Characterization of SCCmec and spa types of methicillin-resistant Staphylococcus aureus isolates from health-care and community-acquired infections in Kerman, Iran

Yaser Fasihi, Somayeh Kiaei, Davood Kalantar-Neyestanaki

To cite this article: Yaser Fasihi, Somayeh Kiaei, Davood Kalantar-Neyestanaki (2017) Characterization of SCCmec and spa types of methicillin-resistant Staphylococcus aureus isolates from health-care and community-acquired infections in Kerman, Iran, Journal of Epidemiology and Global Health 7:4, 263–267, DOI: https://doi.org/10.1016/j.jegh.2017.08.004

To link to this article: https://doi.org/10.1016/j.jegh.2017.08.004

Published online: 16 April 2019
Characterization of SCCmec and spa types of methicillin-resistant *Staphylococcus aureus* isolates from health-care and community-acquired infections in Kerman, Iran

Yaser Fasihi, a, Somayeh Kiaei, a, Davood Kalantar-Neyestanaki b, a

a Student Research Committee, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

b Department of Microbiology and Virology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

**ARTICLE INFO**

**A B S T R A C T**

Spread of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates is a worldwide problem. Molecular typing is a useful tool to understand MRSA epidemiology. Herein, we determined vancomycin-resistant, SCCmec and spa types among MRSA isolates recovered from healthcare and community-acquired infections in Kerman, Iran. A total of 170 *S. aureus* isolates were collected from different patients who were admitted to affiliated hospitals of Kerman University of Medical science. MRSA and vancomycin-resistant *S. aureus* (VRSA) isolates were detected by phenotypic methods. Polymerase chain reaction (PCR) technique was used for detection of *mecA*, *vanA* and *vanB* genes. Staphylococcal cassette chromosome (*mec*SCCmec) and spa typing were used for molecular typing of among MRSA isolates. Overall, 53% of isolates were considered as MRSA. Two MRSA isolates were resistant to vancomycin and *vanA* was detected in only one of VRSA isolates. SCCmec type III belonged to spa types 1030 and 1459 which they were the dominant spa types among community-associated MRSA (CA-MRSA) and healthcare-acquired MRSA (HA-MRSA) isolates. Our findings showed that the SCCmec type I and III spread from hospital settings to community, although the SCCmec type IV spread from community to healthcare systems. We have also reported VRSA isolates from hospitalized patients, therefore, appropriate policies should be enforced in order to prevent the spread of antibiotic resistance isolates in hospitals settings.

© 2017 Ministry of Health, Saudi Arabia. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

*Staphylococcus aureus* is a Gram-positive bacterium that is the most common cause of hospital and community-acquired infections [1]. Methicillin-resistant *S. aureus* (MRSA) isolates have been reported from several countries worldwide and now have become a global epidemic [2,3]. MRSA isolates are generally multi-drug resistant strains and therapeutic options for these isolates are dramatically reduced [4]. Vancomycin is an important antibiotic to treat MRSA isolates, so the emergence of vancomycin-resistant *S. aureus* (VRSA) strains are global serious threats to the public health [4,5]. However, VRSA isolates were reported in Iran and other countries [6,7]. Two mechanisms, including cell wall changes and acquired of *van* genes were involved in resistance to vancomycin in *S. aureus* [1]. Several methods such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), SCCmec and spa typing are usually used for molecular typing of MRSA isolates. PFGE and MLST methods are too expensive and time consuming methods, but SCCmec and spa typing are easy to interpret [8]. The staphylococcal cassette chromosome *mec* (SCCmec) mobile element is responsible for methicillin-resistant in *S. aureus* and at least 11 (I-XI) major types of SCCmec have been reported in *Staphylococcus* species [2,3,9]. SCCmec type I, II and III are the most common SCCmec types in healthcare-acquired MRSA (HA-MRSA), although, SCCmec type IV is a prominent SCCmec type among community-associated MRSA (CA-MRSA) [1,2]. Protein A (Spa) is one of the virulence factors on the surface of *S. aureus*, that prevents the phagocytosis of the bacteria by the immune system [10,11]. A hypervariable region in *spa* gene in the name of Xr, is used for MRSA typing [2,3,10]. There are no reports about spa types among clinical isolates of MRSA in our region. The aim of this study was to determine of antibacterial susceptibility patterns, SCCmec and spa types among clinical isolates of MRSA from healthcare and community-acquired infections in Kerman, Iran.
2. Material and methods

