Short Communication

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Frequency of the toxic shock syndrome toxin-1 gene in methicillin-susceptible and -resistant *Staphylococcus aureus* isolates from teaching hospitals in Shiraz, Iran

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ABSTRACT

Introduction: *Staphylococcus aureus* produces a range of virulence factors such as toxic shock syndrome toxin-1. **Methods:** In this cross-sectional study of 345 clinical *S. aureus* isolates, the presence of the *tst* gene was assessed by polymerase chain reaction (PCR). **Results:** The study revealed 53/345 (15.4%) isolates were positive for the *tst* gene. The *tst* gene was present in 18.1% of methicillin-susceptible *S. aureus* (MSSA) isolates and 11.6% of methicillin-resistant *S. aureus* (MRSA) isolates ($p = 0.136$). **Conclusions:** These results reveal the remarkable risk of *S. aureus* infections in hospitals, regardless of methicillin-resistance status.

Keywords: Toxic shock syndrome toxin-1. *Staphylococcus aureus.* Iran.

Staphylococcus aureus is a common threat to hospitalized patients and is responsible for a variety of infections, ranging from superficial skin and soft tissue infections to toxic shock syndrome and severe systemic infections(1). Furthermore, methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major concern in the hospital environment. Methicillin resistance in *S. aureus* is conferred by penicillin binding protein 2a (PBP2a), encoded by the *mecA* gene located on a genetic element called the staphylococcal cassette chromosome (SCC)(2). *Staphylococcus aureus* produce a remarkable range of secreted virulence factors that facilitate their pathogenicity, such as toxic shock syndrome toxin-1 (TSST-1)(1). TSST-1 is known as a superantigen for its ability to non-specifically stimulate activation of T lymphocytes(3). TSST-1 is encoded by *tstH*, which is located on the bacterial chromosome within a 15.2-kb mobile genetic element; it has been associated with several acute or chronic human diseases, including toxic shock syndrome (TSS) (3). TSS is an acute and potentially fatal illness characterized by high fever, diffuse erythematous rash, desquamation of the skin 1 to 2 weeks after the onset (if not fatal before this time), hypotension, and involvement of three or more organ systems(3).

Characterizing the differences in the pathogenicity and virulence patterns of MRSA and methicillin-susceptible *S. aureus* (MSSA) could help manage hospitalization time and mortality(1) (3). A recent study of clinical MRSA isolates from, Shiraz, Iran demonstrated the prevalence of several toxin genes, including *tst*(1). The aim of this study was to compare the prevalence of the *tst* gene in clinical MSSA and MRSA isolates in Shiraz, a major city in southwest Iran.

This cross-sectional study was conducted in 2012-2013 at the Namazee and Faghihi hospitals in Shiraz. These are major tertiary care hospitals with 1,000 beds; both are affiliated with the Shiraz University of Medical Science. Samples included 345 *S. aureus* isolates obtained from various clinical specimens such as blood, pus, wounds, and urine. Specimens were collected from different wards in each hospital. Duplicate isolates and specimens labeled as 'outpatient' were excluded. The isolates were identified as *S. aureus* by conventional microbiologic procedures (colony morphology, Gram staining, catalase activity, growth on mannitol salt agar, DNase test, and tube coagulase). Preliminary identification of MRSA and MSSA was based on resistance to cefoxitin (30µg) (MAST company, United Kingdom) by the disc diffusion assay according to Clinical and Laboratory Standards Institute (CLSI) guidelines(4). *Staphylococcus aureus* ATCC 25923 (an MSSA) was used as the control for antibacterial susceptibility testing. Confirmed isolates were stored at -80°C for long-term preservation.

