Jundishapur J Microbiol. 2017 February; 10(2):e40592. doi: 10.5812/jjm.40592.

Published online 2016 December 21. **Research Article**

Molecular Characterization and Distribution of Class 1 Integron-Bearing Methicillin Resistant *Staphylococcus aureus* Strains in Burn Patients, Tehran, Iran

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Received 2016 July 03; **Revised** 2016 November 29; **Accepted** 2016 November 29.

Abstract

Background: Methicillin resistant *Staphylococcus aureus* (MRSA) is an increasingly common hospital pathogen in burn patients, which is known to cause over 50% of burn related deaths. One of the serious threats associated with clinical isolates of MRSA is multi-drug resistance, which is associated with integrons.

Objectives: The aim of this study was to determine the distribution and molecular types of MRSA in burn patients and their carriage of integrons.

Methods: During a 7-month period, 106 MRSA isolates were collected from burn wounds of patients admitted to a referral burn hospital in Tehran. Antimicrobial susceptibility testing (AST) was performed for 12 antimicrobial agents. Polymerase chain reaction (PCR) was used to detect *nucA*, *mecA*, *pvl* and tsst-1 genes, and class 1 and 2 integrons. Multiplex PCR technique was used to determine the Staphylococcal cassette chromosome *mec* (*SCCmec*) types of MRSA strains. All isolates were genotyped by staphylococcal protein A (*spa*) typing.

Results: AST showed the lowest rate of resistance to quinupristin-dalfopristin (19.8%), mupirocin (31.3%), and rifampicin (37.7%). All isolates were susceptible to vancomycin, teicoplanin, and linezolid. Multi-drug resistance was observed in 97% of isolates. The most *SCCmec* type was *SCCmec* type III (98.1%) while only 2 (1.9%) MRSA isolates harbored *SCCmec* type IV. *SCCmec* types I, II, and V were not detected. The study revealed the presence of class 1 integron in 58 (54.7%) isolates and class 2 integron in 3.8% of isolates. Six different *spa* types of t030 (66%), t037 (14.2%), t065 (9.4%), t1358 (4.7%), t937 (3.8%), and t084 (1.9%) were identified amongst the isolates.

Conclusions: The study revealed a high prevalence of multi-drug resistance (MDR), class 1 integron, *SCCmec* type III, and *spa* type t030 amongst MRSA associated with burn wounds in an Iranian hospital. The existence of *SCCmec* type III in burn patients emphasizes the nosocomial origin of these strains.

Keywords: *SCCmec* Type, spa Type, MRSA, Integron, Burn Patient

1. Background

Nosocomial infections is a major cause of morbidity and mortality in burn patients and are a great concern of global public health in both developing and developed countries. Due to loss of the functional skin barrier, burn patients are prone to general systemic disorder, bacterial colonization and various infections (1).

More than 70% of deaths in burn patients occur due to bacterial infections. It is well established by many published data that *Staphylococcus aureus*, in particular methicillin resistant *S. aureus* (MRSA) strains, is one of the most common pathogens and have become increasingly common in burn wounds (1, 2). The first MRSA isolate was reported in 1961 from the UK (3). Since then, studies have revealed a steady increase in the incidence of infections caused by MRSA. Resistance to methicillin is mediated by *mecA* gene, which is carried within a large heterologous mobile genetic element called Staphylococcal cassette chromosome *mec* (*SCCmec*). So far, 11 different types of *SCCmecI* (*SCCmec*I-XI) have been classified based on their structural organization and genetic content. *SCCmec* types I to III are the most prominent types in nosocomial MRSA (4).

During the past several decades, in spite of the introduction of a variety of new therapeutic agents, MRSA

strains still show a remarkable ability for rapid dissemination of resistance; to the extent that, currently, one of the threats to public health is the increasing prevalence of multi-drug resistance (MDR) amongst MRSA isolates (5). An increase of resistance not only leads to increased economic burden, but also has limited the treatment options for burn wound infections.

