DISTRIBUTION OF GENES ENCODING TETRACYCLINE RESISTANCE AND AMINOGLYCOSIDE MODIFYING ENZYMES IN *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM A BURN CENTER

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SUMMARY. *Staphylococcus aureus* (*S. aureus*) is one of the most common organisms associated with infections among burn patients and has shown a frequent and rapid development of antibiotic resistance. The presence of genes encoding aminoglycoside modifying enzymes (AME) and tetracycline resistance were detected by PCR and multiplex-PCR. Among the 151 *S. aureus* isolates recovered from the burn patients, 96 (63.6%) were detected to have *mecA* gene. The rate of tetracycline resistance genes associated with *mecA* was 61% (92/151). Forty nine isolates (32.4%) contained *tetM*, 26 (17.2%) possessed only *tetK* and 21 (13.9%) contained both *tetM* and *tetK*. The presence of the *aac(6')-Ie-aph(2'')-I* gene was determined in 18 isolates, *aph(3')-IIIa* in 8 isolates, both the *aac(6')-Ie-aph(2'')-I, aph(3')-IIIa* and the *ant(4')-Ia* genes in 69 isolates, both *aac(6')-Ie-aph(2'')-I* and *ant(4')-Ia* in 6 isolates, and both the *aph(3')-IIIa* and the *ant(4')-Ia* genes in 8 isolates. Most of the strains which harboured the *mecA* gene also contained the *tet* and AME genes.

Keywords: *Staphylococcus aureus,* burn, antibiotic resistance

Introduction

Infection is one of the most serious problems in patients hospitalized with thermal injury, and still remains a leading cause of death among these patients.[1] Infections caused by antibiotic resistant bacteria should be considered as a potential risk and their resistance pattern should be identified as soon as possible. *Staphylococcus aureus* is known to be one of the major causes of infections acquired in hospitals and communities worldwide, and is one of the most common organisms associated with infections among burn patients.[2, 3] Since the introduction of antibiotics in medicine, *S. aureus* has shown a frequent and rapid development and spread of antibiotic resistance, and has developed resistance to all types of antibiotics.[4]

Tetracyclines are broad-spectrum antibiotics used in the treatment and prevention of bacterial infections.[5] Most tetracycline resistant bacteria have acquired tetracycline resistance genes (*tet*). Two main mechanisms of resistance to tetracycline have been described in *S. aureus*: active efflux, resulting from the acquisition of the plasmid-located *tetK* and *tetL* genes and ribosomal protection by elongation factor-like proteins that are encoded by chromosomal or transposonal *tetM* or *tetO* determinants.[6, 7]

Aminoglycosides are broad-spectrum antibiotics that are used in combination with other antibiotics such as β-lactams for treatment of *S. aureus* infections.[8] Inactivation of aminoglycoside antibiotics by aminoglycoside modifying enzymes (AME), such as aminoglycoside phosphotransferase (APH), acetyl-transferases (AAC), and nucleotidyl-transferase (ANT) enzymes, is the most common mechanism of aminoglycoside resistance. [8, 9] The most common AME encoding genes among *S. aureus* are *aac(6')-Ie-aph(2'')*, *aph(3')-IIIa*, and *ant(4')-Ia*, which can be harbored on plasmid or chromosome and are often harbored on transposable elements.[10]

The results of an earlier investigation at the burn unit in Tehran have shown that the incidence of *S. aureus* is

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high and the majority of the isolates were resistant to tetracycline and aminoglycoside antibiotics phenotypically.[3] This study was conducted to investigate the incidence of tetracycline and aminoglycoside resistance determinants among a collection of *S. aureus* strains isolated from burn patients.

Materials and methods

Bacterial isolates

A total of 151 non duplicate (i.e., only one isolate per patient) and non-consecutive *S. aureus* isolates were collected, during September 2010 and June 2012, from clinical samples of burn patients, admitted to the burn ward at Shahid Motahari Hospital, Tehran, Iran. Isolates were identified to the species level by Gram staining, production of coagulase, catalase, DNase and oxidation, and fermentation of mannitol.

Antimicrobial susceptibility testing

Tests for susceptibility to oxacillin (1 µg), gentamicin (10 µg), amikacin (30 µg), kanamycin (30 µg), netilmicin (30 µg), tetracycline (30 µg), doxycycline (30 µg), and minocycline (30 µg) were performed by the disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI).[11] *S. aureus* ATCC 25923 was used as the control strain.