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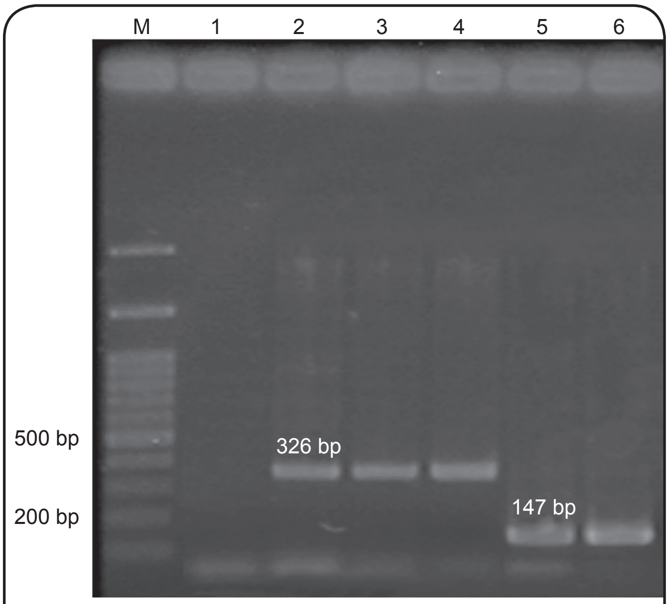
Genomic deoxyribonucleic acid (DNA) was extracted by using the small-scale phenol-chloroform extraction method(5). DNA concentrations were determined by spectrophotometry at A_{260} based on µg/mL concentration. In this study, DNA sample quantities ranged from 10 to 1,000ng. DNA was preserved at -20°C. The phenotypically confirmed MRSA isolates were subsequently tested for the presence of *mecA* as described by Zhang et al.(6). All MRSA and MSSA isolates were also assayed for the presence of the *tst* gene by using previously described primers(7). MRSA reference strains JCSC/4469 were used as positive *mecA* and *tst* gene controls. Reference strains were kindly provided by Professor Alborzi Clinical Microbiology Research Center, Shiraz, Iran. Polymerase chain reaction (PCR) amplifications were performed using a DNA Thermal Cycler 5530 (Eppendorf, Germany). PCR products were mixed with 1µl loading buffer and separated by 1.5% agarose gel electrophoresis at 75V for 90min. The gel was stained with ethidium bromide (Merck, Germany) for 15min and observed under the UV trans-illuminator. Statistical analysis was performed using SPSS software, version 19.0. Chi-square or Fisher's exact tests were performed to analyze the results. P <0.05 was regarded as significant.

Of the 345 *Staphylococcus aureus* isolates included in this study, 42.3% were found to be MRSA by cefoxitin disk and *mecA* PCR; 57.7% were methicillin-susceptible isolates. Namazee hospital yielded 69 (44.8%) MRSA and 85 (55.2%) MSSA isolates. Faghihi hospital yielded 77 (40.3%) MRSA and 114 (59.7%) MSSA isolates. There was no significant difference in the rates of MRSA and MSSA between hospitals.

PCR assays revealed 53 (15.4%) isolates positive for the *tst* gene. A representative gel image of *tst* gene detection by PCR is shown in **Figure 1**. The distribution of *tst* among the *S. aureus* isolates is presented in **Table 1**. Despite the high frequency of *tst* in the MSSA versus MRSA isolates, statistical analysis showed no significant differences. The prevalence of toxin genes in different clinical specimens is shown in **Table 2**. The main clinical sources of *tst* were sputum, wound, and skin specimens, each of which yielded 11 positive samples. Ear specimens showed 2 out of 2 samples were positive for the *tst* gene. The *tst* gene distribution was more variable among clinical specimens in MSSA isolates, since *tst* was detected in 10 different specimens. The *tst* gene was detected in more isolates from Faghihi hospital (n=33) than from Namazee (n=20), but the difference did not reach statistical significance.



M: 100bp ladder; **lane 1:** negative control (distilled water); **lane 2:** positive control for *tst* (326bp); **lane 3 and 4:** *tst*-positive clinical specimens; **lane 5:** positive control for *mecA gene* (147bp); **lane 6:** *mecA*-positive clinical specimen.

FIGURE 1 - A representative gel image of *mecA* and *tst* gene detection by PCR.

In this study of southwest Iranian hospitals, we identified 53 (15.4%) isolates positive for the gene encoding TSST-1. In the north (Tehran) and north-east (Tabriz) of Iran, the *tst*-positive rates are 12.8% and 17.4%, respectively(8) (9). Studies in Turkey and Colombia showed *tst*-positive rates of 14.2% and 18%(10) (11).

The frequency of the *tst* gene was numerically higher in our MSSA isolates than in the MRSA isolates (18.1% vs. 11.6%), although the difference was not significant. Jiménez et al. also showed a greater frequency of *tst* in MSSA versus MRSA isolates from pediatric patients(11). Sila et al(12). from the Czech Republic reported the 6% of *tst*-carrying MSSA were more virulent than 2% of *tst*-carrying MRSA(12).