2.1. Clinical isolates and patient conditions

Total of 170 non-duplicate *S. aureus* isolates were collected from different patients who admitted to hospitals of Kerman University of Medical Science, Iran, from February 2014 to December 2015. Clinical isolates were identified as *S. aureus* by biochemical methods and then, isolates were confirmed by detection of *nuc* gene. The PCR amplifications for the *nuc* gene were carried out as described previously [12,13]. We defined 'community acquired' (CA) and 'healthcare associated infections or hospitalized patients' (HA) according to the current CDC criteria [14].

2.2. Antibiotic susceptibility testing and detection of MRSA and VRSA isolates by phenotypic methods

Antibiotic susceptibility of isolates were determined by disk diffusion method on Mueller–Hinton agar (MHA; CONDA, Co, Spain) according to recommendations of the Clinical and Laboratory Standards Institute (CLSI), using the antibiotics (MAST, Co, UK) as follows: linezolid (30 μg), erythromycin (15 μg), clindamycin (2 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), tetracycline (30 μg) and trimethoprim/sulfamethoxazole (1.25/23.75 μg) [15]. The MRSA phenotype was detected by susceptibility of isolates to cefoxitin (FOX: 30 μg) on MHA according to recommendations of the CLSI. Resistance to vancomycin was screened by agar dilution methods on Brain-heart infusion agar medium (BHI; CONDA, Co, Spain) according to recommendations of the CLSI. Resistance to vancomycin was screened by agar dilution methods on Brain-heart infusion agar medium (BHI; CONDA, Co, Spain) as well as broth micro dilution method was employed to determine the minimum inhibitory concentration (MIC) for vancomycin [15]. *S. aureus* strain ATCC 25923 and *Enterococcus faecalis* strain ATCC 29212 were used as control in susceptibility test [15].

2.3. Detection of mecA, vanA and vanB genes by PCR

Polymerase chain reaction was used for detection of *mecA*, *vanA* and *vanB* among MRSA and VRSA isolates. The total DNA of bacteria were extracted by using Exgene™Clinic SV Kit (GeneALL, Co, Seoul, Korea) according to manufacturer's instructions. The PCR amplifications for the *mecA*, *vanA* and *vanB* genes were carried out as described previously [12,16]. The oligonucleotide primers used for amplification of the *mecA*, *vanA* and *vanB* genes were listed in Table 1.

2.4. SCCmec typing

We used a multiplex PCR, described by Boye et al. to SCCmec typing (SCCmec type I-V) of *mecA* positive isolates [17]. Amplification of SCCmec genes was performed in a final volume of 25 μL containing: 12.5 μL Red Master Mix PCR (Amplicon, Co, Denmark), 0.2 μL of each primer with concentration of 10 pmol/μL, and 2 μL of DNA template top up to 25 μL. PCR protocol was carried out in a thermal cycler (Biometra T1 Thermocycler) with initial denaturation at 94 °C for 4 min, followed by 30 cycles of denaturation at 94 °C for 60 s, annealing 55 °C for 60 s, extension at 72 °C for 1 min and was followed by a final cycle of extension for 4 min at 72 °C. PCR products were detected by electrophoresis by using agarose 2% in 0.5 × TBE buffer, stained by Green Viewer dye (Green Viewer™, Parstous Biotechnology, Co, Iran) and gel image obtained by using a gel documentation system (Gel Doc, UVltedc, Co, United Kingdom).

2.5. Spa typing

The hypervariable X region of the *spa* gene was amplified by using spa-1113f (5'-TAA AGA CGA TTC TCC GGT GAG C-3') and spa-1514r (5'-CAG CAG TAGTGC CGT TG TCT-3') primers in thermal cycler (Biometra T1 Thermocycler) according to Ridom SpaServer recommendations. PCR products were sequenced (Macrogen, Co, Korea) and then assigned through the Ridom web server (http://www.ridom.de/spaserver/).

