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Original Article

Molecular Investigation of Staphylococcal Cassette Chromosome mec *(SCCmec)* Elements Isolated from Intensive Care Unit

Fahimeh Nourbakhsh[1] Ph.D., Vajiheh Nourbakhsh[2] MSc., Samaneh Borooni[3] MSc., Elaheh Tajbakhsh[4*] Ph.D., Dana Daneshmand[5] Ph.D.

[1]Medical Toxicology Research Centre, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[2]Hazrat Fatemeh Zahra Hospitals, Depending on the Treatment and Management of Social Security in Isfahan, Isfahan, Iran.
[3]Department of Biology, Nourdanesh Institutes of Higher Education, Meymeh, Isfahan, Iran.
[4]Departments of Microbiology, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.
[5]Nosocomial Infection Research Center, Isfahan University of Medical Science, Isfahan, Iran.

ABSTRACT

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Background and Aims: Based on the results, *Staphylococcus aureus* is one of the serious infectious agents found in community and hospitals with remarkable potential for high morbidity and mortality around the globe. The present study was carried out for molecular investigation of methicillin-resistant *Staphylococcus aureus* strains and Staphylococcal Chromosomal Cassette mec *(SCCmec)* phenotypes isolated from the intensive care unit in Hazrat Fatemeh Zahra hospital of Isfahan.

Materials and Methods: A total of 76 clinical wound samples were collected from Hazrat Fatemeh Zahra Hospital in Isfahan and evaluated by polymerase chain reaction (PCR) methods. The Methicillin resistance *Staphylococcus aureus* (MRSA) screening was performed by genotypic and phenotypic methods; also antibiotic resistance pattern was determined by using the disk diffusion method and related genes by PCR.

Results: Totally, 53 (69.7%) out of 76 clinical samples were positive for MRSA. Of the 76 MRSA strains, 39 (63.51%) were PVL positive (51.3%). The most commonly infected samples were collected from wounds (40.8%). The most commonly detected antibiotic resistance genes were *mecA* (89.61%), *tetK* (88.23%), *tetM* (49.15%) and *msrA* (46.93%). Resultantly, it was shown that MRSA has the highest level of resistance against methicillin (98%), penicillin (97.24%), tetracycline (89.64%). It was also revealed that the most commonly detected *SCCmec* types in the MRSA strains are types II (14.53%) and III (16.82%).

Conclusions: In summary, this paper argues that the orderly surveillance of hospital-associated infections and initial management and supervision of the antibiotic resistance patterns are required to control the prevalence of MRSA.

[*]**Corresponding Author:** Departments of Microbiology, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran. **Email:** ee_tajbakhsh@yahoo.com

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Introduction

Research on bacterial resistance has a long tradition, especially antibiotic resistance pattern in *Staphylococcus aureus* (*S. aureus*) as an important pathogen which can colonize in the community and hospital surfaces or organs [1]. There are several common kinds of infections as reported by *S. aureus* including various soft-tissue, systemic, and endocarditic infections as well as urinary tract infections **(**UTIs) [2]. Due to the widespread prevalence of infections caused by *S. aureus* and also resistance to methicillin, genetic background and the *SCC mec* typing have become especially important [3, 4].

It has been indicated that methicillin or aminoglycosides are antibiotics their resistance patterns of which quickly increases [5]. Methicillin resistance *S. aureus* (MRSA) is mediated by *mecA* gene and *staphylococcal* cassette chromosome mec (*SCCmec*) as a mobile genetic element (size: 21-67 kbp).

As it is indicated, *SCCmec* are classified into 8 different types (I–VIII). The literature review reveals that *SCC mec* type I was specified in 1961 in UK, type II in Japan, and types III, IV and V were distinctive in New Zealand. Several studies related to *SCCmec* elements have demonstrated that hospital acquired-MRSA (HA-MRSA) are detected in type III of *SCCmec* but community acquired-MRSA (CA-MRSA) are susceptible to various widespread antibiotics [6, 7].

