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Detection of Toxic Shock Syndrome Toxin (*tsst*) Gene Among *Staphylococcus aureus* Isolated from Patients and Healthy Carriers

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Abstract

Background: *Staphylococcus aureus* is the major causative agent of hospital-acquired and community-acquired infections. These bacteria produce a wide variety of exotoxins, including Toxic Shock Syndrome Toxin (TSST) and virulence factors, which are thought to contribute to its pathogenic potential.
Objectives: The aim of this study was to identify *tsst* gene in *S. aureus* isolated from patients and healthy carriers.
Methods: In this cross-sectional study, a total of 60 human *S. aureus* isolates were collected from individuals referred to Shahid Beheshti hospital (patients, n = 40) and healthy farm workers (n = 20) in Hamadan province of Iran. Thereafter, DNA samples were extracted using the phenol-chloroform method and the samples were investigated for *tsst* gene using a specific PCR assay.
Results: The DNA fragment corresponding to the *tsst* gene (326 bp) was observed in 45% (9 out of 20) of *S. aureus* isolated from healthy farm workers; while, 22.5% (9 out of 40) of patients' isolates were found to be positive for *tsst* gene, which indicated that in total 30% of the isolates possessed this gene.
Conclusions: The results of the present study showed the high prevalence of the *tsst* gene among *S. aureus* isolated from healthy farm workers and patients. Therefore, appropriate precautions must be considered to decrease the risk of transmission of such isolates to other humans.

Keywords: Hamadan, *Staphylococcus aureus*, *tsst* Gene

1. Background

Staphylococcus aureus is the main etiological agent of various diseases, including skin and soft-tissue infections (SSTIs), endovascular infections, urinary tract infections (UTIs), bacteremia, and sepsis in both hospitalized and non-hospitalized humans, which is frequently reported from different parts of the world (1).

One of the important prerequisites of infections by these bacteria is its ability to establish a human commensal. *Staphylococcusaureus* is commonly resident in anterior nares of people. It has been reported that 10% to 40% of the population carries this organism (2). Nasal carriers are more susceptible to nosocomial infections than non-carrier individuals and increased risk of infection with these bacteria has been documented in patients with end-stage renal failure after surgery (3).

Staphylococcus aureus produces a wide variety of exoproteins that contribute to its pathogenicity. However, only a small number of its isolates produce additional exoproteins, such as *Toxic Shock Syndrome Toxin* (TSST), which belongs to pyrogenic toxin superantigens (PTSAgs) (4). Furthermore, TSST is a protein with 22-kD molecular weight, which is encoded by the *tsst* gene (5). This protein affects cells of the immune system and stimulates release of interleukin-1, interleukin-2, tumor necrosis factor-alpha (TNF-a) and nonspecific T cell proliferation, which may lead to a severe and potentially fatal disease in humans, known as toxic shock syndrome (TSS) (6-8).

2. Objectives

The present study was conducted to investigate TSST-encoding gene (*tsst*) in *S. aureus* isolated from both healthy carriers and patients in Hamadan.

3. Methods

3.1. Isolation of Staphylococcus aureus

The present cross-sectional study was performed on *S. aureus* isolated from patients and healthy carriers. Also, useful data, including gender, age, and tobacco use were collected through a questionnaire from these people.

Patients: forty *S. aureus* isolates were obtained from different clinical samples (skin, tracheal tube, blood, urine, and sputum) of individuals referred to Shahid Beheshti hospital of Hamadan for a period of 9 months from January 2014 to September 2015. These isolates were identified based on common biochemical tests.

Healthy carriers: overall, 100 specimens were collected from nasal cavity of healthy carriers, who worked in dairy farms using sterile cotton swabs moistened in sterile normal saline. Age, gender, and previous tobacco usage among all of the sampled people were also recorded to see if these characteristics had a relationship with *S. aureus* carriage. The tips of the swabs were placed in a tube (containing 1 mL of phosphate-buffered saline) and 100 μL of this suspension was spread onto mannitol salt agar medium followed by incubation at 37°C for 48 hours (which resulted in the isolation of 20 *S. aureus* isolates).

Finally, all of the isolates (n = 60) were molecularly confirmed to be *S. aureus*, using a species-specific Polymerase Chain Reaction (PCR) (data are not shown).

3.2. Extraction of DNA Samples

Bacterial DNA was extracted from an overnight tryptic soy broth (TSB, Merck) culture of each isolate using the phenol-chloroform method (9).

