

Original Article

Characterization of *Staphylococcal* Cassette Chromosome Mec Elements in Biofilm-producing *Staphylococcus Aureus*, Isolated from Hospital Infections in Isfahan

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ABSTRACT

Article history

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Key words Biofilm producer Methicillin resistance *Sccmec typing Staphylococcus aureus* **Background and Aims:** *Staphylococcus aureus* is one of the important pathogens around the world. The present investigation was carried out to study the distribution of *Staphylococcal* Cassette Chromosome Mec (SCCmec) types and antibiotic resistance properties in methicillin-resistant *Staphylococcus aureus* isolated from Isfahan hospitals.

Materials and Methods: A total of 250 clinical specimens were collected from three major Isfahan hospitals. The samples were cultured, and biofilm producer isolates were subjected to several polymerase chain reaction methods. The patterns of antibiotic resistance were studied using the disk diffusion method.

Results: In the present study, 110 out of 250 samples (44%) were found to be positive for *Staphylococcus aureus*, and all the isolates produced the biofilm in different levels. The most commonly infected samples were collected from wounds (44.5%). The incidence of *mecA*, *tetK*, *ermA*, *ermC*, *tetM*, *aacA-D*, *linA*, *msrA*, *vatA*, *vatC* and *vatB* antibiotic resistance genes were 93.6%, 34.84%, 28.20%, 29.30%, 21.87%, 18.71%, 9.48%, 8.65%, 7.18%, 4.43% and 3.71%, respectively. The distribution of *SCCmec* III (42) was found to be the most type out of 103 *mec* positive strains.

Conclusions: In the prsent study, the highest resistance belonged to methicillin (90.2%), erythromycin (89.7%), ciprofloxacin (89.5%) and penicillin (88%) and the lowest resistance was reported for vancomycin (10%) and nitrofurantoin (8%). These infections with these strains require more advanced hospital care with an emerging demand for the novel antibiotics.

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Introduction

The increasing prevalence of bacterial resistance to commonly used antibiotics may result in an insufficient array of substances to combat some bacterial infections, demonstrating importance of antibiotic resistance pattern in Staphylococcus aureus (S. aureus) that has long been recognized as an important pathogen of hospital acquired infections. S. aureus can be colonized both in the community and hospital settings [1]. Infections caused by this bacterium are mainly treated with methicillin or aminoglycosides that its resistance pattern increased every day. Methicillin resistance in S. aureus (MRSA) is mediated by a penicillin binding protein (PBP)2a encoded by the mecA gene [2-4]. Normally, PBPs show a high affinity for binding to the beta lactam ring. MRSA strains present mecA gene and another kind of protein (PBP2a) that generate lower affinity for binding to methicillin. Subsequently, some antibiotics like methicillin will lose their ability for cell wall destruction as well as their efficiency on bacteria. According to other studies, 50% to 90% of S. aureus strains isolated from hospital infections were resistant to methicillin [5, 6]. S. aureus is introduced as the most common cause of skin and soft-tissue infections (e.g. furunculosis, impetigo, superficial and surgical wounds, and abscess), as well as systemic infections (e.g. pneumonia, urinary tract infections-UTIs and endocarditis) [7-13]. Biofilm producing is regarded as one of the important factors in order to increase antibiotic resistance pattern. The pathogenicity of S. aureus depends on various bacterial surface

components and extracellular proteins. The pathogenesis of a particular *S. aureus* strain is attributed to combination of extracellular factors and such properties as adherence and biofilm formation. Different proteins of the family of microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) which involved in S. aureus adhesion, and affected on important locus that involved biofilm producing genes [9].