Previous studies have emphasized the presence of a wide pattern of resistance to other various therapeutic options such as β-lactamase, macrolides, lincosamides, and mupirocin [7]. Several studies have reported about *msrA* and *msrB* (related to macrolides), *ermA*, *ermB* and *ermC* (related to macrolide, lincosamide, streptogramin B), *mecA* (related to methicillin), *ant* (4′)-*Ia*, *aac* (6′)- *Ie/aph*(2″), and *aph*(3′)-*IIIa* (related to aminoglycosides modifying enzymes), *mupA* (related to mupirocin) and *tet* (related to tetracycline) [8]. Concerning the prevalence of drug resistance, MRSA infections have increased the number of related reviews. Hence, we decided to investigate antimicrobial resistance pattern of *mecA* gene and *SCCmec* phenotypes recovered from the intensive care unit (ICU) in Isfahan, Iran [9, 10].

Materials and Methods

Bacterial isolates

Overall, 76 clinical strains were collected from various samples such as wound (n= 31; 40.8%), blood (n= 5; 6.6%), ear (n= 2; 2.6%), pus (n= 8; 10.5%), body catheter (n= 16; 21%), and urine (n=14; 18.5%) out of hospitalized patients in the ICUs. Furthermore, patient's samples were immediately transported to the laboratory for performing additional tests. All the samples were directly cultured into 7% sheep blood agar (Merck, Darmstadt, Germany) and distinguished by the conventional microbiological methods [11). Further, molecular studies have been performed for drug resistance studies [12].

Antibiotic susceptibility testing

Susceptibility to penicillin (10 u/disk), imipenem (10 µg/disk), cefazolin (30 µg/disk), cefalotin (30 µg/disk), ceftriaxone (30 µg/disk), gentamicin (10 µg/disk), ciprofloxacin (5 µg/disk),

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clindamycin (2 µg/disk), azithromycin (15 µg/disk), erythromycin (15 µg/disk), mupirocin (30 µg/disk), rifampicin (5 µg/disk), tetracycline (30 µg/disk), trimethoprim (5 µg/disk), vancomycin (30 µg/disk), nitrofurantoin (300 µg/disk), and methicillin (30 µg/disk) was determined using the Kirby-Bauer disk diffusion technique in accordance with the clinical and laboratory standards institute (CLSI) [11, 13].

DNA extraction and genes amplification

A typical colony of the biochemically identified *S. aureus* was cultivated in 1 mL tryptic soy broth (TSB) for 24 h at 37˚C. The bacterial genomic DNA of *S. aureus* strains were extracted with a QIAGEN plasmid Minikit (Fermentas, Germany) as recommended. The presence of *mecA, tetK, tetM, ermA, ermC, aacA-D, linA, msrA, vatA, vatC* and *vatB* genes was identified using the Kumar technique [2, 13]. A polymerase chain reaction (PCR) primer for amplification of antibiotic resistance genes in *S. aureus* strains and SCCmec typing was selected by references [4, 6].

Multiplex PCR amplification for *SCCmec* typing

Different *SCCmec* types determined by specific primers, are listed in table 1. PCR amplification was performed in a volume of 50 ml with Emerald Amp MAX PCR Master Mix (Takara, Japan) for all PCR reactions [14]. The DNA Thermal Cycler 480 (Applied Bio systems, Foster City, CA, USA) was programmed as follows: the first denaturation at 94 8C for 5 min, denaturation at 94 8C for 30 s, annealing at 55 8C for 30 s, and an extension at 72 8C for 60 s for 40 cycles and at last the final extension at 72 8C for 4 min. PCR products were analyzed by electrophoresis on agarose 1.5% with SYBR safe staining according to kit protocol [15, 16].

Results

According to the results, 53 (69.7%) out of 76 clinical samples were methicillin resistance. Furthermore, 39 (63.51%) of MRSA samples were PVL positive (51.3%). It was shown that there are significant differences between the types of infections and incidence of MRSA (p=0.049).

