Nasal colonization of SCCmec II, III and tst-I positive Methicillin resistance Staphylococcal aureus isolated from patients in a hemodialysis unit, Tehran, Iran

Abstract
Patients with end-stage renal disease undergoing dialysis are at the high risk of infections or colonization with MRSA. We evaluated the status colonization of two hemodialysis patients and determined their phenotype by using oxacillin and cefoxitin disks and also studied their genotypes by using PCR. We found the MRSA strains in our cases were healthcare associated harboring SCCmec II & III and tst or PVL gene positive. This report emphasizes the importance of S. aureus status colonization in this group due to MSSA and MRSA isolates can harbor the mobile genetic element of tst gene and also the acquisition of the SCCmec for changing MSSA to MRSA is possible.

Keywords: Staphylococcus aureus, Hemodialysis, SCCmec, mecA, tst gene, PVL, exfoliative gene

Introduction
Infections due to Staphylococcus aureus account for a major cause of morbidity and mortality in hemodialysis patients. Colonization of S. aureus is associated with a four-fold higher risk of bloodstream infection. Hemodialysis patients suffer from a high rate of infection or colonization with MRSA which lead to increased rate of mortality, length of hospital stay, and healthcare costs compared to those infected with methicillin-susceptible S. aureus. Methicillin susceptible S. aureus changes to MRSA by acquisition of staphylococcal Cassette Chromosome mec (SCCmec) a genomic island that encodes methicillin resistance. The tst gene, a mobile genetic element, encodes Toxic shock syndrome toxin 1 (TSST-1) a super-antigenic toxin secreted by both MRSA and MSSA. Besides, Panton-Valentine leukocidin is a gamma-toxin mostly produced by CA-MRSA. The aim was to determine the presence of mecA, lukS/lukF-PV (PVL), eta & etb genes (exfoliative toxin A & B) and tst genes (TSST-1) in two S. aureus colonized patients.

Material and methods
Following our previous research regarding nasal carriage of S. aureus in hemodialysis units, we re-evaluated two of those patients who were colonized with MRSA in the last time and at the time of re-evaluation in order to determine the presence of mecA, lukS/lukF-PV (PVL), eta & etb genes (exfoliative toxin A & B) and tst genes (TSST-1) by PCR. All mecA-positive isolates were classified to staphylococcal cassette chromosome mec (SCCmec) types by multiplex PCR.

Bacterial strains
The nasal samples of patients were cultured in blood agar and manitol salt agar medium, and identification of S. aureus was done using Gram staining and conventional biochemical tests. Finally, the isolates were stored in tryptic soy broth containing 15% glycerol, and stored at -70°C until future processing.

Phenotypic determination of MRSA
The disk diffusion method was used for the phenotypic detection of MRSA. The oxacillin (OX) and cefoxitin (FOX) discs on Mueller-Hinton agar plates containing 4% NaCl were used. A zone of inhibition of < 21mm for oxacillin and <13mm for cefoxitin were considered MRSA (10). Staphylococcus aureus ATCC700698 was used as the positive control for MRSA strains. DNA extraction and polymerase chain reaction
Genomic DNA of MRSA strains were extracted using the commercial kit InstaGene Matrix (BioRad; USA) with the addition of lysostaphin (Sigma–Aldrich; USA) to a final concentration of 15μg/ml. Then, the concentration of DNA was assessed using a spectrophotometer. Genomic DNA was extracted using a commercially available DNA extraction kit (QiaAmp DNA Mini Kit) based on the manufacturer’s instructions. For confirmation of MRSA, the mecA gene was detected by PCR with the specific primers listed in the Table. Staphylococcus aureus ATCC700698 was used as the positive control for mecA gene detection.

SCCmec typing
SCCmec typing was performed for all MRSA isolates by multiplex PCR. Primer sequences have been demonstrated in the Table. SCCmec types were identified by comparing the banding patterns of MRSA to ATCC 10442 (SCCmec type I), N315 (SCCmec type II), 85/2082 (SCCmec type III), MW2 (SCCmec type IV), WIS (SCCmec type V) as reference strains.

Case 1
A 59- year old man was on maintenance hemodialysis for 1.2 year. He has used a fistula as his venous access and suffering from the co-morbidities of diabetes mellitus and hypertension. He did not have a history of admission, surgery, skin and soft tissue infections or taking antibiotics over the previous three months. His MRSA isolate was positive Methicillin resistant Staphylococcus aureus (MRSA) nasal isolate with the presence of mecA, lukS/lukF-PV (PVL), eta & etb genes (exfoliative toxin A & B) and tst-I gene positive. This report emphasizes the importance of S. aureus status colonization in this group due to MSSA and MRSA isolates.
harbored mecA gene, SCCmec type III and tst gene but was negative for exfoliative toxins (eta & etb) and PVL.