2.6. Statistical analysis

Statistical analysis was performed with SPSS (v.22.0) statistics software. We used the Fisher's exact test for the comparison of data. A significant difference was statistically accounted at a p-value of <0.05.

3. Results

Total of 170 *S. aureus* isolates, 46(37%) were isolated from outpatients (CA) and 124(73%) isolated from inpatients (HA) who were 1–95 years old. Among 46 CA isolates 21 (45.7%) and 25 (54.3%) were collected from male and female respectively, and among 124 of HA isolates 78(63%) and 46(37%) were collected from male and female, respectively. The clinical isolates were obtained from different sources, including urine 61 (35.8%); wound 50 (29.4%); blood 23 (13.5%); bronchoalveolar lavage (BAL) 15 (8.8%); cerebrospinal fluid (CSF) 2 (1.2%) and other fluid body 19 (11.2%). The antimicrobial susceptibility test results were presented in Table 2. All of the isolates were susceptible to linezolid and two isolates were resistant to vancomycin. As shown in Table 2, the statistical correlation was not observed in the rate of antimicrobial resistance in CA comparison with HA isolates (p > 0.05). Fifty-three percent (n = 90) isolates were considered as MRSA by using phenotypic method and *mecA* gene was detected in for 86 (95.5%) out of fifty-three isolates. Total of 86 *mecA* positive isolates, SCCmec types I, II, III and IV were identified in 25(29%), 2(3%), 40 (46.5%) and 15(17.5%) of MRSA isolates, respectively and four isolates were not typeable. SCCmec type III was the most prevalent SCCmec type in HA-MRSA (53%) and CA-MRSA (30.7%) isolates. SCCmec type II was not detected among CA-MRSA isolates and SCCmec type IV was identified in 11(19.3%) of HA-MRSA isolates. Distribution of antibiotics resistance and SCCmec types among

| Target gene | Primer name | Oligonucleotide sequence (5'→3') | Product size (bp) | Reference |
|-------------|-------------|----------------------------------|------------------|-----------|
| mecA             | MECA-F  | TCCAGATTACAACCTCTACCAGG          | 162              | (12)      |
|                 | MECA-R  | CACTTCTACCTTGTTAAGG             |                  |           |
| nuc             | Nuc-F    | GCCATTGACGTGATACGGTT             | 279              | (13)      |
|                 | Nuc-R    | AGGCAAGCTTACGCACTAAGG           |                  |           |
| vanA            | VanA-F   | CGTAAATGAAATAGTGGCAAATT         | 1030             | (16)      |
|                 | VanA-R   | CCGTTTACGCCTTAATAGCAGTT         |                  |           |
| vanB            | VanB-F   | GTGACAAACCGCGAGGGA              | 433              |           |
|                 | VanB-R   | CGGCCATCCTCTCGAAAAAA            |                  |           |
HA-MRSA and CA-MRSA have been shown in Table 3. MRSA isolates were classified in 17 distinct spa types, including t012, t018, t021, t030, t037, t081, t084, t267, t325, t459, t632, t853, t1358, t2894, t4718, t16099, t16100. The spa type t030 was the most common spa type among HA-MRSA and CA-MRSA isolates. In this study, two new spa types, including t16099 and t16100 were submitted in http://www.ridom.de/spaserver and we report (ed) these spa types for the first time in worldwide. The spa types t012, t018, t021, t632, t853, t2894 and t4718 were just observed in HA-MRSA and spa types t084, t16099 and t16100 were just identified in CA-MRSA. Among mecA positive isolates, the spa types t030, t459, t267 and t021 were as follow 39(45.5), 20(23.2%), 4(4.6%) and 4(4.6%), respectively. One of the VRSA isolates from the surgical wound sample was showed a positive result for vanA gene with MIC >64 μg/ml to vancomycin and which belonged to SCCmec III and also spa type t459. However, the other VRSA isolate from of urine sample of from a burn patient showed a negative result for van genes with MIC = 32 μg/ml to vancomycin and which belonged to SCCmec I and spa type t030. Distribution of SCCmec types, spa types and source of samples in HA-MRSA and CA-MRSA have been shown in Table 4. In this study, there was not a significant relationship through antibiotics resistance, SCCmec, spa types and the age and gender of patients (p > 0.05).