Table 1. Oligonucleotide primers for amplification of SCC*mec* types in *Staphylococcus aureus* strains isolated from Isfahan hospitals

Types	Primer Sequence (5'-3')	Size of product (bp)
***SCCmec* I**	F:GCTTTAAAGAGTGTCGTTACAGG R: GTTCTCTCATAGTATGACGTCC	613
***SCCmec* II**	F: CGTTGAAGATGATGAAGCG R: CGAAATCAATGGTTAATGGACC	398
***SCCmec* III**	F: CCATATTGTGTACGATGCG R: CCTTAGTTGTCGTAACAGATCG	280
***SCCmec* Iva**	F: GCCTTATTCGAAGAAACCG R: CTACTCTTCTGAAAAGCGTCG	776
***SCCmec* IVb**	F: TCTGGAATTACTTCAGCTGC R: AAACAATATTGCTCTCCCTC	493
***SCCmec* IVc**	F:ACAATATTTGTATTATCGGAGAGC R: TTGGTATGAGGTATTGCTGG	200
***SCCmec* IVd**	F: CTCAAAATACGGACCCCAATACA R: TGCTCCAGTAATTGCTAAAG	881
***SCCmec* V**	F: GAACATTGTTACTTAAATGAGCG R: TGAAAGTTGTACCCTTGACACC	325

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Antibiotic susceptibility pattern showed the highest level of resistance against methicillin (98%), penicillin (97.24%), tetracycline (89.64%). Molecular detection of antibiotic resistance genes showed the frequency of *mecA* (89.61%), *tetK* (88.23%), *tetM* (49.15%) and *msrA* (46.93%). It was also revealed that, *SCCmec* types III (16.82%) and II (14.53%) are the most commonly detected *SCCmec* types. There was significant difference in incidence percentage and number of types of infections (p=0.029). Significant difference was also reported as for antibiotic resistance genes compared with the type of infection (p=0.035).

Antibiotic susceptibility pattern in various types of clinical infections is shown in table 2.

According to the results, *mecA* (89.61%) and *tetK* (88.23%) were the most commonly-detected antibiotic resistance genes the lowest patterns of which were related to *vatC (*1.2%) and *vatB* (1%) genes. Other antibiotic resistance genes showed frequency with *msrA* (46.93%), *aacA-D* (18.82%), *tet M* (50.17%), *ermA* (29.20%), *ermC* (29.20%), *vatA* (1.7%), *linA* (9.48%). Recognition procedures of *SCCmec* typing are shown in table 3. It was also identified that the most commonly detected *SCCmec* types in the MRSA strains are types II and III.

Table 2. Antibiotic susceptibility pattern in various types of clinical infections

Antimicrobial agents	Types of Infection %						
	Wound	**Blood**	**Urine**	**Pus**	**Ear**	**Catheter**	**Total%**
Penicillin	48.7	20. 1	12	10	3	3.44	97.24
Imipenem	21	3	1	2	5.5	10	42.5
Cefazolin	12	9	8.1	7	5	10.9	52
Cefalotin	23.1	12.3	8.7	8	4	2.6	58.7
Ceftriaxone	32.1	4.6	20.1	2	1	5.6	65.4
Gentamicin	42.5	12	8.4	3	2.1	4.1	72.1
Ciprofloxacin	34.8	21	10.2	8.1	3.2	10	87.3
Clindamycin	7	2.8	8.2	1	0	2	21
Azithromycin	20.3	7	6.3	9.2	8	2.2	53
Erythromycin	41	21	9	7.3	1.1	4	83.4
Mupirocin	7	2	3	1	2	3.2	18.2
Rifampicin	3	2.3	2	7	1	2	19.3
Tetracycline	40.8	23	13	3	2	7.84	89.64
Trimethoprim	5	1	3	7	2	4	22
Methicillin	60	3	21	10	1	3	98
Vancomycin	1	0	0	0	0	0	1
Nitrofurantoin	1	1	3	0	0	0	5

Table 3. Frequency and recognition procedures of *SCCmec* typing

Infection	Number	TypeI	TypeII	TypeIII	Type VaI	Type VbI	Type VcI	Type VdI	Type V
Wound	21	1	3	15	-	-	1	1	-
Blood	5	2	7	8	-	-	-	-	-
Urine	11	2	10	11	1	-	-	1	-
Pus	8	-	1	-	-	-	-	-	2
Ear	2	-	1	-	-	-	-	-	-
Catheters	5	1	-	1	-	1	-	-	-
Total	53	6	22	35	1	1	1	2	2