Although we identified no significant overall difference in the prevalence of *tst* gene between MRSA and MSSA isolates. We did observe a significant difference in isolates from blood, with 20% of MRSA versus 7.7% of MSSA isolates carrying the

TABLE 1 - The distribution of *tst* in clinical isolates of *Staphylococcus aureus* from two hospitals.

Isolates	MRSA		level of significance^a	MSSA		Level of significance^b	Level of significance^c
Hospitals	n	%		n	%		
Faghihi	11	14.3	p = 0.428	22	19.3	p = 0.744	p = 0.481
Namazee	6	8.7		14	16.5		p = 0.235
Total	17	11.6		36	18.1	p = 0.136^d	

MRSA: methicillin-resistant *Staphylococcus aureus;* **MSSA:** methicillin-susceptible *Staphylococcus aureus;* ^{a}estimated p value for MRSA; ^{b}estimated p value for MSSA; ^{c}estimated p value for MRSA vs. MSSA at each hospital; ^{d}estimated p value for all MRSA vs. MSSA isolates.

TABLE 2 - The prevalence of *tst* in *Staphylococcus aureus* isolates according to clinical specimen.

	Faghihi Hospital		Namazee Hospital		
	MRSA	MSSA	MRSA	MSSA	Total
	number positive/total	number positive/total	number positive/total	number positive/total	number positive/total
Sputum	3/35	4/16	0/23	4/19	11/93
Urine	0/9	4/26	0/5	1/11	5/51
Blood	2/9	2/14	2/11	0/12	6/46
Wound	2/8	1/13	3/10	5/10	11/41
Nose	0/2	1/16	1/1	2/8	4/27
Skin	4/8	7/17	NS	NS	11/25
Throat	0/1	1/4	0/3	0/4	1/12
Eye	NS	0/1	0/3	1/5	1/9
CSF	NS	NS	0/2	1/3	1/5
Ear	NS	2/2	NS	NS	2/2
Total	11/77	22/114	6/69	14/85	53/345

MRSA: methicillin-resistant *Staphylococcus aureus;* **MSSA:** methicillin-susceptible *Staphylococcus aureus;* **NS:** no sample; **CSF:** cerebrospinal fluid.

tst gene. Peck et al(13). reported that TSST-1 producing MRSA isolates were more prevalent in blood samples(13).

Although we identified sputum, wounds, and skin as three major sources of TSST-1 producing isolates, wounds are the most common source for isolation of TSST-1 producing *S. aureus*. Teyhoo *et al.* from Iran and Daghistani et al. from Jordan reported substantial rates of *tst*-harboring *S. aureus* isolates from wound samples(9) (14).

In this study, the skin was most frequently associated with *tst*-carrying isolates, with twice the rate of detection (44%) in comparison to sputum and wounds. This high rate of TSST-1 producing *S. aureus* isolates may be considered a risk factor for vulnerable groups such as newborns and patients with atopic dermatitis(15). As virulence factors accelerate colonization and invasion through minor breaks in the skin and mucus membranes, the subsequent spread of TSST-1 producing *S. aureus* to the bloodstream could have a critical influence on clinical outcomes(14).

MRSA isolates are known to be more antibiotic resistant than MSSA isolates and antimicrobial resistance may generally be considered an important factor for the clinical outcome of *S. aureus* infection as well(11). However, a higher rate of TSST-1 carrying isolates was detected among MSSA isolates compared to that for MRSA isolates, although the difference was not statistically significant in our study. So, MSSA isolates might be more virulent in term of TSST-1 than MRSA isolates. We suggest the most dangerous *S. aureus* are not necessarily those with the most antibiotic resistance. Antibiotic resistance is thought to impose a fitness cost associated with a reduction in toxin production(3) (11), it is not surprising that our MRSA isolates showed less toxin rate.

In summary, we have shown a frequency of up to 15% *tst*-positivity in MSSA and MRSA isolates and suggesting methicillin resistance has no effect on acquisition of the virulence gene. We conclude that the risk of infection with *S. aureus* should not be assigned only based on methicillin resistance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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