The mecA genes, specially called Staphylococcal Cassette Chromosome Mec (SCCmec) elements, are conducted from a special region which are currently classified into types I, II, III, IV and V based on the nature of the mec and ccr gene complexes, and are further classified into subtypes according to differences in their J region DNA [14-18]. Complete identification of bacterial genetic background and the SCCmec element is considered extremely important in regard with molecular typing especially in MRSA [19-21]. It is noticeable that mecA gene is transferred by SCCmec as a mobile genetic element with the size of 21-67 k bp, that is exclusively found in the Staphylococcus genus. A complex of the SCCmec gene contains mec complex, ccr complex and the Junkyard area. Due to different allotypes and complex classes, five (I-V) main types of SCCmec elements were classified in the resent study [22]. SCCmec type I was determined in United Kingdom for the first time in 1961, type II was identified in Japan and types III, IV and V were recognized in New Zealand. According to different types of SCCmec

elements, hospital acquired-MRSA (HA-MRSA) and community acquired-MRSA (CA-MRSA) are known as independent deviations. CA-MRSA strains are susceptible to most widespread antibiotics in comparison with HA-MRSA and more antibiotic resistances can also be detected in *SCCmec* type III. Therefore, in the present study aimed to investigate antimicrobial resistance pattern as well as to determine frequency of the *mecA* gene and different *SCCmec* types in an epidemiologic research [23].

Materials and Methods

Bacterial isolates

Required samples or all samples required and Staphylococcus identification were collected during one year from three hospitals of Isfahan in Iran. A total of 250 clinical samples from various types of infections were selected, among which 110 isolates were Staphylococcus aureus, including blood (n=17), bed sore (n=6), wound (n=49), abscess (n=3), tracheal secretion (n=25), catheter (n=4), joint fluid (n=3) and cerebrospinal fluid (CSF) (n=3). All the samples were directly cultured into 7% sheep blood agar (Merck, Darmstadt, Germany) and incubated aerobically at 37°C for 48 h. After incubation, suspicious colonies were examined using techniques appropriate for diagnosing Staphylococcus spp.(microscopical morphology, catalase and coagulase production). The 110 isolates were distinguished by the conventional microbiological methods too, such as growth on blood agar, mannitol salt agar, DNase tests and coagulase. The API-20-Staph system kit (bio Merieux, France) was used for the final confirmation. After growth, staphylococci were

identified on the basis of colony characteristics, Gram staining, pigment production, hemolytic and the following biochemical reactions: catalyses activity, coagulated test (rabbit plasma), Oxidase test, glucose O/F test, resistance to bacitracin (0.04 U), mannitol fermentation on Mannitol Salt Agar (MSA) (Merck, Darmstadt, Germany), urease activity, nitrate reduction, novobiocin resistance, phosphatase, Deoxyribonuclease (DNase) test and carbohydrate fermentation tests [24]. Five MRSA strains were used for the standard strains, consisting of NCTC10442, N315, 85/2082, CA05, and WIS (WGB8318) [25].

Phenotypic assay on slime production

The slime production assay was performed by cultivation of the S. aureus strains on Congo Red Agar (CRA) plates as described in the different studies. The CRA plates were incubated at 37°C in aerobic conditions for 24 h, followed by storage at room temperature for 48 h. The formation of reddish black colonies with a rough, dry, crystalline consistency on CRA plates was considered to be indicative of slime production. Nonsmile-producing strains produced pinkish red, smooth colonies with a darkening at the center. To complete this method, microtiter plate assay was used. In this way, polysterne plate was applied, 20 µL of isolates were added, and then incubated for 48 h at 37°C, washing with Phosphate-buffered saline. Safranin was utilized for staining the isolates in the polysterne plates, and finally ethanol was used to release biofilm producer isolates. Absorbance was read in 490 nm. Analysis of biofilm producing is shown in

different levels in table 1. These isolates were selected to determine the antibiotic resistance pattern [9-11].