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Discussion

All clinical samples were obtained from Hazrat Fatemeh Zahra Hospital in Isfahan, and were evaluated by PCR methods. The MRSA screening was performed by genotypic and phenotypic methods; also antibiotic resistance pattern was determined by using the disk diffusion method and related genes by PCR. According to the results, in environmentally-infected hospitals, antibiotics are used at a highly irregular manner in ICUs. Similar studies with various results, all of which indicating high drug resistance, have been reported previously by different authors [17, 18]. Because of the lack of sampling limits and section specific conditions we decided not to investigate patient endotracheal tube specimens. Totally, 53 (69.7%) out of 76 clinical samples were positive for MRSA and also out of 76 MRSA strains, 39 (63.51%) were PVL positive.

These results indicate that refractory Staphylococcal diseases are highly prevalent and can threaten patients and even healthy individuals in hospitals. Staphylococcus strains of our investigation had the highest levels of antibiotic resistance against methicillin, erythromycin, ciprofloxacin and penicillin. The lowest resistance was identified for vancomycin and nitrofurantoin.

A similar conclusion was reached by Nourbakhsh and co-authors that showed the highest resistance belonging to methicillin, erythromycin, ciprofloxacin and penicillin and the lowest relating to vancomycin and nitrofurantoin [4]. They demonstrated that *SCCmec* III is the most type out of 103 *mec* positive strains. Results of both studies revealed significant relation between frequency of antibiotic resistance genes and the prevalence of *mecA*. We also distinguished statistically significant relationship between various types of isolated samples, the wound isolates and frequency of methicillin resistance *S.aureus*. Previous studies by Ebadi have emphasized 75.7% of detected samples being pertinent to MRSA in comparison with the present study by which the researchers identified 32.1% of samples as being positive for *SCCmec* type I [15].

Similar studies have been carried out by Udo showing 32% incidence rate of the MRSA strains thus revealing overuse of antibiotics in the group [19]. Molecular characterization, evolution, and epidemiology of *S.aureus* by Lakhundi in 2018 reports that new They reported an increase in *S.aureus* pollution as well as the transfer of pollution from the environment to the community clones of *S.aureus* [20]. Because of the importance of investigating drug resistance in *S.aureus*, studies have been conducted on new methods of diagnosis, investigation and web-based tool for typing staphylococcal infections [21].

A similar conclusion was drawn by Dhawan revealing that CA-MRSA (83%) is the most popular *SCCmec* genotype with frequency of *SCCmec* types II [22] thus being similar to those of Alon D et al stating that type II *SCCmec* is the most predominant type of

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mec element [23]. In another research by Halaji et al, Panton-Valentine Leukocidin in Methicillin-Resistant *S.epidermidis* and *SCCmec* was examined in particular. In this research, it was identified that 12.70 (17%) of MRSE isolates carry PVL gene, thus being similar to our results with 51.3% PVL positive [24]. According to our results, *SCCmec* V and *SCCmec* became particularly significant. Similar results have been reported as to other species subtypes such as *SCCmec* V and *SCCmec* IVc, types Iva and types IVb. Similarly, D'Souza indicated that from 97 *mecA*-positives *S.aureus*, 25% were positive for SCC*mec* III and 34% for *SCCmec* IV. In addition, 73% of *SCCmec* IV were reported as multidrug resistant (MDR) strains [25]. These results all indicate a high prevalence of drug resistance.

Conclusion

Our data revealed that *mecA* gene and resistance to methicillin (98%), penicillin (97.24%), tetracycline (89.64%) are the most commonly detected characteristics of the MRSA strains isolated from hospitals infections and also the *SCCmec* type III was predominant among ICU patients. The results of this study demonstrated that regular surveillance of hospital-associated infections and monitoring antibiotic sensitivity patterns are required. In summary, this paper argued that, special care is necessary to control drug resistance.

Conflict of Interest

The authors report no conflicts of interest.

Acknowledgments

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