

Genotypic characterization of *Staphylococcus aureus* isolated from a burn centre by using *agr*, *spa* and SCCmec typing methods

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Abstract

Infections caused by *Staphylococcus aureus* remain a major global healthcare problem. We aimed to find the common lineages of *S. aureus* strains circulating in a burn hospital in Tehran. A total of 167 isolates of *S. aureus* obtained from patients, healthcare workers (HCWs) and environment in Shahid Motahari burn hospital were genotyped by using *spa*, *agr* and staphylococcal cassette chromosome *mec* (SCCmec) typing methods. Antimicrobial susceptibility testing was performed by using the disc diffusion method. The frequency of methicillin-resistant *S. aureus* (MRSA) was 64.7% ($n = 108$), with distribution frequencies among patient, HCW and surface isolates of 64.2% ($n = 79$), 50% ($n = 7$) and 73.3% ($n = 22$), respectively. SCCmec type III (75%, $n = 81$) was found to be the most frequent SCCmec type among MRSA isolates, followed by SCCmec type I (20.4%, $n = 22$) and SCCmec type IV (1.8%, $n = 2$). The remaining MRSA isolates (2.8%, $n = 3$) were nontypeable by this method. About 78.4% ($n = 131$), 10.2% ($n = 17$) and 4.8% ($n = 8$) of all isolates were characterized as *agr* types I, II and III, respectively, and the other isolates (6.6%) were nontypeable. *spa* types t030 and t037 constituted the first and second most predominant *spa* types found in 56.4% ($n = 57$) and 25.6% ($n = 26$) of isolates, respectively. We also report here a novel *spa* type, t16471. The most prevalent genotypes of the isolates found among patient, surface and HCW samples were SCCmec type III/t030, t037/*agr* type I. Continuous tracking of epidemic isolates and better hospital infection control policies are recommended to efficiently prevent the spread of bacteria to inpatients.

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Keywords: *agr* typing, Iran, MRSA, SCCmec typing, *spa* typing

Original Submission: 10 June 2018; **Revised Submission:** 25 July 2018; **Accepted:** 3 August 2018

Article published online: 11 August 2018

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Introduction

Burns are among the devastating forms of trauma and burn wound infections are the most common complications after burn injury. Multidrug-resistant *Staphylococcus aureus* strains are

considered to be one of the common Gram-positive early colonizers of burn wounds [1,2]. Methicillin, a semisynthetic penicillin, first entered clinical use in 1960 for the treatment of infections caused by β -lactamase-producing staphylococci. However, only 1 year later, *S. aureus* strains that showed resistance to methicillin were reported from clinical infections. Since then, methicillin-resistant *S. aureus* (MRSA) strains have appeared in countries worldwide and continue to be one of the most common pathogens causing healthcare-associated infections (HAIs) [3]. HAIs caused by antibiotic-resistant bacteria are known to be associated with prolonged hospitalization as well as higher healthcare costs and mortality rates in burn patients, and according to a US Centers for Disease Control and

Prevention report, 5% of all HAIs are caused by MRSA [4,5]. The prevalence of MRSA varies from 20% to 90% in Iran [2]. MRSA is usually spread by direct contact with an infected wound or via contaminated hands. Previous studies have shown that about 33% and 2% of people carry *S. aureus* and MRSA in their nares, respectively [6].

MRSA usually carries molecular determinants that confer resistance to a wide range of antibiotics, and treatment of infections caused by these bacteria are challenging [1,7]. A methicillin-susceptible *S. aureus* (MSSA) strain becomes MRSA by the acquisition of a genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*), carrying the *mecA* gene, regulatory genes, recombinase genes and a variety of accessory genes. Molecular epidemiologic methods for the continuous surveillance of MRSA clones and dissemination of the strains can be used in order to control the infections [8,9]. Over the past two decades, a variety of methods, such as pulsed-field gel electrophoresis, multilocus enzyme electrophoresis, multilocus sequence typing, accessory gene regulator (*agr*) typing and *spa* typing, have been used for genotypic characterization of MRSA strains and other bacteria [10]. Previous studies in Iran have reported SCC*mec* type III/to30, t037 as the most common genotypes among MRSA isolates [2]. As common typing methods, pulsed-field gel electrophoresis and multilocus sequence typing are considered to be labor-intensive and expensive; instead, other techniques such as *spa*, *agr* and SCC*mec* typing methods could be used for reliable and accurate typing of MRSA [11].

