Gene Reports 12 (2018) 289–293

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

Determination of antimicrobial resistance pattern and molecular characteristics of methicillin-resistant *Staphylococcus aureus* strains isolated from patients in a teaching hospital of Isfahan, Iran

Mojtaba Shahin[a], Sharareh Moghim[b], Seyed Rohollah Havaei[c], Fahimeh Ghanbari[d], Seyed Asghar Havaei[b,e,*]

[a] Department of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[b] Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[c] Department of Endodontics, Dental School, Khorasgan University, Isfahan, Iran
[d] Department of Molecular Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[e] Nosocomial Infection Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

ARTICLE INFO

Keywords:
Antibiotic resistance
MRSA
MDR
SCC*mec* typing

ABSTRACT

Background: *S. aureus* seems to play a significant role in the pathogenesis of infection in hospitals setting. This study aimed to the investigation of the prevalence of *S. aureus isolates* and the determination of antibiotic susceptibility pattern and the different types of *SCCmec* in MRSA isolates obtained from a clinical sample in Isfahan, Iran.

Methods: This study was performed during January 2013 to March 2014 at tertiary care hospitals. *S. aureus* isolates were collected from the clinical samples. Determination of antibiotic susceptibility pattern was done by the disc diffusion method. Also, *SCCmec* typing was performed by PCR method.

Results: A total of 150 *S. aureus* isolates were collected from the different clinical sample. Among them, 41.3% (62/150) isolates were MRSA. The most sensitivity for MRSA isolates was to vancomycin with 90% sensitivity and the high rate of resistance was toward tetracycline in MRSA and MSSA isolates. All of MRSA isolates were MDR. The rate of SCC*mec* types IV, III, I and II among MRSA isolates were 44.4%, 40.7%, 11.1% and 3.7, respectively.

Conclusions: The results of the current study showed that *S. aureus* especially MRSA isolates with a high rate of resistance still remains an important healthcare problem. Majority of the isolates were community-acquired and belonged to Scc*mec* type.

1. Introduction

Staphylococcus aureus is one of the leading pathogens of nosocomial infections worldwide and is responsible for a wide spectrum of diseases, ranging from bloodstream infections (BSIs), respiratory tract infections (RTIs) and skin and soft tissue infection (SSTI) (Hansra and Shinkai, 2011; Tong et al., 2015). These infections have high morbidity and mortality, especially when *S. aureus* has acquired resistance to methicillin (Raygada and Levine, 2009). Methicillin as a β-lactamase-resistant antimicrobial agent was first introduced in 1959 for treatment of *Staphylococcal* infections. However, in 1961, the first methicillin-resistant *Staphylococcus aureus* (MRSA) strain was identified in the United Kingdom (Enright et al., 2002). The resistance to methicillin is caused by acquiring the *mecA* gene. This gene encodes penicillin-binding protein 2a, (PBP2a or PBP2′) that has a low affinity for all beta-lactam antibiotics. For this reason, even in the presence of beta-lactam antibiotic, *S. aureus* can survive for a long time (Alfatemi et al., 2014; Fishovitz et al., 2014; Stapleton and Taylor, 2002). The *mecA* gene is inserted into a mobile genetic element called *staphylococcal* cassette chromosome mec (SCC*mec*). SCC*mec* is a mobile genetic and a vehicle

List of abbreviation: Bloodstream infections, (BSIs); skin and soft tissue infection, (SSTI); respiratory tract infections, (RTIs); meticillin-resistant *Staphylococcus aureus*, (MRSA); penicillin-binding protein 2a, (PBP2a or PBP2′); staphylococcal cassette chromosome mec, (SCC*mec*); coagulase-negative *staphylococci*, (CoNS); hospital-associated MRSA, (HA-MRSA); community-associated MRSA, (CA-MRSA); Panton-Valentine leukocidin, (PVL); Clinical and Laboratory Standards Institute, (CLSI); The minimum inhibitory concentration, (MIC)

