

## Research Article

# SEA, SEB and TSST-1 Toxin Gene Prevalence in *Staphylococcus aureus* Isolated from Fish

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**Abstract**

*Staphylococcus aureus* is one of the most common causes of food borne diseases worldwide. It can produce various enterotoxins including SEB, SEA and TSST-1. The aim of this study was to assess *sea*, *seb* and *tst* genes in *S.aureus* isolated from fish. A total number of 300 fish samples, including 150 fresh and 150 frozen samples, were collected from Tehran Seafood Market in 2013. Isolation and identification of *S.aureus* from the samples were carried out using conventional methods such as enrichment and culture and assessment of the highlighted genes using polymerase chain reaction. In total, 33.3 and 48.7% of the fresh and frozen samples were contaminated with *S. aureus*, respectively. Furthermore, 50.4% of the total isolates ( $n = 123$ ) contained *sea*, 26.8% *seb* and 4.1% *tst* genes. The maximum gene prevalence belonged to *sea* in *S.aureus* isolated from fresh and *seb* from frozen fishes. A significant portion of the fish samples were contaminated with *S. aureus*; mostly in frozen samples. The high rate of contamination possibly was linked to insanitary handling, storing and/or processing step of the fishes. Prevalence of enterotoxigenic genes within the isolates was relatively high; except for *tst* gene. In conclusion, the high contaminated fish samples with *S.aureus* in the current study reflex the need for hygienic surveillance system to limit food contamination with *S.aureus* and its possible outbreaks.

**ABBREVIATIONS**

SEA: Staphylococcal Enterotoxin A; SEB: Staphylococcal Enterotoxin B; TSST: Toxic Shock Syndrome Toxin

**INTRODUCTION**

Fish is one of the main dishes worldwide, particularly in countries with seashores such as Asian and Far East countries. Bacterial contaminated fish could cause gastrointestinal diseases in humans [1]. A common bacterial contaminant, *Staphylococcus aureus*, is responsible for a significant number of food-borne infections. The most important symptoms include vomit, nausea and fever. Cramp abdominal, diarrhea, dysentery, colitis and gastroenteritis are less common [2-4]. This microorganism can produce heat-stable enterotoxins that are reasons for a majority of food poisoning outbreaks caused by *S.aureus* [5]. There are

more than 20 types of staphylococcal enterotoxins; from which, the most associated with food poisoning are SEA, SEB, SEC, SED and SEE. Toxic shock syndrome toxin (TSST) is a *S.aureus* toxin which causes shock and anaphylaxis through oral or systematic contamination, especially in immunocompromised patients [6-8]. Contamination by *S.aureus* often occurs through contaminated food or via physical contact [9]. The aim of the current study was to discover *sea*, *seb* and *tst* genes in *S. aureus* isolated from fish in Tehran, Iran.

**MATERIALS AND METHODS****Sample collection**

A total number of 300 samples, including 150 marine and 150 farmed fish (fresh and frozen), were collected from Tehran Seafood Market, 2013-2014. These fishes were carried from Persian Gulf, Oman Sea and Caspian Sea to the market by ground

transportation systems. Samples were transferred in cold chain from the market to Food Microbiology Laboratory at the School of Public Health, Tehran University of Medical Sciences, and stored at 4 °C until use. Iran National Standard Protocol No. 356 was used for the isolation of bacteria in this study [10].

### Bacterial isolation

One gram of the fish meat was cut using sterile blade and transferred into 9 ml of 0.1% peptone water and mixed well. Mixture was incubated at 37 °C for 24–48 h. One hundred micro liter of this mixture were cultured on Baird-Parker agar (containing 0.1% potassium telluride solution and egg emulsion) and incubated at 37 °C for 24–48 h. Three to five small, black shiny colonies with transparent zone indicating *S.aureus* were selected and tested for coagulase activity based on Iran National Standard Protocols No. 5272 and No. 6806-3 [11,12]. In the current study, *S.aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were used as positive and negative controls.

