

Original Article

The First Report of t8463 and t605 *spa* Types in Methicillin-resistant *Staphylococcus aureus* isolated from ICUs in Rasht, Iran

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ABSTRACT

Staphylococcus aureus is the most important human pathogen, in community and hospital-acquired infections. The colonization rate of this organism is high in medical personnel and devices. Considering the importance of carriers in the transmission of *S. aureus* infection, this study investigated the origin of methicillin-resistant *S. aureus* (MRSA) isolated from Velayat and Poursina Hospitals of Rasht. In an eight-month period, a total of 500 samples were collected from hospitalized patients, healthcare personnel, various surfaces, air, and medical devices within the intensive care units (ICUs) of Velayat and Poursina Hospitals. After the identification of MRSA strains by microbiological and biochemical standard methods, the DNA of the isolates was extracted. The *spa* typing of MRSA strains was done after determining the sequence of amplified protein A genes by polymerase chain reaction (PCR) using specific primers. Among 500 samples, 45 (9%) samples were infected with *S. aureus* and 31 (68.9%) MRSA strains were identified from different ICUs. For the first time, *S. aureus* was divided into three types with the help of the *spa* technique in the ICU of Rasht hospitals. Among the examined samples, the t14870 *spa* type prevailed (95.5%), which had been found in previous studies in different regions of Iran. However, two types, t8463 (2.2%) and t605 (2.2%), were obtained for the first time in Iran, which were MRSA and obtained from the noses of patients. The high frequency of *S. aureus* isolates in (ICUs) and among healthcare personnel significantly contributes to the transmission of infections within the hospital setting.

Keywords: Methicillin resistant *S. aureus* (MRSA), ICU, *Staphylococcus* protein A, *spa* typing.

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1. Introduction

In the last several decades, *Staphylococcus aureus* has been one of the most important human pathogens and the leading cause of acquired infections in the community and hospital. *S. aureus* causes a wide range of diseases in humans, from mild skin infections to life-threatening diseases. This pathogen exists in the nasal and intestinal cavities of people and is colonized in approximately 50% of healthy people in the community and hospital personnel without symptoms (1). Although there are bacteria in the body of healthy people, antibiotic resistance can lead to severe infections and even death. *S. aureus* becomes resistant to most antibiotics in a short period and causes many treatment problems. There are many virulence factors associated with *S. aureus*, including pathogenicity and bacterial colonization (2). Methicillin-resistant *S. aureus* (MRSA) is the cause of various types of antibiotic-resistant infections and is one of the most important and common resistance patterns among *S. aureus* strains. This resistance is caused by the presence of the *mecA* gene, which is chromosomally encoded (2,3). The *mecA* gene and its expression-regulating genes and additional sequences form a gene complex called the *mecA* complex on a very large gene island called Staphylococcal Cassette Chromosome *mecA* (SCC*mecA*), which can transfer between different strains and leads to the spread of resistance (4). The prevalence of MRSA varies widely in different geographical regions and has shown a significant increase worldwide in recent years. The prevalence of MRSA strains in Asian countries such as China, Korea, and Taiwan is more than 70%; in North America, it is more than 50%; in Europe, it is 20%; and in Iran, it is on average 50% (5). Different regions of Iran have also been reported in various ranges between 20 and 90 percent for the prevalence of MRSA (6). The prevalence of MRSA is higher, especially in nosocomial infections, patient care centers, patients with open wounds, patients with prostheses, and also in immunodeficient patients. Despite the antibiotic resistance pattern in MRSA isolates and the widespread appearance of multi-drug resistant (MDR) MRSA, it is important to periodically evaluate the antibiotic resistance pattern using sensitivity tests (7). Genotyping can be extremely useful for identifying the source of infection and controlling it. Also, typing of *S. aureus* isolates is a suitable method for epidemiological purposes. One of the methods to describe the specific genetic traits of MRSA is to determine the type of SCC*mec* based on the type of recombinase enzyme encoded by *ccr* genes and *mecA* complexes. So far, 11 different types of SCC*mec* have been known, of which five types SCC*mec* I-V are dominant in Iran (8). Another method is *spa* typing. The *spa* gene encoding protein A (Staphylococcal protein A; *spa*) is one of the determinants of differentiation, which has the x polymorphism in a short sequence region, and different studies have identified various patterns of this gene. It can also be used to determine the specific identity of this bacterium (9). As the most important human pathogen, the cause of community- and hospital-acquired