Biofilm producing	Optical Density (OD)	Cut-off				
Strong	OD>0.332	OD>4*ODC ²				
Average	0.166 <od<=0.332< th=""><th>2*ODC<od<=4*odc< th=""></od<=4*odc<></th></od<=0.332<>	2*ODC <od<=4*odc< th=""></od<=4*odc<>				
Weak	0.083 <od<=0.166< th=""><th>ODC<od<=2*odc< th=""></od<=2*odc<></th></od<=0.166<>	ODC <od<=2*odc< th=""></od<=2*odc<>				
Anny attachment	OD<=0.083	OD<=0.083				

Table 1. Biofilm analysis in *S aureus* isolates

Antibiotic susceptibility testing

Biofilm-producing S.aureus isolates were selected and the antibiotic resistance pattern was performed by disc diffusion method on Mueller-Hinton agar. S. aureus isolates were tested for susceptibility to penicillin (10 u/disk); imipenem (10 µg/disk); cefazoline (30 µg/disk); cefalotin (30) $\mu g/disk$); ceftriaxone (30 $\mu g/disk$); gentamicin (10 µg/disk); ciprofloxacin (5 µg/disk); clindamycin (2 µg/disk); azithromycin (15 μ g/disk); erythromycin (15 μ g/disk); mupirocin (30 µg/disk); rifampicin (5 µg/disk); tetracycline (30 µg/disk); trimethoprim (5 $\mu g/disk$); vancomycin (30 µg/disk) and nitrofurantoin (300 µg/disk) by the Kirby-Bauer disk diffusion method (MAST, Merseyside, England). According to Clinical and Laboratory Standards Institute (CLSI) 2011, S. aureus ATCC25923 was used as the control strain [26].

DNA extraction and *Staphylococcus* confirmation

A typical colony of the biochemically identified *S. aureus* was cultivated in 1 mL TSB for 24 h at 37°C. The bacterial genomic DNA of *S. aureus* strains was extracted via a QIAGEN plasmid Minikit (Fermentas, Germany) as recommended.

Antibiotic resistance genes amplification

The presence of mecA (encoding methicillin resistance), tetK, tetM (tetracycline (macrolideresistance). ermA, ermC lincosamide-streptogramin B resistance). aacA-D (aminoglycoside resistance), linA, msrA, vatA, vatC and vatB (streptogramin A resistance) genes were analyzed using the Kumar technique [27]. List of primers are demonstrated in table 2. The multiplex polymerase chain reaction (PCR) were performed in a total volume of 25 µL, including 2 mM of MgCl2, 50 mM of KCl, 10 mM of Tris-HCl (pH 9.0), 0.1% of Triton X-100, 150 µM of dNTPs each (Fermentas, Germany), 2.5 µL of PCR buffer (10X), 25 pmoL of each primers, 2 U of Taq DNA polymerase (Fermentas, Germany), and 4 μL (40-260 ng/ μL) of the extracted DNA template of the Staphylococcus isolates. The four set of primer pairs were used in each reaction mixture. The thermal cycler was adjusted as follows: 94°C 5 min., 30 cycles of 1 min. at 95°C for the denaturation step and 1 min. at 55°C for the annealing-extension step followed by the final extension at 72°C for 90 seconds. This study was approved by Ethics Committee of Islamic Azad University of Shahrekord

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branch. All ethical issues were considered, according to which this study was performed

obtaining the permission of hospitals.

Genes	Primer Sequence (5'-3')	Length(bp)	Reference
aacA-D	F: TAATCCAAGAGCAATAAGGGC	227	(35)
	R : GCCACACTATCATAACCACTA	227	
tet K	F: GTAGCGACAATAGGTAATAGT	260	(32)
	R : GTAGTGACAATAAACCTCCTA	300	
tet M	F: AGTGGAGCGATTACAGAA	150	(25)
	R : CATATGTCCTGGCGTGTCTA	158	
msrA	F: GGCACAATAAGAGTGTTTAAAGG	040	(24)
	R: AAGTTATATCATGAATAGATTGTCCTGTT	940	
	F: AAGCGGTAAACCCCTCTGA	100	(35)
ermA	R: TTCGCAAATCCCTTCTCAAC	190	
arran C	F: AATCGTCAATTCCTGCATGT	200	(35)
ermC	R: TAATCGTGGAATACGGGTTTG	299	
vatA	F: TGGTCCCGGAACAACATTTAT	269	(35)
	R: TCCACCGACAATAGAATAGGG	208	
vatB	F: GCTGCGAATTCAGTTGTTACA	126	(35)
	R: CTGACCAATCCCACCATTTTA	150	
vatC	F: AAGGCCCCAATCCAGAAGAA	167	(34)
	R: TCAACGTTCTTTGTCACAACC	407	
linA	F:GGTGGCTGGGGGGGGTAGATGTATTAACTGG	323	(34)
	R:GCTTCTTTTGAAATACATGGTATTTTTCGA		