### Gene detection

A single polymerase chain reaction (S-PCR) technique was used to detect toxigenes in *S. aureus* isolates. Specific primers used in this study are described in (Table 1). The S-PCR was carried out based on an original protocol by Mazaheri Nezhad Fard et al. [13], as follows: A fresh staphylococcal colony on Baird-Parker agar (Merck, Germany) was suspended in 200 µl of distilled water. DNA was extracted using Viogene DNA Extraction Kit (Viogene-Biotek, Taiwan) according to the user manual and stored at -20 °C until use. To prepare a 25-µl reaction buffer using Hot Star Taq Plus Master Mix kit (Qiagen, Germany), 12.5 µl of 2× PCRmaster mix, 2.5 µl of 10× PCR buffer and 2µl of each primer in 10 pmol concentration were mixed in a sterile microtube. Then, sufficient amount of distilled water was added to the mixture to reach the total volume of 22µl and mixed with 3µl of the DNA template. The primer sequences are described in (Table 1). PCR reactions were thermally cycled using Peqlab Primus 96 thermal cycler (Peqlab, Germany) as follows (modified from Soltan Dallal et al. [14], after an initial denaturation step at 94 °C for 5 min, genes were amplified by 35 cycles; each cycle included denaturation at 94 °C for 45 s, annealing at 55(*sea*), 52 (*seb*) and

51°C (*tst*) for 45 s and elongation at 72°C for 90 s. Amplification was finalized by a final elongation step at 72°C for 7 min. PCR products were detected by electrophoresis in 1 µg/ml ethidium bromide stained 1% agarose gels and visualization under UV light (UVP, France).

### Statistical analysis

Statistical analysis was carried out using SPSS v11.5 software (IBM Analytics, USA). Chi-square test and Fisher's exact two-tailed test were used for statistical analysis, *P*-values less than 0.05 were considered as significant.

## RESULTS AND DISCUSSION

Of 300 fish samples, 33.3% (50 out of 150) and 48.7% (73 out of 150) of the fresh and frozen samples were contaminated with *S.aureus*, respectively. Moreover, 50.4% of the isolates contained *sea*, 26.8% *seb* and 4.1% *tst* genes. Distribution of *sea* gene in *S.aureus* from fresh and frozen samples included 29 (58.0%) and 33 (45.2%) genes, respectively (*P*< 0.001) (Table 2). Distribution of *seb* gene in *S.aureus* from fresh and frozen samples included 12 (24.0%) and 21 (28.8%) genes, respectively (*P*< 0.05) (Table 2). The *tst* gene was prevalent in two *S. aureus* (4.0%) isolated from fresh and three *S.aureus* (4.1%) isolated from frozen samples (*P*< 0.001) (Table 2).

Food safety is one of the most important concerns for every community member. Seafood is one of the fast spoiling food stuff and hence can cause extensive gastrointestinal infections. Therefore, it is very common that seafood safety is a major interest of the food microbiology researchers. In this study, 33.3% (50 out of 150) and 48.7% (73 out of 150) of the fresh and frozen samples were contaminated with *S.aureus*, respectively, Oh et al., (2007) studied the occurrence of toxigenic *S.aureus* in ready-to-eat food in Korea and showed that nearly 9% of 1008 raw fish and fish product samples were contaminated [15]. Atanassova et al., (2008) studied microbiological contamination of 250 sushi samples (a traditional Japanese food) from sushi bars and retailers in northern Germany in 2008 and reported that 11.2% of the fresh and 1.6% of the frozen products were contaminated with *S.aureus* [16]. The rate of contamination in fresh food was

**Table 1:** PCR primers used in the present study.

Gene	Sequence (5'→3')	bp	Ref.
<i>sea</i>	F: CCTTTGGAACGGTTAAAACG R: TCTGAACCTTCCCATCAAAAAC	127	24
<i>seb</i>	F: TCGCATCAAACGACAAACG R: GCAGGTACTCTATAAGTGCCTGC	477	25
<i>tst</i>	F: CATCTACAAACGATAATATAAAGG R: CATTGTTATTTTCCAATAACCACCC	481	26