infections, the high colonization rate in medical personnel and the importance of carriers in the transmission and circulation of infection in the hospital, in this study, the origin of MRSA colonies was determined by the *spa* method from the personnel, patients, surfaces, air, and devices in the ICU, of Velayat and Poursina Hospitals of Rasht.

2. Materials and Methods

2.1. Sample collection

In this cross-sectional study, 500 swab samples were collected from patients, personnel, beds, clothes, and other objects in the ICUs of Rasht Velayat and Poursina Hospitals over eight months, once every two weeks. To take samples from the patients, first, using the information from the patient's file, a checklist containing age, sex, history of antibiotic use, clinical symptoms, and samples were taken depending on the type of infection, from pus, catheters, wounds, urine, blood, eye secretions also hands (palm and dorsum, under the nail, and between the fingers), etc. Also, the air of the ICU ward was sampled using the Andersen pump and *S. aureus* species were investigated using standard microbiological methods.

2.2. Antibiotics susceptibility test

The antibiotic resistance pattern of the isolated strains was investigated using antibiotic discs of erythromycin, clindamycin, gentamicin, linezolid, tetracycline, rifampin, penicillin, and mupirocin according to the Clinical and Laboratory Standards Institute (CLSI). The 30 µg cefoxitin disk was used to evaluate methicillin resistance and detect MRSA strains. The E-test strip was also used for vancomycin. The genome of *S. aureus* strains was extracted from 24-hour bacterial cultures according to the instructions of the manufacturer of the Roche kit in Germany. The oligonucleotide primers were used to carry out the standard PCR reaction to amplify the *spa* gene (Table 1).

2.3. Detection of resistant genes

Polymerase chain reaction (PCR) reaction was performed using 2x Super PCR Master mix (Dye Plus), 10 pmol from each primer, ddH₂O, and 50-100 µmol DNA. The PCR product was electrophoresed on 1.5% agarose gel (10). The different stages involved in PCR reactions include pre-denaturation, denaturation, annealing, extension, and final extension (Table 2). The *S. aureus* LMG 8224 strain was used for the *nucA* gene and the *S. aureus* ATCC 33591 strain was used for the *femA* and *mecA* genes as positive controls.

2.4. The *spa* typing

PCR amplification was performed for *spa* typing of isolates (Table 2). The *spa* typing of MRSA strains was performed after determining the sequence of the amplified protein A gene through PCR (provided by Fazapzoh Company), utilizing specific primers available at <https://spaserver.ridom.de/>). The analysis was conducted using Chromas and Nucleic Acid Sequence Massager software.

Table 1. The sequence of specific primers to perform the PCR technique

Target	Primer sequences (5'→3')		Product size (bp)	Reference
<i>nucA</i>	F	GCGATTGATGGTGATACGGTT	280	(14)
	R	AGCCAAGCCTTGACGAACTAAAGC		
<i>femA</i>	F	AAAAAAGCACATAACAAGCG	132	(15)
	R	GATAAAGAAGAAACCAGCAG		
<i>Spa</i>	F	AGACGATCCTTCGGTGAGC	Variable	(16)
	R	GCTTTTGCAATGTCATTTACTG		
<i>mecA</i>	F	ACTGCTATCCACCCTCAAAC	163	(14)
	R	CTGGTGAAGTTGTAATCTGG		

Table 2. Details of the PCR reactions for each of the investigated genes

Genes Cycles	<i>Spa</i>	<i>mecA</i>	<i>femA</i>	<i>nucA</i>
Pre-denaturation	94°C 5 min	94°C 2min	94°C 2min	95°C 1min
Denaturation	94°C 45 sec	94°C 1min	94°C 1min	94°C 1min
Annealing	60°C 45sec	57°C 2min	58°C 1min	36°C 1min
Extension	72°C 90 sec	72°C 2min	72°C 1min	72°C 2min
Final Extension	72°C 10 min	72°C 10 min	72°C 7min	72°C 7min

2.5. Statistical analysis: Data were analyzed by SPSS version 26 software. The relationship between the variables was checked using the chi-square test and the significance level was considered to be <0.05.