In the present study, we used a combination of different techniques including *spa*, *agr* and SCC*mec* typing to determine the common types of *S. aureus* strains circulating in one burn hospital in Tehran, Iran.

Materials and methods

Bacterial isolates

A total of 405 clinical and nonclinical samples were obtained from patients (pus and blood), nostrils of HCWs (physicians, nurses and service employees) and inanimate surfaces (Computer mice and keyboards, beds, chairs, medical trolleys, phone receivers, faucet handles, door handles, elevator buttons, toilet flush handles) from different wards (emergency, men, women, children and intensive care unit) in the Shahid Motahari burn hospital in Tehran from 2015 to 2016. Duplicate isolates from the same patient were not included in the study. The isolates were identified to the species level using standard biochemical tests including Gram staining, catalase, tube coagulase and DNase tests as well as mannitol fermentation. All *S. aureus* isolates were confirmed by PCR for the presence of the *nucA* gene [10].

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was performed by using the disc diffusion method and in accordance with the Clinical and Laboratory Standards Institute guidelines for the following antibiotics: nitrofurantoin (300 µg), gentamicin (10 µg), mupirocin (20 µg), rifampicin (5 µg), norfloxacin (10 µg), tigecycline (15 µg), ampicillin (10 µg), trimethoprim/sulfamethoxazole (25 µg), chloramphenicol (30 µg), erythromycin (15 µg), clindamycin (2 µg), tetracycline (30 µg), penicillin (10 U), linezolid (30 µg), quinupristin/dalfopristin (15 µg) and imipenem (10 µg) (Mast, Merseyside, UK). Screening for the MRSA isolates was performed using cefoxitin (30 µg) discs on Müller-Hinton agar plates [12].

DNA extraction and identification of MRSA isolates

DNA extraction was performed by using the boiling method as described previously [13]. All phenotypically identified methicillin-resistant isolates were confirmed by PCR for the presence of the *mecA* gene.

SCC*mec* typing

A multiplex PCR with four primer pairs was performed to identify the five main known SCC*mec* types [14]. PCR was performed in a final volume of 25 µL containing 0.5 µM of each primer. The PCR protocol consisted of an initial denaturation step at 94°C for 4 minutes, then 30 amplification cycles at 94°C for 30 seconds, at 55°C for 30 seconds and at 72°C for 1 minute, followed by a final extension step at 72°C for 5 minutes. Amplified products were analysed by electrophoresis on 1% agarose gel containing safe stain.

spa typing

spa typing was performed for MRSA isolates as described by Harmsen et al. [15]. The amplified products were subjected to DNA sequencing by Bioneer (Seoul, South Korea). The obtained sequences were edited by Chromas 1.45 software (Technelysium, Tewantin, Australia; <http://technelysium.com.au/wp/chromaspro/>). The guidelines from the Ridom Spaserver database (<http://spaserver.ridom.de/>) were used to assign the edited sequences to particular *spa* types.

agr typing

The *agr* types of all *S. aureus* isolates (MSSA and MRSA) were determined by the PCR method as described by Shopsis et al. [16].

Results

A total of 167 *S. aureus* strains were obtained in this study, of which 108 (64.7%) were found to be MRSA. The distribution of

TABLE 1. Frequency of MRSA and MSSA in patients, HCWs and surfaces in Shahid Motahari Hospital

Characteristic	No. of samples	MRSA	MSSA	Total
Patients	175	79 (64.2)	44 (35.8)	123 (100)
HCWs	88	7 (50)	7 (50)	14 (100)
Surfaces	142	22 (73.3)	8 (26.7)	30 (100)
Total	405	108 (64.7)	59 (35.3)	167 (100)

Data are presented as n (%).
HCW, healthcare worker; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*.

MRSA isolates among patients, HCWs and surfaces were 64.2% (n = 79), 50% (n = 7) and 73.3% (n = 22), respectively (Table 1). According to antibiotic susceptibility testing results, high rate of resistance to antibiotics such as cefoxitin (99%, n = 107), penicillin (96%, n = 104), norfloxacin (87%, n = 94), gentamicin (86%, n = 93), ampicillin (85%, n = 92), mupirocin and erythromycin (81%, n = 87), clindamycin (75%, n = 81) and rifampicin (65%, n = 70) was observed among MRSA strains. No resistance was observed for linezolid or tigecycline. The most frequent SCCmec type among 108 MRSA isolates was SCCmec type III (75%; n = 81), followed by type I (20.4%; n = 22) and type IV (1.8%; n = 2). The rest of the MRSA isolates (2.8%; n = 3) were nontypeable by this method. Also, by using the agr typing method, 78.4% (n = 131), 10.2% (n = 17) and 4.8% (n = 8) of isolates belonged to agr type I, II and III, respectively, and 6.6% of isolates (n = 11) were nontypeable. The results of SCCmec and agr typing are presented in Tables 2 and 3. spa types t030 and t037 were the first and second most predominant spa types

