Detection of integrons and Staphylococcal Cassette Chromosome (SCCmec) types in *Staphylococcus aureus* isolated from burn and non-burn patients

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Abstract

Background: Methicillin Resistant *Staphylococcus aureus (MRSA)* strains have been recognized as an important reason of infections in health care units. Integrons role in antibiotic resistance box gene transfer has been well recognized which are found in Gram positive bacteria.

Objective: The aim of this study was analyzed of SCCmec typing and determine of integron classes in burn and non-burn specimens.

Methodology: A total of 110 *S. aureus* strains were isolated from burn and non-burn patients. Antimicrobial susceptibility testing**,** detection of *mecA* gene, various SCCmec types and integrons classes were analyzed.

Results: In antimicrobial susceptibility test in burn patients, resistant to both gentamicin and oxacilin and in non-burn patients resistance to oxacilin and cefepime showed the highest ratio In PCR molecular test (80%) and (52.7%) of strains harbored the *mecA* gene. Therefore five different SCCmec types were recognized among our studied strains. Subsequently, integron class I was evaluated as (94.5%) in burn and (12.7%) in non-burn isolates by the multiplex PCR method.

Conclusion: Albeit MRSA strains have the hospital reservoir so may cause serious treats for hospitalized and non-hospitalized patients, hence clinical decision for prevention and treatment may develop due to, *mecA* gene, SCCmec elements and integrons detection in health care units.

Keywords: MRSA, Integron, *mecA* gene, SCCmec types

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Introduction

One of the most serious challenges in hospitalized patients with burn wounds is to be infected with nosocomial pathogens during the hospitalization period. [1] Burn patients are more exposed to infections due to decreased cellular and humoral immunity responses and lack of protective skin barriers. [2] Burn infections maybe initiate by skin flora and then replace by nosocomial pathogens after hospital admission. *Staphylococcus aureus* has been known as an important and universal hospital pathogens, which can cause endemic and epidemic infections in healthcare centers, especially in burn units. [3] Many several pathogenic factors such as biofilm formation, adhesions, superantigenic exotoxins, hemolysins and pore-forming toxins have been described in *S. aureus*. [4] Moreover, resistance to different antibiotic classes with various mechanisms is the significant topic in nosocomial infections. [5] Recently the integrons role in antibiotic resistance transfer has been well recognized. Integrons, the hereditary unit of genes, located in bacterial chromosome, plasmid or transposon with ability of specific antibiotic resistance gene transmission, are found in Gram positive and Gram negative bacteria. According to different integrons classes, type I, II and III are more considerable. [6, 7] Each integrons is consisting of integrase gene *(intI)*, a promoter gene and a proximal primary recombination site *(attI)*. It should be noted that class I and II of integrons are associated with Tn*3* and Tn*7* transposon family respectively. [8, 9]

Resistance to methicillin in *S. aureus* strains is due to expression of an altered penicillin-binding protein (PBP2a) with low affinity for β-lactam antibiotics, encoded by *mecA* gene.[10,11] The *mecA* gene is a part of 21 up to 67 bp genetic mobile elements called Staphylococcal Cassette Chromosome (SCC) with different size and genetic composition among Methicillin-Resistant *S. aureus* (MRSA) strains. [12] Currently various SCCmec elements have been identified and classified into different types such as I, II, III, IV, V, VI and etc. [13,14] MRSA infections originally were accepted as Hospitalized-Acquired or Healthcare-Acquired MRSA (HA-MRSA) but later, the Community-Acquired MRSA (CA-MRSA) occurred with high frequency. [15, 16] On the other hand, the emergence of CA-MRSA infections among burn patients has created a serious challenge in infections control process. [17] HA-MRSA and CA-MRSA can be distinguished from each other due to their SCCmec types basis, therefore SCCmec types I, II, III and VIII are mainly associated with HA-MRSA while CA-MRSA often characterized by SCC*mec* types IV, V, VI and VII. [18] The aim of the present study was to determine the various SCCmec types and integrons classes in *S. aureus* strains isolated from burn and non-burn patients.

Methods:

Bacterial strains and identification

A total of 110 *S. aureus* strains were isolated from April to November 2014. Fifty five isolates were recovered from wounds of burn hospitalized patients in Shahid Motahari Hospital, a referral center for burn patients in Tehran, and other 55 isolates were gathered from wounds of non-burn hospitalized patients in Milad Hospital in Tehran, Iran. In this study the ethical requirements were approved by Iran University Ethics Committee.