3. Results

In the present study, from 500 swabs collected from patients, personnel, and fixed surfaces of the ICUs in the hospitals of Velavat and Poursina in Rasht, 45 (9%) isolates were identified as *S. aureus*. Among all the studied samples, 14 (31.11%) and 26 (57.78%) isolates belonged to females and males respectively. Also, 5 (11.11%) isolates were isolated from surfaces, but none from the air. Among the male participants, 25 (55.5%) were isolated from male patients and 1 (2.2%) from personnel, and among female participants, 8 (17.7%) were isolated from patients as well as 6 (13.3%) were isolated from personnel. Most samples were obtained from the hands, noses, wounds, beds, and clothes of patients and personnel, as well as from other devices in the ward. Out of 45 *S. aureus* isolates, 8 (17.8%) were isolated from the burn ICU at Velavat, 22 isolates (48.9%) from the general ICU, and 15 (33.3%) from the trauma ICU at Poursina Hospital. The most strains were isolated from the hand and nose of the trauma ICU, while the lowest was isolated from the burn ICU. In addition, the strains isolated from the wound were the most frequent in the general ICU, and no strain was isolated from the burn ICU of Velavat Hospital. The most frequently isolated strains came from beds and clothes of patients, uniforms of personnel in general, trauma and burn ICUs at 42.9%, 33.3%, and 23.8%, respectively. There were no isolates of *S. aureus* strains in trauma ICU devices and air, but most samples (three samples) were from general ICU devices. The PCR reaction for the *nucA* gene was carried out using specific primers. Although 65 (13%) of the samples

showed infection with *S. aureus* in the phenotypic test, 45 isolates were confirmed with the *nucA* gene. penicillin, erythromycin, and penicillin antibiotics showed the highest resistance in the General ICU, Trauma ICU, and Burn ICU, respectively. However, linezolid had the lowest resistance in all three sections, and all isolates (100%) were resistant to this antibiotic. Among the isolated strains, 31 (68.9%) isolates were resistant to cefoxitin that all of them were positive for the *mecA* gene in the diagnosis. The MIC results of vancomycin resistance in *S. aureus* isolates indicated that all isolates were sensitive to vancomycin. The strains were divided into two groups: those resistant to methicillin and those susceptible to methicillin. Resistance to all antibiotics in the trauma ICU ward except penicillin and mupirocin was higher than other ICU wards. This study displayed different patterns of antibiotic resistance to various antibiotics (Table 3). The PCR reaction for the *femA* gene, which is a regulatory gene for methicillin-resistant strains, was performed using specific primers. All studied strains, including methicillin-resistant and methicillin-susceptible strains, were examined for the presence of the mentioned gene. This gene was detected in 31 (68.8%) isolates. There was no statistically significant difference between the presence or absence of the *femA* gene in MRSA and MSSA groups using the Chi-square test (P value = 0.540). Finally, all the isolates with the *mecA* gene were the same samples that were phenotypically resistant to the methicillin. Among the examined samples, three different *spa* types were detected in this work that *spa* type t14870 was dominant (95.5%). Also, two types, t8463 (2.2%) and t605 (2.2%), were detected for the first time in Iran and were observed only in two strains, both of which were MRSA and were obtained from the patients' noses (Table 4).