TABLE 2. Results of SCCmec typing among MRSA isolates

SCCmec type	Patient	HCW	Surface	Total
I	16 (14.8)	0	6 (5.6)	22 (20.4)
III	58 (53.7)	7 (6.5)	16 (14.8)	81 (75)
IV	2 (1.8)	0	0	2 (1.8)
Nontypeable	3 (2.8)	0	0	3 (2.8)
Total	79 (73.1)	7 (6.5)	22 (20.4)	108 (100)

Data are presented as n (%).
HCW, healthcare worker; MRSA, methicillin-resistant *Staphylococcus aureus*.

TABLE 3. Genotyping of *Staphylococcus aureus* isolates by agr typing method

agr type	Patient	HCW	Surface	Total
I	94 (56.3)	13 (7.8)	24 (14.3)	131 (78.4)
II	11 (6.6)	2 (1.2)	4 (2.4)	17 (10.2)
III	8 (4.8)	0	0	8 (4.8)
IV	0	0	0	0
Negative	10 (6)	0	1 (0.6)	11 (6.6)
Total (%)	123 (73.7)	15 (9)	29 (17.3)	167 (100)

Data are presented as n (%).
HCW, healthcare worker.

in all patient, HCW and surface isolates, being found in 56.4% (n = 57) and 25.6% (n = 26) of isolates, respectively. Also, spa type t16471 was reported for the first time in the world in this study (Table 4). Distribution of molecular types and different resistant patterns in 101 MRSA isolates are shown in Table 4 (seven isolates were not typed by spa typing).

Discussion

Because of the damage to the first mechanical barrier of the skin, burn patients are more prone to be colonized by different microorganisms. Sepsis is the major complication in burns and is associated with a high rate of mortality in burn patients [3]. Among different infection causing organisms, *S. aureus* and notably MRSA, is the major cause of HAIs as well as infections acquired from the community [17]. Different studies in Iran have shown an increase in the prevalence of MRSA in the country [18]. We found the prevalence of MRSA to be 64.7%, which is comparable to the results obtained by Namvar et al. [18] (65%) and Emaneini et al. (63.6%) [19]. This rate was higher than that reported by Darban-Sarokhalil et al. [2] (35%) and less than that of Song et al. [20] (98%). These discrepancies in the prevalence of MRSA among various studies might be due to the diverse antibiotic use patterns, different infection control policies and lack of supervision on antibiotic use in different centres. In this study, MRSA strains exhibited high rates of resistance to several antibiotics of different classes, notably mupirocin, which is the antibiotic of choice for the treatment of nasally carried staphylococcal infections.

S. aureus (MRSA) was found with high frequency in the hospital environment on different surfaces which can colonize the nares and hands of HCWs and therefore can be considered as an important source of dissemination to inpatients [6,21]. Nowadays typing methods are considered as important tools to assess the prevalence and transmission origin of HAIs [22]. In this study, we used three typing methods (SCCmec, spa and agr typing) to find the common lineages of *S. aureus* strains circulating in one burn hospital in Tehran. While SCCmec types I, II and III are known to be found among the hospital-acquired clones (HA-MRSA), types IV and V are mostly considered to be community acquired [23,24]. In this study, SCCmec types III and I were the most prevalent SCCmec types among MRSA isolates (75% and 20.4%, respectively). It can be concluded from this prevalence that the patients we studied might have acquired the infection from the hospital environment and/or HCWs as SCCmec type III, is related to HA-MRSA isolates. In previous studies from Iran performed by Fatholahzadeh et al. [25], Parhizgari et al. [22], Bayat et al. [26] and Namvar et al. [18], SCCmec type III was found among 98%, 97.5%, 82% and

TABLE 4. Distribution of molecular types and different antibiotic resistant patterns in 101 MRSA isolates