4. Discussion

Infection is the most important factor in increase of morbidity and mortality in hospitalized patients [18]. The spread of multi-drug resistant S. aureus strains have become a serious challenge in community and healthcare systems.

In this study, we investigated the resistance to different antibiotics among S. aureus isolates which were collected from healthcare and community-acquired infections.

MRSA isolates are serious threat for public health [2,3]. The prevalence of MRSA isolates has been reported 20.4–90% in different regions of Iran. Moreover, invasive MRSA infections incredibly affect the hospitalized patients [19–21]. In this study the methicillin-resistance among S. aureus isolates was 53%, which was lower than the other similar reports in Iran, such as Ahvaz (88.6%), and Tehran (75%) [20–21]. The difference between the occurrences of MRSA in Iran, support the use of different policies in infection control, inappropriate use of antibiotics in hospitals and community. Similar to other studies, our findings also suggested that vancomycin and linezolid are the most effective antibiotics against MRSA isolates [2,3,20–23]. This study is the first report of VRSA strain, which was belonged to SCCmec I and spa type t030 from burn center, however this strain was negative for vanA and vanB genes and which belonged MIC = 32 μg/ml to vancomycin. The genetic development of S. aureus isolates with low susceptibility to vancomycin is not well understood, although, cell wall changes may be involved in reduced susceptibility to vancomycin [1,24]. S. aureus isolates with decrease of susceptibility to vancomycin usually have emerged undergoing prolonged monotherapy with vancomycin in patients infected by MRSA, so more surveillance is needed in the use of vancomycin [25].

However, similar to this study, VRSA isolates were reported in Iran and other countries such as Brazil, USA, Korea, South Africa and France, have confirmed that resistance to vancomycin is becoming a global crisis [6,7].

In the present study, resistance to antibiotics in community was high, these findings are very important because the increase of antibiotic resistance in community can be lead to failure in empirical therapy [26,27]. Inappropriate use of antibiotic, ineffective infection control, hygiene practices and extensive use of antibiotics in agriculture are all factors that might be caused the increase of antibiotic resistance in community [26,27]. In this study, resistance to amikacin, gentamicin and trimethoprim/sulfamethoxazole was lower than other antibiotics in CA-MRSA isolates. These findings were similar to several studies in Iran. The resistance to amikacin and gentamicin in four province of Iran has been reported 19.3% from June 2010 to June 2011 among S. aureus isolates [3]. Our findings, showed that the resistance to trimethoprim/sulfamethoxazole was not prevalent, because this antibiotic is not routinely prescribed in health care systems.

Reports showed that SCCmec type III is the most common SCCmec type among HA-MRSA isolates. Namvar et al. in 2014 reported that the SCCmec type III was the predominant type among MRSA isolates which were recovered from burn patients [21]. In other studies, carried out by Montazeri and Japoni-Nejad et al. in Iran, SCCmec III was the most common SCCmec type among HA-MRSA isolates. In similar studies, in Spain, Malaysia and Brazil SCCmec type III had the most frequency among MRSA isolates from various samples [2,20,22,23].

In the present study, SCCmec types I and III which were belonged to spa t030 and t459 were the most common MRSA
types among HA-MRSA and CA-MRSA. In contrast to other studies, spa types, that were reported in this study, were not in agreement with other reports from Iran, moreover, in Iran, most MRSA isolates significantly related to spa types t701, t12311, t021, t037 and t790 [20,22]. These findings showed that different types of MRSA isolates have been spread in Iran. S. aureus with SCCmec types I, II, III are usually considered as healthcare-acquired methicillin-resistant S. aureus (HA-MRSA), while community-associated methicillin-resistant S. aureus (CA-MRSA) isolates are associated to SCCmec types IV and V [1,28]. Our findings confirmed that SCCmec types I and III were the most common types in community. These finding show the spread of HA-MRSA from the hospital setting to the community and spread of CA-MRSA from the community to hospital settings. Therefore, infection control policies play an important role to prevent the transmission of community-acquired infections to patients in hospitals, and vice versa. Since SCCmec III has usually located on large mobile elements usually cannot be transferred among S. aureus by horizontally genes transfer, the CA-MRSA isolates which are carried SCCmec III probably transferring health-care workers spread from hospitals settings to community [1,24,28]. Therefore, detection of carriage of S. aureus among staffs and patients helps us to decrease the spread of MRSA and its infections in the healthcare settings.

