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)[3]. 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[8]. 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.
Genomic deoxyribonucleic acid (DNA) was extracted by using the small-scale phenol-chloroform extraction method. DNA concentrations were determined by spectrophotometry at A260 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. All MRSA and MSSA isolates were also assayed for the presence of the tst gene by using previously described primers. 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.

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. 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). Silva 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.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>MRSA</th>
<th>Level of significance&lt;sup&gt;a&lt;/sup&gt;</th>
<th>MSSA</th>
<th>Level of significance&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Level of significance&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitals</td>
<td>n</td>
<td>%</td>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Faghihi</td>
<td>11</td>
<td>14.3</td>
<td>p = 0.428</td>
<td>22</td>
<td>19.3</td>
</tr>
<tr>
<td>Namazee</td>
<td>6</td>
<td>8.7</td>
<td></td>
<td>14</td>
<td>16.5</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>11.6</td>
<td></td>
<td>36</td>
<td>18.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>estimated p value for MRSA; <sup>b</sup>estimated p value for MSSA; <sup>c</sup>estimated p value for MRSA vs. MSSA at each hospital; <sup>d</sup>estimated p value for all MRSA vs. MSSA isolates.

MRSA: methicillin-resistant Staphylococcus aureus; MSSA: methicillin-susceptible Staphylococcus aureus.
The authors declare that there is no conflict of interest.

CONFLICT OF INTEREST

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