Table 3. Frequency of antibiotic resistance pattern of studied strains isolated from different ICUs

Hospital ICUs Antibiotics	General ICU			Trauma ICU			Burn ICU		
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Erythromycin	78.7	6.7	14.9	84.4	3.1	12.5	68.8	12.5	18.8
Clindamycin	74.5	6.4	19.1	81.2	6.2	12.5	50	6.2	43.8
Gentamicin	42.6	10.6	46.8	50	12.5	37.5	25	18.8	56.2
Linezolid	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
Tetracycline	51.1	0.0	48.9	68.8	0.0	31.2	62.5	0.0	37.5
Rifampin	46.8	2.1	51.1	56.2	0.0	43.8	43.8	0.0	56.2
Penicillin	83.0	0.0	17.0	78.1	0.0	21.9	93.8	0.0	6.2
Mupirocin	36.2	0.0	63.8	25.0	0.0	75.0	37.5	0.0	62.5

Table 4: Details of spa type 45 isolate of *S. aureus*

Isolate number	Site of isolation	Sensitive or resistant to methicillin	Spa type
1	Nose	MRSA	t14870
318	Nose	MSSA	t14870
4	Nose	MSSA	t14870
7	Surface	MRSA	t14870
8	Surface	MRSA	t14870
9	Nose	MSSA	t14870
21	Nose	MSSA	t14870
28	Nose	MRSA	t14870
340	Objects	MRSA	t14870
36	Nose	MRSA	t14870
39	Nose	MSSA	t14870
61	Objects	MRSA	t14870
62	Nose	MRSA	t14870
68	Objects	MRSA	t14870
74	Surface	MRSA	t14870
84	Surface	MRSA	t14870
111	Nose	MRSA	t14870
131	Nose	MRSA	t8463
154	Nose	MSSA	t14870
174	Nose	MRSA	t14870
197	Nose	MSSA	t14870
90	Nose	MSSA	t14870
215	Nose	MRSA	t14870
88	Nose	MRSA	t14870
44	Objects	MRSA	t14870
184	Surface	MRSA	t14870
11	Nose	MSSA	t14870
31	Nose	MRSA	t14870
15	Nose	MSSA	t14870
74	Nose	MRSA	t14870
97	Nose	MSSA	t14870
10	Nose	MSSA	t14870
30	Nose	MRSA	t14870
40	Nose	MSSA	t14870
70	Surface	MRSA	t14870
80	Surface	MRSA	t14870
6	Nose	MRSA	t14870
383	Nose	MSSA	t14870
298	Objects	MRSA	t14870
66	Nose	MRSA	t14870
73	Objects	MRSA	t14870
77	Surface	MRSA	t14870
432	Nose	MRSA	t605
54	Nose	MRSA	t14870
55	Objects	MRSA	t14870

4. Discussion

S. aureus is a bacterium that colonizes on the skin and nasal mucosa and has genes that play a role in increasing antibiotic resistance prevalence. As carrier personnel can transmit the organism and cause severe nosocomial infections in hospitalized patients, it is necessary to prevent and control this infection. Recently, there have been many indications that the environment can play an important role in the spread of *S. aureus*. Due to its ability to adapt to various environmental conditions, including humidity, temperature, levels of ultraviolet radiation, and the type of disinfectant used, the bacterium remains stable in dry environments (2). With the increase of the elderly in society, more people are exposed to surgeries and invasive methods with medical devices, resulting in the spread of diseases in immunodeficient patients; therefore, eliminating *S. aureus* from the hospital environment is important to preventing and reducing nosocomial infections (2). On the other hand, determining the antibiotic resistance pattern of the isolated *S. aureus* and selecting the appropriate drug will reduce treatment costs and promote the rapid recovery of the patient (2). In the present study, 14 (31.11%) *S. aureus* samples were isolated from females, 26 (57.78%) samples collected from males, five (11.11%) isolates were isolated from surfaces, and no isolate was isolated from the air. Among the male participants in the study, 25 were isolated from male patients, and one was isolated from personnel. Among the female participants, eight isolates were obtained from patients and six isolates were obtained from personnel. In this regard, studies have been conducted that show the frequency of *S. aureus* isolated from medical workers was 25.2% in Hamadan (11), 31% in Tehran (12), 42% in Babol (13), and 36% in Ghaemshahr (14). The difference in the number of carriers in different Iranian studies may be attributed to environmental factors such as the long presence of medical staff in hospital wards and the direct contact of medical staff with patients, whose exposure is greater than that of students. Our data showed that the frequency of *S. aureus* in the nose of the carriers was observed in 26 (57.7%) isolates, which was highly similar to the study by Sharma et al (15). Also, there have been different reports of the prevalence of *S. aureus* in health workers in other parts of the world that rate of *S. aureus* in the nose of the carriers was 74.6% which was higher than our study (16). Although the prevalence of *S. aureus* was reported as 18.3% in the nose of healthcare workers (17), another study reported 52.2% of healthcare workers were *S. aureus* carrier (18). It seems that this difference in the frequency of *S. aureus* carriers in the nose of hospital personnel can be related to the differences in infection control committee programs and monitoring systems to identify and treat carriers, as well as the differences in race and geographical area. Our results showed that among *S. aureus* isolates, 68.9% were MRSA. The prevalence of MRSA carriers has been different in areas of Iran, Pourramezan et al. reported 22.5% of MRSA among health-care workers of critical wards at a university

