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Nasal colonization of SCCmec II, III and tst-1 positive Methiticillin 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 SCC*mec 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 SCC*mec* for changing MSSA to MRSA is possible.

Keywords: *Staphylococcus aureus*, Hemodialysis, SCC*mec*, *mecA*, *tst* gene, PVL, exfoliative gene

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Introduction

Infections due to Staphylococcus aureus account for a major cause of morbidity and mortality in hemodialysis patients.[1,2] 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.[3,4] Methicillin susceptible S.aureus changes to MRSA by acquisition of staphylococcal Cassette Chromosome mec (SCCmec) a genomic island that encodes methicillin resistance.[5] 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.[6] Besides, Panton-Valentine leukocidin is a gamma-toxin mostly produced by CA-MRSA.[7] 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,[8] 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 (SCC*mec*) types by multiplex PCR.

Bacterial strains

The nasal samples of patients were cultured in blood agar and mannitol salt agar medium, and identification of *S. aureus* was done using Gram staining and conventional biochemical tests.[9] 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.[11]

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.[12] *Staphylococcus aureus* ATCC700698 was used as the positive control for *mecA* gene detection.[11]

SCCmec typing

SCC*mec* typing was performed for all MRSA isolates by multiplex PCR. Primer sequences have been demonstrated in the Table 1. SCC*mec* types were identified by comparing the banding patterns of MRSA to ATCC 10442 (SCC*mec* type *I*), N315 (SCC*mec* type *II*), 85/2082 (SCC*mec* type *III*), MW2 (SCC*mec* type *IV*), WIS (SCC*mec* 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

harbored *mecA* gene, SCC*mec* type *III* and *tst* gene but was negative for exfoliative toxins (*eta* & *etb*) and PVL.

Case 2

A 58year-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 SCC*mec* 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	Case1	Case2
mecA	F	AGAAGATGGTATGTGGAAGTTAG	583	12	Positive	Positive
	R	ATGTATGTGCGATTGTATTGC				
luk-PV	F	TTCACTATTTGTAAAAGTGTCAGACCCACT	180	13	Negative	Positive
	R	TACTAATGAATTTTTTTATCGTAAGCCCTT				
tst-1	F	TTATCGTAAGCCCTTTGTTG	398	12	Positive	Negative
	R	TAAAGGTAGTTCTATTGGAGTAGG				
eta	F	GCAGGTGTTGATTTAGCATT	93	14	Negative	Negative
	R	AGATGTCCCTATTTTTGCTG				
etb	F	ACAAGCAAAAGAATACAGCG	226	14	Negative	Negative
	R	GTTTTTGGCTGCTTCTCTTG				
SCCmec	Fβ	ATTGCCTTGATAATAGCCYTCT	937	15		
	R α3	TAAAGGCATCAATGCACAAACACT				
	F ccrC	CGTCTATTACAAGATGTTAAGGATAAT	518	16		Positive
	R ccrC	CCTTTATAGACTGGATTATTCAAAATAT				
	F 1272	GCCACTCATAACATATGGAA	415	15-17	Positive	
	R 1272	CATCCGAGTGAAACCCAAA				
	F 5RmecA	TATACCAAACCCGACAACTAC	359	16-17		
	R 5R431	CGGCTACAGTGATAACATCC				
	F	ATCATTAGGTAAAATGTCTGGACATGATCCA	433	13		
	R	GCATCAAGTGTATTGGATAGCAAAAGC				

Discussion

The isolated MRSA of our patients harbored SCC*mec II* & *III* which as expected. The MRSA infections in hemodialysis patients were conventionally considered to be acquired from healthcare (HA), usually with strains harboring SCC*mec* types *II/III*.[18] Moreover, both MRSA clones in our patients were in agreement with the geographic distribution and pattern of MRSA clones in Asian countries.[19] Besides, one of our cases was colonized with MRSA harboring PVL and SCC*mec II*. In other research, many CA-MRSA isolates harbored PVL gene[7] and CA-MRSA harboring SCC*mec IV* & *V* was associated with PVL gene carriage.[20] 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[21,22] and also was detected in both CA-S.aureus and HA- S.aureus.[23] The *tst*-positive strains usually spread silently in hospitals and do not always lead up to clinical symptoms.[24] 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.[25] Besides, nasal colonization of healthcare workers with TSST-1 positive S.aureus was reported[26] and MRSA harboring *tst-1* gene was known as an endemic pathogen in the hospital.[27] We think the isolated *tst*-positive MRSA in our patient was HA-MRSA.

The MRSA colonized in the case I had SCC*mec III* and *tst* gene. A higher mortality rate in patients with *tst-1* positive or SCC*mec II* MRSA isolates was reported.[28] 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[29] 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 SCC*mec* 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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None.

Conflicts of interest

Authors declare that there is no conflicts of interest.

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