

## Molecular Detection of *Staphylococcus aureus* Enterotoxin A and B Genes in Clinical Samples from Patients Referred to Health Centers in Zahedan City

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### Abstract

**Background:** In this study we aimed at detecting the enterotoxin A and B gene of *S. aureus* in clinical samples of patients attending health centers in Zahedan using molecular methods.

**Materials and Methods:** A cross-sectional study was carried out in which 40 samples of *S. aureus* were obtained from patients in a hospital in Zahedan, Iran. Following the biochemical tests, identifications were confirmed by PCR with specific primers.

**Results:** Among 40 clinical isolates of *S. aureus* the frequency of sea gene was 2% and the frequency of seb gene was 8% while the frequency of both sea+seb genes was 3%.

**Conclusion:** Enterotoxin of *S. aureus* is one of the main factors in the pathogenesis of various diseases and production of these toxins increase the incidence of diseases, so rapid treatment is needed for enterotoxin gene expression.

**Keywords:** Enterotoxin; Food Poisoning; *Staphylococcus aureus*; Gene

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### Introduction

Staphylococcal exotoxins from more than 20 different staphylococcal and streptococcal family, that is performance related and share sequence similarity. This bacterial protein known to cause fever and significant human diseases, including food poisoning and toxic shock syndrome is connected. The toxins produced by *Staphylococcus aureus* are for the most part although other species also been shown that enterotoxigenic. (1).

Most genes coding for positions on the mobile elements such as plasmids, bacteriophages or pathogenicity islands located. Thus, horizontal transfer between species is rare. In fact, a recent study showed that the majority of *Staphylococcus aureus* isolates obtained from three separate hospitals more than one enterotoxin (2). The most common staphylococcal enterotoxins SEA and SEB. SEA toxin commonly associated with food poisoning *Staphylococcus*. SEB, while it is associated with food poisoning, has been studied for potential use as a

bioweapon inhalation (3). The aim of this study was to detect enterotoxin A and B genes of *S. aureus* in clinical samples of patients attending a hospital in Zahedan, Iran using molecular methods.

### Materials and Methods

40 samples from 300 persons were collected from a hospital in Zahedan. The samples were quarterly collected from infected men. Ten microliters of each sample were cultured on blood agar. Isolated Gram and catalase positive cocci were further tested for biochemical characterization. Following the biochemical test, *Staphylococcal* isolates were identified using species- specific gene amplification (16 S DNA, Toxin A, Toxin B). Two pair primers used for PCRs are shown in Table1. The total volume of the reaction mixture (25  $\mu$ l), included 2 $\mu$ l of dNTP (200  $\mu$ l, 2.5  $\mu$ l of 10 X Taq buffer containing 15 mM MgCl<sub>2</sub>, 1 $\mu$ l of each oligonucleotide forward and reverse primers(10 pm/ $\mu$ l), 0.35 $\mu$ l of Taq DNA

Polymerase (3 u/μl), 1μl DNA (30 mg/μl) and distilled water (17.2 μl). Pair primer 1 amplified a 270 bp PCR product in the PCR reaction. Pair primer 2 amplified a 477 bp PCR product in the PCR

reaction (6). All experiments and measurements were repeated at least three times. Statistical analyses were performed using SPSS and Excel 2010.