**Case 2**
A 58-year-old man was on maintenance hemodialysis for 2.5 years. He had a fistula as his venous access and was suffering from diabetes mellitus and heart failure. His past medical history included taking antibiotics over the previous three months with no hospitalization, surgery or skin and soft tissue infections. The isolated MRSA harbored PVL, mecA gene and SCCmec type II. The isolate was not producing exfoliative toxin A and B and TSST-1. The Table 1 shows the Oligonucleotide primers, and the characteristic information of our patients.

| Table 1 The oligonucleotide primers and characteristic information, A hemodialysis unit, Tehran, Iran |
|------------------|-----------------|-----------------|
| Target | Primer | Primer sequence (5′ → 3′) | Product Size | Reference | Case 1 | Case 2 |
|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| mecA | F | AGAAAGTGATGTGGAAAGTTAG | 583 | 12 | Positive | Positive |
| | R | ATGTAGTGCCGATGTTGAC | | | | |
| luk-PV | F | TCACTATTTTGAACAAGGCAAGCCACTT | 180 | 13 | Negative | Positive |
| | R | TAAAATAGTTTTTTTACTGCAGCCCTT | | | | |
| tst-1 | F | TTATGTCAGCCCTTTTGTG | 398 | 12 | Positive | Negative |
| | R | TAAAGGATTCTCTTATGGATTAG | | | | |
| eta | F | GCAAGGTGTGTTGATGTTGACATTTTTTATCGTAAGCCCTT | 93 | | Negative | Negative |
| | R | AGATGTCCTATTATTTTGTGCTG | | | | |
| etb | F | ACAAGCAGAAGATACAGCG | 226 | 14 | Negative | Negative |
| | R | GTTTTTGCTGCTTCTTCTTGTG | | | | |
| | Fβ | ATGCGCTTGATAAGCGCTTCTTCTT | | | | |
| | Rα3 | TAAAGGCACTAATGGCAAAACACT | 937 | 15 | | |
| F ccrC | F | CGTCTATGACAGTTGAAGGTAAT | | | | |
| | R ccrC | CCTTTATAGCCTCGGCTATTCAATAT | 518 | 16 | Positive | |
| SCCmec | F 1272 | GCCACTCATAACATATGGA | 415 | 15-17 | Positive | |
| | R 1272 | CATCGGATGAACCAACAAA | | | | |
| | F 5RmecA | TATACCCAAAAACCGCAACTAC | 359 | 16-17 | | |
| | R 5R431 | CGGGCTACAGTGAAACATCC | | | | |
| | F | ATCATAGGTTAAGGCTGGACACATGATCCA | | | | |
| | R | GCATCAAGTGATTGGATAGCAAAACG | 433 | 13 | | |

**Discussion**

The isolated MRSA of our patients harbored SCCmec II & III as which is expected. The MRSA infections in hemodialysis patients were conventionally considered to be acquired from healthcare (HA), usually with strains harboring SCCmec types II/III. Moreover, both MRSA clones in our patients were in agreement with the geographic distribution and pattern of MRSA clones in Asian countries. Besides, one of our cases was colonized with MRSA harboring PVL and SCCmec II. In other research, many CA-MRSA isolates harbored PVL gene and CA-MRSA harboring SCCmec IV & V was associated with PVL gene carriage. Therefore, it seems our clone was HA-MRSA harboring PVL gene.

The MRSA isolated from the case 1 in present study harbored tst-1 gene. The tst gene was identified among MSSA and MRSA and also was detected in both CA-S.aureus and HA-S.aureus. The tst-positive strains usually spread silently in hospitals and do not always lead up to clinical symptoms. However, in an in-vitro study, one strain of MRSA which was isolated from a hemodialysis patient with the catheter-related infection could have expressed the tst gene. Besides, nasal colonization of healthcare workers with TSST-1 positive S.aureus was reported and MRSA harboring tst-1 gene was known as an endemic pathogen in the hospital. We think the isolated tst-positive MRSA in our patient was HA-MRSA.

The MRSA colonized in the case I had SCCmec III and tst gene. A higher mortality rate in patients with tst-1 positive or SCCmec II MRSA isolates was reported. It seems harboring tst gene in case of clinical symptoms would be associated with a poor prognosis. However, none of the tst-positive S.aureus strains was positive for PVL in previous reports that is similar to present report.

**Conclusion**

Because both MSSA and MRSA isolates can harbor the mobile genetic element of tst gene and also the acquisition of the SCCmec for changing MSSA to MRSA is possible, this study has emphasized that how important is to evaluate and detect the colonization status of hemodialysis patients for S.aureus regardless of the resistant pattern.

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Conflicts of interest

Authors declare that there is no conflicts of interest.

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