Although, the mechanisms of resistance amongst bacteria are very different and complex, it has been shown that horizontal gene transfer mediated by mobile genetic elements, e.g., plasmids and transposons, contributes to the spread of antibiotic resistance genes amongst bacteria. However, recently it has been well established that spreading multi resistance in MRSA strains are linked to integrons (5, 6). They are widely known for their role in the spreading of MDR amongst both Gram-positive and especially Gram-negative pathogens (7). The basic structures of these elements consists of two conserved segments (5′and 3′-CS) and an internal variable region (VR) that contains gene cassettes encoding antibacterial resistance determinants. All known integrons are composed of genes such as gene that encode an integrase (intI), recombination site (attI), and an outward orientated promoter (Pc), which directs transcription of the captured genes (8). To date, several classes of integrons have been described based on the homology of their integrase genes. Class 1 integrons are the most ubiquitous amongst both Gram-positive and especially in Gram-negative resistant clinical isolates. Class 2 integrons are less common than class 1 and have been frequently reported in Gram-negative bacteria. Other classes of integrons have rarely been reported (6).

It is well established that the combination of genotyping methods helps to describe different MRSA clones and analysis of the dynamics of *S. aureus* transmission in burn units (9). Despite the fact that *SCCmec* typing is used only for typing in MRSA isolates, protein A gene of *S. aureus* (*spa* typing), as a DNA sequence-based method with moderate discrimination, high throughput and good inter-laboratory reproducibility has emerged as an effective and rapid method for typing of all *S. aureus* isolates based on the number of tandem repeats and the sequence variation in region X of the protein A gene (8).

2. Objectives

The present study could provide data regarding the understanding of the molecular characterization of MRSA isolates by *spa* typing; identify different *SCCmec* types, and also frequency different classes of integron in a referral burn hospital in the capital city of Iran (Tehran).

3. Methods

3.1. Bacterial Strains

Between July 2015 and January 2016, the cross-sectional study was conducted on 106 MRSA isolates collected from burn wound patients who were admitted to a referral burn hospital in Tehran, Iran. Different clinical samples were transported to the laboratory within 4 hours of collection and were processed immediately. Isolation and identification of *S. aureus* isolates was performed based on conventional microbiological procedures such as colony morphology, Gram staining, growth on mannitol salt agar and production of catalase, coagulase, and DNase. MRSA screening was carried out using a cefoxitin disc (30 μg) on Mueller Hinton agar (Merck, Germany) plates supplemented with 4% NaCl and then confirmed by the PCR amplification of the *mecA* gene. Confirmed MRSA isolates were stored in Tryptic Soy Broth (TSB; Merck, Germany) containing 20% glycerol at -70°C for molecular investigation.

3.2. Antimicrobial Susceptibility Testing

Susceptibility testing was performed using a panel of 12 antibiotic disks for all isolates by the Kirby-Bauer disk diffusion procedure according to the guidelines of the clinical and laboratory standards institute (CLSI) (10). Antimicrobial drugs obtained from Mast (Mast Diagnostics, Group Ltd, Merseyside UK) were as follows: teicoplanin (TEC 30 μg), penicillin G (PG 10 U), clindamycin (CD 2 μg), erythromycin (E 15 μg), amikacin (AK 30 μg), gentamicin (GM 10 μg), linezolid (LZD 30 μg), mupirocin (MUP 200 μg), rifampicin (RP 5μg), quinupristin-dalfopristin (SYN 15 μg), and tetracycline (T 30 μg). The minimum inhibitory concentration (MIC) for vancomycin was determined by the E-test strips (AB BIODISK, Sweden) method. Multidrug resistance (MDR) was defined as a resistance of MRSA to 3 or more unique antimicrobial drug classes in addition to beta-lactams. The standard reference strain of *S. aureus* ATCC25923 was used as a quality control (QC) strain in every test run.