3.3. Polymerase Chain Reaction Amplification of the tsst Gene

The target sequence of *tsst* gene was amplified by PCR using primers, which have been previously reported by Mehrotra et al. (Table 1) (10). The total reaction volume (25 μL) contained 2.5 μL of 10X PCR buffer (500 mM, KCL and Tris HCL, pH 8.4), 0.5 μL (12.5 mM) $MgCl_2$, 0.5 μL (200 mM) dNTPs, 0.5 μL (50 pmol) of each primer (SinaClon, Iran), 3.5 μL (120 ng) of extracted DNA, 17 μL distilled water, and 1 U of Taq DNA polymerase (SinaClon, Iran). The PCR amplification was performed under the following conditions: initial denaturation at 95°C for 6 minutes followed by denaturation at 95°C for 1 minute, annealing at 55°C for 35 seconds and extension at 72°C for 1:30 minutes (32 cycles) and a final extension at 72°C for 10 minutes. After amplification, the PCR products were analyzed by electrophoresis on 1.5% agarose gel containing ethidium bromide (0.5 μg/mL).

Table 1. Primers Used for Amplification of the *tsst* Gene

Primer	Sequence 5' - 3'	Product size, bp
***tsst*-forward**	ACCCCTGTTCCCTTATCATC	326
***tsst*-reverse**	TTTTCAGTATTTGTAACGCC	

3.4. Cefoxitin Disk Diffusion Method to Detect Methicillin-Resistant Strains

In the author's previous study, methicillin susceptible isolates were tested by the disk diffusion method using oxacillin disk (Merck, Germany), and the *mecA* gene (methicillin resistance gene) was identified (11). In the present study, sensitive strains to methicillin were confirmed by cefoxitin disk diffusion method (12).

3.5. Statistical Analysis

The results were compared by Chi-square test using the SPSS software, and $P < 0.05$ was considered statistically significant.

4. Results

Patients: a total of 40 *S. aureus* were isolated from the patients (30% from skin, 27.5% from trachea, 25% from urine, 10% from blood, and 7.5% from sputum). The number of *S. aureus* isolated from males (67.5%) was more than that from females, and this was statistically significant (P = 0.02). Besides, most of these isolates (67.5%) belonged to people aged over 50 years old and the statistical analysis showed that there was a significant difference in the isolation of *S. aureus* in various age groups (P = 0.02). However, no difference was observed between tobacco usage and isolation of these bacteria. In addition, the results of cefoxitin disk diffusion method, except in two cases, were the same as the results of oxacillin disk diffusion method, to detect methicillin-resistant isolates.

As shown in Figure 1, *tsst* amplicon of the expected size (326 bp) was detected for some of the patients' and carriers' isolates. Altogether, 9 out of 40 *S. aureus* patients' isolates (22.5%) were found to be positive for the *tsst* gene. All of the *tsst* positive isolates were methicillin-resistant *Staphylococcus aureus* (MRSA) strains except one that was a methicillin-sensitive *Staphylococcus aureus* (MSSA) isolate. Detailed data about isolated *S. aureus* from the patients and carriers are presented in Tables 2 and 3.

Carriers: twenty *S. aureus* were isolated from 100 collected nasal swabs. Unlike patients' isolates, the majority of carriers' isolates (60%) belonged to people aged between 30 and 50 years old. Moreover, 7 isolates (35%) belonged to smokers and Chi-square test revealed that this was statistically significant (P = 0.01). The numbers of *S. aureus* isolated from the 2 groups of individuals (patients and carriers) are compared in Figure 2 based on each category.

The results of PCR assay for 20 *S. aureus* isolated from nasal cavity of healthy carriers indicated that 9 isolates were positive for the *tsst* gene (Figure 1). Nevertheless, unlike patients' isolates, none of these isolates were an MRSA