Table 2. Oligonucleotide primers for amplification of antibiotic resistance genes

Multiplex PCR amplification for SCCmec

typing

S.aureus that were resistant to *mecA* were selected to continue other methods according to the aim of study. Different *SCCmec* types were determined by the specific primers, which were previously described, and listed in Table 2. PCR amplification was performed in a volume of 50 ml by Emerald Amp MAX PCR Master Mix (Takara, Japan) for all the PCR reactions. The DNA Thermal Cycler 480 (Applied Bio systems, Foster City, CA, USA) was programmed as follows: the first denaturation at 94 °C for 5 min,

denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and an extension at 72 °C for 60 s for 40 cycles in the final extension at 72°C for 4 min. PCR products were analyzed by electrophoresis on agarose 1.5% via SYBR Safe staining [28]. Method lists of primers are shown in table 3. Fifteen microliters of PCR products were resolved on a 1.5% agarose gel containing 0.5 mg/mL of ethidium bromide in Tris–borate– EDTA buffer at 90 V for 1 h, using suitable molecular weight markers. Ultimately, the products were examined under ultraviolet illumination.

Types	Primer Sequence (5'-3')	Length(bp)	Reference	
SCCmec I	F:GCTTTAAAGAGTGTCGTTACAGG	612	(25)	
	R: GTTCTCTCATAGTATGACGTCC	015	(35)	
SCCmec II	F: CGTTGAAGATGATGAAGCG	209	(34)	
	R: CGAAATCAATGGTTAATGGACC	398		
SCCmec III	F: CCATATTGTGTACGATGCG	290	(31)	
	R: CCTTAGTTGTCGTAACAGATCG	280		
SCCmec Iva	F: GCCTTATTCGAAGAAACCG	776	(35)	
	R: CTACTCTTCTGAAAAGCGTCG	//0		
SCCmaa Wh	F: TCTGGAATTACTTCAGCTGC	402	(35)	
SCCmec IVD	R: AAACAATATTGCTCTCCCTC	495		
SCCmec IVc	F:ACAATATTTGTATTATCGGAGAGC	200	(35)	
	R: TTGGTATGAGGTATTGCTGG	200		
SCCmaa Wd	F: CTCAAAATACGGACCCCAATACA	001	(35)	
SCCmec Iva	R: TGCTCCAGTAATTGCTAAAG	001		
SCCmaa V	F: GAACATTGTTACTTAAATGAGCG	325	(35)	
	R: TGAAAGTTGTACCCTTGACACC	323	(55)	

Table 3. Oligonucleotide primers for amplification of SCCmec types in S. aureus strains isolated from Isfahan hospitals

Results

Out of 250 clinical samples, 110 were positive for *S. aureus*. All the isolates were biofilm producers in different levels. Superficial and surgical wounds had the highest incidence of *S. aureus* (44.5%), while CSF and joint fluid were the samples that had the lowest incidence (2.8%). Frequency of biofilm producing was arranged as below: wound (78.2%), abscess (10.9%), Tracheal secretion (65.4%), blood (24%), catheter (63%), bed sore (85%), joint fluid (17%), cerebrospinal fluid (13.3%). *MecA* (93.6%) involved the most commonly detected antibiotic resistance genes and the lowest antibiotic resistance pattern belonged to *vatC* (4.43%) and *vatB* (3.71%) genes. Isolates with *mecA* genes were selected for *SCCmec* typing. *S. aureus* isolates of superficial, post-surgical wounds, and respiratory infections had the highest incidence of antibiotic resistance genes. *S. aureus* frequency in different parts of hospitals is demonstrated in table 4.