* Corresponding author at: Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Hezar Jarib St, Isfahan, Iran.
E-mail address: havaei@med.mui.ac.ir (S.A. Havaei).

https://doi.org/10.1016/j.genrep.2018.07.004
Received 26 April 2018; Received in revised form 26 June 2018; Accepted 5 July 2018

for exchanging resistance genes between *Staphylococcus* strains (Halaji et al., 2017; Szczuka et al., 2013). Furthermore, SCC*mec* also is widely distributed among coagulase-negative *staphylococci* (CoNS) species (Halaji et al., 2017). SCC*mec* is composed of different combinations between the *mec* complex and the *ccr* complex. The *mec* complex consists of, *mecA* and two regulatory genes including *mecI* and *mecR1*. The ccr complex encodes recombinase enzymes (*ccrA* and *ccrB* or *ccrC*) responsible for the mobility of the genetic element. To date, from the combination of *ccr* complex with the *mec* gene complex, 11 types (I-XI) SSC*mec* have been reported (2009).

Based on isolation source, MRSA strains were classified into two main groups including hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) (Ebrahim-Saraie et al., 2015).

CA-MRSA infections almost occur in children, young adults, and middle-aged adults (David and Daum, 2010). The hosts of CA-MRSA isolates are different, ranging from healthy people to the individuals with underlying diseases. The CA-MRSA infections are transmitted by direct contact with infected patients, colonized subjects, or a contaminated environment (DeLeo et al., 2010; Al Amiry, 2015).

CA-MRSA strains almost express the genes for the Panton-Valentine leukocidin (PVL) and mainly carry SSC*mec* types IV or V (Valsesia et al., 2010). In contrast, HA-MRSA strains are associated with SSC*mec* type I, II, or III. Unlike to CA-MRSA strains, HA-MRSA strains are mostly resistant to several classes of non- beta-lactam antimicrobials. SCC*mec* typing has provided a strong evidence for distinguishing HA- MRSA from CA-MRSA strains (Moroney et al., 2007; Appelbaum, 2007). The aim of the present study was the investigation of the prevalence of *S. aureus* and the determination of antibiotic susceptibility pattern and different types of *SCCmec* in MRSA isolates obtained from a clinical sample in Isfahan, Iran.

2. Material and method

2.1. Study design and setting

This cross-sectional study was carried out between January 2013 to March 2014 at teaching hospital affiliated to Isfahan University of Medical Sciences (Al-Zahra), Iran. The samples from various clinical specimens were transferred to the laboratory and *S. aureus* isolates were identified by standard microbiological methods such as colonial morphology, Gram staining, Catalase, Coagulase and DNase activities (Mahon and Mahon, 2015). The bacterial isolates were transferred into Tryptic Soy Broth (TSB) (HiMedia, India) containing 20% glycerol and stored at −70 °C.

2.2. Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed by the disc diffusion method on Mueller–.

Hinton agar (Merck-Germany) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) with the following antibiotic discs: oxacillin (1 mg), cefoxitin (30 mg), vancomycin (30 μg), gentamicin (10 μg), clindamycin (2 μg), ciprofloxacin (5 μg), tetracycline (30 μg), levofloxacin (5 μg), rifampicin (5 μg) and trimethoprim/ sulfamethoxazole (co-trimoxazole) (25 μg). *S. aureus* ATCC 25923 was used for quality control (Tenover and Moellering, 2007).

The minimum inhibitory concentration (MIC) of oxacillin and vancomycin was determined by agar dilution method according to the recommendation of CLSI (Cockerill, 2011). Furthermore, the presence of *mecA* gene was confirmed by the amplification of *mecA* gene with specific primers (Havaei et al., 2015).

