Original Article

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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.

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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), radioimmune-assay 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 (Merck, Germany), nutrient agar (Merck, Germany) and then, on mannitol salt agar (Merck, 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*.

Genes	Primer name	Primer sequence (5'-3')	Size of PCR-products
sea	SEA-F	GCAGGGAACAGCTTTAGGC	521bp
	SEA-R	GTTCTGTAGAAGTATGAAACACG	
seb	SEB-F	ACATGTAATTTTGATATTCGCACTG	667 bp
	SEB-R	TGCAGGCATCATGTCATACCA	
sec	SEC-F	CTCAAGAACTAGACATAAAAGCTAGG	271 bp
	SEC-R	TTATATCAAAATCGGATTAACATTATC	
sed	SED-F	CCAATAATAGGAGAAAATAAAAG	378bp
	SED-R	ATTGGTATTTTTTTTCGTTC	
Nuc	Nuc-F	GCGATTGATGGTGATACGGTT	270 bp
	Nuc-R	AGCCAAGCCTTGAACGAACTAAAGC	

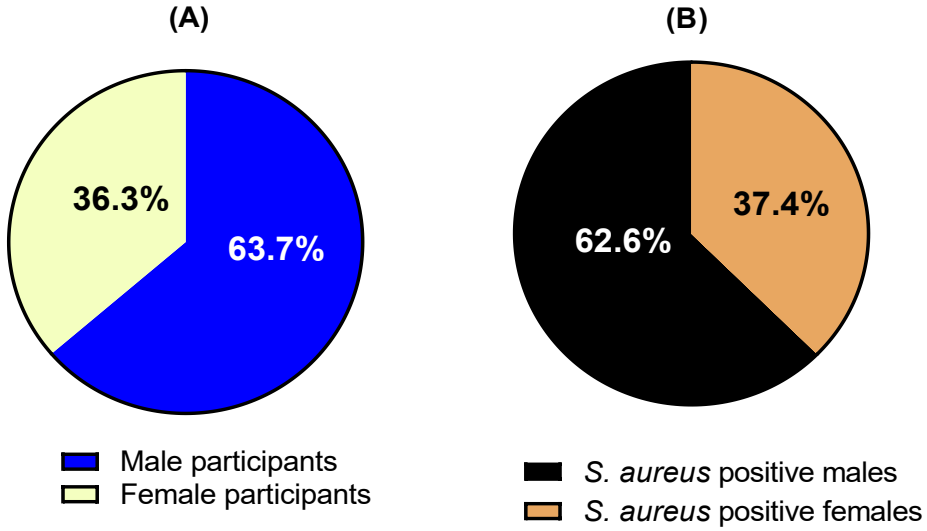

Figure 1. Percentage of male and females participated in the current (A) study and the percentage of *S. aureus* positive patients in male and females (B).

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

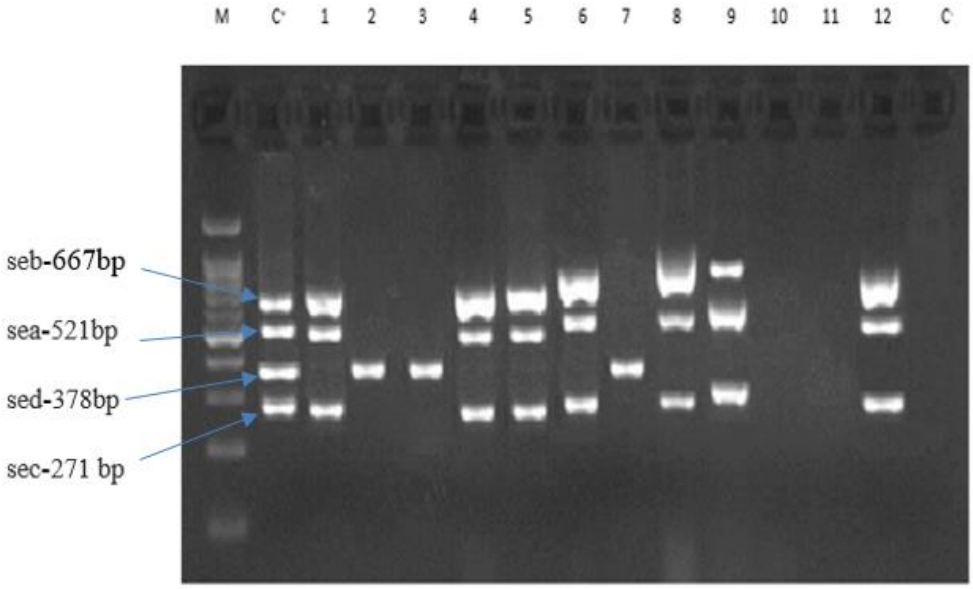

Figure 2. Gel electrophoresis of PCR products in the study (M: DNA marker 100 bp DNA plus (Fermentas, Co, USA), C+: Positive control; *Staphylococcus aureus* subsp. aureus ATCC 29213, C-: Negative control; *Staphylococcus epidermidis* ATCC 14990).

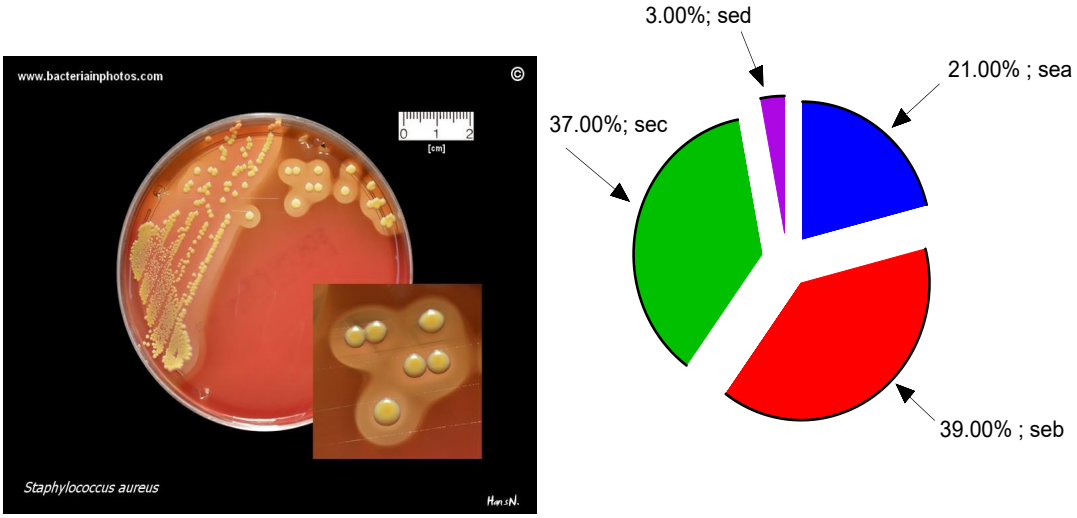

Figure 3. The distribution of genes detected from *S. aureus*. The *S. aureus* culture medium image was obtained from: http://www.bacteriainphotos.com/Staphylococcus_aureus_on_Schaedler_anaerobe_agar.html

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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