3.3. Genomic DNA Extraction

The QIamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) for genomic DNA extraction were used according to the manufacturer's instruction. Lysostaphin (Sigma-Aldrich, USA), to a final concentration of 15 μg/mL, was used for cell wall lysis. DNA purity was determined by taking the optical density (OD) of the sample at 280 nm for protein concentration and at 260 nm for DNA concentration with the help of a spectrophotometer. The ratio OD_{260} /OD_{280} was calculated and a DNA sample within the range of 1.6 - 2 was considered as pure. Samples above this range were considered contaminated with protein and those below by RNA.

3.4. PCR Assays for Detection of Toxin-Encoding Genes

PCR amplification of lukS-PV-lukF-PV (*pvl* genes) and toxic shock syndrome toxin (*tsst*) gene was performed with degenerate primers described by Azimian and Jarraud (11, 12) (Table 1).

3.5. Multiplex PCR Amplification for SCCmec Typing

Different types of *SCCmec* were identified by comparing the banding patterns of MRSA to ATCC 10442 (*SCCmec* type I), N315 (*SCCmec* type II), 85/2082 (*SCCmec* type III), MW2 (*SCCmec* type IVa), and WIS (*SCCmec* type V), as reference strains (Table 1).

3.6. Spa Typing

Different *spa*-types were determined by PCR as previously described (8). The primer sequences are listed in Table 1. PCR products were subjected to DNA sequencing of both strands by Macrogen (Seoul, South Korea). The sequences obtained were edited using the Chromas software (Version 1.45, Australia). Edited sequences were assigned to particular *spa* types according to the guidelines described by the *spa* typing website (http://www.*spa*server.ridom.de).

3.7. Amplification of Integrons

The presence of integron classes in MRSA isolates was investigated by PCR as previously described by Moura et al. (13). The expected amplified products, 280 bp for integron class 1 and 233 bp for integron class 2 were separated on 1% agarose gel (Invitrogen, Carlsbad, CA, USA) in TAE buffer and electrophoresis at 80 V for 1 hour. Bands were visualized using an ultraviolet light (UVI tec, Cambridge, UK) after staining with ethidium bromide (Sigma-Aldrich, USA).

3.8. Statistical Analysis

A statistical analysis was performed using the SPSS software for Windows (version 18.0; SPSS Inc., Chicago, IL).

4. Results

A total of 106 MRSA isolates were investigated. Of these isolates, 85 (80.2%) were men and 21 (19.8%) were women. Results of antimicrobial susceptibility testing of the 106 MRSA isolates revealed that all of the isolates were susceptible to vancomycin (MIC_{50} and MIC_{90} 1 μg/mL), teicoplanin, and linezolid. The most frequent resistance was observed for penicillin (100%), amikacin (83.9%), and erythromycin (84%). The lowest rate of resistance was observed for quinupristin-dalfopristin (19.8%), high-level mupirocin-resistant (31.3%), and rifampicin (37.7%). Antibiotic resistance pattern of 106 MRSA isolates collected from burn patients are presented in Table 2.

In total, 97% of the isolates were MDR. The predominant MDR profiles amongst the isolates included resistance to 7 drugs (23.6%), 6 drugs (62.3%), 4 drugs (1.9%), and 3 drugs (12.3%). *SCCmec* typing showed that 104 (98.1%) of the MRSA isolates harbored *SCCmec* type III while only 2 (1.9%) MRSA isolates harbored type IV. *SCCmec* types I, II, V were not detected in any of the MRSA strains.

All MRSA isolates were successfully *spa* typed and differentiated into 6 different types. The most common *spa* types were t030 (66%) and t037 (14.2%). Other *spa* types found in smaller numbers were t065 (9.4%), t1358 (4.7%), t937 (3.8%), and t084 (1.9%). Two isolates PVL-positive belong to *spa* type t084. Distribution of *SCCmec* types and *spa* types in MRSA isolates with different resistance patterns are presented in Table 3.

The study revealed the presence of class 1 integron in 58 (54.7%) isolates, while only 4 isolates (3.8%) carried class 2 integron. Co-existence of class 1 and 2 integrons was not detected and none of the isolates were positive for class 3 integron. Among the isolates carrying class 1 integron, *spa* types t030 (n = 42, 72.4%), t037 (n = 10, 17.2%), and t065 (n = 6, 10.4%) were more prevalent while all isolates carrying class 2 integrons belonged to *spa* type t030.