<i>spa</i> type (N; %)	No. patients	No. HCWs	No. surfaces	SCCmec type	<i>agr</i> type	Resistance profile	No. isolates
t030 (57; 56.4)	40	5	12	III, I	I	GM, MUP, RP, E, CD, T, IM, PG, NOR	48
t037 (26; 25.6)	23	1	2	III, I	I	NOR, SXT, PG GM, NOR, SXT, CD, E, PG, IM RP, MUP, GM, PG PG, IM, T, CD, E	16
t046 (3; 3)	3	0	0	I	I	E, CD, T, PG, IM, GM, MUP, RP, NOR	3
t4864 (2; 2)	2	0	0	III	II	IM, PG, SXT, NOR, E, T, GM	2
t091 (2; 2)	2	0	0	NT, III	II, I	E, CD, T, PG, GM, MUP, SXT	2
t789 (2; 2)	2	0	0	NT, I	III	C, PG, CD, E	2
t459 (1; 1), t16471 (1; 1)	1, 0	0, 1	0, 0	I, III	I	GM, MUP, RP, NOR, E, CD, T, PG, IM	2
t363 (1; 1)	1	0	0	IV	I	MUP, E, PG, IM	1
t3802 (1; 1), t304 (1; 1)	1, 1	0, 0	0, 0	NT, I	I	PG	2
t937 (1; 1)	1	0	0	I	II	PG, IM	1
t230 (1; 1)	1	0	0	IV	I	MUP, PG, IM	1
t3132 (1; 1), t701 (1; 1)	0, 1	0, 0	1, 0	I, III	II, I	GM, MUP, NOR, E, CD, T, PG, IM	2

C, chloramphenicol; CD, clindamycin; E, erythromycin; GM, gentamycin; IM, imipenem; MRSA, methicillin-resistant *Staphylococcus aureus*; MUP, mupirocin; NOR, norfloxacin; NT, nontypeable; PG, penicillin; RP, rifampicin; SXT, trimethoprim/sulfamethoxazole; T, tetracycline.

56.8% of MRSA strains, respectively. Similarly, Chen et al. [27] and Rodrigues et al. [28] reported SCCmec type III to be the dominant type among colonizers of burn patients.

In this study, 15 different *spa* types were found, among which t030 (56.4%) and t037 (25.6%) were the predominant types. These results are in agreement with other studies from Iran and other Asian countries [26]. In a study carried out in South Africa, t037 was reported as one of the most dominant *spa* types. However, Emaneini et al. [29] reported types other than t030 and t037 as the dominant *spa* types. These discrepancies in prevalence among different studies may be attributed to differences in the type of sample or even the time of sampling and geographical areas. It seems that t037 has been replaced by t030 *spa* type in Iran's hospitals [26,30]. In agreement with our study, several other studies have indicated that t030 and t037 types most commonly belonged to the SCCmec type III and were classified as HA-MRSA. Also, for the first time we found *spa* type t16471, which had not been reported to date in the world in an MRSA strain that had colonized the nose of a HCW. These findings raise serious concerns about the possible spread of MRSA from the hospital environment and HCWs to inpatients in Tehran's hospitals.

One of the crucial regulatory components of *S. aureus* involved in controlling the expression of bacterial virulence factors is accessory gene regulator (*agr*). So far, four *agr* types (I, II, III and IV) have been identified among *S. aureus* strains [31]. In our study, among the 167 *S. aureus* isolates, the predominant *agr* type was type I, with a frequency of 78.4%. This result is consistent with that of studies performed by van Leeuwen et al. [31] (71%), Peerayeh et al. [32] (55.1%) and Indrawattana et al. [33] (58.7%), who reported *agr* type I to be the most frequent *agr* type among *S. aureus* isolates. The prevalence of *agr* type I in our study was more than that reported by Shopsis et al. [16] in America (30.8%) and Ayed et al. [34] in Tunisia (15%). These

dissimilarities may be related to the differences in patient samples as well as in geographic location. Also, because infection control policies might be periodically changing in a healthcare setting, various isolate types might be found during different periods of sample collection.

In summary, we used three different typing methods in order to find the most common lineages of *S. aureus* (MSSA and MRSA) circulating in the hospital and the origin of their spread to patients. According to SCCmec typing results, we found that MRSA isolated from patients, HCWs and environmental surfaces had the same genotype. The most prevalent genotypic background of the isolates was SCCmec type III/t030, t037/*agr* type I, which was found in high frequency in patient, surface and HCW isolates. Therefore, better hospital infection control policies as well as continuous epidemiologic surveillance studies are highly recommended to efficiently prevent the spread of bacteria to inpatients.

Acknowledgement

Supported in part by grant 26563-30-04-94 from the Deputy of Research and Technology, Iran University of Medical Sciences.

Conflict of interest

None declared.

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