Table 4. Frequency of S.aureus isolates in different sections of hospitals

Selected area	Number and Percent of isolates
Orthopedics	35 (35.3)
Internal	33 (33.3)
Intensive care unit	20 (20.2)
Special intensive care unit	11 (11.2)

In the present study, the highest incidence of SCCmec types were SCCmec type III and II. The incidence of IVa, IVc and IVb SCCmec types were proved to be the lowest in S.aureus isolates. As a matter of fact, positive results were reported for all of the types. The study results revealed that most of the S. aureus isolates were resistant to methicillin, penicillin, tetracycline. The highest levels of antibiotic resistance in S. aureus belonged to methicillin (90.2%), erythromycin (89.7%), ciprofloxacin (89.5%) and penicillin (88%). The lowest resistance was observed for vancomycin (10%) and nitrofurantoin (8%). A high incidence of multi-drug resistant (MDR) S. aureus strains was observed in patients' clinical samples. All

of the specimens such as tracheal secretion, bed sore, superficial and surgical wounds and abscesses of the patients were infected with these strains or other isolates were found as high incidence of multi-drug resistant *S. aureus* strains. It should be all the 110 isolates revealed a high virulence. Moreover, frequency of *S.aureus* isolates we detected from different patients, most of which aged 61-70 with 41(37.3%) prevalence that were hospitalized in 3 major hospitals of Isfahan in Iran. In the current study, 73% of patients were women and 27% were the men. Frequency and recognition procedures of *SCCmec* typing is shown in table 5.

Infection	Туре І	Туре П	Туре Ш	Type Va1	Type VbI	Type VcI	Type VdI	Type V	Number
Wound	10	16	15	2	1	1	1	3	49
Blood	2	7	8	-	-	-	-	-	17
Tracheal	2	10	11	1	-	-	1	-	25
Abscess	-	1	-	-	-	-	-	2	3
Joint fluid	-	1	-	-	-	-	-	-	1
CSF	-	-	2	-	-	-	-		2
Catheters	1	-	1	-	-	-	-	-	2
Bedsore	-	1	5	-	-	-	-	-	6

Table 5. Frequency and recognition procedures of SCCmec typing

Discussion

The study findings revealed that 90.3% of all *Staphylococcus* strains had the gene coding resistance against methicillin. In addition to methicillin, the *Staphylococcus* strains had the genes coding resistance against some antibiotics such as macrolides, erythromycin,

lincosamides, aminoglycosides and tetracycline. *Staphylococcus* strains were held to have the highest levels of antibiotic resistance against methicillin (90.2%), erythromycin (89.7%), ciprofloxacin (89.5%) and penicillin (88%). The lowest resistance was reported for vancomycin (10%) and nitrofurantoin (8%). The statistical analysis showed significant differences between the incidence of mecA and other antibiotic resistance genes. In addition, a statistically significant association was detected between the resistance levels s to tetracycline, penicillin, cefalotin, ciprofloxacin, and ceftriaxone with mupirocin, rifampicin, imipenem trimethoprim, and gentamicin antibiotics. Tocharian's study reported that 72% of S. aureus isolates of Lebanon hospitals were methicillin-resistant and 18% of them were resistant to 10-18 antibiotics. Udo also proposed that 1765 (95.6%) inpatients and 81 (4.4%) outpatients of Kuwait hospitals were positive for S. aureus with 32% incidence rate of the methicillin-resistant strains [29-31].