Amplification of mecA, AMEs and tet genes

Total DNA template was extracted from *S. aureus* isolates by the boiling method previously outlined.[12] Under the standard polymerase chain reaction (PCR) conditions, a series of primers (*Table I*) were used, according to the methods described below, for the detection of *mecA*, *tet* and AME genes. Recognition of genes encoding the AMEs enzymes (*aac(6')-Ie-aph(2'')-I*, *aph(2'')-Ib*, *aph(2'')-Ic*, *aph(2'')-Id*, *aph(3')-IIIa*, and *ant(4')-Ia*) and methicillin resistance (*mecA*) was performed as described previously.[9, 10] Amplification of *tet* genes (*tetK*, *tetL*, *tetM* and *tetO*) was performed using CinnaGen PCR master kit (CinnaGen Co.). PCR was performed in a final volume of 25 ml containing 0.5 µM of each primer. The PCR protocol consisted of an initial denaturation step at 94°C for 5 min; 30 amplification cycles at 94°C for 1 min, at 57°C for 1 min (for *tetO*, at 51°C for *tetM*, *tetL*, and *tetK*), and at 72°C for 1 min, followed by a final extension step at 72°C for 5 min. Amplified products were analyzed by electrophoresis on 1% agarose gel containing 0.5 µg/ml ethidium bromide and photographed under UV illumination.

Statistical analysis

Differences in the prevalence of genes among *S. aureus* isolates were calculated using the chi-square test for each gene. A P value of ≤ 0.05 was considered as statistically significant.

Table I - The oligonucleotide primers used for PCR amplified antimicrobial resistance

Gene	Primers (5'-3')	Size of the amplified product (bp)	Reference
mecA	F-TCCAGATTACAACTTCACCAGG R-CCACTTCATATCTTGTAACG	162	9
aac(6')-Ie-aph(2'')-I	F-CAGGAATTTATCGAAAATGGTAGAAAAG R- CACAATCGACTAAAGAGTACCAATC	369	10
aph(2'')-Ib	F-CTTGGACGCTGAGATATATGAGCAC R- GTTTGTAGCAATTCAGAAACACCCTT	867	10
aph(2'')-Ic	F- CCACAATGATAATGACTCAGTTCCC R- CCACAGCTTCCGATAGCAAGAG	444	10
aph(2'')-Id	F-GTGGTTTTTACAGGAATGCCATC R-CCCTCTTCATACCAATCCATATAACC	641	10
aph(3')-IIIa	F-GGCTAAAATGAGAATATCACCGG R-CTTTAAAAAATCATACAGCTCGCG	523	10
ant(4')-Ia	F-CAAACTGCTAAATCGGTAGAAGCC R-GGAAAGTTGACCAGACATTACGAACT	294	10
tetK	F- GTAGCGACAATAGGTAATAGT R- GTAGTGACAATAAACCTCCTA	360	13
tetM	F- AGTGGAGCGATTACAGAA R- CATATGTCCTGGCGTGTCTA	158	13
tetL	F-ATAAATTGTTTCGGGTCGGTAAT R- AACCAGCCAACTAATGACAATGAT	1077	14
tetO	F-AACTTAGGCATTCTGGCTCAC R-TCCCACTGTTCCATATCGTCA	514	15

Results

All the isolates were investigated for the presence of genes encoding tetracycline, aminoglycoside and methicillin resistance. The results are summarized in *Tables II* and *III*.

The *mecA* gene encoding methicillin resistance was determined in 63.6% (96/151) of the *S. aureus* isolates included in this study.

The rate of tetracycline resistance genes associated with *mecA* was 61% (92/151). All the tetracycline resistant isolates, excluding 11 isolates, gave positive results for *tet* genes. Forty nine isolates (32.4%) contained *tetM*, 26 (17.2%) possessed *tetK* and 21(13.9%) included both *tetM* and *tetK* genes. Four isolates were sensitive to tetracycline but positive for the *tet* genes (two isolates contained both *tetM* and *tetK* and two isolates contained only *tetK*). None of the isolates were positive for *tetL* and *tetO* in the PCR assay.

The presence of the *aac(6')-Ie-aph(2'')-I* gene was determined in 18 isolates, the *aph(3')-IIIa* in 8 isolates, both the *aac(6')-Ie-aph(2'')-I, aph(3')-IIIa* and the *ant(4')-Ia* genes in 69 isolates, both *aac(6')-Ie-aph(2'')-I* and the *ant(4')-Ia* in 6 isolates, and both the *aph(3')-IIIa* and the *ant(4')-Ia* genes in 8 isolates. The gene encoding AME was encountered in at least 95 isolates whilst it was absent in 56 of the isolates. Amplification of the *aph(2'')-Ib, aph(2'')-Ic* and *aph(2'')-Id* genes was negative for all the isolates.

Coexistence of the *tet* and AME genes were encountered in 55 of the isolates.

