamination of ICUs with bacterial agents and their possible role in nosocomial infections.

CONCLUSION

Bacterial contamination in the ICUs of Ayatollah Rouhani Hospital in Babol, Iran is high. Most of the surface contamination was related to non-*S. aureus*. The majority of isolated strains was SCC*mec* type I. Type I is associated with HA-MRSA and may be the source of contamination of hospital surfaces, patients infected with MRSA, or MRSA-carrier staff.

Notes

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Ethical Consideration: This study with code 9706021 and ethical code (IR.MUBABOL.HRI.REC. 2018.09.05). All procedures involving human subjects were approved by the institutional ethical committee of University of Medical Sciences in Babol.

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