Similar studies have indicated incidence rate of MRSA, among which Alghaithy reported 61% in Saudi- Arabia, Młynarczyk stated 40% in Warszawie and Rijals' study showed 56.1% in Bokhara. In a study conducted by Virdis, 56% of S. aureus isolates were resistant to one or more antimicrobial agents including kanamycin (28%), ox tetracycline (16%), and ampicillin (12%). The most commonly used antibiotics consisted of oxacillin, nafcillin, cefathiamidine. In fact, vancomycin had the highest resistance to S. aureus strains of the Deng's report [31-36]. Nishijima and Kurokawa reported that the incidence of resistance to penicillin, cephalosporin and clindamycin were 20 to 30% and the occurrence of gentamicin, erythromycin, roxithromycin and meticillin resistance were 55.2%, 39.6%, 39.1% and 21%, respectively.

The *S. aureus* isolates were highly resistant to antibiotics in this study: 36.4% were resistant to streptomycin, 33.6% to ox tetracycline, 29.9% to gentamicin and 26.2% to chloramphenicol, pristinamycin and ciprofloxacin which were in line with the results of the current study [27-34].

Methicillin-resistant S.aureus were detected for SCCmec typing via M-PCR. The II phenotype (32.83%) and III phenotype (64.52%) were the most commonly detected SCCmec types among the mec positive strains of S. aureus . Significant differences were found between the incidence of SCCmec V and SCCmec IVc, SCCmec IVb and SCCmec Iva types. Similar studies have previously been reported, among which D'Souza showed that a total of 97 mecA-positive strains were SCCmec III (25%), 136 were SCCmec IV (34%), and 162 were SCCmec V (41%) which were concordant to the present study results. They also proposed that all the SCCmec III strains, 73% of SCCmec IV and V strains and 72% of SCCmec IV and SCCmec V strains were multidrug resistant [25].

Moussa reported that the most predominant *SCCmec* types among the examined isolates in Saudi Arabia were type V (42.5%), followed by *SCCmec* type III 39 (38.6%) which were similar to this study findings [35].

Conclusion

The present study findings revealed that both *mecA, tetK, tetM, ermA, ermC, aacA-D, linA, msrA, vatA, vatC* and *vatB* genes, also resistance to penicillin, methicillin, tetracycline, ceftriaxone, azithromycin and

tetracycline were introduced as the most commonly detected characteristics of the biofilm producing strains isolated from the hospitals infections.

All the isolates produced biofilm in different levels that is stated as one of the important reasons to create multidrug resistance in the isolates. The high level of *Staphylococcus aureus* resistance against commonly used antibiotics as well as high presence of *SCCmec* types of meticillin-resistance in virulent strains of *S. aureus* represent that infections with these strains require higher levels of hospital cares with an emerging demand for the novel antibiotics. Hence, the clinicians' role in judicious usage of antibiotics is pivotal.

Conflict of Interest

The authors report no conflicts of interest.

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References

- Turlej A, Hryniewicz W, Empel J. Staphylococcal cassette chromosome mec (SCCmec) classification and typing methods: an overview. Pol J Microbiol. 2011; 60(2): 95-103.
- [2]. Ito Y, Ma XX, Takeuchi F, Okuma K, Yuzawa H, Hiramatsu K. Novel type V staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase, ccrC. Antimicrob. Agents Chemotherapy 2004; 48(7): 2637-651.
- [3]. Francis JS, Doherty MC, Lopatin U, Johnston CP, Sinha G, Ross T, et al. Severe community-onset pneumonia in healthy adults caused by methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leukocidin genes. Clin Infect Dis. 2005; 40(1): 100-7.
- [4]. Erol S, Altoparlak U, Akcay MN, Celebi F, Parlak M. Changes of microbial flora and wound colonization in burned patients. Burns 2004; 30(4): 357-61.
- [5]. Merlino J, Watson J, Rose B, Beard-Pegler M, Gottlieb T, Bradbury R, et al. Detection and expression of methicillin/ oxacillin resistance in multidrug-resistant and non-multidrug-resistant *Staphylococcus aureus* in Central Sydney. Aust J Antimicrob Chemotherapy 2002; 49(5): 793-801.
- [6]. Boye K, Barrtels MD, Anderson IS, Møller JA, Westh H. A new multiplex PCR for easy screening of methicillin resistant *Staphylococcus aureus* SCCmec type I-V. J Clin Microbiol. 2007; 13(7): 725-27.
- [7]. Nickerson EK, Wuthiekanun V, Kumar V, Amornchai P, Wongdeethai N, Chantratita KCN, et al. Emergence of community-associated

methicillin-resistant *Staphylococcus aureus* carriage in children in Cambodia. Am J Trop Med Hyg. 2011; 84(2): 313-17.