5. Discussion

The incidence of infections due to *S. aureus*, particularly MRSA strains, in burn patients is increasing worldwide (1, 5). Due to limited data on the distribution of MRSA genotypes isolated from burn patients in Iran, the present study was designed to provide this needed data by investigating MRSA isolated from burn patients in a referral burn hospital in Tehran.

With regards to the continuous changing pattern of antibiotic resistance in MRSA isolates, determination of antibiotic resistance pattern for epidemiological and clinical purposes is an important principle (5). As presented in Table 1, the MRSA isolates were highly resistant to penicillin (100%), erythromycin (84%), amikacin (83.9%), and tetracycline (82.1%), respectively. The antibiotic resistance pattern of MRSA isolates in the present study was parallel with the findings of Parhizgari et al. (14) and Ko et al. (15). Furthermore, similar to other study (11), the susceptibility results revealed that vancomycin, teicoplanin, and linezolid had good activity against MRSA infections. A previous study showed the emergence of MRSA with increased resistance to vancomycin in Iran (11). In line with our findings, Yali et al. (1) and Bahemia et al. (16) showed that none of the *S. aureus* isolates were resistant to vancomycin.

The results may reflect the appropriate prescription of these antibiotics and the successful implementation of surveillance and infection control programs in health care

Table 1. Oligonucleotide Primers Used in This Study

Target	Primer	Primer Sequence ($5' \rightarrow 3'$)	Product Size (bp)
mecA	F	AGAAGATGGTATGTGGAAGTTAG	583
	R	ATGTATGTGCGATTGTATTGC	
luk-PV	F	TTCACTATTTGTAAAAGTGTCAGACCCACT	180
	R	TACTAATGAATTTTTTTATCGTAAGCCCTT	
tsst-1	F	TTATCGTAAGCCCTTTGTTG	398
	R	TAAAGGTAGTTCTATTGGAGTAGG	
intI1	F	CCTCCCGCACGATGATC	280
	R	TCCACGCATCGTCAGGC	
intI2	F	TTATTGCTGGGATTAGGC	233
	R	ACGGCTACCCTCTGTTATC	
spa	F	AGACGATCCTTCGGTGAGC	Variable
	R	GCTTTTGCAATGTCATTTACTG	
SCCmec	Fβ	ATTGCCTTGATAATAGCCYTCT	937
	R α3	TAAAGGCATCAATGCACAAACACT	
	F ccrC	CGTCTATTACAAGATGTTAAGGATAAT	518
	R ccrC	CCTTTATAGACTGGATTATTCAAAATAT	
	F 1272	GCCACTCATAACATATGGAA	415
	R 1272	CATCCGAGTGAAACCCAAA	
	F 5R*mecA*	TATACCAAACCCGACAACTAC	359
	R 5R431	CGGCTACAGTGATAACATCC	
	F	ATCATTAGGTAAAATGTCTGGACATGATCCA	433
	R	GCATCAAGTGTATTGGATAGCAAAAGC	

Table 2. Antibiotic Resistance Pattern of 106 MRSA Isolates Collected From Burn Patients[a]

Antibiotics (Disc Content)	Interpretation of Antibiotic Susceptibility Testing		
	R	I	S
Penicillin G, 10 U	106 (100)	0	0
Vancomycin, 30 μg	0	0	106 (100)
Teicoplanin, 30 μg	0	0	106 (100)
Clindamycin, 2 μg	81 (76.4)	3 (2.8)	22 (20.8)
Erythromycin, 15 μg	89 (84)	1 (0.9)	16 (15.1)
Amikacin, 30 μg	89 (83.9)	4 (3.8)	13 (12.3)
Gentamicin, 10 μg	78 (73.6)	0	28 (26.4)
Tetracycline, 30 μg	87 (82.1)	0	19 (17.9)
Rifampicin, 5 μg	40 (37.7)	3 (2.8)	63 (59.4)
Linezolid, 30 μg	0	0	106 (100)
Mupirocin, 200 μg	33 (31.1)	0	73 (68.9)
Quinupristin-dalfopristin, 15 μg	21 (19.8)	0	85 (81.1)

