Detection of Enterotoxin-Coding Genes of *Staphylococcus aureus* Isolated from Hospitalized Patients Using a Multiplex-PCR Method

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Abstract

*Staphylococcus aureus* (*S. aureus*) is a pathogen in community-acquired or hospital infections. Hence, the identification of this pathogen in clinical samples is a health concern and demands continued surveillance and close monitoring. In the current study, *S. aureus* strains were isolated from various clinical specimens in the Shariati Hospital, Tehran, Iran. Samples were studied to discover *S. aureus* enterotoxin-coding genes A (*sea*), B (*seb*), C (*sec*), and D (*sed*). It was found that 21% enterotoxigenic *S. aureus* harbored *sea* gene, 39% were carried *seb* gene, 37% were positive for *sec*-gene, and 3% were carried *sed* gene. None of all *S. aureus* strains harbored more than one of the enterotoxigenic genes. Based on the data obtained from the current study, it could be suggested that *seb* and *sec* genes are good candidates for the identification of *S. aureus* in clinical specimens. Further investigations are required to discover the association between these genes and the pathogenicity of this bacteria, and finally using these data in clinical settings.

Keywords: *Staphylococcus aureus*, Enterotoxin genes, Pathogenicity, Multiplex-PCR

1. Introduction

*Staphylococcus aureus* is a normal flora found on human skin, anterior nose, and respiratory tract mucosal surfaces (1, 2). This bacteria also could be a significant pathogen in both community-acquired and hospital infections (3, 4). *S. aureus* could cause a wide range of infections such as bacteremia, endocarditis, and osteomyelitis (5, 6).

Enterotoxins are proteins produced by *S. aureus* and *Bacillus cereus*. Enterotoxins from *S. aureus* can cause staphylococcal food poisoning (SFP). The genes of *sea*, *seb*, *sec*, *sed*, are recognized as important staphylococcal enterotoxin-coding genes (7, 8). It has been found that enterotoxins cause around 95% of SFP outbreaks infections. The remaining 5% of contaminations may be related to newly recognized SEs (9). Ses as etiological agents an increasing the severity of *S. aureus* contaminations have not been established.
Enterotoxins of *S. aureus* isolated from human different clinical samples have been formerly identified via various techniques (8, 10, 11). Staphylococcal enterotoxins can be usually identified by immunoassay, e.g., immune diffusion, enzyme-linked immune sorbent assay (ELISA), radioimmunoassay and latex agglutination. However, the availability of these procedures is commonly limited due to economic issues (12-14). Therefore, the DNA-based approach, like polymerase chain reaction (PCR), is presently used as a simple and robust method for identifying enterotoxigenic strains. The current study aimed to recognize *S. aureus* strains (encoding *Nuc* gene) and identify enterotoxin-coding genes (*sea*, *seb*, *sec*, and *sed*) in samples collected from hospitalized patients.

### 2. Material and method

#### 2.1. Sampling

A total of 100 isolates of *S. aureus* from 168 patients suspected of infection were obtained from various human clinical specimens in the Shariati hospitals of Tehran, Iran. These samples were urine (n=30), wounds (n=35), sputum (n=10), blood (n=5), CSF (n=3) and pus from the burn wound (n=17), between June 2014 and July of 2015. In order to isolate *S. aureus* strains, samples were cultured on 5% sheep blood agar (Merk, Germany), nutrient agar (Merk, Germany) and then, on mannitol salt agar (Merk, Germany). Gram staining and culture features (colony morphology, pigmentation, and hemolysis) were used for the diagnosis of all isolates. The API-20-Staph system kit (bio Merieux SA, Marcy1, Etoile, France) and PCR amplification with *Nuc* gene were used for the final confirmation of all strains.

#### 2.2. DNA extraction and *Staphylococcus* confirmation

A typical colony of the biochemically recognized as *S. aureus* was cultured in 1 mL trypticase soy broth (TSB) for 24 h at 37 °C. A total genomic DNA of *S. aureus* isolates was extracted through a QIAGEN plasmid Minikit (Fermentas, Germany).

#### 2.3. PCR-amplification

The multiplex polymerase chain reaction (PCR) were performed in a total volume of 25 μL, including 2 mM of MgCl2, 50 mM of KCl, 10 mM of Tris-HCl (pH 9.0), 0.1% of Triton X-100, 150 μM of dNTPs (Fermentas, Germany), 2.5 μL of PCR buffer (10X), 25 pmol of each primers (Table 1), 2 U of Taq DNA polymerase (Fermentas, Germany), and 2 μL of the extracted DNA template of the *Staphylococcus* isolates. The four set of primer pairs were used in each reaction mixture. The thermal cycler was adjusted as follows: initial denaturation for 5 min at 94 °C, followed by 30 cycles of denaturation (94 °C for 1 min), annealing (56 °C for 1 min), and elongation (72 °C for 1 min). A final step (72 °C for 10 min) was performed after the completion of the above cycles. The amplified PCR products were visualized by standard gel electrophoresis in a 1.5% agarose gel stained by ethidium bromide (5 μg/mL) for 30 min. The gels were photographed under ultraviolet light using the Gel-Doc.