- [8]. Kawaguchiya M, Urushibara N, Kuwahara O, Ito Kobayashi N. M. Mise Κ. Molecular characteristics of community-acquired methicillinresistant Staphylococcus aureus in Hokkaido northern main island of Japan: identification of sequence types 6 and 59 Panton-Valentine leucocidin positive community-acquired methicillin-resistant Staphylococcus aureus. Microb Drug Resist. 2011; 17(2): 241-50.
- [9]. Ammendolia M.G., Di Rosa R., Montanaro L., Arciola CR., Baldassarri, L. Slime production and expression of the slime-associated antigen by *staphylococcal* clinical isolates. J Clin Microbiol. 1999; 37(10): 3235-238.
- [10]. Neela V, Ghasemzadeh MH, Belkum AV, Horst-Kreft D, Mariana NS, Ghaznavi Rad E. First report on methicillin-resistant Staphylococcus aureus of Spa type T037, Sequence type 239 SCCmec type III/IIIA in Malaysia. Eur J Clin Microbiol Infect Dis. 2010; 29: 115-17.
- [11]. Arciola CR., Baldassarri L., Montanaro L. Presence of icaA and icaD genes and slime production in a collection of *staphylococcal* strains from catheter-associated infections. J Clin Microbiol. 2001; 39, 2151-156.
- [12]. Rodrigues MVP, Fortaleza CMCB, Riboli DFVM, Rocha RS, Rocha C, Cunha MLRS. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a burn unit from Brazil. Burns 2013; 39: 1242-249.

- [13]. Boyce JM, White RL, Causey WA, Lockwood WR. Burn units as a source of methicillinresistant *Staphylococcus aureus* infections. J Am Med Assoc. 1983; 249: 2803-807.
- [14]. Matsumura H, Yoshizawa N, Narumi A, Harunari N, Sugamata A, Watanabe K. Effective control of methicillin-resistant *Staphylococcus aureus* in a burn unit. Burns 1996; 22: 283-86.
- [15]. Rutala WA, Setzer KB, Sherertz RJ, Sarubbi FA. Environmental study of a methicillin-resistant *Staphylococcus aureus* epidemic in a burn unit. J Clin.Microbiol. 1983; 3: 683-88.
- [16]. Pournaras S, Slavakis A, Polyzou A, Sofianou D, Maniatis AN, Tsakris A. Nosocomial spread of an unusual methicillin-resistant *Staphylococcus aureus* clone that is sensitive to all non-b-lactam antibiotics, including tobramycin. J Clin Microbiol. 2001; 39: 779-781.
- [17]. Rashid A, Solomon LK, Lewis HG, Khan K. Outbreak of epidemic methicillin-resistant *Staphylococcus aureus* in a regional burns unit: management and implications. Burns 2006; 32: 452-57.
- [18]. De Giusti M. Phenotypic detection of nosocomial mecA positive coagulase negative *staphylococci* from neonates. J Antimicrob Chemother. 1999; 44: 351-58.
- [19]. Pe´richon B, Courvalin P. VanA-type vancomycin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 2009; 53: 4580-587.
- [20]. Malachowa N, DeLeo FR. Mobile genetic elements of *Staphylococcus aureus*. Cell. Mol Life Sci. 2010; 67: 3057-71.
- [21]. Khosravi AD, Hoveizavi H, Farshadzadeh Z. The prevalence of genes encoding leukocidins in *Staphylococcus aureus* strains resistant and sensitive to methicillin isolated from burn patients in Taleghani Hospital, Ahvaz, Iran. Burns 2012; 38(2): 247-51.
- [22]. Shallcross LJ, Williams K, Hopkins S, Aldridge RW, Johnson AM, Hayward AC. Panton-Valentine leukocidin associated *staphylococcal* disease: a cross-sectional study at a London hospital, England. Clin Microbiol Infect. 2010; 16(11): 1644-648.
- [23]. Santucci SG, Gobara S, Santos CR, Fontana C, Levin AS. Infections in a burn intensive care unit: experience of seven years. J Hosp Infect. 2003; 53(1): 6-13.
- [24]. Melles DC, Gorkink RF, Boelens HA, Snijders SV, Peeters JK, Moorhouse MJ, et al. Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. J Clin Invest. 2004; 114(12): 1732-740.
- [25]. D'Souza N, Rodrigues C, Mehta A. Molecular characterization of methicillinresistant *Staphylococcus aureus* with emergence of epidemic clones of sequence