2.3. PCR-based assignment of SCCmec elements

Genomic DNA was extracted from *S. aureus* isolates using High Pure PCR Template Preparation Kit (Roche Diagnosis, Mannheim, Germany) according to manufacturer's procedure. All *mecA* positive isolates were screened for SCC*mec* typing using multiplex PCR method by the previously described primers (Boye et al., 2007; Cockerill, 2011). PCR conditions were as follow: 4 min initial denaturation at 94 °C for 1 cycle, followed by 30 cycles of denaturation (94 °C/30 s), annealing 60 s at 55 °C and extension (72 °C/60 s), and a final extension at 72 °C for 4 min. The PCR products were visualized on a 1% agarose gel stained with KBC power load dye (CinnaGen Co. Iran). Five MRSA strains, NCTC10442 (SCC*mec* I), NCTC N315 (SCC*mec* II), NCTC 85/2082 (SCC*mec* III), NCTC CA05 (SCC*mec* IV) and JCSC3624 (SCC*mec* IV) were used as the standard strains with SCC*mec* elements.

3. Results

Totally 150 non-duplicates *S. aureus* isolates were collected from clinical samples at a studied hospital in Isfahan, Iran. Out of the 150 positive cultures, the most frequent source of bacterial isolation was from SSTIs 53 (35.3%), followed by 33 (22%) isolates from BSIs, 32 (21.6%) isolates from RTI, 10 (6.6%) isolates from UTIs and 22 (14.6%) isolates from other infections.

Exist of *mecA* gene in all isolates was evaluated by susceptibility test and then confirmed by PCR. Among of the 150 *S. aureus* isolates, 62 (41.3%) were MRSA and *mecA* positive According to the results of MIC of oxacillin, 90.3% (56/62) of MRSA isolates were considered as oxacillin-resistance. Moreover, based on agar dilution method, none of the isolates were resistant to vancomycin.

The results of antibiotic susceptibility revealed that the highest in vitro resistance for for both MRSA and MSSA isolates were against to tetracycline with 55.3%. Moreover, except vancomycin, rifampicin and co-trimoxazole were the most sensitive antibiotic against MRSA and MSSA isolates, respectively. The full results of antibiotic susceptibility pattern of *S. aureus* isolates are shown in Table 1.

Table 1
The results of antibiotic susceptibility pattern of *S. aureus* isolates.

Antibiotics	MSSA Total No. 88 No. (%)			MRSA Total No. 62 No. (%)			Total		
	S	I	R	S	I	R	S	I	R
Gentamycin	74 (84.1)	1 (1.1)	13(14.8)	36 (58.1)	2 (3.2)	24 (38.7)	110 (73.3)	3 (2)	37 (24.7)
Ciprofloxacin	76 (86.4)	0	12 (13.6)	25 (40.3)	20 (32.3)	17 (27.4)	101 (67.4)	20 (13.3)	29 (19.3)
levofloxacin	83 (94.3)	2 (2.3)	3 (3.4)	38 (61.3)	11 (17.7)	13 (21)	121 (80.7)	13 (8.7)	16 (10.6)
Clindamycin	82 (93.2)	1(1.1)	5 (5.7)	13 (21)	12 (19.3)	37 (59.7)	95 (63.3)	13 (8.7)	42 (28)
Tetracycline	47 (53.4)	0	41 (46.6)	15 (24.2)	5 (8.1)	42 (67.7)	62 (41.4)	5 (3.3)	83 (55.3)
Co-trimoxazole	83 (94.3)	1 (1.1)	4 (4.6)	39 (62.9)	5 (8.1)	18 (29)	122 (81.3)	6 (4)	22 (14.7)
Rifampicin	85 (96.6)	1 (1.1)	2 (2.3)	35 (56.5)	2 (3.2)	25 (40.3)	120 (80)	3 (2)	27 (18)
Vancomycin	88 (100)	0	0	62 (100)	0	0	150(100)	0	0
VISA (vancomycin agar dilution)	83 ()	5 ()	0	52 (83.9)	10 (16.1)	0	135 (90)	15 (10)	0