All isolates were transferred to the microbiology laboratory in Iran University of Medical Science in Trypticase soy broth (TSB), and confirmed by culturing in conventional media such as (5%) sheep blood agar, nutrient agar and mannitol salt agar. Thereafter Gram staining was performed for bacterial morphology diagnosis. All isolates were identified by standard biochemical tests for *S. aureus* such as catalase, oxidase, coagulase and DNase as described by Koneman *et al*. (1997), and finally stored at -20°C. [19]

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was achieved by using disc diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI 2012) [20] with following antibiotics: ciprofloxacin (5µg), vancomycin (30µg), rifampin (5µg), cefotaxime (30µg), fusidic acid (10µg), fosfomycin (200µg), cotrimoxazol (25µg), cefepime (30µg), gentamicin (10µg), erythromycin (15µg) and oxacillin (1µg) (MAST, Merseyside, England).

Molecular characterization of *mecA* gene and SCCmec elements

A DNA commercial purification kit was used for genomic DNA extraction (QIAGEN, Hilden,

Germany). The PCR molecular test was performed to investigate the *mecA* gene prevalence for all isolates with specific primers as described previously by Merlino *et al* in 2002. The PCR program was performed as follows: initial denaturation at 94°C for 5 min for 40 cycles, consisting of denaturation (94°C for 30 sec), annealing (57°C for 45 sec), and extension (72°C for 30 sec), followed by a final extension at 72°C for 5 min[21]. Thereafter the SCCmec typing was carried out by a multiplex PCR method using specific primers as described by Boye *et al*. in 2007. [22]

PCR identification of integrons class I and II

In this study class I and II of integrons were distinguished with specific primers and programs which were previously used. [23]
The primers used for all PCR amplification are listed in Table 1.

Table 1- The specific primers used for amplification and detection of *mecA* gene, integrons and SCCmec different types.

Primers		Sequences	bp	References
mecA	F	AAAATCGATGGTAAAGGTTGGC	533 bp	21
	R	AGTTCTGCAGTACCGGATTTGC		
SCCmec	Fβ	ATTGCCTTGATAATAGCCYTCT	937 bp	22
	Rα3	TAAAGGCATCAATGCACAAACACT		
	FccrC	CGTCTATTACAAGATGTTAAGGATAAT	518 bp	
	RccrC	CCTTTATAGACTGGATTATTCAAAATAT		
	F1272	GCCACTCATAACATATGGAA	415 bp	
	R1272	CATCCGAGTGAAACCCAAA		
	F5R mecA	TATACCAAACCCGACAACTAC	359 bp	
	R5R431	CGGCTACAGTGATAACATCC		
Integron class I	F	CCTCCCGCACGATGATC	280 bp	23
	R	TCCACGCATCGTCAGGC		
Integron class II	F	GTAGCAAACGAGTGACGAAATG	788 bp	
	R	CACGGATATGCGACAAAAAGGT		

Statistical analyses

Statistical analyses were performed using (SPSS) software version 20. Chi-square test was used to compare the two groups. ($P \leq 0.05$ was considered significant)

Results:

During 8 month period of sampling, 110 *Staphylococcus aureus* strains were collected from the wounds of burn and non-burn patients admitted to Shahid Motahari and Milad Hospitals. The antimicrobial susceptibility test was performed by disc diffusion method. All of the strains were identified as susceptible to vancomycin, fusidic acid and fosfomycin. Resistant to both gentamicin and oxacilin with (67.2%) showed the highest ratio in our burn patients.

Also resistant to ciprofloxacin and cefotaxime were (63.2%), cefepime (60%), erythromycin (58.2%) and cotrimoxazol (54.5%). Resistance to rifampin was less than others, (18.2%).

In contrast in non-burn patients resistance to antibiotic agents was as follows: oxacilin (47.3%), cefepime (45.4%), ciprofloxacin (32.7%), cefotaxime (30.1%) and cotrimoxazol (25.4%). Resistance to gentamicin was (18.2%); erythromycin and rifampin had the lowest percent in comparison to other antibiotics, (14.5%). In antimicrobial resistance pattern by disc diffusion method, (67.2%) of burn and (47.3%) of non-burn strains were resistant to oxacillin, while in PCR molecular test, (80%) and (52.7%) of strains harbored the *mecA* gene respectively.

The *mecA* positive strains showed various types of SCCmec: type I (2.3%), III (56.8%), IV (20.5%) and type V (9.1%). None of the strains harbored type II, however (11.3%) of strains

were not typeable in burn patients. Whereas in non-burn isolates the percentage of SCCmec types were as follows: type I (17.2%), type II (13.8%), type III (37.9%), type IV (27.6%) and type V (3.5%). (Fig.1)

Integron class I was found in (94.5%) of burn isolates and (12.7%) of non-burn ones by the multiplex PCR method. Integron class II was recognized only in one strain of burn isolates.