Table II - Distribution of tetracycline resistance genes among *S. aureus* isolates

	tet genes present			
Resistance patterns	*tetM*	*tetK*	*mecA*	No. of Isolates
T,	+	+	+	21
T,	+	-	+	46
T,	-	+	+	24
T,DXT	-	+	+	1
T,DXT	-	-	-	3
T	+	-	-	3
T	-	+		1
T	-	-	-	8
-	+	+	-	2
-	-	+		2
-	-	-	+	4

T; tetracycline, DXT; doxycycline , MN; minocycline

Discussion

Tetracyclines still remain the first-line treatment for a number of infections in many parts of the world, including Iran.[3, 16, 17] According to the literature review, there is a limited amount of data regarding the prevalence rate of *tet* genes among *S. aureus* strains isolated from burn patients in Iran. In this study, the coexistence rate of *mecA* and *tet* genes was 61%, which is slightly higher than similar reports from Europe (57.1%) and lower than those from Japan (100%).[16-19]

In the current study, the most common tetracycline resistance mechanism is mediated by *tetM* and *tetK* genes, respectively. The resistance mechanism mediated by these genes is predominant among tetracycline resistant MRSA from Turkey, Malaysia, and most European countries, but not so in North America where there is a high prevalence rate of the *tetK* gene among MRSA isolates.[5, 16, 18-21] The *tet* genes are contained within conjugative transposons that can be transferred horizontally and expressed in Gram-positive and Gram-negative bacteria.[22]

Coexistence of *tetM* and *tetK* genes among the *S. aureus* isolates was detected in this study, as well as in the study of Trzcinski et al.[14] Our results are also consistent with those reporting the *tetL* and the *tetO* genes to be rarely detected in *S. aureus* isolates.[18-21]

The *aac(6')-Ie-aph(2'')* gene encodes the AAC(6')-APH(2'') enzyme, a bifunctional enzyme with kinase activity, that inactivates a broad range of aminoglycosides and confers concomitant resistance to gentamicin and the

Table III - Distribution of aminoglycoside resistance genes among the *S. aureus* isolates

	AME genes present				
Resistance patterns	*aac(6')-Ie-aph(2'')-I*	*aph(3')-IIIa*	*ant(4')-Ia*	*mecA*	No. of Isolates
AK, GM, K, NET, TN	+	+	+	+	66
AK, GM, K, TN	+	+	+	+	3
AK, GM, K, NET, TN	+	-	+	+	6
AK, GM, K, NET, TN	+	-	-	+	10
AK, GM, K, NET, TN	-	+	+	+	8
-	-	-	-	+	3
-	+	-	-	-	2

AK; amikacin , GM; gentamicin ,K; kanamycin, NET; netilmicin, TN; tobramycin

majority of aminoglycosides commonly used in medical practice.[8, 9, 23] As also reported in other studies, the *aac(6')-Ie-aph(2'')* gene was the most common AME gene among the *S. aureus* isolates and was found either alone or with other AME or *tet* genes.[9, 23]

Remarkably, most of the strains which harboured the *mecA* gene also contained the *tet* and AME genes. These genes might be located on the same or associated genetic element. In MRSA, methicillin resistance is mediated by *mecA*, which is carried on Staphylococcal Cassette Chromosome mec (SCC*mec*). SCC*mec* type III is the dominant SCC*mec* type in Iran; this type includes not only the *mecA* gene, but also the genes encoding resistance to several non-beta-lactam antibiotic classes, such as tetracyclines and aminoglycosides.[24]

Conclusion

This study has shown that the *S. aureus* isolates recovered from burn patients contain a variety of tetracycline and amino glycoside resistance genes, and *tetM* and *aac(6')-Ie-aph(2'')* genes were the most common *tet* and AME genes among the *S. aureus* isolates, respectively.

RÉSUMÉ. Staphylococcus aureus (S. aureus) est l'un des organismes les plus communs associés à des infections chez les patients souffrant de brûlures et a montré une évolution fréquente et plus rapide de la résistance aux antibiotiques. La présence de gènes codant pour des enzymes de modification de l'aminoglycoside et de la résistance tétracycline a été détectée par PCR et PCR multiplexe. Parmi les 151 isolats de S. aureus récupérés des patients brûlés, 96 (63,6%) contenaient le gène mecA. Le taux de gènes de résistance à la tétracycline associés à mecA était de 61% (92/151). Quarante-neuf isolats (32,4%) contenaient tetM, 26 (17,2%) ne possédaient que tetK et 21 (13,9%) contenaient tetM et tetK. La présence du gène aac(6 ')-Ie-aph ('' 2)-I a été identifiée dans 18 isolats, et le gène aph(3')-IIIa dans 8 isolats. Les gènes aac(6 ')-Ie-aph(2'')-I, aph(3')-IIIa et ant(4')-Ia ont été identifiés dans 69 isolats, aaC (6 ')-Ie-aph (2'')-I et ant(4')-Ia dans 6 isolats, et l'aph (3')-IIIa et ant(4')-Ia dans 8 isolats. La plupart des souches qui hébergeaient le gène mecA contenait également des gènes tet et AME.

Mots-clés: *Staphylococcus aureus*, brûlure, résistance aux antibiotiques

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