[a]Values are expressed as No. (%).

settings. Mupirocin is usually used for the treatment of different types of staphylococcal skin infections. The rate of high-level mupirocin-resistant in this study was 31.1%, that is in accordance with the results of another study from Iran done by Shahsavan et al. (17) (25%) but is higher than what was reported in India (5% (18), Greece (1.6%) (19), and Jordan (2.6%) (20). Unfortunately, in the present study, resistance to mupirocin was relatively high, which could be attributed to the inappropriate use of mupirocin in treatment of skin and soft tissue infections and elimination of MRSA nasal carriers among patients and medical staff. Clindamycin is an effective and reliable agent for the treatments of penicillin-allergic patients. The present study demonstrated a high level of resistance to clindamycin (76.4%), that is similar to the studies of Korea (69%) (2) and USA (65%) (21). The reason for the high rate of clindamycin resistance in this study could be attributed to the extensive use of this antibiotic in the clinic. Furthermore, 73.6% of our isolates were resistant to gentamicin, which was consistent with the findings reported by Yali et al. (1), Abbasi-

Table 3. Distribution of *SCCmec* Types and *spa* Types in MRSA Isolates With Different Resistance Patterns

Resistance Profile	Number of Isolates (%)	*SCCmec* Type (n)	*spa* Types (n)
PG, AK, E, T, GM, RP, MUP	25 (23.6)	III (25)	t030 (20), t037 (5)
PG, AK, E, T, CD, GM	45 (42.5)	III (45)	t030 (29), t037 (9), t065 (7)
PG, AK, T, CD, RP, SYN	13 (12.3)	III (13)	t937 (4), t030 (9)
PG, E, CD, GM, MUP, SYN	8 (7.5)	III (6); IV (2)	t030 (3), t065 (3), t084 (2)
PG, AK, E, CD, RP	2 (1.9)	III (2)	t030 (2)
PG, AP, E, CD	9 (8.5)	III (9)	t030 (3), t037 (1), t1358 (5)
PG, AK, T, CD	4 (3.8)	III (4)	t030 (4)

Abbreviations: PG, penicillin; CD, clindamycin; E, erythromycin; AK, amikacin; GM, gentamicin; MUP, mupirocin; RP, rifampicin; SYN, quinupristin-dalfopristin; T, tetracycline.

Montazeri et al. (22), and Babakir-Mina et al. (23). Our results showed that 37.7% of MRSA isolates were resistant to rifampicin. Other studies have reported different rates of resistance to rifampicin in burn units which varies from 57% in Iraqi Kurdistan (23) to 2% in Germany (24).

The observation of MDR in 97% of the MRSA in this study confirms the huge challenge of MDR in public health. Similar high prevalence of MDR among MRSA have been reported amongst burn-injury patients in China (100%) (6) and Taiwan (75.8%) (5).

SCCmec type III, detected in 98.1% of the MRSA, was the dominant *SCCmec* type in this study and was similar to results reported previously by Parhizgari et al. from Iran (14) and Brazil (25) where high frequency of *SCCmec* type III was prevalent. As stated by other investigators, *SCCmec* types I, II, and III are related to hospital acquired-MRSA (HA-MRSA) while *SCCmec* types IV and V are prominent types in community acquired-MRSA (CA-MRSA) (26). The high frequency of *SCCmec* type III in our study emphasizes the nosocomial origin of these strains in the burn unit. *SCCmec* type IV was identified in 1.9% of the isolates that were also PVL- positive. Based on the previous literature, PVL prevalence varies widely amongst nations, ranging from 2% to 35% among MRSA strains (27).

