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

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ABSTRACT

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, SCC*mec* 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 chromosomemec (SCC*mec*) 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. SCC*mec* type III belonged to *spa* types t030 and t459 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 SCC*mec* type I and III spread from hospital settings to community, although the SCC*mec* 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.

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), SCC*mec* and *spa* typing are usually used for molecular typing of MRSA isolates. PFGE and MLST methods are too expensive and time consuming methods, but SCC*mec* and *spa* typing are easy to interpret [8]. The staphylococcal cassette chromosome *mec* (SCC*mec*) mobile element is responsible for methicillin-resistant in *S. aureus* and at least 11 (I-XI) major types of SCC*mec* have been reported in Staphylococcus species [2,3,9]. SCC*mec* type I, II and III are the most common SCC*mec* types in healthcare-acquired MRSA (HA-MRSA), although, SCC*mec* type IV is a prominent SCC*mec* 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, SCC*mec* and *spa* types among clinical isolates of MRSA from healthcare and community-acquired infections in Kerman, Iran.

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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 follow; 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) 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 SCC*mec* typing (SCC*mec* type I-V) of *mecA* positive isolates [17]. Amplification of SCC*mec* 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 TCC TTC GGT GAG C-3′) and *spa*-1514r (5′-CAG CAG TAGTGC CGT TTG CTT-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, SCC*mec* 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. SCC*mec* type III was the most prevalent SCC*mec* type in HA-MRSA (53%) and CA-MRSA (30.7%) isolates. SCC*mec* type II was not detected among CA-MRSA isolates and SCC*mec* type IV was identified in 11(19.3%) of HA-MRSA isolates. Distribution of antibiotics resistance and SCC*mec* types among

Table 1
List of primers were used in this study.

Target gene	Primer name	Oligonucleotide sequence (5′–3′)	Product size (bp)	Reference
mecA	MECA-F	TCCAGATTACAACTTCACCAGG	162	(12)
	MECA-R	CCACTTCATATCTTGTAACG		
nuc	Nuc-F	GCGATTGATGGTGATACGGTT	279	(13)
	Nuc-R	AGCCAAGCCTTGACGAACTAAAGC		
vanA	VanA-F	CATGAATAGAATAAAAGTTGCAATA	1030	(16)
	VanA-R	CCCCTTTAACGCTAATACGATCAA		
vanB	VanB-F	GTGACAAACCGGAGGCGAGGA	433	
	VanB-R	CCGCCATCCTCCTGCAAAAAA		

Table 2
Antibacterial resistance profile of 170 *S. aureus* isolates recovered from of inpatients (HA-MRSA) and outpatients (CA-MRSA).

Type of isolates	Resistance to antimicrobial agents. n(%)										
	No. of isolates	AK	GM	CD	E	CIP	T	TS	FOX	MRSA	VRSA
HA-MRSA	124(73)	42(34)	50(40.3)	58(46.7)	69(55.6)	64(51.6)	79(64)	37(30)	64(51.6)	64(51.6)	2(1.6)
CA-MRSA	46(37)	11(24)	19(41.3)	24(52)	32(69.5)	19(41.3)	28(61)	17(37)	26(56.5)	26(56.5)	–
Total	170(1 0 0)	53(31)	69(40.5)	82(48.2)	101(59.4)	83(48.8)	107(63)	54(31.8)	90(53)	90(53)	2(1.2)

AK; Amikacin, GM; Gentamicin, CD; Clindamycin, E; Erythromycin, CIP; Ciprofloxacin, T; Tetracycline, TS; trimethoprim/sulfamethoxazole, FOX; cefoxitin.

Table 3
Distribution of MRSA, SCC*mec* types and source of samples among 170 *S. aureus* isolates, n(%).

Source (n)	MRSA	*mecA*	HA-MRSA	CA-MRSA	SCC*mec* type (n)
Urine (66)	38(57.6)	38(100)	15(39.4)	23(60.6)	SCC*mec* I(19), SCC*mec* III(15) & SCC*mec* IV(4)
Wound (50)	26(52)	24(92.3)	25(52)	1(41.6)	SCC*mec* I(4), SCC*mec* II(1), SCC*mec* III(15) & SCC*mec* IV(3)
Blood (23)	8(34.7)	8(100)	8(34.7)	–	SCC*mec* I(2), SCC*mec* III(1) & SCC*mec* IV(5)
BAL*(15)	12(80)	11(91.6)	11(80)	1(8.33)	SCC*mec* III(7) & SCC*mec* IV(3)
CSF*(2)	–	–	–	–	–
Fluid body (14)	5(35.7)	4(80)	5(47.4)	–	SCC*mec* III(4)
Total (170)	90(52.9)	86(95.5)	64(71.1)	26(28.9)	SCC*mec* I(25), SCC*mec* II(1), SCC*mec* III(42) & SCC*mec* IV(15)

* BAL: *Bronchoalveolar lavage*, CSF: Cerebrospinal fluid.

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 $\geq$64 μg/ml to vancomycin and which belonged to SCC*mec* III and also *spa* type t459. However, the other VRSA isolate from of urine sample of from a burn patient showed a negative result for *van* genes with MIC = 32 μg/ml to vancomycin and which belonged to SCC*mec* I and *spa* type t030. Distribution of SCC*mec* types, *spa* types and source of samples in HA-MRSA and CA-MRSA have been shown in Table 4. In this study, there was not a significant relationship through antibiotics resistance, SCC*mec*, *spa* types and the age and gender of patients ($p > 0.05$).

4. Discussion

Infection is the most important factor in increase of morbidity and mortality in hospitalized patients [18]. The Spread of multidrug 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 SCC*mec* I and *spa* type t030 from burn center, however this strain was negative for *vanA* and *vanB* genes and showed 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 SCC*mec* type III is the most common SCC*mec* type among HA-MRSA isolates. Namvar et al. in 2014 reported that the SCC*mec* 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, SCC*mec* III was the most common SCC*mec* type among HA-MRSA isolates. In similar studies, in Spain, Malaysia and Brazil SCC*mec* type III had the most frequency among MRSA isolates from various samples [2,20,22,23].

In the present study, SCC*mec* types I and III which were belonged to *spa* t030 and t459 were the most common MRSA

Table 4
Distribution of SCC*mec* types, *spa* types and origin of samples among HA-MRSA, CA-MRSA and VRSA isolates.

No. of isolates	Origin of sample	SCC*mec* type	*spa* type	CA-MRSA	HA-MRSA
3	Wound	III	t030	1	2
1*	**Wound**	**III**	**t030**	**–**	**1**
3	Urine	I	t459	2	1
2	Urine	I	t84	2	–
7	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
1	Blood	III	t030	–	1
1	Blood	I	t030	–	1
2	Blood	IV	t021	–	1
1	Blood	IV	t018	–	1
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	Urine	I	t1358	–	1
1	Wound	IV	t4718	–	1
1	Wound	IV	t325	–	1
1	BAL	IV	t2894	–	1
1	BAL	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.

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 SCC*mec* 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 SCC*mec* types IV and V [1,28]. Our findings confirmed that SCC*mec* 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, SCC*mec* 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 SCC*mec* 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.

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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.

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