type (ST) 22 and ST 772 in Mumbai, India. J Clin Microbiol. 2010; 48(5): 1806-811.

- [26]. Milheiriço C, Oliveira DC, de Lencastre H. Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome mec type IV in methicillin-resistant *Staphylococcus aureus*: 'SCCmec IV multiplex'. J. Antimicrob. Chemother. 2007; 60(1): 42-8.
- [27]. Kumar R, Yadav BR, Singh RS. Antibiotic resistance and pathogenicity factors in *Staphylococcus aureus* isolated from mastitic Sahiwal cattle. J. Biosci. 2011; 36(1): 175-88.
- [28]. Boyce JM, Papa E, Dickenson R, Medeiros AA. Failure of routine susceptibility tests to detect imipenem resistance among strains of methicillinresistant *Staphylococcus aureus*. Antimicrob. Agents Chemother.1991; 35(7): 1495-497.
- [29]. Tokajian S, Haddad D, Andraos R, Hashwa F, Araj G. Toxins and Antibiotic Resistance in *Staphylococcus aureus* Isolated from a Major Hospital in Lebanon. ISRN Microbiol 2011; 2011: 812049.
- [30]. Virdis S, Scarano C, Cossu F, Spanu V, Spanu C, De Santis EP. Antibiotic Resistance in *Staphylococcus aureus* and Coagulase Negative *Staphylococci* Isolated from Goats with Subclinical Mastitis. Vet Med Int. 2010; 2010: 517060.
- [31]. Udo EE, Al-Sweih N, Dhar R, Dimitrov TS, Mokaddas EM, Johny M, et al. Surveillance of antibacterial resistance in *Staphylococcus aureus* isolated in Kuwaiti hospitals. Med PrincPract 2008; 17(1): 71-5.
- [32]. Alghaithy AA, Bilal NE, Gedebou M, Weily AH. Nasal carriage and antibiotic resistance of *Staphylococcus aureus* isolates from hospital and non-hospital personnel in Abha, Saudi Arabia. Trans R Soc Trop Med Hyg. 2000; 94(5): 504-507.
- [33]. Rijal KR, Pahari N, Shrestha BK, Nepal AK, Paudel B, Mahato P, et al. Prevalence of methicillin resistant *Staphylococcus aureus* in school children of Pokhara. Nepal. Coll J. 2008; 10(3): 192-95.
- [34]. Nishijima S, Kurokawa I. Antimicrobial resistance of *Staphylococcus aureus* isolated from skin infections. Int J Antimicrob Agents 2002; 19(3): 241-43.
- [35]. Moussa I, Kabli SA, Hemeg HA, Al-Garni SM, Shibl AM. A novel multiplex PCR for molecular characterization of methicillin resistant *Staphylococcus aureus* recovered from Jeddah, Kingdom of Saudi Arabia. India J Med Microbiol. 2012; 30(3): 296-301.
- [36]. Momtaz H, Hafezi L .Meticillin-resistant *Staphylococcus aureus* isolated from Iranian hospitals: virulence factors and antibiotic resistance properties. Bosn J Basic Med Sci. 2014; 14(4): 2 19-26.