The genetic diversity of the MRSA isolates was evaluated using *spa* typing. The common *spa* types in MRSA isolates vary in different geographic regions (26). Our analysis of 106 MRSA clinical isolates using *spa* typing revealed 6 different *spa* types with t030 detected in 66% of the isolates as the most prevalent *spa* type. *Spa* type t030 was previously reported in another conducted study done by Japoni-Nejad et al. (28) from Iran and is in agreement with findings of Chen et al. (29) who suggested that strains with t030 was successfully established as the dominant *spa* type in hospitals in China. In contrast to other reports from Iran (14), Saudi Arabia (30), and Malaysia (31), our data demonstrated low frequency of t037 (14.2%) with variability in resistances pattern, among MRSA isolated from burn-injury patients.

In this study, t065 was detected in 9.4% of the isolates. Similarly, Shakeri et al. (32), also reported *spa* type t065 at low frequency (1%). Sangvik et al. (33) reported that t012 (8.8%), t084 (5.6%), and t065 (5.2%) were the most common *spa* types identified in 728 persistent nasal carriers in North Norway. Other *spa* types identified with low frequency in this study included t1358 (4.7%), t937 (3.8%), and t084 (1.9%). The distribution of these *spa* types was in agreement with results of the previous surveys in Iran (28, 32). Although t937 was reported more in clinical *S. aureus* strains, there is evidence that it can be observed in methicillin sensitive *S. aureus* (MSSA) and as well as in strains recovered from animals (34). Additionally, the observed *spa* type distribution of MRSA strain isolates in our study mirrored high genetic diversity of MRSA in burn patients.

As previously stated, integrons are widely known for their role in the dissemination of antibiotic resistance amongst pathogenic bacteria. In this study, class 1 and 2 integrons were detected in 58 (54.7%) and 4 isolates (3.8%) isolates, respectively. These results are in agreement with results of the previous studies in which the detection rate of integron class 1 was more than that for integron class 2 (6, 35). Xu et al. (6)in China, reported class 1 integron in 53% of the *S. aureus* isolates. In contrast to our results, Guney et al. (35) from Turkey revealed that none of the isolates harbored class 1 integron. The high prevalence of class 1 integron in our study strongly supports suggestions that class 1 integron may serve as reservoirs of antimicrobial resistance in MRSA strains.

In conclusion, the present study investigated antibiotic susceptibility data, integron frequency and genetic background MRSA in burn patients that revealed vancomycin, teicoplanin, and linezolid were efficient therapeutic options for MRSA infections. We also confirmed the presence of t030, t037, t065, t1358, t937, and t084 with a high level of MDR in burn-injury patients. High occurrence of MDR in burn units has potentially catastrophic consequences. High frequency of integron class 1 emphasizes

that antibiotic resistance remains a major problem. Accordingly, infection control measures to reduce the spread of multi-resistant strains and also halt the rapid evolution of antibiotic resistance must be prioritized in burn units in Iran.

Acknowledgments

This work was supported by a research grant from the research deputy of Shahid Beheshti University of Medical Sciences (Grant No. 8406).

Footnotes

Authors' Contribution: Study concept and design: Mehdi Goudarzi, Sima Sadat Seyedjavadi and Hossein Goudarzi; acquisition of data: Elham Beiranvand and Maryam Fazeli; analysis and interpretation of data: Mehdi Godarzi and Hossein Goudarzi; drafting of the manuscript: Mehdi Goudarzi, Hossein Goudarzi and Edet E Udo; critical revision of the manuscript for important intellectual content: Maryam Fazeli, Mehdi Goudarzi and Edet E Udo; statistical analysis: Elham Beiranvand and Sima Sadat Seyedjavadi; administrative, technical, and material support: Mehdi Goudarzi, Sima Sadat Seyedjavadi, Maryam Fazeli; study supervision: Maryam Fazeli, Mehdi Goudarzi.

Funding/Support: This work was supported by a research grant from research deputy of Shahid Beheshti University of Medical Sciences (Grant No. 8406). Research deputy has no role in the design of the study or the collection, analysis, and interpretation of data as well as in writing the manuscript.

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