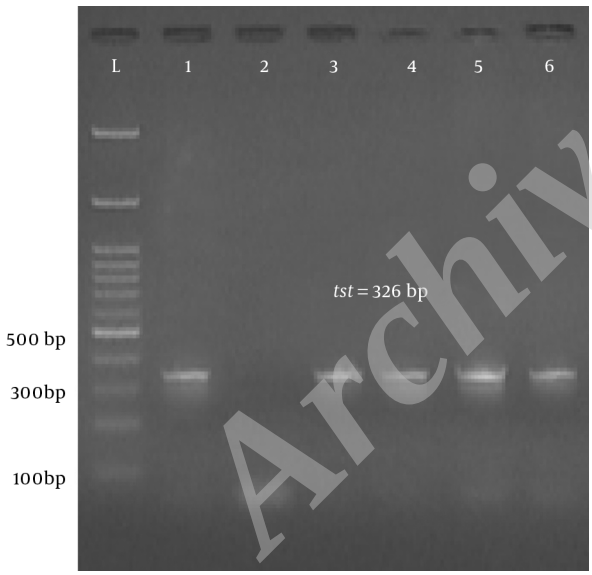
Table 2. Number of *Staphylococcus Aureus* Isolated from Patients

Sample type	No. of Isolates	No. of MRSA	Gender		Age			Tobacco Usage	*tsst* Positive
			Male	Female	30 >	30 - 50	50 <		
Skin	12	7	8	4	2	4	6	1	2
Trachea	11	11	7	4	1	0	10	2	2
Urine	10	10	5	5	1	2	7	0	2
Blood	4	4	4	0	0	3	1	0	2
Sputum	3	2	3	0	0	0	3	1	1
Total	40	34	27	13	4	9	27	4	9

Table 3. Number of *Staphylococcus Aureus* Isolated from Healthy Carriers

Gender	No. of Samples	No. of Isolates	No. of MRSA	Age (Positive Carriers)			Tobacco Usage	*tsst* Positive
				30 >	30 - 50	50 <		
Male	90	20	1	3	12	5	7	9
Female	10	0	0	0	0	0	0	0
Total	100	20	1	3	12	5	7	9

Figure 1. Electrophoresis of the Polymerase Chain Reaction Products Obtained from Amplification of the *tsst* Gene



Lane 1, a *tsst* positive strain (*S. aureus* ATCC 25923); Lane 2, negative control (contained no template DNA); Lanes 3 - 6, *S. aureus* isolates, which were positive for *tsst* gene; Lane L, a 100 bp DNA ladder.

strain. According to the results, the proportion of *tsst*-positive isolates in carriers (45%) was significantly higher with respect to the patients (22.5%) (P = 04).

5. Discussion

Staphylococcus aureus is a common cause of nosocomial and community-associated infections. Diseases with these bacteria increase length of hospital stay, antibiotic use, costs, and mortality. Besides, some of *S. aureus* strains are MRSA, which are known as the most prevalent antimicrobial resistant bacteria isolated in various continents, including Americas, North Africa, Europe, and the Middle- and Far East (13). Fluit et al. reported that *S. aureus* was the most common causative agent of nosocomial pneumonia, and skin and soft tissue infections (1). In the present study, the percentages of isolated *S. aureus* from skin and trachea were higher than the other samples. However, a high percentage of the isolated bacteria also belonged to urine samples. Furthermore, 65% of patients' isolates belonged to elderly suggesting that old people may be at higher risk of infection with these bacteria. In this case, Kang et al. indicated that nosocomial infections with *S. aureus* were more prevalent in the elderly group (14) and the current finding was in agreement with this result. However, some researchers found no significant difference between isolated bacteria and age of the patients (15).

On the other hand, nasal carriage is a risk factor for acquiring nosocomial infection. Von Eiff et al. reported that nosocomial *S. aureus* bacteremia in carriers was attributable as an endogenous source (16). Therefore, elimination of *S. aureus* from nasal carriers is of great help in controlling such diseases. Kluytmans et al. showed that using mupirocin nasal ointment may lead to significant reduction in the rate of surgical-wound infections (17). Some researchers also reported that elimination of *S.*