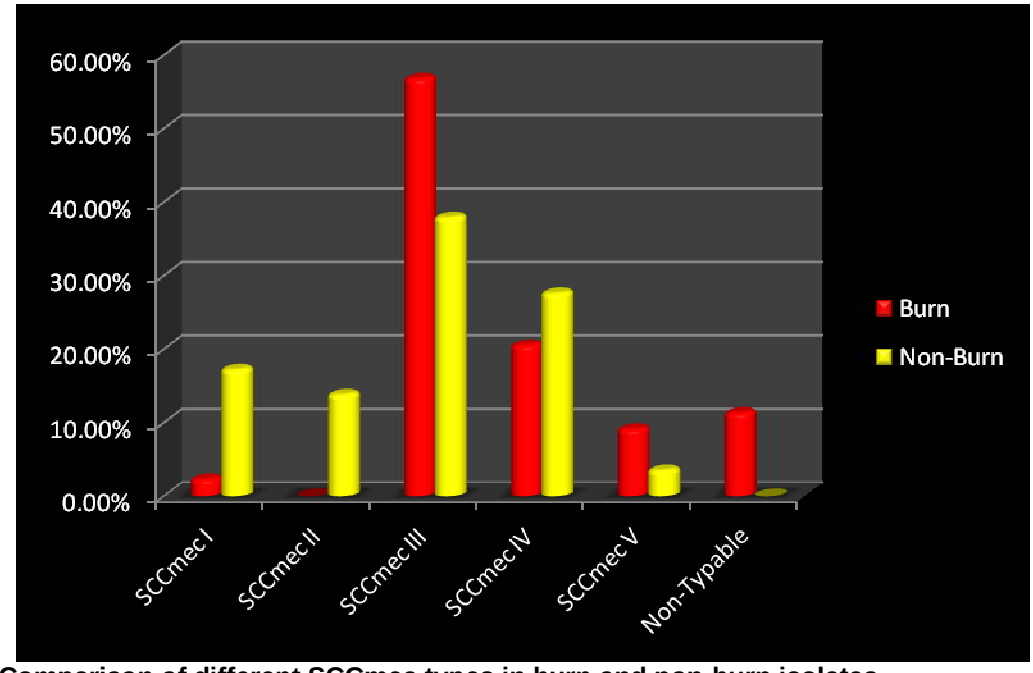

Fig.1- Comparison of different SCCmec types in burn and non-burn isolates.

Discussion:

Burn infections are usually associated with *S. aureus* in high frequency, which can cause important clinical consequences due to the expression of various virulence factors and the presence of antibiotic resistance genes. Many studies have demonstrated the capability of various mechanisms of the drug resistance genes in dissemination of drug-resistant bacteria and chronic infections in hospitalized patients. [24] By the emergence of MRSA, multi drug resistant strains and different SCCmec elements, the *S. aureus* infections and related disease have become momentous. This study describes the prevalence of integrons and SCCmec elements in *S. aureus* strains isolated from burn and non-burn wound infections.

In our study by PCR molecular test (80%) of strains harbored the *mecA* gene in burn isolates, while in antimicrobial resistance profile only 67.2 percent of the strains were resistant to oxacillin. Although in non-burn isolates both *mecA* gene and resistance rate to antimicrobial agents were lower in comparison to burn patients.

By using a multiplex PCR method, detection of various SCCmec elements showed that the SCCmec type III had the highest ratio in both groups. Based on various SCCmec types, our studied isolates were classified in (HA-MRSA) group. These strains showed the most resistance to selective antibiotic agents by disc diffusion method, similar to other studies.[1] According to results, (13.8%) of our non-burn isolates harbored type II of SCCmec, while this type of SCCmec was not found in burn isolates. Bacterial genetic diversity and the emergence of new types of SCCmec may cause these results.

Due to other studies, SCCmec type I provides resistance to β-lactams antibiotics, while type II and III contain multiple resistances to non β-lactam agents and afford a molecular explanation for the multidrug resistance that often documented in MRSA isolates circulating in healthcare environments. [18]

On the other hand the integrons class I and II were determined. Amongst integron classes, which are classified based on integrases gene structure, class I with drug resistance box genes is more frequently in Gram negative bacteria. [25] Dissemination of drug resistance genes between integrons may be caused by integrase functional system through genetic elements arrangements. [26] In Ren *et al*. study in 2012 on *S. aureus* in different clinical specimens, the class I of integrons was higher than class II, in which the class I of integrons in urine was more than other collected specimens, [23] but in our study in two group of burn and non-burn patients the prevalence of class I integron was significant in wound specimen of burn isolates by Chi-square test ($p \leq 0.03$).

It should be mentioned that class II of integron was observed in one strain of burn patients. In addition, a meaningful relation between SCCmec type III and integron class I was evaluated in present study by Chi-square test ($p \leq 0.01$).

In conclusion, clinical strategies for prevention and treatment may develop in accordance to the integrons classes, *mecA* gene and SCCmec element detection in health care units. Hence, the detection of these elements may be regarded as a functional tool for screening the bacterial drug resistance. In our opinion, other important virulence genes which are involved in hospitalized patients should be recognized for *S. aureus* infections control. However the correlation between these virulence factors and integrons classes and the ability of various toxin productions may be evaluated in mentioned groups.

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It should be noted that, one of the main limitations in our study was the specimens' collection from burn patients.

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