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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		