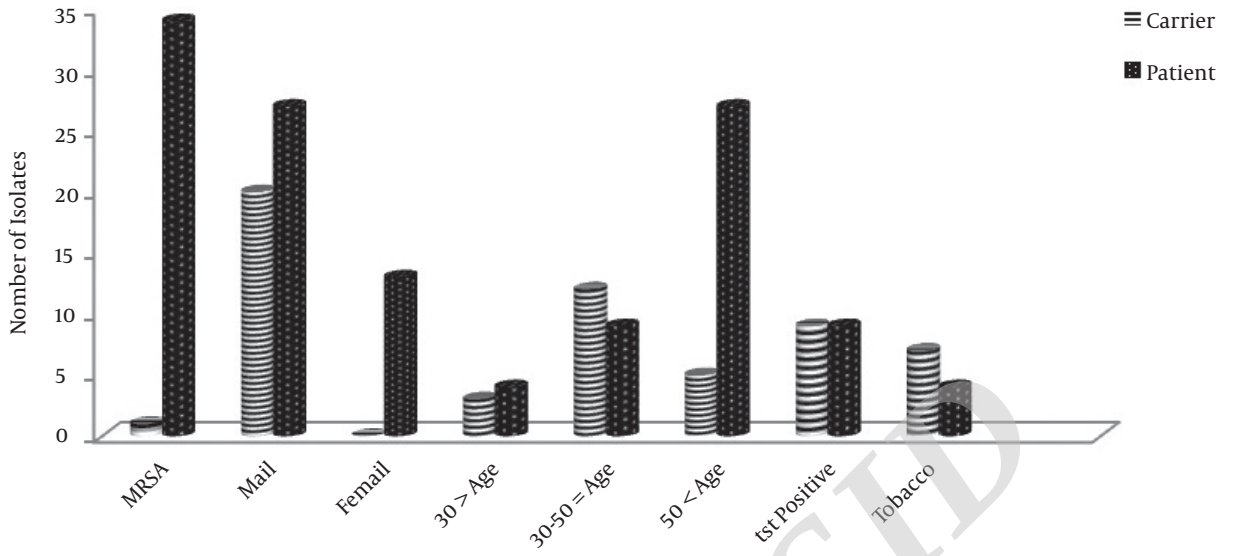


Figure 2. Comparison Chart of Patients' and Carriers' Isolates

aureus nasal carriage with mupirocin may result in disappearance of these bacteria from the other areas of the body (18, 19). HU et al. indicated that many factors, including deformities of the nasal cavity, genetic influences, and bacterial interference, are involved in the colonization of *S. aureus* in the nasal cavity (2). In the current study, the rate of occurrence of *S. aureus* in the nasal cavity of healthy people was 20%, which is somewhat similar to that reported by other researchers, who indicated that carriage rates in the Netherlands was about 24% (3, 20). From another perspective, this is an important issue as the sampled carriers were those who worked in dairy farms and it was previously shown that *S. aureus* isolated from milk samples may also be *tsst* positive indicating the possibility of the transfer of these bacteria between humans and animals (21).

Regarding the important role of TSS toxin in septic shock, the present study was performed to detect tsst gene among *S. aureus* isolated from patients and healthy carriers. The results of the PCR assay demonstrated that 22.5% of patients' isolates contained the *tsst* gene. These *tsst*-positive *S. aureus* were isolated from samples of different origins. However, the results also revealed that the percentage of *tsst*-positive isolates in carrier individuals was almost 2 folds (45%) higher than that for patients, and this difference was statistically significant (P = 0.04).

Different numbers of *tsst*-positive *S. aureus* have been reported in previous studies. Using the PCR method, El-Ghodban et al. recorded that only 3 out of 40 *S. aureus* isolated from clinical sources possessed the *tsst* gene (22). Mehrotra et al. examined 107 *S. aureus* isolated from healthy carriers to determine *tsst* positive samples and showed that 24.3% possessed this gene (10). Also many studies have been conducted on the presence of *tsst* gene in *S. aureus* isolates from Iran. Kord and Amini analyzed 76 *S. aureus* strains isolated from clinical samples. Their results showed that only 8.95% of isolates were positive for the *tsst* gene (23). In another study, performed on 100 MRSA and 100 MSSA isolates in Hamadan, the prevalence of TSST-1 was 11% (24). The current results showed that the frequency of *tsst* gene (30%) in Hamadan was more than that in other areas of Iran. Meanwhile, the results of the present study were in agreement with previous findings, which indicated that many *S. aureus* isolated from carriers contained the *tsst* gene (25).

Although several researchers have reported that *tsst* gene was more prevalent in MRSA than MSSA strains (26, 27), this study indicated that *tsst* gene was more prevalent in MSSA strains suggesting that MSSA strains must also be considered as potential risk of TSS in humans.

5.1. Conclusion

The results of the present study revealed that a high proportion of all *S. aureus* isolates (18 out of 60 isolates; 30%) possessed the *tsst* gene. This should be considered as a major health concern as such isolates may circulate among humans, animals, and environment. Hence, appropriate hygienic measures should be taken to control and prevent such infections.

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