5. Conclusion

Finally, we suggest that new politics for infection control must be used to limiting the spread of MRSA and VRSA isolates in our hospitals settings, because based on our results, many MRSA isolates, which are involved in community-associated infections have originated HA-MRSA and vice versa. However, according to our study, the rate of MRSA isolates is very high in Kerman, Iran, therefore, the emergence of VRSA isolates is a worrying problem and could be caused to decrease the therapeutic options.

Conflicts of interest

None declared.

Funding/support

This research was supported by Kerman University of Medical Sciences and health services with Grant no: 95000434.

Ethic approval Code

This study was approved by ethical committee of Kerman University of Medical Sciences. The Ethic approval Code is IR. KMU.REC.1395.859.

Table 4

| No. of isolates | Origin of sample | SCCmec type | spa type | CA-MRSA | HA-MRSA |
|----------------|-----------------|-------------|----------|---------|---------|
| 3              | Wound           | III         | t030     | 1       | 2       |
| 3              | Urine           | I           | t459     | 2       | 1       |
| 2              | Urine           | I           | t84      | 2       | –       |
| 2              | Urine           | III         | t030     | 5       | 2       |
| 1              | Urine           | I           | t267     | 1       | –       |
| 1              | Urine           | IV          | t16099   | 1       | –       |
| 1              | Urine           | I           | t16099   | 1       | –       |
| 3              | Urine           | III         | t267     | 2       | 1       |
| 9              | Urine           | I           | t030     | 7       | 2       |
| 1              | Urine           | I           | t030     | –       | 1       |
| 1              | Urine           | IV          | t1358    | 1       | –       |
| 1              | Urine           | I           | t325     | 1       | –       |
| 1              | Urine           | IV          | t16100   | 1       | –       |
| 1              | Blood           | IV          | t459     | –       | 1       |
| 1              | Blood           | I           | t459     | –       | –       |
| 1              | Blood           | III         | t030     | –       | 1       |
| 1              | Blood           | I           | t030     | –       | –       |
| 1              | Blood           | IV          | t021     | –       | 1       |
| 1              | Blood           | IV          | t018     | –       | –       |
| 1              | Blood           | IV          | t1358    | –       | 1       |
| 4              | Urine           | III         | t459     | –       | 4       |
| 1              | Wound           | III         | t012     | –       | 1       |
| 1              | Wound           | IV          | t459     | –       | 1       |
| 4              | BAL             | III         | t459     | –       | 4       |
| 1              | BAL             | IV          | t459     | –       | 1       |
| 3              | BAL             | III         | t030     | –       | 3       |
| 1              | Urine           | III         | t632     | –       | 1       |
| 1              | Wound           | II          | t037     | –       | 1       |
| 1              | Urine           | IV          | t459     | –       | 1       |
| 2              | Wound           | I           | t030     | –       | 2       |
| 1              | Wound           | I           | t459     | –       | 1       |
| 1              | Wound           | I           | t081     | –       | 1       |
| 1              | Wound           | I           | t1358    | –       | 1       |
| 1              | Wound           | IV          | t4718    | –       | 1       |
| 1              | Wound           | IV          | t325     | –       | 1       |
| 1              | Wound           | IV          | t2894    | –       | 1       |
| 1              | Wound           | IV          | t325     | –       | 1       |
| 1              | Wound           | III         | t853     | –       | 1       |
| 7              | Wound           | III         | t030     | –       | 7       |
| 2              | Wound           | III         | t459     | –       | 2       |
| 4              | Fluid body      | III         | t030     | –       | 4       |
| Total: 83      | –               | –           | 26       | 57      |

* VRSA isolates.