### Table 1. The designed primers used for the detection of enterotoxin-coding genes in *S. aureus*.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer name</th>
<th>Primer sequence (5’-3’)</th>
<th>Size of PCR-products</th>
</tr>
</thead>
<tbody>
<tr>
<td>sea</td>
<td>SEA-F</td>
<td>GCAGGGACACGCTTGGGC</td>
<td>521bp</td>
</tr>
<tr>
<td></td>
<td>SEA-R</td>
<td>GTTCGTAAGATATGAAACAG</td>
<td></td>
</tr>
<tr>
<td>seb</td>
<td>SEB-F</td>
<td>ACATGTAATTTGTATCCTGGCTG</td>
<td>667 bp</td>
</tr>
<tr>
<td></td>
<td>SEB-R</td>
<td>TGCAAGCATGATGTATACCA</td>
<td></td>
</tr>
<tr>
<td>sec</td>
<td>SEC-F</td>
<td>CTCAAGAACTAGACATAAAAGCTAGG</td>
<td>271 bp</td>
</tr>
<tr>
<td></td>
<td>SEC-R</td>
<td>TTATATCAAATCCGATATACATTAC</td>
<td></td>
</tr>
<tr>
<td>sed</td>
<td>SED-F</td>
<td>CCAAATAATGGGAAAAATAAAG</td>
<td>378bp</td>
</tr>
<tr>
<td></td>
<td>SED-R</td>
<td>ATGGATTTTTTTCTGTTT</td>
<td></td>
</tr>
<tr>
<td>Nuc</td>
<td>Nuc-F</td>
<td>GCGATTGATGGGATACGGTT</td>
<td>270 bp</td>
</tr>
<tr>
<td></td>
<td>Nuc-R</td>
<td>AGCCAAGCCTTGGAAACGACTAAAGC</td>
<td></td>
</tr>
</tbody>
</table>
3. Results

In the current study, 168 patients participated in sampling that comprised 107 males (63.7%) and 61 females (36.3%), 14 to 75 years old (mean age: 43.5±4.2) (Figure 1). One hundred isolates of S. aureus were obtained. S. aureus was isolated mostly from male patients (62.6%, 67/107) (Figure 1). In females, 23 patients were S. aureus positive (37.4%; 23/61) (Figure 1). Multiplex PCR reaction with specific primers for sea, seb, sec and sed genes were performed. A 521 bp, 667 bp, 271 bp, and 378 bp segments were related to the amplification of a specific fragment of sea, seb, sec and sed genes respectively (Figure 2).

There were 21% isolates associated with the sea gene, 48% isolates associated with the seb gene, 37% of the isolates were associated with the sec gene, and 3% of the isolates associated with

![Figure 1. Percentage of male and females participated in the current (A) study and the percentage of S. aureus positive patients in male and females (B).](image1)

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![Figure 2. Gel electrophoresis of PCR products in the study (M: DNA marker 100 bp DNA plus (Fermentas, Co, USA), C+: Positive control; Staphylococcus aureus subsp. aureus ATCC 29213, C-: Negative control; Staphylococcus epidermidis ATCC 14990).](image2)
the sed gene (Figure 3). None of the isolated S. aureus strains harbored more than one enterotoxigenic gene.

4. Discussion

In the current investigation, we have applied a multiplex PCR-based diagnostic protocol to the identification of four enterotoxin-coding genes sea, seb, sec, and sed from S. aureus in hospitalized patients. The most common enterotoxigenic gene in S. aureus strains in our study was seb. This finding is consistent with previous findings that indicated most enterotoxigenic S. aureus strains typically carried seb gene (15-17). However, our results were not in line with some previous reports indicating that most enterotoxigenic S. aureus strains generally carried sea, sec or sed genes (18, 19). In the current investigation, the frequency of enterotoxigenic S. aureus contains seb in the clinical samples was 39% (Figure 3). On the other hand, we found that no enterotoxigenic isolates carried more than one gene. This result is different from other studies which indicated that some enterotoxigenic strains had more than one gene. Naffa et al. (2006) reported the prevalence of sea and sec among 100 Jordanian clinical S. aureus was 15 and 4%, respectively (20). Mehrabi et al. (2015) were reported sea (30%), seb (11.11%), sec (15.55%) and sed (4.44%) in (21). Mehrotra et al. (2000) reported out of 107 S. aureus strains, 21 (19.6%) were positive for sea, 6 (5.6%) were found to be seb positive, 8 (7.5%) were positive for sec, and 2 (1.9%) contained the gene for sed (22). Goto et al. (2007) reported 11 (36.67%) of these 30 strains were positive for the sea gene, 13 (43.33%) for seb, 8 (26.67%) for sec, and 7 (23.33%) for sed (8). Demir et al. (2011) among of 120 S. aureus strains the classical enterotoxin genes sea gene (n: 36, 30%) was the most widespread, followed by sec (n: 9, 7.5%), and sed (n: 6, 5%) (23). Taj et al. (2015) reported the enterotoxin genes sec (n=16) (13.91%), seb (n=13, 11.30%), and sea (n=12, 10.43%) (24). The incidence of enterotoxigenic S. aureus from different clinical specimens in previous studies is in a wide range from 17.8% to 86.6% (16, 19, 20, 25-30). This different prevalence might be due to variances in the ecological source of isolates, the sensitivity of diagnosis procedures, and the type and number of specimens.

Based on the data obtained from the current study, it could be concluded that the detection of enterotoxigenic genes in S. aureus strains by PCR suggests a specific, sensitive, relatively quick, and cheap alternative to other assays. However, the prevalence of S. aureus enterotoxin-encoding genes could be varied in each region or hospital. More investigations in this field could delineate an association between these genes and the pathogenicity of S. aureus bacteria, and finally
using these data in clinical settings.

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**Conflict of Interest**

None declared.

5. References

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