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

S. Abbasian¹, N. N. Farahani¹, Z. Mir¹, F. Alinejad², M. Haeili¹, M. Dahrmardehei³, M. Mirzaii¹, S. S. Khoramrooz⁵, M. J. Nasiri⁶ and D. Darban-Sarokhalil¹

¹Department of Microbiology, Faculty of Medicine, 2Burn Research Center, Shahid Motahari Hospital, Iran University of Medical Sciences, Tehran, 3) Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, 4) School of Medicine, Shahroud University of Medical Sciences, Shahroud, 5) Medicinal Plants Research Center, Faculty of Medicine, Yasuj University of Medical Sciences, Yasuj and 6) Department of Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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.

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

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Corresponding author: D. Darban-Sarokhalil, Department of Microbiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.
E-mail: darban.d@iums.ac.ir

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 (SCCmec), 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 SCCmec 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 SCCmec 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 SCCmec 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.

SCCmec typing
A multiplex PCR with four primer pairs was performed to identify the five main known SCCmec 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 Shopsin 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...
MRSA isolates were found among patients, HCWs, and surfaces (64.2% \((n = 79)\), 50% \((n = 7)\) and 73.3% \((n = 22)\), respectively) (Table 1). According to antibiotic susceptibility testing results, high rates of resistance to antibiotics such as cefoxitin (99%), penicillin (96%), norfloxacin (87%), gentamicin (86%), ampicillin (85%), mupirocin and erythromycin (81%), clindamycin (75%), rifampicin (65%), and linezolid (75%) were 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 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-Sarokhalli 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

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**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 (44.2) | 44 (35.8) | 123 (100) |
| HCWs                    | 88             | 7 (7.9)  | 10 (11.6) | 17 (19.6)  |
| Surfaces                | 142            | 22 (15.5) | 8 (5.7)  | 30 (21.2)  |
| Total                   | 405            | 108 (26.7) | 59 (14.6) | 167 (41.3) |

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

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**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) |
| II          | 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.

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**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 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 type I, which was found in high frequency in patient, surface and HCW isolates. Therefore, better hospital 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.

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### Conflict of interest

None declared.

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

| spa type (N; %) | No. patients | No. HCWs | No. surfaces | SCCmec type | agr type | Resistance profile | No. isolates |
|----------------|--------------|-----------|--------------|--------------|---------|--------------------|--------------|
| t030 (57; 56.4) | 40           | 5         | 12           | III, I       | I       | GM, MUP, RP, CD, T, IM, PG, NOR | 48           |
| t037 (26; 25.6) | 23           | 1         | 2            | III, I       | I       | GM, MUP, RP, CD, T, IM, PG, NOR | 16           |
| t046 (3; 3)     | 3            | 0         | 0            | I            | I       | GM, MUP, RP, CD, T, IM, PG, NOR | 3            |
| t4864 (2; 2)    | 2            | 0         | 0            | II           | I       | GM, MUP, RP, CD, T, IM, PG, NOR | 2            |
| t091 (2; 2)     | 2            | 0         | 0            | III          | II      | GM, MUP, RP, CD, T, IM, PG, NOR | 2            |
| t363 (1; 1)     | 1            | 0         | 0            | I            | I       | GM, MUP, RP, CD, T, IM, PG, NOR | 1            |
| t304 (1; 1)     | 1            | 0         | 0            | II           | I       | GM, MUP, RP, CD, T, IM, PG, NOR | 1            |
| t937 (1; 1)     | 1            | 0         | 0            | III          | II      | GM, MUP, RP, CD, T, IM, PG, NOR | 1            |
| t320 (1; 1)     | 1            | 0         | 0            | IV           | I       | GM, MUP, RP, CD, T, IM, PG, NOR | 1            |
| t3132 (1; 1), t701 (1; 1) | 0.1 | 0 | 0 | I, III | II | GM, MUP, RP, CD, T, IM, PG, NOR | 2 |

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