hospital of Tehran (19) but Abdullahi et al. found MRSA in 75% of healthcare workers in southwestern Iran (20). Similar to the results obtained from our study, Ghasemian et al. identified a high rate of MRSA (74.2%) in patients on hemodialysis (14). Contrary to our results, in a study conducted in Birjand, the prevalence rate of MRSA in the hospital was declared as 39.4%, which is much lower than the prevalence rate obtained from our study (21). These differences can be attributed to the study population, the type of clinical isolates, and the prescription of specific antibiotics in different geographical areas. In other parts of the world, there are reports of different levels of MRSA, in this regard, a study conducted in India on medical students to isolate MRSA revealed that only one of one hundred eighty one medical student was carrying MRSA (15). In accordance with our results, a study conducted in Brazil reported a high frequency of MRSA (72.3%) in the nose of carriers in primary health care units (16), but in Nepal 47.4% MRSA detected in nasal carriage among health care workers and medical students (17). A study in Nepal showed that 36 (92.3%) of 39 *S. aureus* isolated from healthcare staff in the ICUs were declared as MRSA (18). This difference seems to be related to the sample size, the geographical distance between the sampling locations, the type of sample and dissemination of specific clones. The predominant *spa* type in our study was t14870 (95.5%) and two *spa* types including t8463 (2.2%) and t605 (2.2%) were found for the first time in Iran, both of which were MRSA as well as were obtained from the noses of patients. In this regard, Gudarzi et al. performed *spa* typing among 90 samples *S. aureus* which identified five *spa* types including t037, t030, t790, t969, and t044 (22). Similar to the results obtained with our study, some studies in Iran have shown that t14870 is the most common *spa* type among *S. aureus*, although Montazeri et al. in Ahvaz (23) introduced different *spa* types, were mentioned t14870 was predominant *spa* type. Contrary to our results, other studies have introduced other *spa* types as the most common *spa* types in Iran, and in Hashemizadeh et al. different were identified *spa* types (*spa*003, t386, t1877, t314, t030, t186, t1816, t304, t325, t345), of which *spa* type t030 was the most common among only MRSA isolates (24). In another study, Latifpour et al identified 27 different *spa* types in only hospital MRSA isolates, and type t030 was introduced as the most frequent *spa* type (25). To the best of our knowledge, these *spa* types may be different according to different geographical regions in different countries or even different cities, different type of sample (For example, some studies have only investigated *spa* types in MRSA, others in MSSA, and some investigated in clinical samples, but some in healthcare workers). Our results showed a high prevalence of MRSA in the carriers of healthcare workers and three *spa* types were identified in them. Considering the importance of MRSA in hospitals, there is a need for a new control policy to address infections caused by this bacterium in hospitalized patients and hospital personnel. Hence, identification of new *spa* types emphasis on the

reality which periodically performed MRSA surveillance studies in order to identify major molecular *spa* types related to human infection and characterize the new emergence of *spa* types.

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Authors' Contribution

Writing and Investigation: P.K
Methodology, Revision and Supervision: A.M
Revision and Visualization: M.A
Writing and Interpretation of data: G.M
Statistical analysis and Methodology: M.G

Ethics

It is stated that all ethical considerations were taken into account in the preparation of the submitted manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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