**Table 1.** Primer sequences to identify enterotoxin A and B.

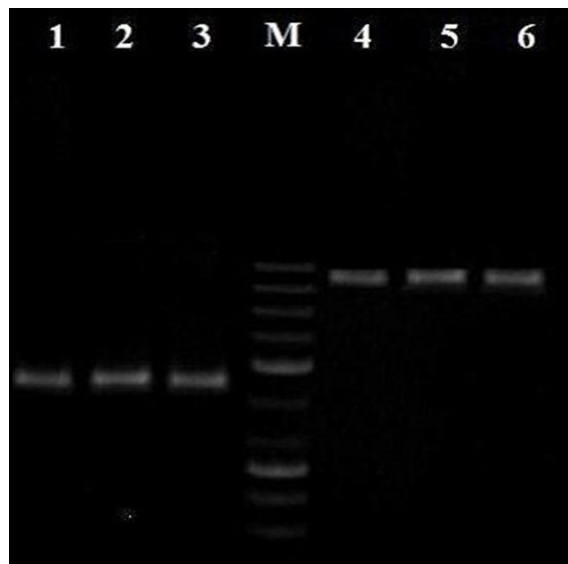
Gene	Primer	Sequence	Product size (bp)
SEA	SEAf	TGT ATG TAT GGA GGT GTA AC	270
	SEAr	ATT AAC CGA AGG TTC TGT	
SEB	SEBf	TCG CAT CAA ACT GAC AAA CG	477
	SEBr	GCA GGT ACT CTA TAA GTG CC	

### Ethics Statement

The study was approved by the Shahid Bahonar University of Kerman Ethics Committee.

### Results

In this investigation, 300 samples were obtained from 300 healthy carriers, of which 40 samples of *S. aureus* were detected biochemically. To detect *S. aureus* types *a*, *b* enterotoxins a total of 40 *S. aureus* strains originating from healthy carriers was tested for enterotoxin production by PCR assay. The specificity of PCR was tested for positive and negative control strains. The six SE-encoding genes (*sea*, *seb*) were detected in the positive control strains and not in the negative control strains (Figure 1).



**Figure 1.** Gel analysis of PCR-amplified toxin gene sequences. Lanes 1, 2, 3 SEA (270bp) and lane 4, 5, 6 SEB (477bp).

Furthermore, 270 and 477 bp segments were related to the amplification of a specific fragment of gene *sea* and *seb* that is responsible for enterotoxin type A and B and 270 bp for staphylococcal enterotoxin A gene (*sea*) (Figure 1, lane 1, 2, 3), and 477 bp for staphylococcal enterotoxin B gene (*seb*) (Figure 1,

lane 4, 5, 6) Results showed that 24 (2%) isolates were associated with the *sea* gene, 15 (8%) isolates were associated with the *seb* gene. Only one of these 3% of the isolates harbored *sea* and *seb*.

### Discussion

In this study the prevalence of toxins produced was low but enterotoxin A had highest prevalence. Today to determine the mechanism and role of enterotoxin and determine its specific receptors great deal of research was done or is being done. Therefore, rapid diagnosis of Enterotoxigenic by standard methods is very important.

Chiang et al. showed that 1.48% of *S. aureus* strains contain the gene encoding for *sea*. Most isolated type was staphylococcal enterotoxin A (6.28%) strains (7). Adwan et al. showed that 37% of *S. aureus* isolates containing the gene encode for enterotoxin type A (8). The results in Anvari et al. showed that 74% of the samples have enterotoxin A (9).

*S. aureus* samples isolated from Motahari Hospital, Tehran showed that of 100 samples of *S. aureus* clinical isolates had 12% of enterotoxin A, 1% of enterotoxin B and 1% enterotoxin A and 2% of enterotoxins A and B (10). Nashev et al. reported the most abundant enterotoxin is enterotoxin A (23%) in *S. aureus* isolates from nasal samples (11).

The most common enterotoxin coding gene in their study was *sea* with a prevalence of 33%, followed by *sec* with 15% prevalence (12). A Turkish investigation showed that only 2.9% of 70 *S. aureus* strains tested were positive for *sea* gene while there were no positive results for other putative genes (13, 14, 15, 16).

### Conclusion

Given the role of enterotoxin in the pathogenicity of *S. aureus* and nosocomial infections in this study, patients can be considered as a source of emissions in the hospital and enterotoxins A and B also play important roles in infectious diseases.

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and helped us to complete this project.

#### Author contributions

All author of this article have same contribution for doing this project.

#### Conflict of Interest

The authors declare no conflict of interest.

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