**Abbreviations:** Amplicon Size; Ref: Reference; F: Forward; R: Reverse

**Table 2:** Distribution of *sea*, *seb* and *tst* genes in *S. aureus* isolated from fishes.

Sample	No.*	<i>sea</i> (%)	<i>seb</i> (%)	<i>tst</i> (%)	<i>P</i> -value
Fresh fish	50	29 (58.0)	12 (24.0)	2 (4.0)	<i>P</i> < 0.001**
Frozen fish	73	33 (45.2)	21 (28.8)	3 (4.1)	<i>P</i> < 0.05**
Total	123	62 (50.4)	33 (26.8)	5 (4.1)	<i>P</i> < 0.001**

**Abbreviations:** \*No. of *S. aureus*; \*\*statistically significant; percentages are rounded to one decimal place

higher than that in frozen food, contrary to the current study. In 2012, Zarei et al., reported that the prevalence of *S.aureus* in 70 fresh salt water raw fish samples included 4.3% of the whole isolates and in 2014, Mus et al., reported that occurrence of *S.aureus* in retail fresh fish included 3.8% (3 out of 78) [17,18]. This difference between results from the current study and those from other studies was seen possibly due to differences between the climates (sometimes up to tens of degrees centigrade) or better food processing and delivery schemes. In the current study, the long distance between the fishing sites and the selling market has made a longer store time. This may have resulted in a rise in the microbial load of the fishes. Furthermore, contamination of vehicles, workers' hands and boots and ice could cause higher contamination rates in frozen samples, as well as traditional fishing styles.

In the current study, 50.4% of the isolates contained *sea* and 26.8% *seb* genes. Literature review revealed that the prevalence of *sea* and *seb* genes in *S.aureus* isolates from this study was much greater than that from other studies. Normanno et al., (2005) studied coagulase-positive staphylococci and *S.aureus* in food products marketed in Italy and showed that 12.5% of the *S.aureus* (1 out of 8) isolated from fish products included *seb* genes; with no *sea* gene found [19]. Puah et al., (2016) investigated various enterotoxigenic genes in *S.aureus* isolated from ready-to-eat foods (sushi and sashimi) and reported that of 32 sushi samples, zero (0%) and one (3.1%) sample contained *sea* and *seb* genes, respectively [20]. Furthermore, of 20 sashimi samples, three (15%) and zero (0%) samples contained *sea* and *seb* genes, respectively. In the present study, 4.1% of the isolates contained TSST-1 gene (4.0% of the isolates from fresh and 4.1% of the isolates from frozen samples). Rhee and Woo (2010) found no TSST-1 gene in 165 *S.aureus* strains isolated from various food samples, 2003-2006 [21]. In the current study, frequency of *sea* genes was higher in *S.aureus* isolated from fresh samples than that from frozen samples while *seb* genes was lower in fresh samples than that of frozen ones. However, repeated freeze and thaw or long-term frozen maintenance of the food samples might result in instability of the bacterial genome and loss of virulence genes [22,23-26]. In general, the wide spread of enterotoxigenic encoding genes whether in fresh or frozen food raises the concern that infections by the *S.aureus* with a high rate of virulence genes may cause severe and long-term diseases in patients.

## CONCLUSION

The high contamination rate of fish samples with *S.aureus* seen in the current study has urged that a serious hygienic surveillance system (e.g. HACCP and ISO standards) is needed to limit food contamination with *S.aureus* and its possible outbreaks. Furthermore, however *S.aureus* isolates partially included the toxin genes, these isolates are supposed to cause further severe infections in the host. In summary, information from this study has provided a better understanding of *S.aureus* spread in seafood in Iran which reflects the current poor process and transport management of the seafood.

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